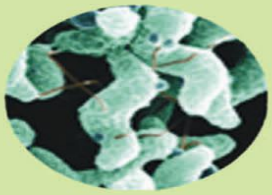
PROCEEDINGS OF UGC NATIONAL SEMINAR

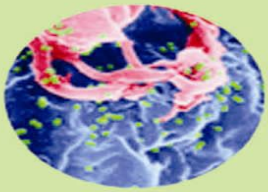
On

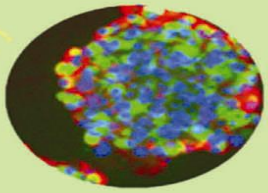
ज्ञान-विज्ञान विमुक्तये

"RECENT TRENDS IN LIFE SCIENCES 2014"

August 7th & 8th 2014







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OBJECTIVES OF THE SEMINAR

- **To develop academic aptitude and scientific temper among the students with regard to life sciences and inspire them to make their career out of it.**
- **To inculcate learning spirit in students and motivate them to keep abreast of advanced knowledge apart maid routine subject.**
- **To bring the students to the closed vicinity of ever developing folds of life sciences and exposing them to current discoveries by providing a common platform for the interaction with eminent scientists, expertise resource persons, researchers and managers of life sciences.**
- **To create awareness about the multifarious applied aspects of life sciences in designing and formulating new products of commercial value.**
- **To prepare the students mindset through intellectual stimulation to take up academic as well as research activities in the unexplored or unfolded areas of life sciences.**
- **To integrate all students, researchers, scientists, academicians, industry policy makers, biology teachers from colleges, university teaching faculties and others interested in life sciences for overall development of the country.**
- **To train and develop skillful and technical human resource to face the emerging challenges in the competitive global scenario where major concern is for quality and cost effectiveness.**
- **To make a right path way between the instruction and job market requirement by building up relevant institutional learning, research innovation and industrial requirement in order to suit the competitive world market.**

MAJOR THEMES

1. **Animal Physiology**
2. **Toxicology**
3. **Pharmacoology**
4. **Exercise Physiology**
5. **Sericulture**
6. **Wild life biology**
7. **Environmental Biology**
8. **Aquaculture**
9. **Ecology**

Key Note Address

Bio Medical Sciences and Biotechnology for Disease Control: A review of Past, Present and Future

By
Prof. K.Sathyavelu Reddy,
Department of Zoology, S.V.University, Tirupati.

Introduction: As we move into the 21st Century it is becoming increasingly clear that biomedical Sciences are entering the most exciting phase of their development. AIDS, tuberculosis and malaria each kill over a million people annually. HIV remains without a cure or vaccine, and is growing rapidly in India and much of the African continent. Antibiotic resistance is a growing concern for organisms such as tuberculosis. Other diseases, such as SARS, Ebola, and flu variations, are also causes for concern. The World Health Organization has warned of a possible coming flu pandemic resulting from bird flu mutations. In 2009, there was an outbreak of swine flu whose country of origin is still unknown.

Industrial countries have not been able to solve the problem of the spiraling costs of health care resulting from technological development, public expectations, and—in particular—the rapidly increasing size of their elderly populations. The people of many developing countries are still living in dire poverty with dysfunctional health care systems and extremely limited access to basic medical care. Against this complex background, today we examine the role of science and technology for disease control in the past and present and assess the potential of the remarkable developments in the basic biomedical sciences for global health care.

Medicine before 20th Century:

Advances in science and philosophy throughout the 16th and 17th centuries led to equally momentous changes in medical sciences. The elegant anatomical dissections of Andreas Vesalius swept away centuries of misconceptions about the relationship between structure and function of the human body; the work of Isaac Newton, Robert Boyle, and Robert Hooke disposed of the basic Aristotelian elements of earth, air, fire, and water; and Hooke, through his development of the microscope, showed a hitherto invisible world to explore. In 1628, William Harvey described the circulation of the blood, a discovery that, because it was based on careful experiments and measurement, signaled the beginnings of modern scientific medicine. After steady progress during the 18th century, the biological and medical sciences began to advance at a remarkable rate during the 19th century, which saw the genuine beginnings of modern scientific medicine. Charles Darwin changed the whole course of biological

thinking, and Gregor Mendel laid the ground for the new science of genetics, which was used later to describe how Darwinian evolution came about.

Science, Technology and Medicine in 20th Century:

Although rapid gains in life expectancy followed social change and public health measures, progress in the other medical sciences was slow during the first half of the 20th century, possibly because of the debilitating effect of two major world wars. The control of communicable disease has been the major advance of the 20th century in scientific medicine. It reflects the combination of improved environmental conditions and public health together with the development of immunization, antimicrobial chemotherapy, and the increasing ability to identify new pathogenic organisms. Currently, live or killed viral or bacterial vaccines—or those based on bacterial polysaccharides or bacterial toxoids—are licensed for the control of 29 common communicable diseases worldwide. The highlight of the field was the eradication of smallpox by 1977. The next target of the World Health Organization (WHO) is the global eradication of poliomyelitis.

Summary of Scientific Medicine in the 20th Century:

In summary, although the 20th century witnessed remarkable advances in the control of communicable disease, the current position is uncertain. The emergence of new infectious agents, as evidenced by the severe acute respiratory syndrome (SARS) epidemic in 2002, is a reminder of the constant danger posed by the appearance of novel organisms; more than 30 new infective agents have been identified since 1970. Effective vaccines have not yet been developed for some of the most common infections—notably tuberculosis, malaria, and HIV—and rapidly increasing populations of organisms are resistant to antibacterial and antiviral agents. Furthermore, development of new antibiotics and effective antiviral agents with which to control such agents has declined. The indiscriminate use of antibiotics, both in the community and in the hospital populations of the industrial countries, has encouraged the emergence of resistance, a phenomenon exacerbated in some of the developing countries by the use of single antimicrobial agents when combinations would have been less likely to produce resistant strains. Finally, public health measures have been hampered by the rapid movement of populations and by war, famine, and similar social disruptions in developing countries. In short, the war against communicable disease is far from over.

Although some of the diseases that produce this enormous burden may be at least partially preventable by the more effective control of risk factors, to what extent such control will be achievable is unclear, and for many diseases these factors have not been identified. In short, scientific medicine in the 20th century, for

all its successes, has left a major gap in the understanding of the pathogenesis of disease between the action of environmental risk factors and the basic disease processes that follow from exposure to them and that produce the now well-defined deranged physiology that characterizes them. It is against this rather uncertain background that the role of science and technology for medical care in the future has to be examined.

Science, Technology and Medicine in the Future:

Without question the fields of molecular and cell biology were the major developments in the biological sciences in the second half of the 20th century. The announcement of the partial completion of the human genome project in 2001 was accompanied by claims that knowledge gained from this field would revolutionize medical practice over the next 20 years. After further reflection, some doubts have been raised about this claim, not in the least the time involved; nevertheless, considerable reason for optimism still exists. Although the majority of common diseases clearly do not result from the dysfunction of a single gene, most diseases can ultimately be defined at the biochemical level; because genes regulate an organism's biochemical pathways, their study must ultimately tell us a great deal about pathological mechanisms.

The genome project is not restricted to the human genome but encompasses many infectious agents, animals that are extremely valuable models of human disease, disease vectors, and a wide variety of plants. However, obtaining a complete nucleotide sequence is one thing; working out the regulation and function of all the genes that it contains and how they interact with each other at the level of cells and complete organism presents a much greater challenge. The human genome, for example, will require the identification and determination of the function of the protein products of 25,000 genes (*proteomics*) and the mechanisms whereby genes are maintained in active or inactive states during development (*methylomics*). It will also involve the exploration of the roles of the family of regulatory ribonucleic acid (RNA) molecules that have been discovered recently. All this information will have to be integrated by developments in information technology and systems biology. These tasks may take the rest of this century to carry out. In the process, however, valuable fallout from this field is likely to occur for a wide variety of medical applications. The first applications of DNA technology in clinical practice were for isolating the genes for monogenic diseases. Either by using the candidate gene approach or by using DNA markers for linkage studies, researchers have defined the genes for many monogenic diseases. This information is being used in clinical practice for carrier detection, for prenatal diagnosis, and for defining of the mechanisms of phenotypic variability.

Stem Cell and Organ Therapy:

Stem cell therapy, or, to use its more popular if entirely inappropriate title, therapeutic cloning, is an area of research in cellular biology that is raising great expectations and bitter controversies. Transplant surgery has its limitations, and the possibility of a ready supply of cells to replace diseased tissues, even parts of the brain, is particularly exciting. Stem cells can be obtained from early embryos, from some adult and fetal tissues, and (at least theoretically) from other adult cells.

Embryonic stem cells, which retain the greatest plasticity, are present at an early stage of the developing embryo, from about the fourth to seventh day after fertilization. Although some progress has been made in persuading them to produce specific cell types, much of the potential for this field so far has come from similar studies of mouse embryonic stem cells. For example, mouse stem cells have been transplanted into mice with a similar condition to human Parkinson's disease with some therapeutic success, and they have also been used to try to restore neural function after spinal cord injuries.

Many adult tissues retain stem cell populations. Bone marrow transplantation has been applied to the treatment of a wide range of blood diseases, and human marrow clearly contains stem cells capable of differentiating into the full complement of cell types found in the blood. Preliminary evidence indicates that they can also differentiate into other cell types if given the appropriate environment; they may, for example, be a source of heart muscle or blood vessel cell populations. Although stem cells have also been found in brain, muscle, skin, and other organs in the mouse, research into characterizing similar cell populations from humans is still at a very early stage

Education:

The central theme of the previous sections is that the potential fruits of the exciting developments in the biomedical sciences will be achieved only if a complete change in attitude occurs on the part of industrial countries, with the evolution of a much more global attitude to the problems of medical research and health care. Change will have to start in the Universities of the industrial countries, which will need to incorporate a more global perspective in medical education so that the next generation of young people is more motivated to develop research careers that take a more international view of the problems of medical research. A major change of emphasis in education will be required and will be difficult to achieve unless those who control the university education and research programs can be convinced that funding is available for further development in these new directions.

Of course, much broader issues involving education need to be resolved for the better exploitation of medical research. People are increasingly suspicious of modern biological science and of modern high-technology medicine, a factor that, together with concerns over the pastoral skills of today's doctors, is

probably playing a role in driving many communities in industrial countries toward complementary medicine. These trends undoubtedly are attributable to inadequacies of medical education and the way that science is taught in schools—reflected by the lack of scientific literacy both in the general public and in governments. If trust is to be restored between the biomedical sciences and the public, significant efforts will have to be made to improve the level of scientific literacy, and a much more open dialogue will need to be developed between scientists and the community. This requirement will be increasingly important as work on basic biomedical sciences impinges on areas such as gene therapy, stem cell research, and the collection of large DNA databases to be used for both research and therapeutic purposes in the future.

Summary:

Research in basic human biology and the biomedical sciences is entering the most exciting phase of its development. However, it is difficult to anticipate when the gains of this explosion in scientific knowledge will become available for the prevention and treatment of the major killers of mankind. Thus, medical research must strike a balance between the well-tried approaches of epidemiology, public health, and clinical investigation at the bedside with the application of discoveries in the completely new fields of science that have arisen from the genome revolution.

If this balanced approach toward the future provision of health care is not to continue to worsen the gap between North and South, however, a complete change of attitude is necessary toward health care research and practice on the part of the industrial countries. A major effort will be required to educate all parties—international nongovernmental organizations, governments, universities, and the private sector—in global health problems. Equally important will be a major change of emphasis in the Universities of industrial countries toward education programs in science and medicine to provide medical scientists of the future with a more global perspective of health and disease. If this transformation can be achieved—if it can form the basis for the establishment of networks for sustainable research programs between universities and related bodies in the North and South—much progress will be made toward distributing the benefits of biomedical research and good practice among the populations of the world. However, the great potential of advances in the biomedical sciences for global health will not come to full fruition without much closer interaction between the fields of basic and clinical research and the fields of public health, health economics, and the social sciences.

Plenary Talk - 1

NOVEL STRATEGY FOR ENHANCING THERAPEUTIC EFFICACY OF STANDARD CHEMOTHERAPEUTIC AGENTS IN TREATING CANCER

Morbidities associated with cancer treatment is a serious concern. Improving the therapeutic efficacy of current standard chemotherapeutic agents is urgently needed. We are working on a strategy to improve the therapeutic efficacy of certain chemotherapeutic agents by testing combination therapies involving a small molecule, Tolfenamic Acid (TA). TA targets Specificity protein (Sp) transcription factors that play critical role(s) in the growth and metastasis of cancer. Sp proteins also regulate the expression of Survivin, a member of Inhibitor of Apoptosis Protein family that is associated with resistance to chemo- and radiation therapies and impacts the prognosis. Our pre-clinical using the models for both adult (esophageal, ovarian, pancreatic and prostate) and pediatric (leukemia, medulloblastoma and neuroblastoma) malignancies demonstrated promising results. TA inhibits cancer cell growth through inducing apoptosis and causing cell cycle arrest. We also found that by suppressing survivin, TA augmented the response of human cancer cells and mouse tumors to radiation therapy by inducing radiosensitization. We also tested the efficacy of TA in a combination therapy along with chemotherapeutic agents (eg., 5FU, Cisplatin, Gemzar, Toptecan and Vincristine). These combination therapies resulted in higher therapeutic response in pre-clinical studies suggesting that TA sensitizes the malignant cells to chemotherapeutic agents. These findings are crucial in developing novel strategies for treating human cancers.

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Presenting Author

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Dr. Basha is working on investigational new drugs focusing on developing novel therapeutic strategies for treating various human cancers. He received young scientist travel award from Asian Pacific Society for Neurochemistry, four research presentation awards from the Society of Toxicology and a research presentation award (1st place) at the International Conference on Drug Discovery & Therapy. Dr. Basha delivered several invited talks in Canada, Dubai, Korea, India, and USA. He co-authored about 60 peer reviewed publications and serving as a reviewer for more than 25 iournals. He served as Guest Editor for two iournals and co-Editor for a book.

Plenary Talk - 2

Animal cell Culture - Production of Biotherapeutics

K.R.S.Sambasiva Rao
Department of Biotechnology, Acharya Nagarjuna University
Nagarjunanagar, Guntur – 522 510, A.P., India

The large scale *in vitro*culture of animal cells including human cells for manufacture of various biotherapeutics and for screening of several new drugs has taken up a pivotal role in the field of biotechnology. And the cell culture technology has become a basis for development of vaccines. The *in vitro* culture of cells for the production of highly effective vaccines against a wide variety of human and animal viral diseases is now playing a significant role in human health. The recombinant DNA technology has made possible to transfer a gene from one species into the cells of another and these transformed cells can produce the encoded gene products for therapeutic use. And in the case where animal proteins cannot be synthesized in bacteria, such proteins can be expressed successfully and potentially in cultured animal cells. In the early eighties the majority of biotherapeutics produced were being from *E. coli* (86%), whereas in early ninety this figure had been dropped to around 40%, whereas 50-60% were being produced using animal cells such as Chinese Hamster Ovary (CHO) cells and Baby Hamster Kidney (BHK) cells. Majority of therapeutic products which are being used for the treatment of cardiovascular diseases (tPA, reteplase), cystic fibrosis (DNases), anemia (erythropoietin), haemophilia (coagulation factors VIII and IX), cancer and viral infections (interferons and interleukins), multiple sclerosis (interferon-beta2) and dwarfism (human growth hormone) were being produced using animal cell culture technology. Monoclonal antibodies which are being used for treatment of various diseases, required in relatively very large amounts and demand large scale manufacturing. Despite the fact that the animal cells considered as production factories for various therapeutics, the major disadvantage is that the production cost is relatively very high and only small production scale when compared with bacteria or yeast cells. The *in vitro* large scale culture of animal cells need complex and specific balanced culture media which contain substrates such as glucose, amino acids, vitamins, salts, trace elements and others. And the large scale culture for industrial purpose is mainly performed in specific bioreactors. Currently, a major development is to design large scale cultivation procedures using the media free of serum additives or protein growth factors is considered to be safer as the transfer of biological contamination can be prevented. Particularly, potential contamination of biopharmaceutical end products with agents that may transfer the factors which transfer the various contaminating factors can be avoided by developing protein/serum free media for related cell culturing processes during large scale culture.

Invited Talk – 1

Metastasis and Angiogensis in Cancer

Dr. C. Thirunavukkarasu*M. Sc., Ph. D., FABMS.,*
Assistant Professor (*Stage II*)
Department of Biochemistry and Molecular Biology
PondicherryUniversity
Puducherry, India.

Cancer is the leading cause of death globally and accounts for 13% of all lethality as per WHO report. The mechanism of initiation and spread of cancer in the living system is quite complicated. The defects in suppressor genes and unregulated growth of cells leads to their dedifferentiation and uncontrolled proliferation. The aggressive properties of cancer cells are marked by its potential of metastasis and angiogenesis. The progression of cancer growth initiates from a primary tumor, where the cells undergo proto-oncogene activation further aggravated by defects in tumor suppression (p53 gene expression).

The primary tumor cells continue to replicate locally at the site of formation in an uncontrolled fashion. The tumor cells overcome the spatial restriction and invade locally by mesenchymal transition mode. Epithelial mesenchymal transition (EMT) aids normal embryogenic morphogenesis of cancer stem cells, which is mediated by the transcription factors ZEB1 and ZEB2. The EMT triggers MMPs which aid in dissolution of β-integrins and cahderins and dissociation of cancer cells within epithelial cell sheets to individual cells, which exhibit multiple mesenchymal properties including aggressive invasiveness. The secretion of SDF1 by stromal cells induce MMP2 and MMP9 by CXCR4 positive cancer cells, which drives the migration of cancer cells.

The locally invading cancer cells further spread by intravasation into lymphatic or vascular circulation, induced by TGFβ and enhanced by tumor associated macrophages (TAM) and epithelial growth factor (EGF). The metastasis from primary site is further promoted by secretion of vasculo endothelial growth factor (VEGF) secreted by the tumor cells to promote formation of new blood vessels. These blood vessels are more tortuous and prone to leakage thus promoting the transport and dislodging of cancer cells to secondary site. Additionally neovasculature is also essential for nutrient supply, drainage of metabolites and supply of humoral factors and immune cells to the tumor growth site.

Metastasis and angiogenesis are thus indispensible for sustenance and effective spread and establishment of cancer. Hence modern therapeutic strategies are being developed targeting the factors involved in neoangeogenesis and EMT. A deeper insight into these pathways and their regulation would pave way in multitargeted therapeutic design to an effective cure for cancer.

Invited Talk - 2

WHITE BLOOD CELLS SAFEGUARD OUR HEALTH

Prof.S.Krupanidhi
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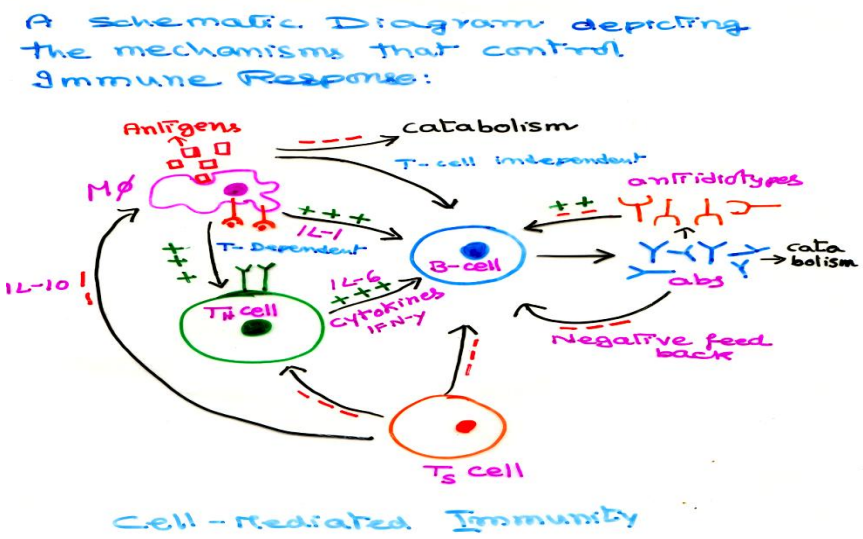
Vertebrate blood is composed of both cellular and fluid components. The cellular components include RBCs and WBCs. The former is involved in the transportation of oxygen from the ventilating region to the deeper parts of organs and tissues. The latter are more important primarily because of their defined role in safeguarding the host organism. Their normal count varies between 4000 to 11,000/cmm. Doctors look at WBC numbers for diagnosis:

- If number goes up there is some kind of infection and surgery might be needed.
- Clinics will count the number of WBC's in a blood sample, this is called differential count.
- A decrease in the number of white blood cells is leukopenia
- An increase in the number of white blood cells is leukocytosis.

WBCs take their origin from the haemotopoietic tissue in the bone marrow and mature in lymph glands. They are boon to our health. The WBCs count determines the state of our health. They are classified as granulocytes and lymphocytes. Granulocytes are: neutrophils, eosinophils, basophils, mast cells and monocytes (which mature as macrophages). They are involved in innate immunity i.e., they encounter pathogens destined to be characterized by pathogen associated molecular patterns (PAMPs) and further the cascade of reactions prompt them to devour the pathogen and process its antigens. To perform this action of safeguarding the host, granulocytes need not be taught. They are all equipped with the receptors popularly known as Toll Like Receptors.At least 5 to 20 bacteria would be killedin the life time of a monocyte (within 10 days).

The lymphocytes are involved in adaptive immunity. They are B cells and T cells. Natural killer (NK) cells also come under lymphocytes. B cells are so called because they are invented in relation to the Bursa Fabricius in birds. It is a gland in the cloaca of birds, whose removal made birds to deprive the potential of adaptive immunity. B-cells respond to T helper cells and as a consequence of promptingB-cells secrete immunoglobulinsthat neutralize the toxic influence and invasion of pathogens. The T-cells are so named because these lymphocytes undergo maturation in the thymus gland. T-cells are very important in the adaptive immunity because they receive the information about bacterial peptides from macrophages and convey to B-cells to synthesize the tailored-made immunoglobulins, as an end product of adaptive immunity which takes care of the pathogen invasion.



Types of WBCs	Normal prevalence in human blood
• Neutrophils	• 62%
• Eosinophils	• 2-3 %
• Basophils	• 0.4 %
• Monocytes	• 5%
• Lymphocytes	• 30 %
– NK cells	– 7%
– Th cells	– 46%
– Tc cells	– 19%
– B-cells	– 23%

CD4 T cells (T helper cells) are the target cells for the HIV. Hence, there is the depletion of T helper cells in AIDs patient. As a result the patient looses the capacity of adaptive immunity and therefore, he is vulnerable to mycotic infections like candida, etc. Yet another incident where the kidney transplant is made to tolerate/accept upon reducing the number of CD 8 T cells (Tc cells). To facilitate the same, anti CD3 treatment regimens are being given in addition to the immunosuppressive drugs. One more inherent immunodeficiency namely X-Linked Agammaglobulinemia (XLA) a condition unable to produce antibodies that are needed to defend against bacteria and viruses. A genetic mistake in a gene called Bruton's Tyrosine Kinase (BTK) deprives B cells from developing normally which causes infection due to*Streptococcus*, *Staphylococcus* and *Haemophilus*.

Therefore, essentially the varied WBCs and their relative roles in combating pathogens and ultimately in supporting life made them an important discipline in both clinical and medical sciences and also became a decision making in the diagnosis of diseases and disorders.

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Invited Talk -3

Stem cell therapy for peripheral artery disease

AlavalaMatta Reddy
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Peripheral artery disease (PAD) is a kind of established inflammatory disease mainly caused by atherosclerosis where arteries become narrow, hardened and occluded. Patients with severe stage of PAD may develop critical limb ischemia (CLI) with symptoms of rest pain and non-healing ulcerations. Despite of available treatment options like endovascular therapy and surgical revascularization, many CLI patients still could not overcome major amputation of legs, cardiovascular diseases or death. Gene therapy with vascular endothelial growth factor (VEGF) is the first step of modern therapeutic options for vascular regeneration, but the results are not promising. In these patients, stem cell therapy is a novel and promising option to promote vasculogenesis, improve vascular growth factors and also tissue perfusion. Mesenchymal stem cells (MSCs) are able to differentiate into a variety of cell types including vascular cells. Mononuclear cells (MNC) component isolated from bone marrow, peripheral blood and umbilical cord blood is the common source of MSC for regenerative medicine. A long-term clinical trial of therapeutic angiogenesis by transplantation of granulocyte colony-stimulating factor (GMCSF) stimulated peripheral blood stem cells (PBSC) in critical limb ischemia patient resulted in limb salvage, decreased pain, improved ankle brachial index and also enhanced peak systolic velocity. Advances in stem cell therapy and angiogenic factors show promise to vascular regeneration therapy, although further basic studies are required to understand molecular mechanisms in detail.

Key words: Critical limb ishchemial, Peripheral blood stem cells, Mesenchymal stem cells, Vasculogenesis

Invited Talk –4

BIO MEDICAL WASTE MANAGEMENT SCENARIO IN INDIA WITH SPECIAL REFERENCE TO ANDHRAPRADESH STATE.

G.Latha[1], **M.Rajasekhar**[2], DV.Ramana[1]

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2. Department of Zoology, S.V.University, Tirupati

Hospital waste has been considered as bio hazardous waste. Improper methods of waste segregation, transportation and disposal are the causatives for several infectious diseases to the biota and poses risk to the environment. Hospital waste has not received enough attention to dispose and incinerate in India. The objective of the present study is to evaluate the growth of health care facilities, amount of hospital waste generated and the usage of Common Biomedical Waste Treatment Facilities (CBMWTF) by health care facilities in India. A comprehensive study has been carried out in Andhra Pradesh. It was estimated that 2,10,680kgs /day is being left untreated in India. Average hospital waste generated in South India is 1.04 kgs /bed/ day. The average waste generation per bed per day is very less in central India. It was identified that CBMWTF's are working well all over the India. All Government Health Care Facilities are properly using CBMWTF's in Chittoor district when compared to Private Health Care Facilities for their waste disposal. Waste handling practices, segregation methods, and usage of CBMWTF in Chittoor District were thoroughly evaluated and found significant results. It is concluded that the hospital waste generation rate is high and increasing year to year. Waste handling practices and segregation methods need to be improved among all private Health Care Facilities. Health risk of occupational exposure and incidence of infections related to hospital waste were at concerned levels. Education, training and practice guidelines for waste management are very much needed.

Key words: Hospital Waste, Bio hazardous waste, Waste Management Practices, Health care waste, Medical waste

Invited Talk - 5

Prospects in Diatom research for new bioactive compounds and biofuels.

Thomas Kiran.M.

Chief technology officer, Kadambari Consultants Pvt. Ltd., Hyderabad, India

In recent years, there has been tremendous interest in microalgal metabolites among researchers such as natural products chemists, pharmacologists, and biochemists. There are two major reasons for this surge of interest. First, it has been recognized that microalgae can be a source of new types of metabolites or potential drugs. In the past, drug searches have been focused mostly on organisms such as actinomycetes, fungi, and higher plants. Here, people are increasingly isolating known compounds or close analogues of known compounds, and the task is becoming more and more repetitive and wasteful. Meanwhile, microalgae have yielded new types of structures not found in higher plants or other traditional drugs sources. The second reason that microalgae have been attracting so much attention is the realization that they may be a primary source of some exciting molecules found in marine invertebrates. In the past two decades, a great number of new structures with unique biological activity have been found in marine invertebrates. Many of them are potential therapeutic drugs, but their supplies are very limited. Of all the micro algal species diatoms is a major group having enormous potential in neutraceutical and biofuel industry.

A number of commercial developments have occurred in microalgal biotechnology in recent years. New products are being developed for use in the mass commercial markets as opposed to the health food markets. These include algal derived long chained polyunsaturated fatty acids, docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) for use as supplements in human nutrition and animals, pigments in food and pharmaceutical industry, aquaculture and poultry, fertilizers and agrochemicals, for effluent treatment and algae for other bioactive compounds (Borowitzka, 1992, Lebeauetal., 2003). Diatom extracts demonstrated anti-tumoral activities against human lung cancer and also had anti-HIV effects (Berge, 1997, 1999).

In this article we want to explore the enormous potential of microalgae as precursors to life saving bioactive compounds, Natural food in Aquaculture, Bioremediating agents and precursors for biofuel production. This will provide a new insight on nano technology assisted Growth, Biomass production, Lipid production and bioactive molecule production in indigenous microalgae.

Invited Talk - 6

Challenges of Ionizing Radiation on Tumor Treatment and Role of Angiogenesis

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Ionizing radiation is a non-specific, but most widely used therapeutic method for cancer treatment. However a minor fraction of tumor cell population manages to survive after radiation. Radiation efficacy depends on adequate oxygen supply. Rapid growing tumors cause hypoxia that up regulates many pro-survival pathways. At clinical doses, radiation activates inflammatory pathways and causes oxidative stress that plays a positive role during angiogenesis. Selective targeting of signaling mechanisms may radiosensitize tumors.

Invited Talk - 7

Assessment of Microbial Contamination of Ethiopian Currency Notes

Harikrishna Ramaprasad Saripalli[1], Lydia Swapna Nandam[2], Zenebe Teka[3]&Haftom Kebede[4]
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ABSTRACT

Microbial contaminations of the Ethiopian currency have been receiving very poor attention.The objective of this study was to investigate the extent of degree of bacterial contamination of Ethiopian paper currency notes. A total of 25 old paper currency notes and 15 fresh paper notes, with five representatives of each denomination, were collected from artisans (supermarkets, bakeries, butcheries, taxi drivers, milk sellers, merchants, fruits and vegetable sellers and bajaj drivers) and commercial bank of Ethiopia respectively. By means of broth wash and appropriate culturing the microbes were isolated, characterized, and then identified to genus level. Results showed that all the samples had bacterial growth of which count varied based on the degree of dirtiness of the birr. Old paper notes bear much more microbial colonies and gram positive bacteria were much higher in number than those of the gram negative ones. But there was no any relationship between the degree of contamination and denominations which probably might have resulted from the same constructional substance of the paper notes. As confirmed by biochemical tests the following pathogenic and potentially pathogenic bacterial genera were isolated from the currency notes: *Escherichia coli, Staphylococcus spp., Bacillus, Klebsiella, Streptococcus, Serratia, Salmonella, Pseudomonas, Citrobacter, Shigella, Listeria, Enterobacter* and *Micrococcus*. The only two fungal genera found on Ethiopian paper notes investigated here were *Aspergilus* spp and *Penicillium* spp. Moreover the estimated number of microbes on each paper note is also given. The Ethiopian currency notes are found contaminated with pathogenic or potentially pathogenic microbes; in rare cases these contaminated paper currencies could act as a vehicle of transmission of diseases. So, an efficient public awareness and raising conciseness seem to be necessary. Therefore, it seems that creating public awareness on this issue may help minimize the occurrence of disease from microbial contamination.

Key words: Microbial Contamination, Ethiopian currency notes, Public health

INTRODUCTION

The Ethiopian birr (Amharic) is the nomenclature of currency in Ethiopia. Before 1976 dollar was the official English translation of birr. Today it is officially referred to as birr in English as well. In 1931 the Emperor of Ethiopia, Haileselassie formally requested that the international community use the name Ethiopia instead of Abyssinia and the issuing bank of Abyssinia also became the bank of Ethiopia. The Ethiopia birr is the second most used currency in Africa with 88 million users after Nigerian Naira. One hundred eighty six billion birr was in circulation in 2008(en.wikipidan. org).

Paper currency is widely exchanged for goods and services in Ethiopia and in most other countries worldwide. In 1999 the United States department of Treasury printed more than 35 billion one dollar bills each with life span of about 18 months. In recent study 94% of United States one dollar bills were found to be contaminated with potentially pathogenic microorganisms. The possibility that currency notes might act as vehicles or fomites for transmission of pathogenic microorganisms was suggested in 1970s (Pope *et al*., 2002, Pinner, *et al*., 1996).

Bacteria have been shown to be spread from person to person via contact with fomites. Paper currency is commonly and routinely passed among individuals. Thus bacteria could be spread on the surface of paper currency. Paper currency can be contaminated by droplets during coughing, sneezing, touching with previously contaminated hands or other materials and placement on dirty surface and it is commonly handled by various categories of people during transaction. Contamination of objects by pathogenic microorganisms is much public health concern that can be source of transmitting pathogens (Pope, *et al*., 2002).

Paper currencies are widely used and each currency is exchanged many times during the time it circulates. If some of these papers are contaminated with pathogenic bacteria, there is a possibility to spread these microorganisms, to uncontaminated ones.

Comprising a large number of organisms, microbes are found everywhere including on paper notes frequently exchanged among different people of different social classes and

occupations; even among children. Therefore contaminated paper notes can result in spreading dangerous diseases caused by pathogens. People with different skins, sweat pH, skin secretions, activities and diseases (skin, digestive and respiratory tract diseases) bear too many different and probably disease causing microbes which can be transferred easily via handling money (Abrams BL and Waterman NG, 1972).

There are several studies on the microbial contamination of currency notes worldwide (Abrams BL and Waterman NG, 1972; El- Dars FM and Hassan WM, 2005; Goktas P and Oktay G, 1992; Jakir hosen M *et al*., 2006; Khin NO *et al*., 1989; Oyero OG and Emikpe BO, 2007; Pope TM *et al*., 2002; Shekarforoush Sh *et al*., 2009; Umeh EU *et al*., 2007 and Xu J, Moore JE and Millar B Ch, 2005) and many pathogens have been identified including *Staphylococcus, Escherichia, Klebsiella, Shigella, Salmonella, Bacillus, Pseudomonad, Diphtroids, Citrobacter* and so on. However, degrees of contamination and types of microbes may be area-dependent because of the texture of paper notes, sanity condition and microbe endemism (El, Dars, FM and Hassan WM, 2007).

Microbial contamination of paper money is not only confined to developing nations. Several studies from the United States reported contamination of coins and paper bills and identification of the presence of pathogenic microbes like *Staphylococcus aureus, E.coliKlebseille, Enterobacter* and others (Abrams BL and Waterman NG, 1972).

A study in Egypt also reported that 65% of the paper bills had bacteria like *Staphylococcus albus, Staphylococcus aureus* and *Klebseille pneumonia*. In addition to this various pathogenic microbes associated with tuberclosis, meningitis, pneumonia, tonsillitis, peptic vlcers, genitial tract infections, gastroenteritis and throat infection had been identified in damaged or spoiled notes held together with bits of sticky tapes (Goktas P and Oktay G, 1992).

In Bangladesh, research studies reported high rates of microbial contamination of currency notes in circulation. The micro organisms implicated included members of family Enterobacteriaceae, *Mycobacterium* sps.,*Vibrio* sps., *Bacillus* species, *Staphylococcus* species, *Micrococcus* species and *Corynebacterium* species. Most likely contaminants of paper money

are environmental organisms such as gram positive flora (especially bacillus species) and those arising from human normal skin flora such as *Staphylococcus aureus* (Charnock. C, 2005).

Since money is very important for human life as it facilitates the needs and currency notes have vital role for exchange of goods and services worldwide, these paper notes and coins can be contaminated by microbes when they come in to contact with skin, anal region, wounds, nasal secretions generated by sneezing, coughing and some persons entering these paper notes and coins in to their mouth. So this microbial contamination of currency notes could lead to transmission of diseases from person to person that causes different types of diseases such as tuberculosis, pneumonia and other communicable diseases. These can be spread by different means such as food, water, air, soil etc., and thus have risk on public health of the world population. To minimize the risk of microbial contamination by paper currency notes, it requires special consideration to conduct complex studies and to prove the pathogenic nature of the microbes.

The main concern of this study was to confirm whether the Ethiopian paper currency notes are contaminated by bacteria & fungi; then to isolate, characterize, identify the microbes and then raising public awareness during currency handling.

MATERIALS AND METHODS

Sample collection:

A total of 25 readily available old paper notes (paper notes in circulation) and 15 intact fresh paper notes (paper notes newly minted and were not entered in currency system) in Axum, Ethiopia were used in this study. The notes selected for the study comprised nine series of 5 currency notes each. Three of which included Birr 100 (units of currency in Ethiopia is Birr) fresh notes and the rest included old notes of Birr 1, Birr 5, Birr 10, Birr 50 and Birr 100 (table 1). Samples were collected wearing sterile gloves from different occupation groups such as supermarkets, bakeries, butcheries, taxi drivers, milk sellers, merchants, fruits and vegetable sellers, Bajaj drivers and so on and fresh paper notes from Commercial Bank of Ethiopia,

Axum Branch, Ethiopia. Each paper note was kept in separate sterile nylon bag until the preparations took place.

Table 1. Numbers of old and fresh paper notes examined for each denomination

Type of Currency Notes	Birr 1	Birr 5	Birr 10	Birr 50	Birr 100	Birr 100 (1)	Birr 100 (2)	Birr 100 (3)
New Notes	0	0	0	0	0	5[b]	5[b]	5[b]
Old notes	5[a]	5[a]	5[a]	5[a]	5[a]	0	0	0

[a]Paper notes collected in sterile condition from artisans

[b]Paper notes collected in sterile condition from bank treasury

Table 2. Data acquired on old paper notes: the number of colonies on each paper note, the types of gram positive and gram negative bacteria and the number of fungal colonies.

Old Currency Note Birr 1								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$8x10^3$	1	1	-	1	-	5	$9x10^3$
2	$6x10^3$	2	2	-	-	2	-	
3	$15x10^3$	9	3	2	-	-	1	
4	$7x10^3$	3	1	1	-	-	2	
5	$9x10^3$	6	3	-	-	-	-	
Old Currency Note Birr 5								
S No	No. of colonies (CFU)	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$21x10^3$	12	9	-	-	-	-	$24x10^3$
2	$58x10^3$	4	-	1	-	30	23	
3	$4x10^3$	4	-	-	-	-	-	
4	$32x10^3$	26	3	2	1	-	-	

5	$5x10^3$	2	3	-	-	-	-	
Old Currency Note Birr 10								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$44x10^3$	1	38	-	5	-	-	$23x10^3$
2	$3x10^3$	1	2	1	-	-	-	
3	$3x10^3$	-	3	-	-	-	-	
4	$28x10^3$	4	21	-	1	-	2	
5	$37x10^3$	35	2	-	-	-	-	
Old Currency Note Birr 50								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$29x10^3$	-	28	-	-	-	1	$20x10^3$
2	$23x10^3$	8	10	-	-	2	3	
3	$15x10^3$	12	3	-	-	-	-	
4	$4x10^3$	1	3	-	-	-	-	
5	$29x10^3$	28	1	-	-	-	-	
Old Currency Note Birr 100								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$14x10^3$	10	4	-	-	-	-	$15x10^3$
2	$15x10^3$	9	6	-	-	-	-	
3	$10x10^3$	10	-	-	-	-	-	
4	$12x10^3$	6	6	-	-	-	-	
5	$13x10^3$	6	1	-	-	-	6	

Microbe isolation, culturing and characterization:

For microbial isolation each paper note was treated by 100 cc broth wash. Using a sampler 0.1 cc of the microbe bearing liquid was transferred into sterile appropriate media (Abrams BL, Waterman NG, 1972; Goktas P and Oktay G, 1992; Khin NO *et al*.,1989 and Pachter B R *et al*., 1997). The number of colonies were then counted using colony counter after

incubation for 72 hours at 37ºC and pure colonies were isolated by using streaking and restreaking method where pure colonies can be identified by using colony morphology, growth patterns, colony pigmentation, etc. Once pure colonies were isolated, they were categorized into gram positive and gram negative by using the gram-staining technique. After gram staining the colonies were identified to genus level by using biochemical tests. Here to identify the microbes to genus level Mac-conkey agar, blood agar, and chocolate agar were used for the bacterial colonies. For the fungal identification, potato dextrose agar (PDA) was used and they were identified based on their morphological features, growth pattern, spore formation and others. The total number of cells on each paper note was calculated by using the formula which was given below; the results were tabulated in table2 and table3.

$$\text{Total Number of Cells} = \text{Number of Colonies} \times \frac{1}{\text{Dilution Factor}}$$

DISCUSSION

Noting the significant occurrence of microbes on old paper notes and their negligible occurrence on the fresh ones gave clues that their sources should be the places where the paper notes were collected. The skin, respiratory system, digestive system and also other places where notes are deposited or come into occasional contact such as earth, mud and so on, are the major sources of the microbes.

Owing to their constructional substance (which is pure cotton), the Ethiopian paper notes` cannot be suitable media for microbial growth, and multiplication. So notes may play a major role as a vehicle and a surface area for microbial transfer. It means that frequency and duration of presence of a specific microbe should not be expected to prevail the others. Naturally, microbes found on paper notes come from the sources with which they come into contact.

Since Paper notes are commonly contaminated with frequently high pathogenic or potentially pathogenic microbes, they may cause serious diseases. As reported earlier, there is no strong relationship between demonstrations and degrees of contamination, probably because of the same substance (pure cotton). However, use of polymer paper notes may decrease contamination to a large extent; it has been well shown that currency provides a surface area for microbe establishment and functions as a strong vehicle in their transmition. The microbial load of the currency note may vary depending on the source of the sample. For example, the highest intestinal pathogen contamination was reported in butcheries and the

lowest isolates were observed on paper notes obtained from office workers. As a result, the poorer the sanity condition, the more the contamination will be. The matter of great importance is that not only children and foods are susceptible to microbial infections, but also vulnerable people and patients of weakened immune system as well as healthy people are prone to serious dangers, due to presence of different and abundant pathogens on paper notes.

Table 3. Data acquired on fresh paper notes: the number of colonies on each paper note, the types of gram positive and gram negative bacteria and the number of fungal colonies.

New Currency Note Birr 100								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$2x10^3$	-	-	-	-	2	-	$2x10^3$
2	0	-	-	-	-	-	-	
3	$3x10^3$	1	-	-	-	1	1	
4	$2x10^3$	-	2	-	-	-	-	
5	$3x10^3$	-	-	-	-	3	-	
New Currency Note Birr 100								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$4x10^3$	-	-	-	-	2	2	$2x10^3$
2	0	-	-	-	-	-	-	
3	$3x10^3$	-	-	-	-	2	1	
4	0	-	-	-	-	-	-	
5	$3x10^3$	2	-	-	-	1	-	
New Currency Note Birr 100								
S No	No. of colonies CFU	Gram +ve		Gram –ve		Fungi		Average No. of colonies
		Bacillus	Coccus	Bacillus	Coccus	Asp.	Pen.	
1	$3x10^3$	-	-	-	-	1	2	$3x10^3$
2	$2x10^3$	-	-	-	-	-	2	
3	$2x10^3$	-	-	-	-	-	2	
4	$3x10^3$	-	-	-	-	-	3	
5	$5x10^3$	-	-	-	-	2	3	

The pathogenic or potentially pathogenic bacterial genera found on these Ethiopian currency notes, namely *Escherichia coli, Staphylococcus spp., Bacillus, Klebsiella, Streptococcus, Serratia, Salmonella, Pseudomonas, Citrobacter, Shigella, Listeria, Entrobacter*, and *Micrococcus* (which are similar to previous studies in different countries) may cause a wide variety of diseases from food poisoning, wound and skin infections, respiratory and gastro intestinal problems to life threatening diseases such as meningitis, septicemia and the like. The pathogenic genus *Aspergillus*, reported earlier on the Nigerian, Iranian and other countries currency, was found frequently on the paper notes investigated in this study even on the intact newly minted ones. The inhalation of its spores may lead to severe pulmonary aspergillosis. Moreover the *Penicillium* spp., are occasional causes of infection in man, the resulting disease is called penicilliosis. The above mentioned diseases and many others threaten Ethiopian Children, hospital hosts, immunocompromised patients and even healthy people who are in contact with paper notes.

Therefore, the following recommendations and suggestions have been given to reduce currency contamination and improve public health:

1. Enhancement of public awareness of currency contamination through media in order to keep currency clean, out of children reach and away from foods and more care is taken while handling money

2. Use of washable polymer paper notes

3. Disinfection of paper notes in banks by ultra violate light, supersonic and chemicals (Singh *et al*., 2002).

4. Of course electronically transaction replaces the traditional methods of trading in which paper notes play a key role. The later can greatly decrease wasting national resources on replacing tattered paper notes.

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Phytochemical analysis and antihepatitic activity of phenolic constituents *of Phyllathus niruri*

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Abstract

Phyllanthus niruri is a traditional Indian medicine belonging to the Phyllanthacae family. The aim of this study was to identify the potential compounds responsible for antihepatitic activity of Phyllanthus niruri. The Ethyl acetate, Hexane of phyllanthus was used to know the phytochemicals and phytochemical contents. Our phytochemical research of these extracts led to the isolation of various bioactive constituents. The chemical structures will be determined by spectroscopic analyses. Among phytochemicals phenols etc. The main phytochemicals are alkaloid, terpenoids, flavonoids etc. Of them Phenol content was more in Ethyl acetate than Hexane extract.

Key words: Phyllanthus niruri, phytochemicals, Hepatitis

Introduction:

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds (Krishnaiah et al., 2007). Many plant families have been reported to have ethno medicinal application. Phyllanthus niruri is a plantwidely found in India and is popularly used for the treatmentof renal pathologies, particularly urolithiasis. Themedicinal properties of this plant have been associated withsome of its active components such as lignans, glycosides,alkaloids, ellagitannins, terpenes and phenylpropanoids,besides flavonoids and polyphenols, such as quercetin, rutin and gallic acid (GA). Although many in vitroand in vivo antioxidant effects of P. niruri extracts havebeen shown (Bagalkotkar et al., 2006; Amin et al.,

2012), which seem to be determined by itspolyphenolic components (Manach et al., 2005), the bioavailability of thosecomponents and their impact on human health need furtherinvestigation.

Phytoconstituents present in the P.niruri responsible for its pharmacological properties includelignans, alkaloids, flavonoids, benzenoids, coumarins,tannins, diterpenes, triterpenes, sterols, phytallates and lipids.

Considering all these facts, the present study was designedto investigate the presence of various phytochemicals in the two different extracts of Phyllanthus niruri, a plant which evokes various therapeuticeffects.

Materials and Methods

Solvent Extraction

The whole plant materials was air dried until all the water molecules evaporated and plants become well dried for grinding. After draying, the plant material were grinded well using mechanical blender into fine powder and transferred in to air tight containers with proper labelling for future use. The Ethyl acetate, Hexane extracts was prepared by 100gms of powdered plant material soaked in 500ml of different solvents in room temperature at 72h. The extracts were filtered through muslin cloth and through what men filter paper (Grade 1). Extracts are concentrated by using water both contains rotary evaporator. Total yield of plant extract ranges from 5 -6% respectively.

Phytochemical Screening

In the present study, solvents like Ethyl acetate, Hexane are used to extract the phytochemicals from *Phyllanthus niruri* by using standard protocols.

1. Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of reddish brown precipitate (or) coloration.

2. Test for Terpenoids (Salkowski Test)

0.5 gram of each extract was added to 2 ml of chloroform. Concentrated sulphuric acid(3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

3. Test for Tannins

About 0.5 gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

4. Test for Saponins (Foam test)

To 2ml of extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

5. Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or coloration).

6. Test for Cardiac glycosides (Keller Kelliani's test)

5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

7. Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

8. Test for reducing sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

9.Test for Flavonoids

To portion of the dissolved extract, a few drops of 10 % ferric chloride solution were added. A green or blue colour indicates the presence of phenolic nucleus.

10. Test For Resins [8]

10 ml of petroleum ether extract was obtained in a test tube, the same amount of cupper acetate solution was added and the mixture was shaken vigorously and allowed to separate, a green colour indicates the presence of resins.

11. Sterols and Steroids (Salkowski's Test)

One ml of extract was treated with 2 ml of chloroform and equal amount of concentrated sulphuric acid was added, upper layer is turns to red indicates the presence of the sterols and steroids.

Results

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, reducing sugar, flavonoids, alkaloids and phlobatannins were present in the samples.

Investigations on the phytochemical screening of *P. niruri* Ethyl acetateextract revealed the presence of alkaloids, terpenoids, cardiac glycosides, phenols, flavonoids, resins, steroids. However in the Hexane extract only alkaloids resins, steroids are seen. These phytochemicals are biologically active. (Table 1).

The presence of these phytochemicals has been attributed to the bioactive principles responsible for ethnopharmalological activities of most medicinal plant. This dictates why efforts have been expanded in studies aimed at elucidating their levels in medicinal plant (Edeoga et al.,2005). The medicinal values of plants are dictated by their phytochemicals and other chemical constituents (Fallah et al., 2005). The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported.

Discussion

The investigation of plants as potential sources of new drugs to treat cancer, AIDS diabetes, parkinson's and malaria requires the search of as many resources as possible, the discovery of Phytochemical compounds with, for example, cytotoxic and /or anti-tumour activity could lead to the production of new drugs for the treatment of various diseases. Therefore, the development of appropriate extraction methods in order to obtain plant extracts with as many phytochemical compounds as possible is important.

Table1. Phytochemical screening of *P.niruri* in different solvent extracts

S.No	Phytochemical Constituents	Hexane extract	Ethyl acetate extract
1.	Phenolic Compounds	_	+
2.	Saponins	_	_
3.	Flavonoids	_	+
4.	Terpenoids	_	+
5.	Alkaloids	+	+
6.	Tannins	_	_
7.	Cardio glycosides	_	+
8.	Steroids	+	+
9.	Reducing Sugars	_	_
10.	Anthraquinones	_	_
11.	Resins	+	+

+Present; -Absent

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of (Varadarajan et al., 2008) pharmacologically active chemical compounds.

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceuticals research. Approximately 3000 plants species are known to have medicinal properties in India.The Rigveda (3700 B.C.),

mentions the use of medicinal plants. Our traditional systems of medicines, viz., Ayurveda, Yunani, Siddha and Homeopathy etc. use herbs for treatment. It is estimated that 40% of the world populations depends directly on plant based medicine for their health care. The present study was under taken to identify the phytochemicals present in PN extracts with suitable solvents such as Hexane and ethyl acetate.

The plant have traditionally provided a source of hope for novel drug compounds, as plant herbal mixture have made large contributions to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment.

In the present investigation, Phytochemical screening of *Phyllanthusniruri* has been done in Hexane and ethyl acetate. Table.1 represents the results of Phytochemical screening of PN. PN contains phenolic compounds, flavonoids, terpenoids, alkaloids, cardio glycosides, steroids, resins but in Hexane extract only alkaloids, steroids and resins.

Flavonoids also known to have a wide array of therapeutic activities as antihypertensive, anti-rheumatism, antimicrobial, diuretic and antioxidants (Trease & Evans 2002).

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The various extracts PN have revealed the presence of Triterpenoids, Steroids, Glycosides, Alkaloids, Flavonoids. Alkaloids, flavonoids were found to in PN. From this analysis, PN ethyl acetate have more constituents compared to Hexane extract. The results of preliminary phytochemical analysis are shown in Table 1.

Alkaloid has nemerous functions and among them foremost is their analgesic, antispasmodic and bacteriological effects. Alkaloids with medicinal properties and are used in

the management of cold, chronic catarrh, persistent headaches and migraine. The antibacterial properties of tannins have been documented (Gill 1992). Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. It has been reported to have analgesic properties.

The Phytochemical screening and quantitative estimation of PN yields the most promising secondary metabolites such as alkaloids, flavonoids, phenol, proteins, amino acids tannin, and carbohydrates. They were known to show the medicinal activity as well as physiological activity . Alkaloid, tannins, terpenoids, flavonoids and phenol are found abundant in the ethanol samples, it should be noted that phenol components are of importance and interest in pharmacy due to their relationship with cancer activity.

These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides and alkaloids.

From the present study, we can deduce that, PN may serve as constituents of animal and human diet supplying the body with minerals, proteins and energy. The presence of secondary metabolites that are biologically important contributes to its medicinal value and thus can be potential sources of useful drugs.

Conclusion

Results obtained in this study have considerable value with respect to Phytochemical screening of phenols and flavonoids. These results suggest that ethyl acetate and Hexane are used for isolation of novel bio active compounds in ethno medicinal and development of potential drugs.

In this study PN have a various chemical groups in their chemical composition. The importance therapeutic and medicinal of PN extracts, it would be interesting to purify and identify different molecules and tested it in vitro and in vivo by exploiting on animal models, to specify this therapeutic application.

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Aluminium-induced oxidative stress in kidney tissue of rats: protective effect of vitamin E

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Abstract

The present study was aimed to investigate the ameliorative effects of vitamin E against aluminium-induced nephrotoxicity in rats. Healthy rats were allocated into four groups (n=6/group). Rats in group I served as controls and rats in groups II (aluminium exposed), III (Vitamin E administered) and IV (aluminium + vitamin E exposed) were treated as experimental animals. Significant increase ($p<0.01$) in the lipid peroxidation levels with a significant decrease ($p<0.01$) in the activity levels of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase were observed in the kidney tissue of aluminium exposed rats over controls. Furthermore, glutathione content was also significantly reduced ($p<0.01$) in kidney tissue of aluminium exposed rats. On the other hand, administration of vitamin E significantly ($p<0.01$) reversed the changes induced by aluminium in the kidney tissue of rats as compared to aluminium alone exposed rats. No significant changes were observed in the selected biochemical variables in vitamin E alone treated rats over controls. However, a significant increase ($p<0.05$) in the glutathione content was observed in vitamin E treated rats over controls. From the results it can be concluded that aluminium induced nephrotoxicity mediates oxidative stress in rats. On the other hand, vitamin E through antioxidant property mitigates aluminium induced oxidative stress in the kidney tissue of rats. Further, we also extended our studies using in silico analysis to understand the exact link between vitamin E, aluminium and glutathione related enzymes. The results obtained will be discussed.

1. Introduction

Aluminium (Al) is a common element available in the earth's crust which constitutes about 8%. Al occurs naturally in silicates, cryolite, and bauxite rock. It is believed that the use of aluminium and its compounds increased worldwide because of their wide applications in a range of activities including building, canning, automobile, tanning, paper and glassware industries. Moreover, it is also used in medicines such as antacids and also cosmetics such as perfumes and also as food additives. Recently, International Agency for Research on Cancer classified Al as a carcinogenic to humans. Human studies indicated that occupational exposure to Al causes neurological disorders, pulmonary diseases, liver disorders, bladder cancer and fertility

problems. Previously it has been shown that Al could be an etiologic factor for amyotrophic lateral sclerosis, Alzheimer's and Parkinson's disease (Krewski et al., 2009). Although a range of toxicological studies indicated the adverse effects of Al, studies related to the Al-induced nephro-toxicity are little exploited (Krewski et al., 2007). Kidneys play a major role in the elimination of Al (approximately 95%) from the body (Sargazi et al., 2006). Studies of Roy et al. (1991) reported that Al exposure causes renal tissue degeneration including deterioration of renal cortex tubules and recently, studies of Mahieu et al. (2003) indicated that Al affects renal metabolism and alters tubular transport mechanisms. Many studies also indicated that Al induced toxicity mediates oxidative stress by altering pro- and anti-oxidant status in mammalian experimental models (Krewski et al., 2007). Recently, studies of Kutlubay et al. (2007) indicated that Al accumulates in kidneys and provokes generation of reactive oxygen species thereby affects vital components of cell such as lipids, proteins and nucleic acids thereby negatively targets structural and functional integrity of kidneys. However, the exact mechanism(s) of action of Al-induced nephrotoxicity in altering pro- and antioxidant status are not well defined.

Vitamin E is a classical antioxidant that plays an important role in counterattacking lipid peroxidative products including reactive oxygen species (Traber et al., 2007). Because of its potential antioxidant property many studies evaluated the protective effects of vitamin E against a range of free radical induced pollutants (Jalili et al., 2007; Al-Attar et al., 2011).

Considering the facts that 1) studies related to the mechanism(s) of action of Al-induced nephrotoxicity are little exploited 2) Al generates reactive oxygen species in biological tissues including kidneys and 3) vitamin E is a classical antioxidant that is widely used against oxidative stress induced pollutants, the present study was aimed to investigate the protective effects of vitamin E against Al-induced oxidative stress in kidneys of rats.

2. Materials and methods

2.1. Procurement and maintenance of experimental animals

Male healthy rats of Wistar strain purchased from Indian Institute of Sciences, Bengaluru India were used in the present study. Rats were housed in polypropylene cages (18" x 10" x 8") lined with sterilized paddy husk, and provided filtered tap water *ad libitum* and standard rat chow (purchased from Sai Durga Agencies, Bengaluru, India). All the rats were maintained in a well regulated environment (temperature 25 ± 2°C; 12-hour light and 12-hour dark cycle, humidity 50 ± 10%) in the animal house facility at Watson Life Sciences, Tirupati.

2.2. Chemicals

Aluminium chloride and Vitamin E were selected as test chemicals. They were purchased from Sigma Chemical Company, St Louis, MO. Chemicals such as glutathione oxidized and NADPH were also purchased from Sigma Chemical Company, St Louis, MO. All other chemicals used in the present study with highest purity were obtained from local commercial sources.

2.3. Experimental design

Twenty four healthy rats with a body weight of 110 ± 10 g were selected for the present study. The animals were randomly allocated into four groups of six rats each. Animals in group 1 served as controls and animals in groups 2, 3 and 4 were treated as experimental groups. The experimental period was 30 days. The selection of doses of test chemicals was based on previous studies.

Group 1: unexposed rats

Group 2: rats exposed to Al (orally through gavage) alone at a concentration of 150 mg/Kg/day

Group 3: rats exposed to vitamin E (I/P) alone at a concentration of 15 mg/Kg/day

Group 4: rats received both Al+vitamin E at same doses

The body weights of rats were recorded before and after completion of experimental period. After completion of the experimental period, all the rats were euthanized by cervical dislocation. Kidney was isolated immediately, weighed to the nearest milligram using Schimadzu electronic balance and after weighing, the tissues from all the control and experimental groups were carefully stored at -80°C until further use.

2.4. Biochemical assays

Kidneys homogenates were obtained by using tissue homogenizer. Kidney homogenates (10% w/v) were prepared using a homogenizing buffer (100 mM KCl containing 0.3 mM of EDTA; pH: 7). All the homogenates were centrifuged at 1500 rpm for 30 min at 4°C to separate cell debris. Further, supernatant was centrifuged at 16000 rpm for 1 hr at 4°C. The supernatant was used for biochemical analysis. The biochemical parameters selected and performed according to standard protocols (1) Thiobarbituric acid reactive substances (TBARS) (lipid peroxides) were estimated according to method of Okawa et al. (1979). (2) Reduced glutathione was estimated by the method of Sedlak and Lindsay (1968). (3) The activity of superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (1972). (4) The activity of

glutathione peroxidase (GPx) was assayed according to the method of Rotruck et al. (1973). (5) Catalase (CAT) activity was assayed by the method of Takahara et al. (1960). (6) The activity of glutathione-**S**-transferase (GST) was measured by using the method of Habig et al. (1974). (7) Glutathione reductase (GR) activity was assayed as described by the method of Dulber and Anderson (1981). The protein concentrations were determined using Lowry et al. (1951).

2.5. In silico analysis

Gamma-glutamyl cysteine ligase (gGCL) plays an important role in glutathione metabolism. Therefore, it was selected as the test protein. The protein sequence of GGCL from Rat was retrieved in fasta format from NCBI database (http://www.ncbi.nlm.nih.gov/) with the AC No: NP_036947.1 and it was subjected to BLAST similarity search against PDB data base to find out the possible template. The template with maximum identity was chosen and its sequence and structure were obtained. Sequence alignment was carried out between the GGCL and template sequences in PIR format, incorporated with python script and successfully run through modeller to construct the homology model of GGCL. The generated model was evaluated with Ramachandran plot by submitting to Procheck and ProsaWeb analysis server. The residues that are observed in the disallowed regions were subjected to loop modelling through ModLoop server (https://modbase.compbio.ucsf.edu/modloop/). The gGCL model obtained from the homology modelling process was loaded into MOE workspace and the missing hydrogen atoms were added. The structure is subjected to protonation at a temperature of 300K, pH of 7 and 0.1 mol/L in the generalized born implicit solvent. The electrostatic potential terms were applied with a distance cut off of 15Å and dielectric constant of 1. Further the protonated structure was energy minimised in the MMFF94x force field to an RMS gradient cut off of 0.05. The final optimized conformation of the protein was saved and used for further studies.

The site finder module of MOE was used to predict the potential possible binding sites on the gGCL. The probing was done with hydrophobic and hydrophilic terms by means of alpha spheres to find out the tight atom packing centres. The potential binding sites were defined by these alpha spheres and their dummy atoms were defined to create the centroid of the sphere. From the list of possible binding sites predicted, the site covering the maximum residues among all was considered to perform the docking simulations.

2.6. Molecular Docking

Molecular docking simulations were carried out with GSH, Vit-E and Al into the above mentioned binding site to know their binding mode and affinity ranges for gGCL using MOE docking module. The optimized conformation of the gGCL was loaded into MOE workspace and the binding site was defined. Triangle matcher docking placement methodology was used by aligning ligand triplets of atoms on triplets of alpha spheres of binding site and a population of possible docking conformations were generated. These docked conformations were ranked by London DG scoring function where the free binding energies of the ligands from a given pose were calculated. Multiple docking conformations were generated for each ligand and among them, the conformation with lowest docking score was considered for the analysis of molecular interactions and docking energies.

3. Results

No significant changes were observed in body weights of rats in controls and experimental groups (data not shown). No clinical signs of toxicity were also observed in any of the groups. None of the rats were excluded from the present study.

Significant increase in the lipid peroxidation (LPx) levels were observed in Al exposed rats over controls. No significant changes were observed in LPx levels in vitamin E exposed rats over controls. However, a significant decrease was observed in the LPx levels in Al+vitamin E exposed rats over Al alone exposed rats (Table 1). On the other hand, significant decrease ($p<0.01$) in the activity levels of antioxidant enzymes such as glutathione peroxidase, glutathione reductase, glutathione-S-transferase, superoxide dismutase and catalase were observed in the kidney tissue of aluminium exposed rats over controls. Furthermore, glutathione content was also significantly reduced ($p<0.01$) in kidney tissue of Al exposed rats. On the other hand, administration of vitamin E significantly ($p<0.01$) reversed the changes induced by Al in the kidney tissue of rats as compared to Al alone exposed rats. Interestingly, a significant increase in the glutathione content was observed in vitamin E treated rats over controls.

Further, *in silico* analysis were performed to understand the role of vitamin E in accelerating glutathione system. Homology model of the gamma-glutamyl cysteine ligase catalytic subunit (GGCL) was constructed using Modeller tool and its stereo chemical quality was evaluated by Ramachandran plot and ProsaWeb analysis. The best quality model was Energy minimized in the MOE working environment and a stable conformation was attained.

The potential binding site of GGCL was predicted using MOE site finder and docking simulations were carried using MOE-Dock module using GSH, Vitamin E and Al as ligands against the GGCL to find their possible mode of binding orientations and affinity ranges. The docking scores were observed as -11.193, -9.830 and -3.017 Kcal/mol for GSH, Vitamin E and Al respectively indicating that GSH has more affinity among and Al did not showed any molecular interactions with the GGCL but has a less affinity of binding energy (Figure 1 and Table 2).

4. Discussion

The results of the present study indicate that 1) Al negatively targets antioxidant enzymes thereby leads to imbalance in the pro- and anti-oxidant status as evidenced by significant increase in the LPx levels in kidney tissue of rats 2) vitamin E administration ameliorates the activity levels of antioxidant enzymes thereby reduces the LPx levels in the kidney tissue of Al exposed rats and finally 3) Al induced changes in the glutathione system at least in part does not mediate gGCL and surprisingly, vitamin E accelerated glutathione system might target gGCL in rats as evidenced by docking studies.

It is well known that a balance between pro- and antioxidant system is crucial in the regulation of physiological functions. Antioxidant enzymes and antioxidants such as glutathione continuously monitor and regulate the levels of free radicals thereby protect cells and cell organelles. In general antioxidant enzymes can be broadly categorized into primary and secondary enzymes. The antioxidant superoxide dismutase (SOD) converts the superoxide anions (O_2^-) into hydrogen peroxide (H_2O_2), which can easily diffuse through the cell membranes into the cytoplasm where the antioxidants catalase and glutathione peroxidase reduce the hydrogen peroxide and thereby detoxify ROS. Secondary enzymes in antioxidant defense include those of glutathione metabolism. Glutathione-**S**-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) and cellular components damaged by ROS attack, to nucleophilic xenobiotics, which leads to their detoxification. NADPH-dependent glutathione reductase (GR) replenishes the GSH substrate for glutathione peroxidase (GPx) and glutathione-**S**-transferase (GST) from oxidized glutathione (GSSG).

Table 1: Effect of vitamin E on lipid peroxidation and selected antioxidant enzymes in the kidney tissue of Al intoxicated rats

Parameter	Control	Experimental groups		
		Al-exposed	Vitamin E	Al exposed + Vitamin E
Lipid peroxidation (µmol of malondialdehyde formed /mg protein/hour)	$7.35^{a} \pm 1.21$	$16.25^{b} \pm 2.38$	$6.47^{a} \pm 1.08$	$13.49^{d} \pm 1.17$
Superoxide dismutase (U/mg protein)	$13.96^{a} \pm 0.29$	$6.80^{b} \pm 0.21$	$14.72^{a} \pm 0.27$	$9.31^{d} \pm 0.31$
Catalase (µmol of H_2O_2 decomposed/min/mg protein)	$40.8^{a} \pm 3.2$	$20.61^{b} \pm 4.35$	$42.56^{a} \pm 2.71$	$31.95^{d} \pm 3.29$
Glutathione peroxidase µmol of glutathione oxidized/(min mg of protein)	$4.2^{a} \pm 0.72$	$2.4^{b} \pm 0.25$	$5.7^{a} \pm 0.51$	$3.62^{d} \pm 0.29$
Glutathione-*S*-transferase nmol of CDNB formed/(min mg of protein)	$5.1^{a} \pm 0.49$	$2.2^{b} \pm 0.2$	$6.1^{a} \pm 0.76$	$3.31^{d} \pm 0.32$
Glutathione reductase (nmol of GSSG utilized/min/mg of protein)	$30.51^{a} \pm 3.02$	$22.51^{b} \pm 1.23$	$32.31^{a} \pm 2.65$	$26.51^{d} \pm 2.61$
Reduced glutathione (mg/100 g tissue)	$6.12^{a} \pm 0.6$	$2.41^{b} \pm 0.2$	$8.1^{c} \pm 0.29$	$4.88^{d} \pm 0.4$

Values are mean ± S.D. of six individual rats.
Mean values in a row with different letters differ significantly from each other at $p<0.01$.

In the present study, Al exposure significantly reduced the activity levels of SOD and catalase in the kidney tissue of rats. This might indicate the accumulation of superoxide anions which in turn suggests failure of dismutation of superoxide anions by SOD and improper removal of hydrogen peroxide. Glutathione system is also one of the major antioxidant systems that counter attacks ROS. GSH acts as substrate for GPx and has a direct antioxidant function by reaction with superoxide, hydroxyl radical and singlet oxygen, leading to the formation of oxidised glutathione and other disulfides (Kosower and Kosower, 1978). In the present study, Al exposure significantly reduced the levels of reduced glutathione levels in the kidney tissue of rats. GPx is crucial to mitigate the levels of hydroperoxides with conversion of GSH to GSSG (Meister and Anderson, 1983). Al exposure also significantly reduced the GPx levels in the kidney tissue of rats as compared to controls and this might be at least in part due to 1) since

GSH acts as the substrate for GPx, the decreased availability of GSH in the tissues of Al exposed rats might have resulted in a decline in the activity of GPx and 2) lower activity of catalase leads to accumulation of hydrogen peroxides which eventually inactivates GPx (Searle and Wilson, 1980). GR and GST are also crucial enzymes which play vital roles in the formation of reduced glutathione and mitigation of free radicals by conjugation with GSH, respectively. In the present study, Al exposure caused a significant decrease in the activity levels of GR and GST in the kidney tissue of rats. It is well known that GSSG is converted to glutathione by GR in the presence of NADPH as co-factor. Thus, the decreased availability of GSSG might in turn causes reduction in the activity levels of GR in the kidney tissue of Al exposed rats. Furthermore, GST catalyzes the conjugation of GSH with nucleophilic xenobiotics or with cellular components damaged by ROS attack, which leads to their detoxification. Thus, decreased activity of GST in the kidney tissue of rats during Al intoxication might be due to the decreased availability of GSH. Previously several studies indicated that Al alters pro- and antioxidant status (Chinoy and Memon, 2001; Omar et al., 2003; El-Demerdash, 2004; Newairy et al., 2009).

The role of vitamin E or **α**-Tocopherol as antioxidant is well established. It has ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against the injury of oxidation. Moreover, it has been indicated that vitamin E inhibits peroxidation of membrane lipids by scavenging lipid peroxyl radicals and is converted into a tocopheroxyl radical as a consequence (Arita et al., 1998). In the present study, administration of vitamin E significantly reduced the LPx levels in the kidney tissue of Al intoxicated rats. Surprisingly, vitamin E significantly enhanced the activity levels of selected antioxidant enzymes in the kidney tissue of Al exposed rats. The results are in consonance with earlier reports (El-Demerdash, 2004; Qureshi et al., 2009). No significant changes were observed in the activity levels of antioxidant enzymes and LPx levels in the kidney tissue of rats administered with vitamin E alone. However, the glutathione content in the kidney tissue of vitamin E administered rats were significantly increased as compared to controls. This suggests that vitamin E has triggering effect on glutathione system. These results prompted us to know whether vitamin E has affinity towards rate limiting enzyme of glutathione system, Gamma-glutamyl cysteine ligase (gGCL). To understand the interactions of vitamin E and glutathione system, we performed in silico analysis.

The template to construct the homology model of the rat gGCL was selected based on BLAST similarity patterns. BLAST results indicated that glutamate cysteine ligase of *Saccharomyces cerevisiae* showed the maximum identity (42%) and query coverage of 95%. Therefore, it was chosen as a template to build protein structure of rat gGCL using modeller software. A total of 20 gGCL homology models were constructed using Modeller and among them, the structure with the lowest DOPE (Discrete Optimized Protein Energy) score (-71329.296) was chosen as best model for further analysis (Figure 1). The stability of the protein was determined using Ramachandran plot statistics. The plot revealed that 89.3% of the residues in most favoured regions, 8.6% in additional allowed regions, 1.1% in generously allowed regions and 1.1% in disallowed regions (6 residues: SER 115, ARG 321, SER 337, CYS 339, GLU 361 and GLN 607). These residues were converted into loops using Modloop server and the looped structure was further evaluated with Ramahcandran plot, where no residues were observed in disallowed regions (Figures 2 and 3). The Prosaweb analysis of the gGCL structure also showed a Z-score of -9.05 indicating the reliability of selected model (Figure 4) based on the accuracy of phi and psi backbone dihedral angles.

Table 2: Molecular docking analysis depicting interactions between modeled protein and selected ligands

S.no	Ligand	Docking Score (Kcal/mol)	No H-Bonds	Interactive residues	H-bond length (Å)
1	GSH	-11.193	3	E52	2.3
				E96	3.0
				E96	3.1
2	Vit-E	-9.830	3	R410	2.2
				E425	2.3
				R427	3.0
			Arene cationic interaction	R427	-
3	Al	-3.017	-	-	-

Figure 1: Homology model of gGCL constructed using Modeller.

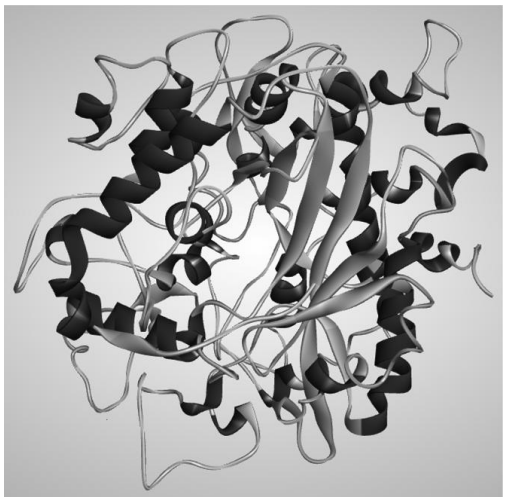


Figure 2: Ramachandran plot showing the stereochemical quality of the model where no residues were observed in the disallowed region.

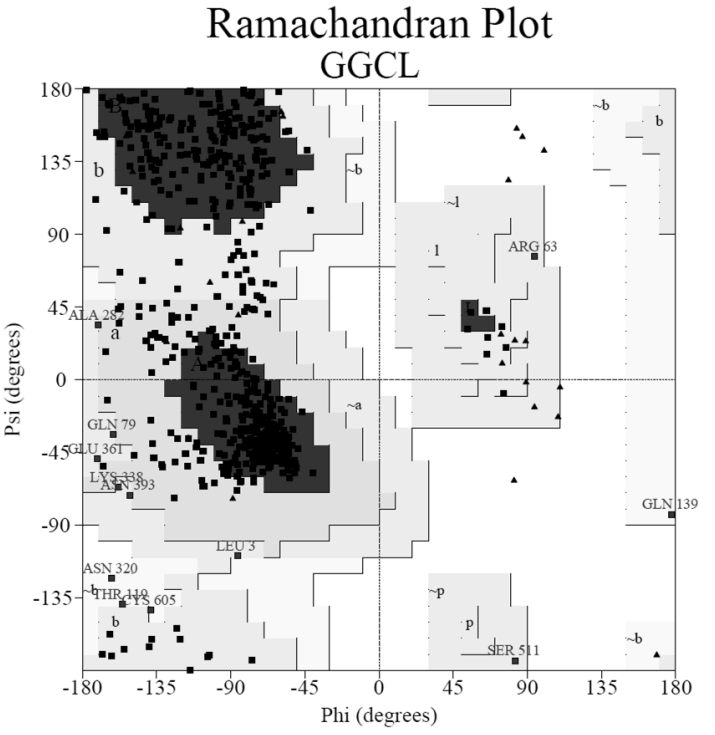


Figure 3: Superimposition of looped segments of GGCL structure before (dark) and after (ash to white) looping process. The residues observed in disallowed regions were denoted at the bottom of the loops.

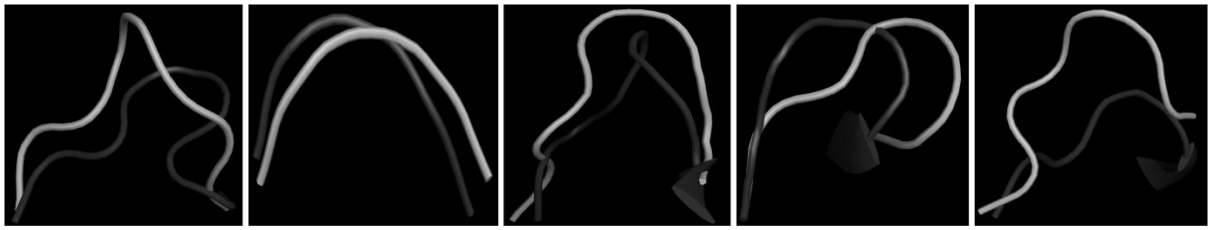


Figure 4: Prosaweb analysis of the GGCL structure showing the Z-score in the native X-ray crystallographic regions.

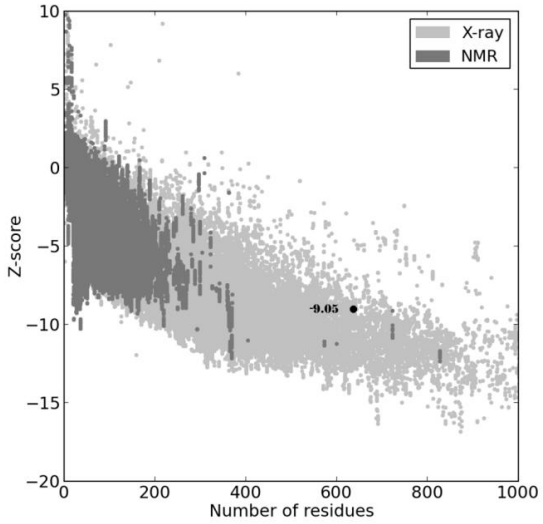


Figure 5: (A) gGCL structure showing the predicted potential binding sphere. Arrow indicates regions on the binding sphere are the reactive centres of the cavity. (B) the amino acid residues of gGCL observed in the predicted binding sphere.

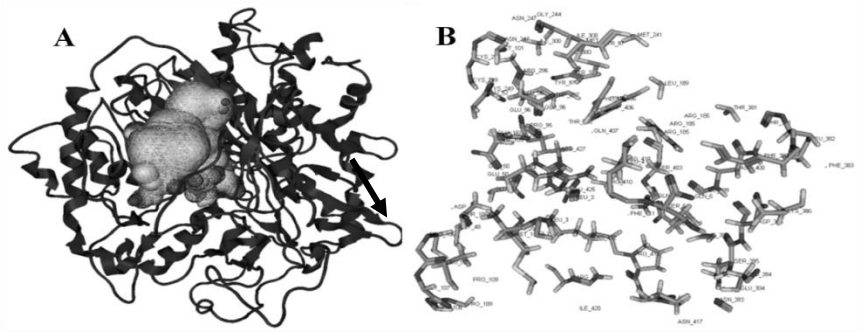
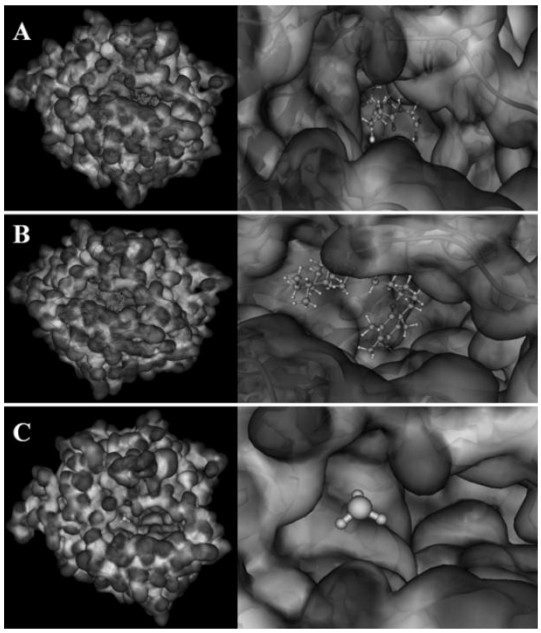


Figure 6: Molecular docking of (A) GSH (B) Vit-E and (C) Al in the binding sphere of gGCL. Hydrophobic, polar and solvent exposed binding regions of gGCL involved in ligand affinity.



The final structure is energy minimised and refined in the MOE workspace and proceeded for the next important task i.e the prediction of possible potential binding sites in gGCL model. This is the critical task which forms the basis to predict and analyse the interaction properties of the GGCL with small molecules. The hydrophobicity and hydrophillicity are the main parameters along with the electrostatic potentials of the structure that drives its molecular interactions. Small molecule ligands contain their own hydrophilic/hydrophobic, electrostatic and steric properties and the same make their interactions more specific in a defined binding sphere. By virtue of these qualities a potential binding sphere was identified in the gGCL model where a highly accurate and specific interactions are expected (Figure 5).

The molecular docking simulations of GSH, Vitamin E and Al in this binding site encouraging the accuracy in the prediction of binding sphere where the ligands were observed to be sit in the cavity in perfect orientations. The molecular interactions of the ligands are more defined and specific with binding sphere. GSH is interacting with the E52 and E96 residues

forming three hydrogen bonds. The –COOH group present at one end of GSH is interacting with the –COOH group of glutamate residue at 52nd position. Another glutamate residue at 96th position is also interacting in the same manner with its –COOH group but forming two hydrogen bond interactions with two –NH groups of GSH. Whereas Vitamin E is interacting with gGCL binding by means of R410, R427 and E425 residues forming three hydrogen bonds one with each. Here the interaction is confined to a specific region of the Vitamin E molecule where the ring structure is playing major role in forming the interactions. The –OH group of the aromatic ring alone is interacting with all the three residues of the binding site and one more interesting point is this aromatic ring is also forming one arene cationic interaction with R427 residue. All these interaction make them to orient in the cavity in a specific way. Here Al is not forming any interactions with the binding site but showing a sort of affinity for gGCL structure which is defined by its docking energy i.e. -3.017 Kcal/mol. Whereas GSH and Vitamin E are showing the docking energies of -11.193 and -9.830 Kcal/mol respectively indicating that GSH is showing more affinity than Vit-E for GGCL (Figure 6 and Table 2).

5. Conclusion

The present study demonstrated that vitamin E administration in combination with aluminium minimized its hazards. In addition, vitamin E proved to be beneficial in decreasing the levels of free radicals and triggering the activity levels of SOD, CAT and glutathione system. Consequently, exposure to aluminium should be minimized and caution should be paid towards the sources of aluminium in foods, water and personal-care products. Furthermore, using diets rich in vitamin E could be beneficial in mitigating aluminium toxicity.

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Deterioration of physicochemical and antioxidant properties of Malaysian Tualang honeys on long term storage

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Abstract

Honey is a rich source of natural nutrients. Its production is a slow, natural process, the pace of which varies with seasonally. However, based on recent reports, we hypothesize that the long-term storage of processed honey, even under the most appropriate storage conditions, results in a deterioration of its quality. To test our hypothesis, we collected Tualang honey samples harvested during the years 2005, 2008, 2009 and 2010 and tested various parameters including physicochemical properties and also performed comparative analyses of antioxidant capacities to assess its medicinal values. Our results indicate that, upon long-term storage, the quality of honey samples deteriorates, as observed in our TH 2008 and TH 2005 year honey samples, which showed unacceptable quality based on the recommended criteria of free acidity (71.34±1.31 meq/kg), moisture (27.72%), diastase activity (3.38±0.34 Goth scale) and HMF (449.89±3.23 mg/kg) by Codex and European Commission Regulation. A significant (p<0.05) decrease in antioxidant properties were also observed. In the present study, we show that, even after appropriate processing, most of the quality parameters of honey decrease, which suggest that these parameters could otherwise be used as markers to assess the age of the honey.

Keywords: Honey, Physicochemical properties, HMF, Antioxidant capacity

1. Introduction

Oxidative stress occurs in a system when free radicals and reactive oxygen species (ROS) overwhelm its endogenous and exogenous antioxidant defenses beyond the ability of the system to neutralize and eliminate them(Ortial et al., 2006). However, increases in exogenous antioxidants are believed to alleviate any oxidative damage and help balance cellular free

radicals and ROS. Honey has been studied extensively because of its physicochemical, antimicrobial, antioxidant, antiviral, antitumor and anti-inflammatory properties (Viuda-Martos et al., 2008; Yolanda et al., 2011). Recent investigations of several natural compounds have revealed that honey is one of the most nutritious products that exists in the form of a traditional remedy containing monosaccharides, amino acids, proteins (including enzymes), organic acids, vitamins, minerals, various phytochemicals and many biologically active entities (Ahmed et al., 2007).

Honey is obtained from a variety of sources all across the world, and each variety varies in its composition and hence, in its beneficial properties. Recently, we performed comparative analyses using honey collected from various sources and have successfully shown that Malaysian tualang honey is among the best honey available based on its high antioxidant and free radical-scavenging ability (Kishore et al., 2011). However, in addition to the source, the physicochemical and the antioxidant capacities of honey samples are highly dependent on the storage conditions during climatic changes (Bath & Singh, 2000). Therefore, a lack of knowledge about proper storage conditions may ultimately reduce the physicochemical properties of honey and subsequently reduce the medicinal properties and the quality of this foodproduct. Apparently, although Malaysian tualang honey is among the best honey, the extreme temperatures in Malaysia (temperatures of up to 40°C) may have drastic effects on the quality of honey during storage prior to consumption.

To the best of our knowledge, there has only been one study on the effect of storage on the physicochemical and antioxidant properties of honey samples (Nombre et al., 2010). The current study specifically addresses the effect of long-term storage on processed honey by evaluating medicinal properties of Malaysian tualang honey that was harvested over several years (collected during 2005, 2008, 2009 and 2010). The findings of the present study will help to provide appropriate guidelines for processing and storage of Malaysian tualang honey and for identifying markers to assess the age of honey.

2. Methods and materials

2.1 Chemicals and instruments

All chemicals, reagents and solvents used were of analytical grade and were purchased from Merck (Germany). Standards used in the antioxidant assays were L-ascorbic acid, gallic

acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu's phenol reagent (Sigma; St. Louis, MO, USA).

2.2 Honey samples

Malaysian Tualang honey samples harvested from the nectar of *Apis dorsata bee's nectar* during 2005, 2008, 2009 and 2010 from Tualang trees in the rain forest of Kedah (peninsular Malaysia) were included in this study. The honey was intitially filtered to remove solid particles by Federal Agricultural Marketing Authority (FAMA) of Malaysia and was subjected to gamma irradiation at 25 kGy at Sterilgamma (M) Sdn. Bhd. prior to being submitted to us for analyses.

2.3. Physicochemical analyses

Moisture, color, pH, free acidity, electrical conductivity, ash content, reducing sugars and sucrose content were analyzed in all honey samples using respective AOAC **(**Association of Analytical Communities) methods(AOAC, 2000).

2.3.1.Diastase activity

Diastase activity, which is a measure of the hydrolytic activity of the enzymes in honey, was determined using the Horwitz et al., method (1980).

2.3.2. Hydroxymethylfurfural

HPLC method

The HPLC method used was a modification of the basic method published by the International Honey Commission in 1999, as described by Khalil et al., (2010). The hydroxymethylfurfural (HMF) content of a sample was calculated by comparing the corresponding peak areas of the sample with those of the standard solutions of HMF (Sigma–Aldrich, USA) after correcting for the honey dilution. There was a linear relationship (R^2 = 0.9998; Fig. 1) between the concentration and the area of the HMF peak, and the results are expressed in mg/kg.

2.4. Total phenolic and biochemical analyses

2.4.1. Determination of total phenolic content

The total phenolic content of honey samples was determined using the Folin-Ciocalteau method (Singleton et al., 1999). The total phenolic content is expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

2.4.2. Determination of total flavonoid content

The total flavonoid content of the honey samples was determined using the method described by Woisky and Salatino (1998). Total flavonoids were calculated as mg quercetin equivalents (QE)/100 g of honey.

2.4.3. Evaluation of total antioxidant capacity using the phosphomolybdenum method

The total antioxidant activity of tualang honey samples was evaluated using the green phosphomolybdenum complex method (Prieto et al., 1999). The total antioxidant capacity was calculated as ascorbic acid equivalents (mg AAE/ g honey).

2.4.4. Determination of ascorbic acid content

The standard 2, 4-dinitrophenylhidrazine method was used to determine the ascorbic acid content of the honey samples (Omaye et al., 1979).

2.4.5. Determination of protein content

The protein content of honey samples was measured using the Bradford assay (Bradford, 1976).

2.5 Analyses of antioxidant activities

2.5.1. Free Radical scavenging activity of DPPH

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical-scavenging effect of honey samples was measured using the method described by Hatano et al., (1988)and the radical-scavenging activity is expressed as IC_{50} (mg/mL).

2.5.2. Ferric reducing/antioxidant power assay (FRAP)

The antioxidant capacity of tualang honey samples was estimated according to the procedure described by Benzie and Strain (1996). Ferrous sulfate was used for the calibration curve (0-500 μM), and the results, which were obtained from triplicate analyses, are expressed in μM of ferrous sulfate per 100 g of honey (μmol Fe (II)/100 g of honey).

2.5.3. Superoxide anion radical-scavenging activity

Superoxide radicals generated by the xanthine-xanthine oxidase system were determined spectrophotometrically by monitoring the product of the reaction with nitroblue tetrazolium salt (NBT) (Robak & Gryglewski, 1988). The results are expressed as the concentration of the test sample required for a 50% reduction in the absorbance of the control in mg/mL (IC_{50}).

2.5.4. Peroxynitrite radical-scavenging activity

Peroxynitrite-scavenging activity was determined according to methods described by Koppenol et al., (1996). The IC_{50} value was determined and expressed as mg/mL.

2.6. Statistical Analyses

Three different harvests for each year were analyzed for each parameter, and the data are expressed as the mean ± standard deviation (SD) (n=6). Each sample was analyzed in duplicate, and the statistical differences represented by letters were obtained through a one-way analysis of variance (ANOVA). Differences between the means at the $p<0.05$ confidence level were considered statistically significant. Correlations were obtained using Pearson's correlation coefficient (r) in bivariate linear correlations using the SPSS statistical program.

3. Results and Discussion

3.1.1. Physicochemical properties

Physicochemical parameters that have been previously studied were divided based on origin and their involvement with conductivity, coloration, sugar and quality control, which include moisture, HMF and diastase activity. The obtained data are presented in Table 1.

3.1.2. Physicochemical parameters based on origin

Honey pH

Regardless of geographical origin, honey is acidic in nature, and its acidity influences its texture and stability as well as its storage conditions (Williams et al.,2009; Saxena et al., 2010). The pH of honey is also a useful indicator of any possible microbial contamination. As shown in Table 1, the pH was statistically different ($p<0.05$) for samples collected during different years and for samples that were stored for different periods of time, even though the pH range obtained remained within the limits specified by the EU Council (2002) and Codex Alimentarius (2001). The pH of Tualang honey samples varied from 3.02 to 4.14 with gradual differences observed over extended periods of storage.

Color intensity

The color intensities of Tualang honey significantly ($p<0.05$) increased during storage. The most recent honey samples, which were harvested in 2010 and 2009, had an intensity varying from 214.17±3.05 to 281.50±4.23 mAU. For the 2008 and 2005 samples, the extremely dark intensities (412±2.97 and 679.83 mAU) from the Millard reaction were perhaps due to the formation of colored pigments and higher colloidal content. Similar observations have been made in previous studies involving Italian, Slovenian and Malaysian honeys (Beretta et al., 2005; Bertoncelj et al., 2007; Khalil, et al., 2011).

Electrical conductivity

An electrical conductivity of <0.8 mS/cm is a good indicator of the botanical origin of honey as well as its mineral content and increases upon storage. Accordingly, in the present study, the electrical conductivity (mS/cm) values increased significantly from 0.30 for the freshest harvest (2010) to 1.13 for the oldest harvest (2005). The high conductivity was perhaps due to an increase in the organic ionizable fraction of the honey. This is further explained by a high acidity (Persano Oddo et al., 2008). Previous studies have indicated that electrical conductivity does not depend exclusively on mineral content but increases with an increase in ash, organic acids, proteins, and some complex sugars content as well (Terraberint et al., 2003).

Free Acidity

The maximum free acidity permitted by Codex Alimentarius honey standards is 50meq/kg. In the present study, honey harvests from 2010 and 2009 yielded free acidity values between 17.82 and 23.30 meq/kg, which are well within international regulations and thus, indicate the absence of an undesirable fermentation. However, the other two older harvests from 2008 and 2005 had a free acid content of 56.76 and 71.34 meq/kg, respectively, and thus, they exceed the acceptable range proposed by Codex Alimentarius (2001). Higher levels may results from a possible microbial alteration as a consequence of higher temperatures during prolonged storage (Kaur-Bath & Narpinder, 2000). In the present study, the values indicate that the sugars present in the samples have undergone fermentation in the presence of yeasts, and it is well known that during fermentation, glucose and fructose are converted into carbon dioxide and alcohol. In the presence of oxygen, the alcohol is further hydrolyzed and converted into acetic acid, which contributes to the free acid content of the honey (Ouchemoukh et al., 2007). These findings, along with the results from the current study, clearly indicate that the moisture content in Tualang honey samples is influenced by the botanical source, geography, climatic conditions, season and storage.

Ash content

Ash represents the direct measure of the inorganic residues after honey carbonization and indicates the possible botanical origin of honey (Malika et al., 2005). The ash content in all Tualang honey samples studied varied widely between the 2010, 2009, 2008 and 2005 samples and measured 0.35, 0.38, 0.51 and 0.68 g%, respectively. The 2008 and 2005 samples had the highest ash contents,where as 2005 sample is above the 0.6% limit (Codex Alimentarius Commission, 2001). Honey normally has low ash content and, in the current study, the high

variability observed in the honey's ash content may indicate that the harvest processes and/or the beekeeping techniques used by the producers were non-uniform. Nevertheless, it has also been proposed that the ash content of honey depends on the floral origin of the material collected by the bees during foraging on the flora (Ojeda De Rodriguez, Sulbaran De Ferrer, Ferrer, & Rodriguez, 2004).

Sugar content

Monosaccharides, such as glucose and fructose form the major carbohydrate content (60%) of honey (Mendes, Brojo Proenca, Ferreira & Ferreira, 1998). Although the ratio of the two monosaccharides mainly depends on the source of the nectar, it varies from 0.9 to 1.4 (fructose/glucose) and is a good indicator for explaining the structure and crystallization of honey. The concentration of sucrose should be less than 5%. In the present study, this ratio fell within the Codex Alimentarius (2001) limits for all honey samples, which suggested no variation over the period of storage. Any observed changes in the concentrations are due to the hydrolysis of sucrose by the enzyme invertase or sucrase, which release the monosaccharides fructose and glucose. Similar results were observed by Cantarelli et al., (2008).

3.1.3. Physicochemical parameters based on quality control

Moisture content

The moisture content of honey significantly affects its quality during storage as it slows fermentation and granulation to provide a longer shelf life. However, moisture content also depends on various factors, such as the harvesting season, degree of maturity reached in the hive, and climatic factors (Finola, Lasagno & Marioli, 2007). Two of the four Tualang honey samples, especially from 2010 and 2009, yielded moisture contents between 10.29% and 16.25%, which indicate a proper degree of maturity and that the beekeepers had used the optimal time for extraction. The values were within the allowed limit of 21% moisture content permitted by the EU Council (2002) and Codex (2001). Nevertheless, two of the samples, from 2008 and 2005, had moisture contents of 22.77 and 27.72%, respectively, which are slightly above 20% and were probably due to a prolonged storage period and prior extraction of honey from hives.

Diastase activity

Diastase activity has been found to be mainly influenced by honey storage and heat. It facilitates the conversion of starch to maltose and is added by bees during honey production. Although there is a large natural discrepancy of this parameter in honey, a minimum value of 8 on the

Gothe scale is expected of a high quality honey (Bogdanov et al., 1999). In the current study, diastase activities were determined to be 19.53, 14.80, 5.78 and 3.38 (Goethe unit) for the 2010, 2009, 2008 and 2005 honey samples, respectively ($p<0.05$). The diastase activity decreased after storage for at least 24 months with respect to fresh samples, which has been observed in previous studies (Sahinler, 2007). This further substantiates that a measure of diastase activity in honey can be an effective indicator of the storage time/freshness of the honey.

HMF Content

The HMF content in fresh honey is generally very low; however, honey with a HMF content up to 80 mg/kg is still considered to be fresh (Codex Alimentarius, 2001; EU council, 2002).Higher HMF values suggest probable exposure to heat and/or prolonged storage periods, which result in a caramelization of carbohydrates, termed the Maillard reaction, as well as fructose decomposition (Ruoff et al., 2007). Based on the HPLC method (Table 1), the HMF concentrations in the tualang honey samples stored for 1 and 2 years (the 2010 and 2009 tualang honey samples) exhibited initial HMF contents of 6.92±0.02 and 13.20±1.60 mg/kg, respectively. However, Tualang honey samples stored for more than 2 years (2008 and 2005) had the highest HMF values, 109.61±0.45 and 449.89±3.23 mg/kg, respectively, ($p<0.05$). It is obvious that heating is not the only factor influencing HMF formation in honey, *i.e*., the honey's composition, pH and floral source also contribute. Our results are consistent with Khalil et al., (2010), who found that the amount of HMF in Tualang honey stored for more than one year was significantly higher compared to newly harvested honey.

3.1.2. Total antioxidant capacity

The influence of storage on total antioxidant capacity is shown in the table 2. The total antioxidant capacity assay is mainly based on the reduction of Mo (VI) to Mo (V) by the extraction and subsequent formation of a green phosphate/Mo (V) complex. A high absorbance value for a sample indicates a strong antioxidant activity. During the present study, the fresh Tualang honey samples (2010 & 2009) were observed to function more effectively as antioxidants and had significant ($p<0.05$) total antioxidant capacities. The results of the total antioxidant capacity assays indicate that during storage the antioxidant capacities of the samples decreased considerably with age. A good correlation between total phenolics and total antioxidant capacity was observed (r = 0.987) (Kishore et al., 2011), and this may be the reason for the greater total antioxidant capacity displayed by the fresh honey samples.

Table 1: Comparative physico-chemical properties of tualang honey samples on storage

Physico-chemical analysis	Tualang honey 2010	Tualang honey 2009	Tualang honey 2008	Tualang honey 2005
Moisture (%)	10.29	16.25	22.77	27.72
Colour (mAU)	214.17±2.05[a]	281.50±4.42[b]	412.00±3.45[c]	679.83±4.58[d]
pH	4.15±0.12	3.84±0.14	3.52±0.12	3.06±0.19
Electrical conductivity (mS/cm)	0.307±0.006	0.529±0.009	0.802±0.011	1.134±0.043
Free acidity (meq/kg)	17.82±0.98[a]	23.30±0.63[b]	56.76±0.83[c]	71.34±1.31[d]
Ash (%)	0.35±0.03	0.38±0.03	0.51±0.04	0.68±0.04
Fructose (%)	46.13	39.90	34.72	29.70
Glucose (%)	41.92	36.83	32.50	26.60
F+G	88.05	76.73	67.22	56.30
Sucrose (%)	3.68	3.10	1.45	0.22
Diastase activity (Gothe scale)	19.53±0.93[a]	14.80±0.49[b]	5.78±0.56[c]	3.38±0.34[d]
HMF (mg/kg)	6.92±0.02[a]	13.20±1.60[b]	109.61±0.45[c]	449.89±3.23[d]

According to the European Codex Honey Standards (**Bogdanov et al., 1999**), a well processed and ready to be consumed honey must contain the following characteristics: maximum moisture content of 20–21 g/100 g of honey, electrical conductivity < 0.8 mS/cm, reducing sugars content >65 g/100 g, sucrose content <5 g/100 g, free acidity <50 milliequivalents/kg, diastase number >8G, 5-HMF content < 80 mg/kg of honey. Significantly different values are represented by different letters.

3.2. Total phenolic and biochemical activities

3.2.1. Total phenolic and flavonoid content

Phenolics and flavonoids are the most widely detected secondary metabolites distributed in honey samples, and it has been reported that the antioxidant activity of phenolics is mainly due to their redox properties and abilities to serve as hydrogen donors and singlet oxygen quenchers (Chuanphongpanich et al., 2006). According to the results shown in Table 2, the fresh honey samples, which had strong antioxidant activities, also had high total phenolic and flavonoid contents. However, in the older honey samples, these values deteriorated during storage and varied significantly (p<0.05) between samples. The average content of total phenolics and

flavonoids obtained for our honey samples is similar to those of other honey samples from various floral sources, which have been reported in the literature (Meda et al., 2005).

Table 2: A compilation of data from tualang honey samples: Total phenolic, flavonoid, ascorbic acid content, antioxidant and free-radical scavenging activity of tualang honey samples.

Biochemical parameter	Tualang honey 2010	Tualang honey 2009	Tualang honey 2008	Tualang honey 2005
Total Phenolic content (mg GAE/100 g)	85.52±2.69[a]	76.72±1.28[b]	53.03±1.63[c]	23.13±0.66[d]
Total Flavonoid content (mg QE/100 g)	51.92±1.31[a]	43.91±1.97[b]	27.37±1.07[c]	12.52±0.89[d]
Total Antioxidant capacity (mg AAE/g honey)	54.27±1.24[a]	50.37±0.97[a]	33.32±0.85[b]	14.75±0.65[c]
Ascorbic acid content (mg/100 g of honey)	37.04±0.86[a]	31.15±0.70[b]	24.45±0.60[c]	10.02±1.12[d]
Protein content (mg BSA/100 g of honey)	79.16±0.99[a]	76.42±1.68[a]	50.57±0.82[b]	37.41±1.22[c]
DPPH radical-scavenging activity (IC_{50} values mg/mL)	6.16±0.43[a]	6.69±0.36[a]	9.71±0.36[b]	15.02±0.45[c]
FRAP (µmol Fe(II)/100 g of honey)	124.04±2.93[a]	112.17±2.49[b]	74.80±2.68[c]	38.79±1.42[c]

Data are expressed as means±SD. GAE, gallic acid equivalents; QE, quercetin equivalents; AAE, ascorbic acid equivalents; BSA, bovine serum albumin equivalents; IC_{50} , 50% inhibitory concentration; FRAP, Ferric reducing/antioxidant power; SD, standard deviation. Significant *P* values are presented ($p<0.05$). Significantly different values are represented by different letters.

3.1.3. Ascorbic acid and Total Protein content

Honey also contains ascorbic acid, which is an important water-soluble antioxidant and a cofactor for several enzymes. The obtained values for ascorbic acid and total protein content levels for the Tualang honey samples are presented in Table 2. The ascorbic acid levels (mg/100 g of honey) of the Tualang honey samples varied from 37.04 (year 2010) to 14.75 (year 2005). A reduction in ascorbic acid content was observed in older honey samples, and this reduction was mainly due to the sensitive nature of ascorbic acid towards water, heat and oxygen content. In addition to these factors, different processing and storage techniques and tropical conditions may have also led to the higher losses because of a higher oxidation rate of ascorbic acid (Padayatty et al., 2003).

Fig.1. Linear relationship between the concentration of HMF and peak area.

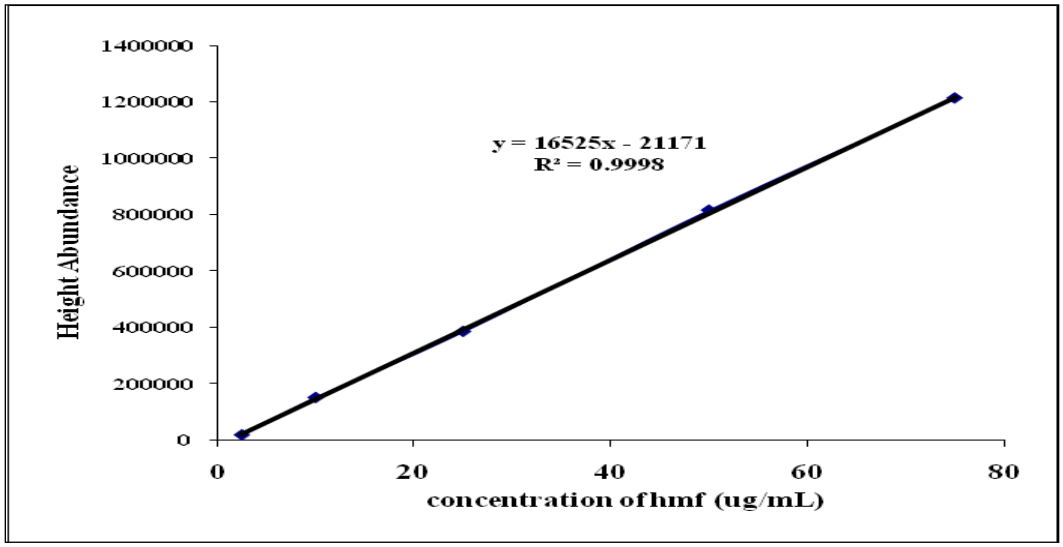
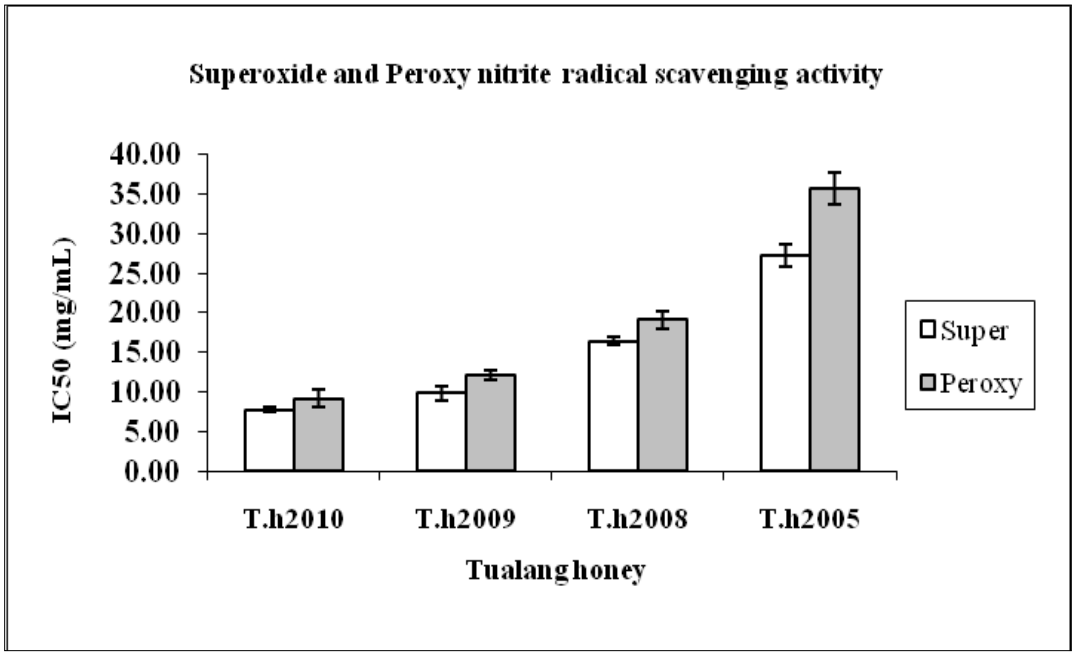


Fig.2. Superoxide anion radical and Peroxy nitrite radical scavenging activities of tualang honey samples.



Data are expressed as mean ± SD. IC_{50}represents the mg/mL concentration providing 50% inhibition of superoxide anion radical and peroxynitrite radical scavenging. Ascorbic acid (IC_{50}, 2.3 mg/mL) was used as areference antioxidant for comparison. Significant P values are presented ($P<0.05$).

In the current study, the protein content (mg BSA/100 g of honey) of the Tualang honey varied from 79.16 (year 2010) to 37.41 (year 2005), which was determined using bovine serum albumin (BSA) as a standard. The protein level of honey is dependent on the type of flora as well as the enzymes introduced by the honey bees themselves, in addition to enzymes in the nectar (Alvarez-Suarez et al., 2010).

3.2. Analyses of antioxidant activities

3.2.1. DPPH radical-scavenging activity

DPPH is a relatively stable nitrogen-centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and has been widely used to evaluate the antioxidant activity of various natural products (Amarowicz et al., 2004). The IC_{50} (mg/mL) values of the honey samples (Table 2) varied from 6.16 (year 2010) to 15.02 (year 2005). The fresh honey samples showed a greater scavenging activity when compared to the older honey samples, and this greater activity was mainly due to the deterioration of the antioxidants, especially phenolics and flavonoids, present. A significant difference (p<0.05) in the radical-scavenging activities between the honey samples was also observed. The results obtained in this study are comparable to the DPPH radical-scavenging abilities of Tualang honey samples obtained from previous studies (Mohamed et al., 2010; Kishore et al., 2011).

3.2.2. Ferric reducingantioxidant power assay

The FRAP assay results for the Malaysian Tualang honey samples (μmol Fe (II)/100 g of honey) tested are displayed in Table 2, and the obtained results varied from 124.04 (year 2010) to 38.79 (year 2005). Storage had a significant (p<0.05) effect on ferric ion-reducing activities, a relatively higher absorbance value indicates a higher reduction rate of ferric ions to ferrous ions. In the current study, the observed range of FRAP values was comparable to the reducing capacity range of Slovenian honey samples (Bertoncelj et al., 2007) and raw Milleflori honey (Blasa et al., 2006). A positive linear correlation between the DPPH assay and the total polyphenol content (r = 0.975) to the FRAP method and the phenolic content (r = 0.991) was observed. This correlation indicates that the phenolics are the major antioxidant compounds responsible for the antioxidant activities of fresh honey samples.

3.2.3. Superoxide anion radical and Peroxynitritescavenging activity

Superoxide anions are precursors of other reactive oxygen species, which include hydrogen peroxide, hydroxyl radicals, and singlet oxygen. The further reaction of these superoxide radicals

with nitric oxide results in the formation of cytotoxic peroxynitrites, which oxidize cellular components, such as proteins, lipids, and nucleic acids (Balavoine and Geletti, 1999). Peroxynitrites are relatively stable but once protonated, they form highly reactive peroxynitrous acid. The superoxide anion and peroxynitritescavenging activities of the Tualang honey samples were investigated, and the results were compared with those of reference antioxidants. A higher extract concentration required to scavenge radicals indicates a lower antioxidant activity. Based on the results (Fig. 2), it is evident that the fresh honey samples (years 2010 & 2009) showed significant superoxide anion and peroxynitritescavenging activities when compared to the older stored honey samples.

Conclusions

Both the physicochemical properties and the phenolic compound composition of honey are responsible for its quality and antioxidant activity, thus rendering it a healthy food and source of antioxidants. Our findings suggest that the long-term storage of honey, even under the appropriate temperatures and storage conditions, decreases the quality in a variety of aspects and parameters and ultimately leads to the loss of its major nutritive and medicinal values. Based on our findings, we also suggest that highly sensitive parameters, such as moisture, free acidity, diastase activity, HMF content, total phenolics and antioxidant activity, can be used as markers for approximating the shelf life and age of honey. However, further extensive studies need to be performed to determine the most sensitive markers and to specify any guidelines for the assessment of the age and quality of the honey.

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Therapeutic potential of ginger on cardiac antioxidant defense system and blood glucose levels in experimental diabetic rats

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ABSTRACT

Antioxidant defense system plays a key role in diabetic complications. The effects of ginger ethanolic extract on ruined antioxidant system in heart tissue remains unclear in diabetic rats. In this study, we investigated the impact of oral administration of ginger ethanolic extract on antioxidant parameters in experimental diabetic rats. Thirty rats were equally divided into five groups, including normal control (NC), diabetic control (DC), ginger treatment (Gt), diabetics treated with ginger (D+Gt) and diabetics treated with glibenclamide (D+Glb). The parameters like XOD, AA, blood glucose and body weight levels. XOD, AA are significantly decreased in heart of diabetic rats, which indicates impaired antioxidant status. However, we found that the administration of ginger to diabetic rats restored the altered antioxidant status that is almost similar to ginger treated group. Further, evidences from our histopathological studies also prove that ginger protects the heart and liver tissues from oxidative stress in diabetic rats.
Keywords: Diabetes, antioxidant enzymes, heart, rats.

Introduction:

Diabetes is one of the most pressing global health problems and it is estimated that the prevalence of diabetes will be more than 300 million in 2025 (Ojewole, 2006). Increased oxidative stress and changes in antioxidant capacity was observed in both clinical and experimental diabetes and thought to be the etiology of diabetic complications (Baynes, 1991). Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances and some may inhibit insulinase activity (Bopanna et al., 1997). Some plants are involved in the stimulation of cells to produce more insulin and others may increase cells in the pancreas by activating regeneration of pancreatic cells.

Traditionally, medicinal plants are extensively used in India due to their low cost, easy accessibility to everyone and perceived fewer side effects and their medicinal use has been gradually increasing in developed countries. In many respects, the mechanism of action of the herbal drugs differs from that of the synthetic drugs (or) pure compounds. *Zingiber officinale* (Roscoe), commonly known as ginger is one of the commonly used spices in India and around the world. Ginger has been used to treat a number of diseased conditions including headache, cold, arthritis, postoperative nausea and vomiting, motion sickness, and reduces symptoms in patients with nausea of pregnancy. Ginger has hypoglycemic, insulinotropic, hypolipidemic and antioxidant properties. Ginger is used to treat alcoholism, bronchitis, cancer, ulcer and dyspepsia (Shanmugam et al., 2010). A preliminary study has reported that ginger contains a large number of bioactive compounds like polyphenols, alkaloids, saponins and tannins. Some of the chemical constituents isolated from ginger include (6) - gingerol, α-zingiberene, phenolic compounds, essential oilsand oleoresin resins (Van Wyk and Wink, 2003). We had recently reported the renal protective effect of ginger in alcoholic subjects (Shanmugam et al., 2010).

So for there was limited work on the cardiac protective effect of ginger in diabetic rats. Hence, the present study was carried out to know the impact of ginger on cardiac antioxidant defense system parameters against STZ induced diabetic rats.

Materials and Methods:

Animals

Male albino Wistar rats, body weight of 180±200 grams, were used in this study. The rats were housed in clean polypropylene cages having 6 rats per cage under hygienic conditions and maintained under temperature controlled room (27 ± 2^0C) with photoperiod of 12 hours light and 12 hours dark cycle. The rats were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and approved by the Institutional Animal Ethical Committee No: 09(iv)/a/CPCSCA/IAEC/07-08/SVU/Zool/KSR-CHRK/ dated: 26/6/08. S.V.University, Tirupati, Andhra Pradesh. The rats were allowed standard rat pellet diet (Lipton India Ltd., Mumbai, India) and water *ad-libitum* for the duration of the experiment.

Chemicals

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fischer (Pittrsburg, PA, USA), Merk (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (50 mg/kg body weight) in 0.1 M cold citrate buffer (pH: 4.5). The animals were allowed to drink 15% glucose solution overnight to overcome the drug- induced hypoglycemia. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The animals are acclimatized one week in diabetic condition after one week ginger treatment was given to the diabetic rats for 30 days.

Preparation of Ginger extract

The fresh rhizomes of Ginger were locally purchased in Tirupati in the month of July 2009. Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the plant material and the extraction was continued. The process was repeated three times and the three extractions were pooled together, combined filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanol extract was air-dried; finally giving 180g of light brown, powdery, crude ethanol extract of *zingiber officinale* dried rhizomes. Without any further purification the plant crude ethanol extract was used in the study. Dose equivalent to 200 mg/kg body weight of the crude extract was calculated and suspended in 2% v/v Tween 80 solution for the experiment.

Experimental design

The rats were divided into 5 groups of six in each group and treated as follows:

Group I – Normal Control (NC): Six rats were received 0.9% of Nacl / kg bodyweight via Orogastric tube for a period of 30 days.

Group II – Diabetic Control (DC): Sixrats were used as diabetic control by the induction of STZ intraperitonial injection of 50mg/kg body weight after fasting.

Group III – Ginger treatment (Gt): Six rats were received the ethanolic extract of ginger, 200mg/kg bodyweight via orogastric tube for a period of 30 days.

Group IV – Diabetic + Ginger treatment (D+Gt): Six diabetic rats were received the ethanolic extract of ginger, 200mg/kg bodyweight via orogastric tube for a period of 30 days.

Group V - Diabetic + Glibenclamide treatment (D+Glb): Diabetic rats treated with glibenclamide (600 µg/kg body weight) in aqueous solution for a period of 30 days.

The animals were sacrificed after 24 hours of the last treatment by cervical dislocation and the heart and liver tissue was excised at 4°C. The tissues was washed with ice-cold saline, immediately immersed in liquid nitrogen and stored in the deep freezer at -80˚C for further biochemical analysis and enzymatic assays. Before assay, the tissues were homogenized under ice-cold conditions. Selected parameters were estimated by employing standard protocols.

Biochemical parameters estimation

Cardiac Xanthine Oxidase and Ascorbic acid levels are estimated by by Omaye et al. (1971) & Srikanthan & Krishnamurthy (1955).

All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as a standard. The blood glucose levels were measured by using an Accucheck glucometer (Roche – Germany).

Histopathological examinations of Heart and Liver

The heart and liver tissues were isolated immediately after sacrificing the animal and washed with ice-cold saline andthey were then fixed in 10% formalin solution. Sections of 3µm thickness were stained with haematoxylin and eosin (H&E) for histopathological examination.

Statistical analysis

The data are expressed as Mean values with their SD. Readings of the five different groups were compared using one-way ANOVA analysis with Dennett's multiple comparision test. Statistical analysis was performed using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA). Using M.S. Office, Excel Software the data has been analyzed for the significance of the main effects (factors), and treatments along with their interactions. Differences were considered significant at (*$p < 0.001$), ($^{\Psi}p < 0.01$).

Results

Blood glucose and Body weight changes

Blood glucose levels were found to be significantly increased after STZ-induction and the administration of ginger, decreased the blood glucose levels significantly ($^{\Psi}p<0.01$) in diabetic rats than that of diabetic control group (Fig-1). These decreasing levels of blood glucose may be due to the regeneration of β - cells of the pancreas, which were destroyed by STZ.

A significant decrease in body weight in the diabetic control rats was observed than normal control rats. Whereas with ginger treatment, the bodyweight was significantly ($^{\Psi}p<0.01$) increased in diabetic rats (Fig-2).

Results of XOD, AA in the Heart tissue of all Experimental groups

The ethanolic extract of ginger was selected for antioxidant activity in diabetic rats. We observed significant (*$p < 0.001$) decrease in the levels of XOD, AA in heart tissue of diabetic rats when compared to normal control (NC). After administration of ginger to diabetic rats the XOD, AA levels are significantly ($^{\Psi}p<0.01$) increased. The results have shown in (Fig-3).

Histological study

Histopathological investigation of heart tissue that also provided an essential evidence for the biochemical analysis. The heart of normal control (NC) and ginger treated (Gt) rats shows striations along the length of the normal muscle fibres (NMF) and the normal nuclei (N) of the cardiac muscle cells lie in the middle of the cells, several cardiac fibres are seen branching (CFBr) and a prominent perinuclear region is seen around several nuclei. Where as in the heart

tissue of diabetic control rats (DC), we observed, hyalinization of muscle fibres, with focal cellular infiltration or necrosis of muscle fibres (NMF), congestion (C), slight infiltration (SI) and slight vacuolation (SV). However in ginger treated diabetic rats (D+Gt), the heart tissue showing normal nucleus (N), and we also observed mild swelling of muscle cells (MSMC) and rearrangement of focal cardiac muscle fibres (RCMF) this also reveals normal architecture of the myocardium with intact muscle fibres. Thus ginger has some protective effect on the myocardium against STZ-induced diabetis. Where as in Diabetic rats treated with glibenclamide (D+Glb), the heart tissue showing normal nucleus (N) and normal cardiac fibers (NCF) and normal nuclei (N) of the cardiac muscle cells lie in the middle of the cells.

Table 1:- Effect of ginger and glibenclamide on XOD levels and AA levels in diabetic rats

	Xanthine Oxidase (XOD) (µ moles of uric acid/gram)	**Ascorbic acid (AA)** (µ moles of ascorbic acid/gram)
Group I (NC)	0.81± 0.0141	2.15±0.166
Group II (Gt)	0.981± 0.083	2.19±0.142*
Group III (DC)	1.53±0.033*	1.56±0.163
Group IV (D+Gt)	1.028±0.019*	1.84 ±0.176*
Group V (D+Gli)	1.20±0.012	1.98±0.207

All the values are mean, ± SD of six individual observations,
*significant at $p < 0.001$

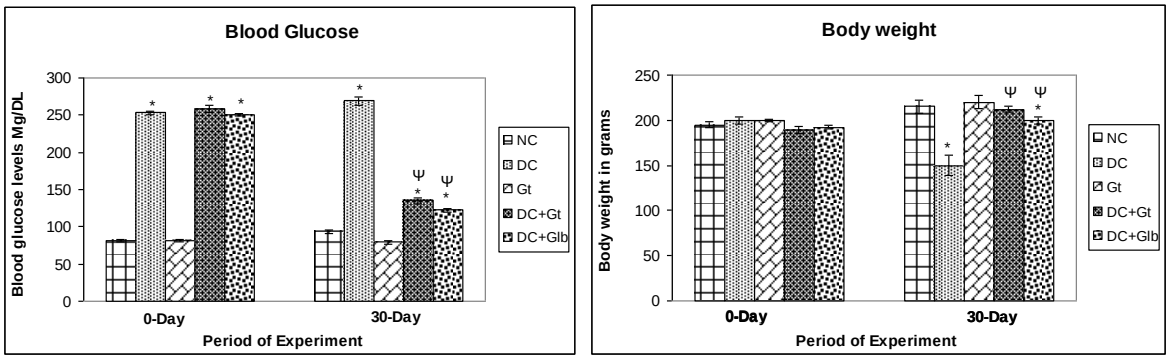
Discussion

The present study investigates the effects of ginger antioxidant property in STZ- induced diabetic rats. Oxidative stress is suggested to be a potential contributor to the development of

complications in diabetes. Increased free radical production or reduced antioxidant defense responses, both of which occur in the diabetic state may give rise to increased oxidative stress (Halliwell and Gutteridge, 1990). Consequences of oxidative stress are cell injury, i.e. damage lipids, antioxidant enzyme system, disruption in cellular homeostasis and accumulation of damaged molecules (Jakus, 2000).

In Streptozotocin-induced diabetic rats, we observed increased blood glucose levels. The elevation of glucose in STZ-treated rats was due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA (Yamamoto et al., 1981). Where as with ginger treatment in diabetic rats, we observed that blood glucose levels are decreased. This may be due to the free radical scavenging and hypoglycemic activity of ginger. Ginger inhibits lipid peroxidation and prevents streptozotocin-induced oxidative stress and protects β-cells from diabetic shock.

Figure 1: The effect of ginger on Blood glucose levels and Body weight changes in diabetic rats. The values are significant compared to the following: control ($^{*}p < 0.001$), diabetic ($^{\Psi}p < 0.01$)



We have registered a decrease in body weight in STZ diabetic rats. The characteristic loss of body weight associated with STZ-induced diabetes is due to increased muscle wasting in diabetes (Ravi et al., 2004). This indicates polyphagic condition and loss of weight due to excessive break-down of tissue proteins in diabetic rats (Chatterjea and Shinde, 2002). Hakim et al., (1997) have stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats (Rajkumar et al., 1991). When ginger was administered to diabetic rats, the body weights seemed to be increased, as was

the ability to reduce hyperglycaemia. However, it could not normalize the body weight completely. The treatment with ginger showed a significant increase in body weight in diabetic rats. The administration of ginger to STZ diabetic rats reduced blood glucose levels, in accordance with earlier reports (Al-Amin et al., 2006).

XOD serves as a sensitive marker of oxidative stress and it plays an important role in maintaining the integrity of the cell system. XODis involved in several reactions in the body and is one of the most prominent non-enzymatic antioxidants. In the current study, A XOD levels was decreased in heart tissues of diabetic rats. Depletion of tissue XOD levels enhances cellular damage caused by oxidative stress. Significant depletion of XOD & AA (*$p<0.001$) in diabetic rats suggests its increased utilisation against reactive oxygen species. However, ginger treatment in diabetic rats, reversed the XOD & AA to normal levels, this shows that ginger has an antioxidant property. Reports are available on the antioxidant effect of ginger by decreasing lipid peroxidation, increasing XOD & AA level and maintaining normal levels of antioxidant enzymes. A number of researchers have reported that 6-gingerol, tannins, polyphenolic compounds (coumarins), flavonoids and triterpenoids of ginger have antioxidant properties in various experimental models (Shanmugam et al., 2010).

The diabetogenic potential of STZ is responsible for a progressive development of the hepatic and cardiac tissues lesions. In the present study the protective effects of ginger in experimental groups of rats were established by histological study. Figure-5 illustrates histopathological investigation that also provided an essential evidence for the biochemical analysis. In the normal control rats the heart, shows striations along the length of the normal muscle fibres (NMF), the normal nuclei (N) of the cardiac muscle cells lie in the middle of the cells, several cardiac fibres are seen branching (CFBr) and a prominent perinuclear region is seen around several nuclei. Where as in the heart tissue of diabetic rats hyalinization of muscle fibres, with focal cellular infiltration or necrosis of muscle fibres (NMF), congestion (C), slight infiltration (SI) and slight vacuolation (SV). However in ginger treated diabetic rats the heart tissue showing normal nucleus (N), and we also observed mild swelling of muscle cells (MSMC) and rearrangement of focal cardiac muscle fibres (RCMF). This reveals normal architecture of

the myocardium with intact muscle fibres thus ginger has some protective effect on the myocardium against STZ-induced diabetes. Where as in D+Glb treated rats the heart tissue showing normal nucleus (N) and normal cardiac fibers (NCF) and normal nuclei (N) of the cardiac muscle cells lie in the middle of the cells.

In conclusion, our results demonstrated that STZ is capable of causing marked oxidative stress in addition to deplete the antioxidants and inhibiting the activities of antioxidant parameters. Finally, the results of our work reveals that ginger would be a valuable source of anti-diabetic and antioxidant agents, with a potential use in pharmaceutical preparations. In all, we suggest that the ginger diets have health promoting effects especially in metabolic disorders. Moreover further studies of ginger extracts and *in-vivo* studies of inhibition are warranted.

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Bioaccumulation of heavy metals in fish (Rastrelliger) collected fromRoyapuram and Kovalam waters of Chennai, India

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ABSTRACT

A study was done to know the concentration of heavy metals in the edible muscle of fishes (Rastrelliger) collected from the coastal water of Royapuram and Kovalam of Chennai area. Royapuram coast is associated with industries. Kovalam is located far away from Royapuram area and is an unpolluted with reference to industries. The fishes were collected. The tissues are dried and digested and the samples are prepared. The sample solutions were tested to find the concentration of heavy metals using Atomic Absorption Sphectrophotometer. The concentration of heavy metals followed the following ranking in the fishes collected from Royapuram and Kovalam regions.

Royapuram: Fe > Zn > Cu >Mn. Kovalam: Fe > Cu > Zn >Mn .The concentration of heavy metals was found to be more in the fishes collected from Royapuram than Kovalam. Nickel and Lead was found to be below detectable limits from the fishes collected from both the areas.

KEY WORDS: Heavy metals; Rastrelliger; Royapuram; Kovalam; Chennai; Bioaccumulation

INTRODUCTION

According to the Food and Organization, the percapita availability of food in India, in terms of calories is low. Indian diet is basically cereal based. Thirty seven percent of the population in India live below the poverty line and majority of them suffer from malnutrition. Most malnutrition in India is protein energy malnutrition, resulting from lack of energy food. Food production from agricultural sources cannot keep pace with the ever increasing population. The sea is known for its productivity, with rich and diversity of organisms. The sea is exploited for different edible sources of food like fishes, Lobsters, Crabs, Oysters, Cephalopods, Holothurians, Sea weed (Rao,1995)[1] etc for their nutritive values and for other purposes.

Among all the marine resources, fish and crustaceans rank the majority. Fish serves as food for mankind. Fish food is highly significant in that it helps to build up the health of the public. The importance of fish as food lies not merely in its rich protein content, but also in its quality. Protein of fish origin contains many essential amino acids such as arginine, histidine, isoleucine, lysine, methionine, phenylalinine, threonine, tryptophan and valine(Shanmugam, 1992)[2]. In shallow coastal and shelf waters the amunt of organic matter is high(Eastherson and Mahadevan,1980)[3].

The consumption of food can improve the health of the people. At the same time they can accommodate the pollutants in their body because of the pollution of their habitat. The ocean has often referred to as the ultimate sink. The Indian coastal waters indicates that petroleum hydrocarbons constitute one of the major pollutants followed by heavy metals and chlorinated hydrocarbons(James,1990)[4]. Heavy metals finally reaches the fishes through the food chain and accumulate in the tissues. The major toxic effects of heavy metals include the blocking of the functional groups and molecules of biological importance like enzymes, polynucleotides or transport systems for essential nutrients and ions.

Now a days the marine region particularly the coastal area has been affected by the pollution from different sources. In the marine environment, the near shore regions are of concern due to the fact that they are the sites of the worlds most critically polluted areas(Ramachandran,1990)[5].

The data and researches on the accumulation of heavy metals in pelagic fishes in the coastal waters of Chennai is still short.

Rastrlliger kanagurta Cuvier is commonly called Indian Mackerel belongs to the family Scombridae. It is widely spread in the Indo-pacific region. It is pelagic form and its is a planktonic feeder. On average this fish accounts for about eight percent of the country's marine fish landings.

MATERIALS AND METHODS

Sample collection and preparation

The fish samples were collected from the coastal waters of Royapuram and Kovalam. The same species of fishes were collected from the Royapuram and kovalam areas which are of same size and length(18-20cms). The collected fishes were then placed in an icebox. Then they were transported to the laboratory and preserved at 4C for further analysis. Removal of the muscles from the species were done within twenty four hours of the collection. The fish samples were brought to the room temperatures. Then the samples were rinsed with distilled water to remove sands and debris. The muscle from the fish samples were removed using sterile scissors, knife and forceps. The petridishes were labeled before dissection process. The muscle tissue is

removed and kept in the labeled petridish and were dried in the hot air oven at 110 C for 24 hours.

Heavy metal Analysis

Digestion Method

One gram of the dried sample tissue was taken, to which concentrated sulphuric acid and concentrated perchloric acid were added in the ratio of 3:1. Then the mixture was exposed to slight temperature. After this 0.05 N HNO3 was added and made up to a constant volume.

Analysis

The prepared solutions were aspirated in a Instrumental Laboratory(Vido-12) Atomic absorption spectrophotometer to find out the concentration of heavy metals Copper, Iron, Manganese, Nickel, Lead and Zinc. Blanks and standards were run simultaneously along with the sample solutions.

RESULTS AND DISCUSSION

The mean concentrations of the heavy metals Copper, Iron, Manganese and Zinc in the samples collected from Royapuram were 0.635 ppm, 4.23 ppm, 0.251 ppm and 0.796 ppm. The heavy metals lead and Nickel were found to be below detectable limits.

The mean concentrations of the heavy metals Copper, Iron, Manganese and Zinc in the samples collected from Kovalam were 0.461 ppm, 3.52 ppm, 0.132 ppm and 0.393 ppm. The heavy metals lead and Nickel were found to be below detectable limits.

The S.D values of the heavy metals Copper, Iron, Manganese and Zinc in *Rastrelliger kanagurta* from Royapuram coastal area were $\pm$ 0.359; $\pm$ 1.222; $\pm$0.244; $\pm$0.963 respectively where as the same species from the Kovalam area showed $\pm$ 0.099; $\pm$2.775; $\pm$0.047; $\pm$0.112 respectively.

All the values between the concentrations of the same heavy metals from the species between Royapuram and Kovalam areas showed that the values were significant at 0.05% confidence level.

In the fishes *Rastrelliger kanagurta* the heavy metal Iron concentration was found to be higher when compared to that of other heavy metals. The heavy metals Lead and Nickel were found to be below detectable limits. The heavy metals Cu, Fe, Mn and Zn are generally required for normal physiological function, but needed only in traces.

The present findings are similar to the studies by Maheswari Nair *et al.*,where the content of Iron and Zinc is more followed by Copper and Manganese. The concentration of heavy metals in the samples from Royapuram were higher than the specimens collected Kovalam area. This can be due to the fact that Royapuram is nearer to Ennore area which is highly polluted with industries. Even Royapuram area has industrial complex which release their wastage in to the sea.

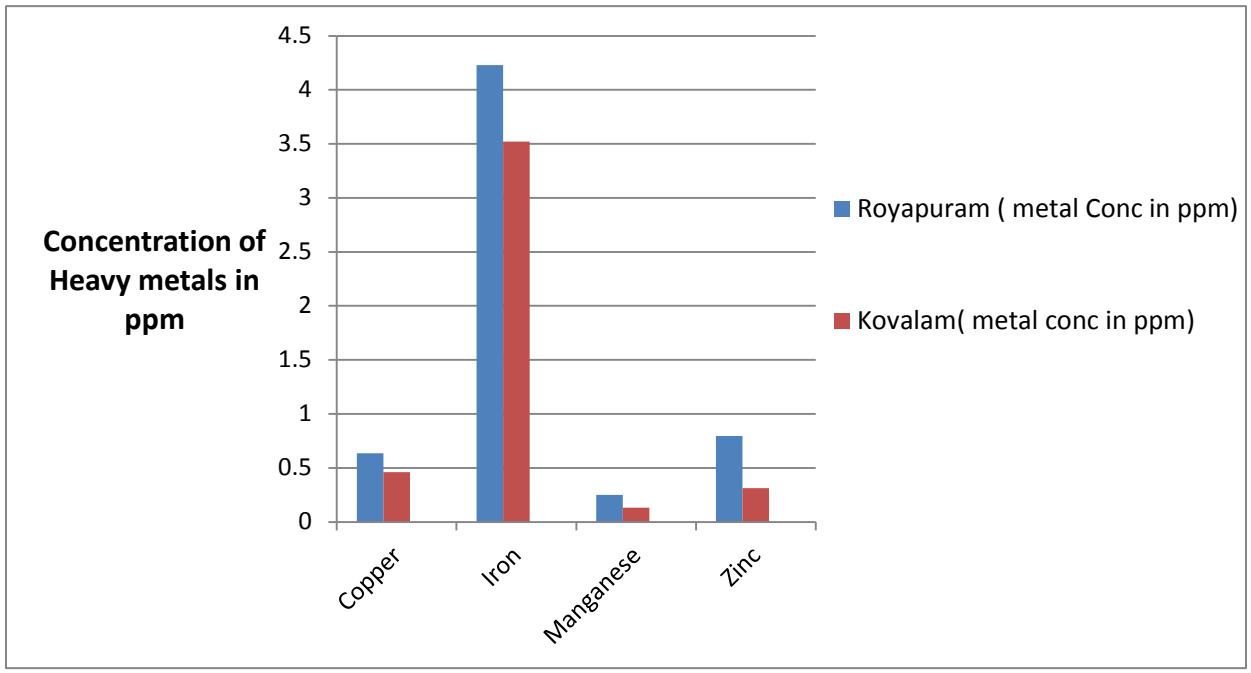
The findings of Jaffar and Ashraf (1988)[6] showed similarity with the present findings where the concentration of Iron is more followed by Zinc, Copper, Manganese etc. The entry of heavy metals into the fishes may also be through phytoplanktons and zoo planktons. Marine diatoms showed the concentration of heavy metals where Iron content is higher than the other heavy metals (Umamaheswara Rao and Mohanchand 1990)[7].

George and Kurieshy (1979)[8] reported the concentration of Iron more than Zinc followed by other metals in Zooplankton from Bay of Bengal.

The concentrations of the heavy metal could have been high if the experiments could have done in monsoon season because during monsoon season the pH and the salinity decreases so it may facilitate the dissolution of precipitated form of the metal(hydroxide, carbonate or chloro complexes) and increase the amount of ionic species in the solution. The most broadly accepted paradigm is the bio availability of metal in the form of free metal iron(Sunda and Thoutte 1978)[9]. During the summer season the concentration of the heavy metals is low. It may be due to the low bio availability of the metal ions in the sea water due to high salinity and pH. The concentration of manganese in the organisms were very low that they cannot even be predicted. It may be due to the fact that marine animals, have very low tendency to accumulate manganese. The muscles of fishes seem to accumulate less amount of heavy metals than the liver, kidney(Jaffar and Ashraf 1988)[6].

The research show that Royapuram area is polluted than the Kovalam area due to Industrialization and urban activities.

The chart below shows the concentration of heavy metals Copper, Iron, Manganese and Zinc in Rastrelliger kanagurta from Royapuram and Kovalam areas.



CONCLUSION

The comparison and analysis revealed the fact that the accumulation of heavy metals in muscle tissues of Rastrelliger kanagurta collected from Royapuram and Kovalama areas are low. The results reveal, that the amount of heavy metal accumulated in the muscle tissues were comparatively less which may not affect the consumer at higher levels of food chain. It can be concluded that it is safe for consumption.

The study also reveals that the level of pollution is more at Royapuram which is surrounded by Industries when compared to Kovalam coastal waters which is free from Industries.

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A Survey of Medicinally Important Plants in around Venkatapur mandal ofBhadrachalam area; Khammam District; (A.P)

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Abstract

A survey on Ethnomedicinal plants was conducted in Venkatapuram- -mandal of Bhadrachalam area in the month of June-2014. The tribal people of thatarea revealed 27 medicinal plants and their utility. One among them is Dioscorea-hispida, apart from ethnomedicinal value, it is fatal, when it is taken in the raw form.The chemical nature of the tuber is evaluated in the ethnobotanical laboratory of SR&BGNR G.D.C Khammam. The documented medicinal plants were tabulated alphabetically(family wise).

Key Words:-Ethnomedicinal plants, Dioscorea hispida(Tuber).

Introduction:- Khammam District in Andhrapradesh is one of the state, rich with forest wealth. The tribal people like; Koyas, Lambadas and Konda Reddys are living in this forest by adopting podu cultivation.The forest is predominated by Dry-deciduous, Moist-deciduous, riparian, Scrub and grass land vegetation. Whenever the tribal people gets diseases, they may approach the (Vejjolu) or (Health healers) . They used to give herbal medicine for immediate cure. Instead of relying on modern medicine, still, the tribes are depending on the age old traditional medicine.

Some of the medicinal plants may also used for edible purpose. At the time of cooking of the above foods, if enough precautionary measures are not taken, it becomes fatal.

Material & Methods:- An ethnobotanical survey was conducted by a team of SR&BGNRGovtCollege, Khammam to unearth the traditional knowledge of tribal people at Venkatapuram mandal of Bhadrachalam area in the month of June -2014 by using questioniers , oral interviews, the team members collected 27 ethnomedicinal plants information. The collected plants were pressed and make it in the form of herbarium and kept in the ethnobotanical laboratory of SR&BGNRGovtCollege. For every specimen, proper voucher number was given. survey of the literature shows that, ethnomedicinal information of kondareddis is poor. A few persons like Koppula.HemadriV.S.Raju, Sateesh Sutari, C.S.Reddyetc are concentrated on ethnobotanical studies. The collected plant specimens were identified by using the flora of Bombay& The flora of Andhrapradesh by T.Pullaiah.

Results and Discussion:- The present work reported a total of 27 plants belonging to various families are to be used for medicinal purpose. During survey, The research team found that one

of the tuberous plant Dioscorea hispida was eaten by the local tribes and subjected to death. The tuber of the plant was collected and examined in the ethno –botanical laboratory of SR&BGNR GDC Khammam, and came to know that an AlkoloidDioscorine-a, 4-epidioscorine causes neuromuscular blockage. This result the death of the patient.

Dioscoria hispida:- It is a twining vine, arising from tuberous roots, and reaching a length of several meters. Stems are covered with spines. Leaves are 3-foliolated. Panicle is axillary, slender, hairy,12 to 20 cm long. Flowers are small, unisexual, male flowers with 6-stamens, female flowers similar to males, 3-winged, 3-celled ovules, 2 in each cell.Fruit is a capsule, oblong and about 5cm long. Flesh and sap of tubers are yellowish. The tuber is growing luxuriantly in the Venkatapuram mandal of Khammam District.

Constituents:- Tubers yield alkaloid dioscorine-a and 4-epidioscorine and neuromuscular blocking agent. A saponin glycoside, diosgenin is also reported. Study of the mineral content reports the tubers are a good source of phosphorus, Calcium& Iron.A mature gadung tuber weighs up to 15kg, each 100 gm of tuber (wet bases)yields 20gms of carbohydrates,78gm of water, 1.81gm of protein, 0.16 gm fat,0.93 gm of fiber & 0.69 ash.

Properties:- Yellow flesh tubers reported to have better taste than white flesh tubers.Flesh and sap of the tubers are yellowish; studies suggest that the tuber is aantihelmintic, antioxidant, anti-inflamatory, analgesic and anti-tumor activities.

Toxin removal:- Without removing the toxin, if the fresh tubers are eaten, it becames, fatal, by affecting on the nerves & muscles. It should be cut into pieces and thoroughly washed before eating. Is better to soak the tuber in the salt water for one or two days for removing the toxins.

Ethnomedicinal importance:- It is used to cure Arthritis, Rheumatic pains & Sprains.

1) Used as poultice of freshly pounded material or decoction as external wash.
2) It is also used to killed the worms in whitlow, sores, boils. etc.
3) The decoction of the tuber is used as alternative and diuretic in chronic rheumatism.
4) Tendril of yam have been used as de-worming medicine.
5) Yellow juice from the flesh and sap of tubers is used for bleaching clothes & abaca fibers.

ETHNOMEDICINAL PLANTS AND THEIR USES

S.No	Botanical name	Family	Vernacular name	Part used	Ethnic use
1	Abrus precatorius	Fabaceae	Gurija	Seed	Abortifacient
2	Acalypha indica	Euphorbiaceae	Penta puti	Whole plant	Skin diseases
3	Alangium salvifolium	Alangiaceae	ooduga	Bark	Snake bite
4	Anogeissus Acuminate	Combretaceae	Pasi	Leaf	Cough
5	Argemone Mexicana	Papavaraceae	Mulu pucc ha	Whole plant	Skin diseases
6	Atlantia monophylla	Rutaceae	Adavi nimma	Root	Paralysis
7	Blumea bifoliata	Asteraceae	Kukka pogaku	Root	Cough
8	Boerhavia diffusa	Nyctaginaceae	Atika mamidi	Whole plant	Fever, tonic
9	Boswellia serrata	Burseraceae	Anduga	Bark	Fever, tonic
10	Casearia nigrescens	Salicaceae	Kanmeswaram	Bark	Fish poison
11	Cissus vitiginea	Vitaceae	Adavi draksha	Fruit	Healing power
12	Crotalaria verrucosa	Fabaceae	Tella janumu	Root	Snake bite
13	Curculigo orchioides	Hypoxidaceae	Nela thadi	Twiner	aphrodisiac
14	Dioscorea hispida	Dioscoreaceae	Chenna gadda	Tuber	Arthritis, Fever
15	Gmelina arborea	Lamiaceae	Gummadi teku	Bark	Anti-inflammatory , veterinary
16	Indigoferacassioides	Fabaceae	Karu kandi	Root	Scorpion sting
17	Millingtonia hortensis	Bignoniaceae	Boda malle	Bark	Bronchitis
18	Morinda pubescens	Rubiaceae	Togara mogili	Bark	Appetite, stimulant, anthrax
19	Mucuna pruriens	Fabaceae	Dula gondi	Seed	Abortifacient, aphrodisiac
20	Phyllanthus amarus	Phyllanthaceae	Nela usiri	Whole plant	Jaundice
21	Pueraria tuberose	Fabaceae	Nela gummadi	Twiner	All diseases
22	Sida cordata	Malvaceae	Gaya paku	Root	Tonic
23	Soymida febrifuga	Meliaceae	Somi	Bark	Tonic,Diuretic
24	Strychnos nux-vomica	Loganiaceae	Visha mushti	Bark	Snake bite,anti-septic
25	Strychnos potatorum	Loganiaceae	Chilla	Seed	Eye diseases
26	Trichodesma indicum	Boraginaceae	Gabba	Leaf	Wounds
27	Waltheria indica	Malvaceae	Nalla benda	Leaf	Insect bite

Figure:- Tribal man explaining the ethno--medicinal importance of the tuber

Figure-2. Interaction with tribal people.

Figure-3.Dioscorea hispida (Tuber)

Figure-4. Natural habitat of Dioscorea hispida.

Conclusion:- Traditional knowledge of plants in many tribal communities is changing because of rapid socio-economic and cultural changes. This is particularly true in Koya &Konda Reddy tribes in Khammam District of A.P. Documentation of this knowledge is valuable for the communities and their future generations, and for scientific consideration of wider uses of traditional knowledge. The wealth of this tribal knowledge of medicinal plants points to a great potential for research and the discovery of new drugs to fight diseases, obtaining foods and other uses. So further scientific assessment of these medicines for phytochemical, biological & clinical studies is however greatly needed.

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Exercise physiology – Effects of cardiovascular functions

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Abstract

Physiology is the study of mechanical, physical and biochemical functions of the body. Exercise physiology is a branch of physiology, about the study of the cells and tissues of the body functioning and how exercise alters the structure and function of the body. Regular exercise is now used as a therapy during rehabilitation from various injuries or illness and it is used as a preventive strategy to delay the onset and progression of atherosclerotic cardiovascular disease, cancer, type2 diabetes and back pain.

The study of the cardiovascular system refers to the heart and all the vessels through which the blood flows. Knowledge of exercise physiology is essential for implementing strategies to develop optimal physical performance among individuals. Regular exercise makes the more efficient at pumping blood and delivering oxygen to the exercise muscles. Releases of adrenaline and lactic acid into the blood result in an increase of the heart rate (HR). Cardiovascular physiology examines how oxygen and other nutrients are transported by cardiovascular system and used by the muscles during exercise. The primary purpose of the system is to deliver nutrients to and remove metabolic waste products from tissues.

Physical activity has been shown to have beneficial effects on glucose metabolism, skeletal muscle function, ventilator muscle strength, bone stability, locomotor coordination, psychological well-being, and other organ functions. It also reduces surgical risks and strengthening the immune system. However, in the context of this review, we will focus entirely on the cardiovascular system. The aim of this review is on about the physiological and biochemical mechanisms involved in mediating exercise-induced cardiovascular effects.

Keywords: physiology, exercise physiology, cardiovascular

INTRODUCTION

Exercise Physiology : Exercise physiology is a branch of physiology, which studies how exercise alters the structure and function of the body. Exercise is now used as a therapy during

rehabilitation from various injuries or illness and it is used as a preventive strategy to delay the onset and progression of atherosclerotic cardiovascular disease. Exercise testing using a treadmill or bicycle ergo meter is commonly used in the cardiology department to assess the exercise tolerance and diagnosis of ischemic heart disease. However, exercise tolerance and capacity depends on multiple factors such as age, sex, physical/ mental conditioning, medications, disease status, etc.

During exercise, multiple physiological changes are encountered. In general cardiac output (CO) increase is due to a larger increase in heart rate (HR) and a smaller increase in stroke volume (SV). During exercise, the CO may increase to a maximum value of 35 L/min (baseline 5 L/min). Most of the increased cardiac output goes to the exercising muscle and part of it goes to the skin (to dissipate heat) and heart. The increase in flow to these organs is due to vasodilatation and the flow to the gastrointestinal organs and kidneys decreases (secondary to increased sympathetic activity).

The total peripheral resistance to blood flow is reduced due to the arteriolar vasodilatation in the skeletal muscle, skin, and cardiac muscle. The net result is a decrease in total peripheral resistance (TPR). In addition, there is increased venous return because of increased muscular activity, which in turn further increases the cardiac output. The cardiovascular and muscular changes in blood flow with exercise is also controlled by various factors including but not limited to exercise centres in the brain, local chemical changes in the muscle, mechanoreceptors and chemo receptors in muscle, arterial baro receptors but not limited.

Cardiovascular changes with isometric exercise

Cardiovascular changes during isometric exercise differ from those during dynamic exercise. Static exercise causes compression of the blood vessels in the contracting muscles, leading to a reduction in the blood flow in them. Therefore, total peripheral resistance, which normally falls during dynamic exercise, does not fall and may, in fact, increase, especially if several large groups of muscles are involved in the exercise. The activation of the sympathetic system with exercise thus leads to an increase in HR, cardiac output, and BP.

Because the total peripheral resistance does not decrease, the increase in HR and cardiac output is less and an increase in the systolic, diastolic, and mean arterial pressure is more compared with

those seen with dynamic exercise. Because BP is a major determinant of after load, the left ventricular wall stress, and thus the cardiac workload, is significantly higher during static exercise compared with the cardiac workload achieved during dynamic exercise.

Exercise places an increased demand on the cardiovascular system. Oxygen demand by the muscles increases sharply. Metabolic processes speed up and more waste is created. More nutrients are used and body temperature rises. To perform as efficiently as possible the cardiovascular system must regulate these changes and meet the body's increasing demands

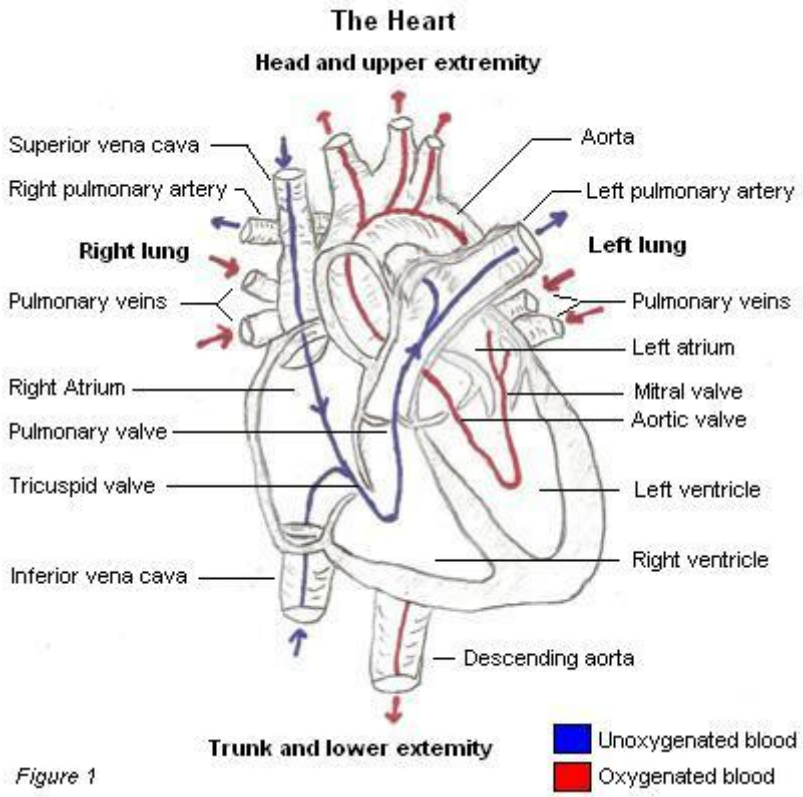

Figure 1

Below we will examine the acute or immediate response to exercise and also the long-term adaptations that take place in the cardiovascular system with repeated exercise. The most important aspects of the cardiovascular system to examine include:

Heart rate: Resting heart rate averages 60 to 80 beats per min in healthy adults. In sedentary, middle aged individuals it may be as high as 100 beats per min heart rate increases rapidly with the onset of activity, providing exercise intensity remains constant, heart rate will level off. This is known as steady-state heart rate where the demands of the active tissues can be adequately met by the cardiovascular system.

Stroke volume: Stroke volume is the amount of blood ejected per beat from left ventricle and measured in ml/beat. Stroke volume increases proportionally with exercise intensity. In untrained individuals stroke volume at rest it averages 50-70ml/beat increasing up to 110-130ml/beat beat during intense, physical activity. In elite athletes resting stroke volume averages 90-110ml/beat increasing to as much as 150-220ml/beat

Cardiac output: Cardiac output is the amount of blood pumped by the heart in 1 minute measured in L/min. It is a product of stroke volume and heart rate (SV x HR). If either heartrate or stroke volume increase, or both, cardiac output increases also. Cardiac output increases proportionally with exercise intensity - which is predictable from understanding the response of heart rate and stroke volume to activity. At rest the cardiac output is about 5L/min. During intense exercise this can increase to 20-40L/min.

Blood Flow: The vascular system can redistribute blood to those tissues with the greatest immediate demand and away from areas that have less demand for oxygen. Because at rest 15-20% of circulating blood supplies skeletal muscle. During vigorous exercise this increases to 80-85% of cardiac output. Blood is shunted away from major organs such as the kidneys, liver, stomach and intestines. It is then redirected to the skin to promote heat loss.

Blood pressure: At rest, a typical systolic blood pressure in a healthy individual ranges from 110-140mmHg and 60-90mmHg for diastolic blood pressure. During exercise systolic pressure, the pressure during contraction of the heart (known as systole) can increase to over 200mmHg and levels as high as 250mmHg.

Blood: During resting conditions the oxygen content of blood varies from about 20ml of oxygen per 100ml of arterial blood to 14ml of oxygen per 100ml of venous blood (2). The difference in oxygen content of arterial and venous blood is known as a-vO2 difference.

As exercise intensity increase the a-vO2 difference increase also and at maximal exertion the difference between arterial and venous blood oxygen concentration can be three times that at a resting level. Although no extra red blood cells have been produced, the greater concentration of haemoglobin per unit of blood significantly increases the bloods oxygen carrying capacity**.**

The short & long term effects of exercise on the cardiovascular system

The cardiovascular system undergoes drastic changes during and immediately after intense exercise. Even more importantly, the cardiovascular system makes long term and beneficial adaptations to the demands of a regular exercise regimen.

During exercise, the cardiovascular system is called upon to meet the increased needs of the body in many ways. The cardiovascular system rushes oxygen to hard working muscles, returns used blood to the lungs to be re-oxygenated, and delivers fuel to the active tissues of the body.

Short-Term Effects

1. One of the short-term effects of exercise is an increase in hart rate. Actually heart rate will begin to rise before the start to exercise. As brain realizes you are going to work out and releases adrenaline to speed up our heart in preparation for the upcoming exertion. This is called "anticipatory response". Heart rate will continue to rise in direct proportion to the intensity of exercise until maximum heart rate is achieved.

2. Stroke volume- the amount of blood pumped out of the left ventricle by each beat increases by up to 80 ml per beat.

3. Cardiac output- the volume of blood the heart pumps in a period of one minute-increases from the typical 5 liters per minute, to up to 40 liters per minute, during strenuous exercise.

4. Shot-term changes in blood flow. At rest, the muscles require only about 15 to 20 percent of the total amount of blood circulating through the body. During exercise the hardworking muscles demand more oxygen from the cardiovascular system, up to 80 percent. In response, blood is shunted away from the digestive organs, kidney and liver and redirected to the skeletal muscles. Blood flow to the skin also increases. The blood vessels serving the skin dilate to allow more blood to the surface of the body. This helps to cool the body during exercise.

5. Blood pH-the level of acidity in our blood– becomes more acidic. Body uses by-products of carbon dioxide to buffer the hydrogen ions in your bloodstream. Hydrogen ions are electrically charged particles in your body. Because the greater the number of hydrogen ions the higher the acidity. Because breathe faster during cardiovascular exercise, expel carbon dioxide faster than you would normally. This gives the hydrogen ions time to accumulate.

Long-Term Effects

1. Decrease in resting heart rate. Because the rigors of regular exercise require so much work from the cardiovascular system, sedentary periods become even easier for the heart by comparison. The heart eventually becomes more efficient, and no longer needs to beat as quickly to supply the body with blood while at rest.

2. Stroke volume increases at rest. Resting heart rate is able to slow down because the heart is now trained to pump a larger quantity of blood with every beat.

3. Improved circulation. In response to the need to supply the muscles with more oxygen during exercise, the body increases its number of capillaries, the smallest blood vessels in the body. Existing capillaries also open wider.

4. Blood pressure decreases by up to 10 mmHg. An mmHg is a unit used for measuring pressure levels.

5. Blood volume increases. The body produces a greater number of red blood cells in order to keep the muscles supplied with oxygen during heavy exercise.

Conclusion

The study is all about the exercise physiology and its effects on cardiovascular system advances on the understanding of the influence of physical activity as non-pharmacological treatment in the prevention and control of cardiovascular disease, in particular to hypertension. Thus, regular physical exercise is a good recommendation because it presents real gains in reducing blood pressure in hypertensive patients, particularly when combined with pharmacological treatment and even healthy living life.

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EFFECT OF ANTIJUVNILE HORMONE PRECOCENE-II ON THE OVARION DEVELOPMENT OF *CHILO PARTELLUS*

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ABSTRCT

The length and width of the oocyte of normal pupae and adults of *Chilopartellus* increased gradually, where as in the precocene – II treated pupae and adults there was prominent decrease in the length and width of the oocyte.

KEY WORDS:*Chilopartellus*, Precocene-II, Juvenile hormone (JH), ovaries.

INTRODUCTION:

In insects deposition of yolk or vitellogenesis is under hormonal control. Depending on the species, JH and/or ecdysteroids may play important role in the control of vitellogeninbiosynthesis**.**

Vitellogenin is synthesized extraovarially and then become the major egg yolk protein i.e,vitellin. Vitellogenesis is the period of rapid oocyte growth during which yolk is deposited. Vitellogenin is synthesized in the fat body of females prior to the onset of yolk deposition in the oocytes. These female specific proteins are taken up by the developing oocytes deposited, in a slightly modified form, as vitellins, the major yolk proteins.

MATERIALS AND METHODS:

Insect culture:

The stem borer *Chilo partellus* is the most destructive pest of Jowar, one of the main millet crops grown in arid zones. For experimental purpose the above insect was reared in the laboratory on artificial diet of sorghum leaf powder and chick pea flour at a temperature of 27 ± 1^0 C and RH 65±5%.

The insect passes through five larval instars and then pupates. Freshly pupated pupae were treated with 5 micro grams/ 2 micro liters of precocene-II on its abdominal region. Parallel controls were treated each time and the experiments were triplicated. Ovaries of the precocene-II

treated, 2nd, 3rd and 4th instar, resultant precocious adults and the resultant adults from the treated pupae, dissected out to observe the gross morphology of oocytes.

RESULTS &DISCUSSION:

CONTROL:

PUPAE:

There was a gradual increase in the ovary and oocyte length and width during the pupalperiod.On the 1st day the length and width of the oocyte was 170.5×155.0µm.On the 2nd day of the pupal period the oocyte was 201.5×170.5µm. On the 3rd day of the pupal period the length and width of the oocyte was 232.5×170.5µm. There is further increase in the length and width of the oocyte. It was 263.5×186.0µm. On the 5th day of the pupal period the length and width of the oocyte was 294.5×263.5µm.

ADULT:

In the freshly emerged adult the length and width of the Oocyte was 325.5×325.5µm respectively. On the 2nd day of the adult life the length and width of the oocyte was 346.5×341.0µm.

TREATED:

EFFECT ON OVARIES:

In the resultant adults following Precocene-II treatment of 2nd,3rd,4th instar larvae, the ovaries remained immature and the ovaries appeared thin and filamentous ,vitellogenesis was disturbed.

TREATMENT OF PUPAE:

The length and width of the oocyte was less, when compared with the controls. In the freshly pupated pupa the length and width of the oocyte was 116.3×170.5µm. On the 2nd day of the treated pupae the length and width of the oocyte was 108.0×155µm. On the 3rd day the length and width of the oocyte was 93.0×139.5µm. There was a further decrease in the length and width

of the oocyte was 77.5×124µm. On the 5thday of the pupal period the ovaries were thin and filamentous, the length and width of the oocyte decreased to 62.0×108.5µm.

ADULT:

The ovaries were filamentous and the oocyte was 46.0×77.5µm. On the 2ndday of the adult life the length and width further decreased to 31.0×62.0µm.

DISCUSSION:

Precocious adults eclosed following Precocene-II treatment of 2nd, 3rd and 4th instar larvae , possessed thin filamentous ovaries , that remained essentially undeveloped throughout their short lives. This result gave conclusive proof, that the corpora allata were inactive, since the corpora allata are known to be the source of gonadotropic (juvenile) hormone.

All these findings point towards a lowering of the reproductive potentiality of females as a result of Precocine-II treatment.

TABLE-I

Oocyte development in control Pupae and adults of Chilo partellus			
Age of Pupae and moths(h)	No of larvae treated with acetone	No. of Pupae servived	Length and Width of the Oocyte
Pupae			
24 hours	20	20	170.5 x 155.0 µm
48 hours	20	20	201.5 x 170.5 µm
72 hours	20	20	232.5 x 170.5 µm
96h ours	20	20	263.5 x 186.0 µm
120 hours	20	19	294.5 x 263.5 µm
Adults			
24 hours	20	19	325.5 x 325.5 µm
48 hours	20	20	346.5 x 345.0 µm

TABLE-II

Inhibition of Oocyte development by Precocene-II in Chilo partellus (Pupae & Adults)			
Age of Pupae and moths(h)	No of larvae treated with Precocene-II	No. of Pupae servived	Length and Width of the Oocyte
Pupae			
24 hours	20	16	116.3 x 170.5 µm
48 hours	20	17	108.0 x 155.0 µm
72 hours	20	18	93.0 x 138.5 µm
96h ours	20	18	77.5 x 124.0 µm
120 hours	20	19	62.0 x 108.5 µm
Adults			
24 hours	20	16	46.0 x 77.5 µm
48 hours	20	18	31.0 x 62.0 µm

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Seri culture –a preferred rural employment and enterprise

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What Is Silk?

Man is always inquisitive for silk products. SILK - The Queen of Textiles, spells luxury, elegance, class and comfort. Mankind has always loved this shimmering fibre of unparalleled grandeur from the moment Chinese Empress Shiling Ti discovered it in her tea cup. It withstood many a daunting challenges from other natural and artificial fibres and yet, remained the undisputed Queen of Textiles since centuries. Exquisite qualities like the natural sheen, inherent affinity for dyes and vibrant colours, high absorbance, light weight, resilience and excellent drape etc. have made silk, the irresistible and inevitable companion of the eve, all over the world.

What is Sericulture?

Sericulture is an agro-based industry. It involves rearing of silkworms for the production of raw silk. Chemically speaking, silk is made of proteins secreted in the fluid state by a caterpillar, popularly known as 'silkworm'. These silkworms feed on the selected food plants and spin cocoons as a 'protective shell' to perpetuate the life. Silkworm has four stages in its life cycle viz., egg, caterpillar, pupa and moth. Man interferes this life cycle at the cocoon stage to obtain the silk, a continuous filament of commercial importance, used in weaving of the dream fabric.

Why Seri Culture?

- High employment potential
- Provides vibrancy to village economies.
- Low gestation, High returns.
- Women friendly occupation.
- Ideal programme for weaker sections of the society.
- Eco friendly activity.
- Satisfies equity concerns.

Types of Silk;

There are five major types of silk of commercial importance, obtained from different species of silkworms which in turn feed on a number of food plants. These are:

- Mulberry
- Tropical Tasar
- Oak Tasar
- Eri
- Muga

Mulberry silk

The bulk of the commercial silk produced in the world comes from this variety and often silk generally refers to mulberry silk. Mulberry silk comes from the silkworm, **Bombyxmori L.** which solely feeds on the leaves of mulberry plant. These silkworms are completely domesticated and reared indoors. In India, the major mulberry silk producing states are Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Jammu & Kashmir which together accounts for 92 % of country's total mulberry raw silk production.

Tropical Tasar:

Tasar (Tussah) is copperishcolour, coarse silk mainly used for furnishings and interiors. It is less lustrous than mulberry silk, but has its own feel and appeal. Tasar silk is generated by the silkworm,**Antheraeamylitta** which mainly thrive on the food plants Asan and Arjun. The rearings are conducted in nature on the trees in the open. In India, tasar silk is mainly produced in the states of Jharkhand, Chattisgarh and Orissa, besides Maharashtra, West Bengal and Andhra Pradesh. Tasar culture is the main stay for many a tribal community in India.

Oak Tasar:

It is a finer variety of tasar generated by the silkworm,**Antheraeaproyeli J.** in India which feed on natural food plants of oak, found in abundance in the sub-Himalayan belt of India covering the states of Manipur, Himachal Pradesh, Uttar Pradesh, Assam, Meghalaya and Jammu &Kashmir. China is the major producer of oak tasar in the world and this comes from another silkworm which is known as **Antheraeapernyi.**

Eri Silk

Also known as Endi or Errandi, Eri is a multivoltine silk spun from open-ended cocoons, unlike other varieties of silk. Eri silk is the product of the domesticated silkworm, **Philosamiaricini** that feeds mainly on castor leaves. Ericulture is a household activity practiced mainly for protein rich pupae, a delicacy for the tribal. Resultantly, the eri cocoons are open-mouthed and are spun. The silk is used indigenously for preparation of chaddars (wraps) for own use by these tribals. In India, this culture is practiced mainly in the north-eastern states and Assam. It is also found in Bihar, West Bengal and Orissa.

Muga Silk

This golden yellow colour silk is prerogative of India and the pride of Assam state. It is obtained from semi-domesticated multivoltinesilkworm,**Antheraeaassamensis.** These silkworms feed on the aromatic leaves of Som and Soalu plants and are reared on trees similar to that of tasar. Muga culture is specific to the state of Assam and an integral part of the tradition and culture of that state. The muga silk, an high value product is used in products like sarees, mekhalas, chaddars, etc.

Silk Manufacturing Process

There are many steps involved in silk manufacturing.

- Cultivation of Cocoons (Sericulture)
- Filature Operations
- Manufacturing of Silk Yarns
- Finishing of Silk Fabrics

Manufacturing Process for Silk Yarns

Silk is a fine translucent fiber produced from the silkworm. The manufacturing process of silk starts with the rearing of cocoons. There are many varieties of the silkworm from which the silk fiber can be obtained. However, it is found that the fiber obtained from the larva of Bombyxmori is of commercial value. The process of obtaining silk fiber from Bombyxmori requires careful nourishment of the cocoons which is put through the spinning process.

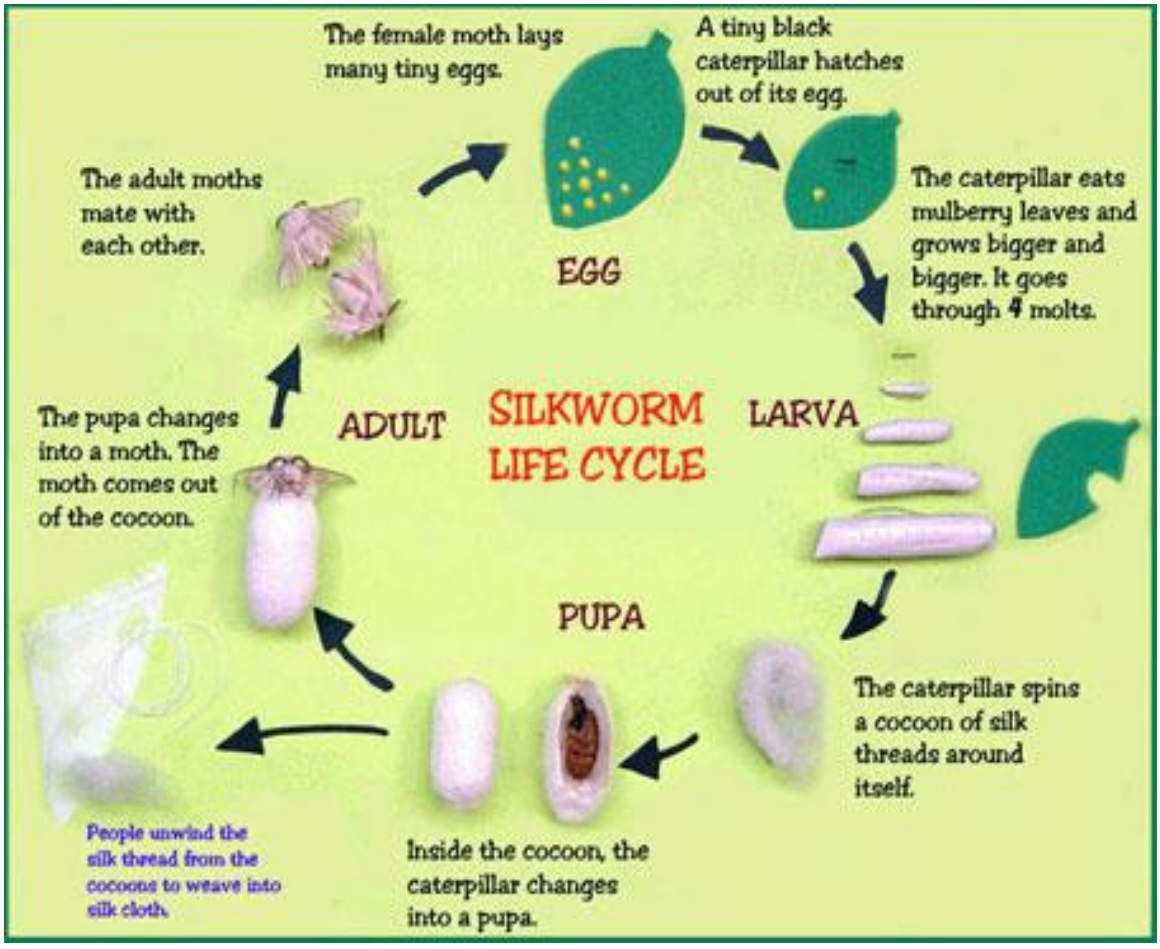
Cultivation of Cocoons:

The process of cultivating the silkworm for the production of raw silk is called as sericulture. Silk fiber is a continuous filament fiber consisting of the fibroin, which is connected together with the silk gum, sericin. This natural protein or fibroin is secreted from two salivary glands.

There are four stages in the life cycle of the moth which are as follows:

1. The egg, which develops into a larva, or caterpillar – the silkworm
2. The silkworm, which spins its cocoon for protection, to permit development into the pupa
3. The pupa emerges from the cocoon as the moth.
4. Female moth lays eggs, so continuing the life cycle.

They may be hatched three times a year. The female moth lays around 350 to 400 eggs and the moths die soon after. As they are subject to hereditary infection, the eggs from infected moths are destroyed which results into production of fine silk. Larvae of about 3mm are hatched from the eggs. For about 20 to 30 days, they are carefully nurtured and are fed five times a day on chopped mulberry leaves. Ideal temperature 23c-27c, humidity is 65%-80%.The silk moth lays eggs,



Mating

The silk-moths cannot fly because their bodies are too heavy in relation to their wings. Male's silk-moths die soon after mating while one single female silk-moth lies from 300 to 500 tiny eggs before she dies. The eggs hatch into the silkworms in spring time and the complete cycle takes approximately about two months.

- In 11thday, just after emergence mating with male and female by both are coupling.
- Mating for about one day every 30 minutes ejaculate sperms.

Decoupling

- After 1 ½ hours. Because need three ejaculations.

Egg laying

- Silk moths lay eggs on specially prepared paper by using paper card method.

Chemical treatment of eggs

I) Formaldehyde treatment (2%)-When immersing eggs are disinfected.

II) Acid treatment (1.1 5g Hcl)-eggs are immersed for 5 minuets

III) Washing in running water

IV) Air drying-keeping under the fan

Incubation

I) 1st day to 7thkeep the eggs rapped in a tissue paper.

II) 8th day-black boxing, eggs are rapped with black paper or put in to black box to prevent irregular hatching.

Hatching

- 11th day morning all the eggs are gray in color with dark spot.
- Expose eggs to light and then eggs are hatched around an hour.
- Silkworm are white but this stage black in ant stage
- .Which hatch into an ant called as larva about 1/8 inches (3mm) in length.
- This can be done at breeding station or rearing station.

Brushing

- Keep the eggs cards are rearing trays and removal of warms from egg shells/cards.
- Then giving 1st feed (1cm strips of lender mulberry leaves).warms are very attractive to mulberry leaves because it contain citral.

Warm rearing station

I) feeding

II) Cleaning

III) Increasing space

Feeding the larva

- The larva at this stage has voracious appetite and requires careful nourishment. They are fed 5 times a day on chopped mulberry leaves.
- After four changes of skin or molting the worm reaches full growth of about 3 ½ inches (9cm) long.
- At this stage the interest in the food ceases and is ready to spin its cocoon.
- The silkworm begins to secrete a protein like substance through a small opening under the caterpillar's jaws which is called as the spinneret.
- The silk solidifies when it comes in contact with the air.
- Pupation-On 27th day they leave the leaves and look for a corner to build a cocoon. Within twenty – four hours and in three days the cocoon is complete to a size and shape of a peanut shell.
- The filament is in the form of a double strand of fibroin, which is held together by a gummy substance called sericin, or silk gum.
- As this cutting through damages the cocoon, the filament cannot be unwound in one long thread. The life cycle is terminated at this point by a process known as stoving, or stifling.
- If left undisturbed, the pupae inside the cocoon develop into a moth within two weeks. To emerge, the moth breaks open the cocoon by secreting an alkaline liquid that dissolves the filament.

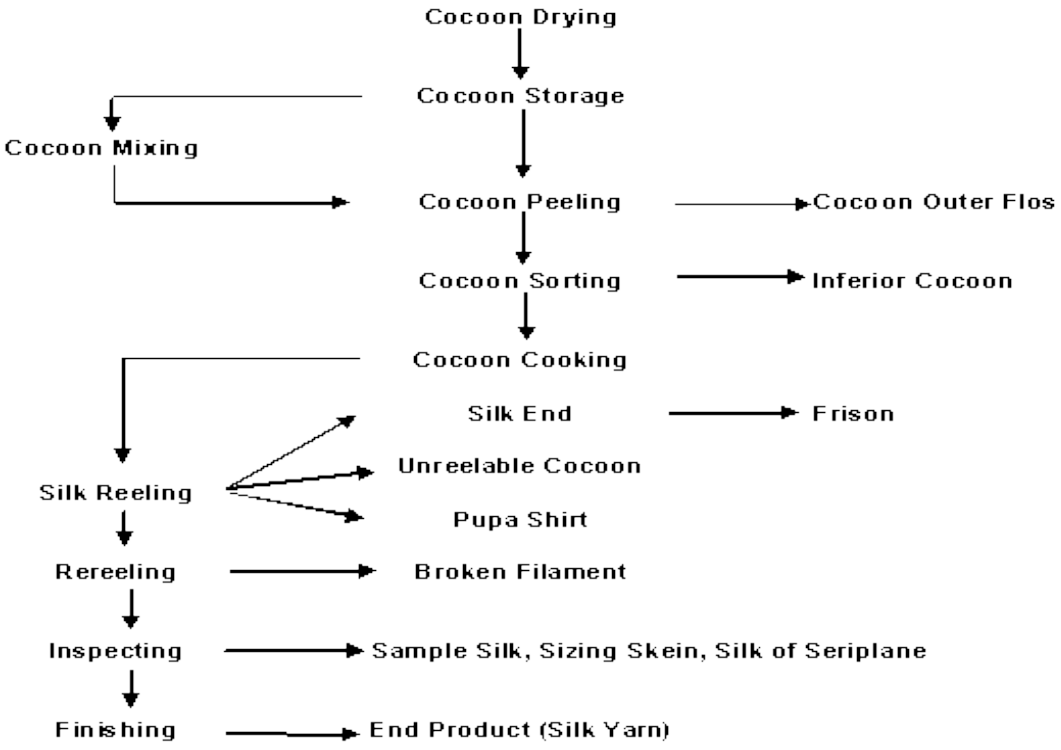
Processing & grading

- The silk yarn production begins with selecting for their quality.

- The cocoons will be sorted into normal and abnormal cocoons.
- The fresh cocoons normal are reelable to produce raw silk but the abnormal cocoons are unreelable.
- The abnormal cocoons are double cocoons, perforated cocoons, internally and externally soiled cocoons, thin-end cocoons, thin-middle cocoons, malformed cocoons, etc.

After the first selection of cocoons,

- They have to be dried.
- The first goal of cocoon drying is the protection of cocoon quality, to preserve conditions for reeling cocoons and prevent damage that might be caused by long periods of storage.
- Drying kills the pupa and evaporates moisture that would otherwise ruin cocoons.
- Dried cocoon storage conditions are designed to keep the raw material for long periods without any damage from moulds and pests.
- Cocoon storage should preferably be built with double walls. Cloth or polyethylene bags are recommended as containers for dried cocoon storage.
- In most modern factories which aim at producing high-grade raw silk, the cocoons are graded on visual inspection or by mechanical tests are actually mixed as in the required proportions. This is called cocoon mixing or blending and is done to ensure speed and uniformity of reeling as well as to obtain the desired effect in raw silk. The reelable cocoons have then to undergo the reeling processing, after which they are either processed further to raw silk or to thrown silk. The unreelable silk is processed further to spun silk.



INDIAN SCENARIO

In India, sericulture is not only a tradition but also a living culture. It is a farm-based, labour intensive and commercially attractive economic activity falling under the cottage and small-scale sector. It particularly suits rural-based farmers, entrepreneurs and artisans, as it requires low investment but, with potential for relatively higher returns. It provides income and employment to the rural poor especially farmers with small land-holdings and the marginalized and weaker sections of the society. Several socio-economic studies have affirmed that the benefit-cost ratio in sericulture is highest among comparable agricultural crops.

Item	Mulberry sericulture	Sugarcane	Turmeric
Total input costs	48,659	30,575	29,610
Gross returns	96,132	60,200	55,317
Net returns	47,476	29,625	25,707
CB ratio	1:1.98	1:1.97	1:1.02
Crop period	1 year	1 year	4 – 5 months

In India, mulberry silk is produced mainly in the states of Karnataka, Andhra Pradesh, Tamil Nadu, Jammu & Kashmir and West Bengal, while the non-mulberry silks are produced in Jharkhand, Chattisgarh, Orissa and north-eastern states. India has the unique distinction of being the only country producing all the five known commercial silks, namely, mulberry, tropical tasar, oak tasar, eri and muga, of which muga with its golden yellow glitter is unique and prerogative of India. China leads the world with silk production of 1,26,000 MT or 82.41% of the produce. India is the second largest producer of silk in the world with silk production of 23,679MT and has 15.49 % share in global raw silk production. All the countries except China and India have been witnessing a declining trend in raw silk production in the last two decades.

ANDHRAPRADESH SCENARIO

Andhra Pra1desh has many traditionally rich silk Handloom Weaving Centres like Dharmavaram, Pochampally, Peddapuram, Gadwal, Narayanpet, Cuddapah, etc., Thetraditional,

heavy, broad bordered rich with buttasarees of Dharamavaram have worldwide popularity. Similarly the tie & dye Jamadhani fabrics & silk. Sarees produced inPochampally are World renowned and are earning lot of Foreign exchange. There are35,000 Nos. of Silk Handlooms in Andhra Pradesh. The highest concentration is inDharmavaram (Ananthapur) Cuddapah and Pochampally in Nalgonda District.Sericulture is an agro based labour intensive industry providing gainful employment mostly to rural people. Andhra Pradesh occupies 2ndposition in the country in production of silk.

HERBAL IMMUNOSTIMULANTS FOR SUSTAINABLE AQUACULTURE

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Aquaculture is a viable and legitimate sub-sector in India's agriculture industry that produces food for human consumption and stock enhancement, generating substantial employment and economic prosperity inRural and coastal communities. Recently aquaculture is beset by disease and environmental problems. Disease is a major constraint in cultured species around the world. Production losses due to diseases outbreaks are a major threat to the sustainability of this sector The diseases are caused mainly by virus, bacteria, fungi and parasites. The world's demands for high quality aquaculture products make control of diseases increasingly important. Protecting the fish from diseases can be done by two ways .one is by strengthening the self immune power of the organism and the second is through medication .But the use antibiotics and chemotherapy has been criticized because their use has created with resistance bacteria, and toxicity both in fish and environment. Another alternate for sustainable aquaculture is herbal immunostimulants .

In recent years there has been a significant interest in the preparation, experimental and clinical characteristics of biological active substances namely immunostimulants. Immunostimulants are substances which enhance the humoral and cellular immune response both in specific and non specific way. These are the products from natural or synthetic origin having different mechanism of action immunostimulants activate bodies natural resistance in order to cope with various pathogenic ,bacterial and viral disease some herb al extracts have potential application in fish culture primly because they can be easily obtained , not expensive and act against broad spectrum of pathogen .

some medicinal plant extracts or products have been used to control fish and shellfish diseases. In this regard the medicinal plant extracts and their products act as immunostimulants modulating the immune response to prevent and control fish and shellfish diseases. The immunostimulants mainly facilitate the function of phagocytic cells, increase their bactericidal activities, and stimulate the natural killer cells, complement, lysozyme activity, and antibody responses in fish and shellfish which confer enhanced protection from infectious diseases.

Currently increased consumer demand for perfection in fish and shellfish farms has put new dimensions to the quality, safety, elimination of concomitant pollutants, antibiotics, and carcinogens during the production process. In this context plants or their byproducts are preferred since they contain several phenolic, polyphenolic, alkaloid, quinone, terpenoid, lectine, and polypeptide compounds many of which have been shown to be very effective alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds. In aquaculture the herbal medicines are also known to exhibit anti-microbial activity, facilitate growth, and maturation of cultured species; besides under intensive farming the anti-stress characteristics of herbs will be of immense use without posing any environmental hazard. Administration of herbal extracts or their products at various concentrations through oral (diet) or injection route enhance the innate and adaptive immune response of different freshwater and marine fish and shellfish against bacterial, viral, and parasitic diseases. Even an overdose of immunostimulants may induce immunosuppression without side effects but helps to reduce the losses caused by disease in aquaculture..

Characteristics of an ideal immunostimulant:

1. It should be non-toxic even at a high dose rate.

2.It should stimulate a wide range of non-specific immune responses against bacteria,fungi,virus,protozoa and helminthes.

3.It should be capable of amplifying primary and secondary immune responses to infectious agents.

4.Breakdown products of compound concerned should be either inactive or readily biodegradable in the environment.

5.It should be having defined chemical composition or biological activity.

6.It should be active by oral route and should be stable both in its native state and after incorporating into food and water.

7. It should be inexpensive

Main objectives of Immunostimulation:

1.Promoting a greater and more effective sustained immune response to tho se infectious agents producing subclinical disease without risks of toxicity,carcinogenicity or tissue residues.

2.Hastening the maturation of non-specific and specific immunity in young susceptible animals.

3.Enhancing the level of duration of specific immune response, both cell mediated and humoral,following vaccination.

4.Overcoming of immunosuppressive effects of stress and of those infectious agents that damage or interface with the functioning of cells of immune system.

5. Selectively stimulating the relevant components of the immune system or non-specific immune mechanism that preferentially confer protection against micro-organisms.

SOME PLANT ORIGINATED IMMUNOSTIMULANTS

Natural plant product promote various activities such as Antistress, Growth promotion, Appetite stimulation,

Immunostimulation, Aphrodisiac and Antimicrobial properties Due to the active principles such as alkaloids,

flavanoids pigments, phenolics, terpenoids, steroids and essential oils.

1.Ocimum sanctum (Tulsi) : Leaves of Ocimum sanctum contain water-soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophylllene that may act as an immunostimulant. Leaf extract of Ocimum sanctum affected both specific and non-specific immune responses and disease resistance against Aeromonas hydrophila. It stimulated both antibody response and neutrophil activity

2. Phyllanthus emblica (Amla) : Phyllanthus emblica has antioxidant activity, anti-fungal activity, antimicrobial activity and anti-inflammatory activity. Amla fruit pulp contains large proportion of vitamin C, which has also been identified as animmunostimulant.

3. Azadirachta indica (Neem) :Azadirachta indica is a highly esteemed ‘‘wonder’’ tree of India that is widely dispersed throughout the country. Biomedical research has revealed that neem possesses anti-human immunodeficiency virus, anti-tumor andantimicrobial activities. Azadirachtin, a triterpenoid derived from A. indica, enhanced respiratory burst activities, the leukocyte count

4. Solanum trilobatum (Purple Fruited Pea Eggplant) : The herbal extract of Solanum trilobatum contains compound like Sobatum, b-solamarine, solaine, solasodine, glycoalkaloid, diosogenin and tomatidineSolanum trilobatum possesses a broad spectrum of antibiotic, antibacterial and anticancer activity. A study aimed at assessing the effects of the water- and

hexane-soluble fractions of S. trilobatum on the nonspecific immune mechanisms and disease resistance

5. Eclipta alba (Bhringraj) : Eclipta alba a herb belonging to Asteraceae, is widely available and distributed throughout India. This plant has been reported to possess several medicinal properties. The methanol extracts of the whole plant of Eclipta albasignificantly increased the phagocytic index, antibody titer and WBC count .

6. Zingiber officinale (Ginger) : Roots and the obtained extracts of Zingiber officinale contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity.

7. Echinacea (purple coneflowers) and Allium sativum (garlic)

Echinacea and Allium sativum improve the gain in body weight, survival rate and resistance against challenge infection of Aeromonas hydrophila. Both compounds showed extended effects after withdrawal and improved resistance to cold stress during the winter season

8. Camellia sinensis (Green tea) : Green tea (GT) extracts contain a unique set of catechins that possess biologic activity in antioxidant, antiangiogenesis, and antiproliferative assays that are potentially relevant to the prevention and treatment of various forms of cancer

9. Aloe vera : Oral administration of aloe vera in common carp can enhance some of specific and non specific immune responses. This appears to be achieved primarily by increasing lysozyme activity, serum bactericidal power and the total protein and IgM levels.

10. Nyctanthes arbortristis (Night-flowering Jasmine) : Nyctanthes arbortristis (L) is widely used plant in the traditional medicinal systems of India. It possesses hepatoprotective, antileishmanial, antiviral and antifungal activities. extract-supplemented diet appears to be the optimal regimen for maximal disease resistance .

11. Fermented vegetable product (FVP) : The phagocytic activities and superoxide generation of peritoneal induced leukocytes were significantly higher in fish

12. Other plant extract : Plant extract of Cyanodon dactylon, Aegle marmelos, Tinospora cordifolia, Picrorhiza kurooa and Eclipta alba were used to increase the immunity

Advantages of Herbal Immunostimmulents.

- These are available in plenty and cheap
- Their action is effective
- No adverse effect on natural ecosystem.
- They act as the substitution for feed,
- They act as growth promoter.

- They acts as anti bacterial substances.
- They acts as anti fungal agents.

Conclusion : Herbal immunostimulants have the ability to reduse the stress effects and have a potential application in fish culture, primarly because they can be easily obtained,not expensive and acts against broad spectrum of pathogens.Most of the herbal extracts can be given orally, which is the most convenient method of immunostimulation.It will be best if we use herbal immunostimulants instead of chemicals.UsingS of chemicals to control diseases leads to effluent remittance in the fish muscle which may cause side effects to the consumer.As this biological and eco-friendly approach is not known to many of the farmers, awareness programs should be done to popularize these herbal immunostimulants.It is better to conduct result demonstration to disseminate this herbal approach rapidly among the farmers to control the diseases.A better disease control management remains key for the further development of aquaculture in general, and specifically a more sustainable industry SO USE of herbal immunostimulants for sustainable aquaculture is the need of the hour.

Effect of 2-Methyl furan on sperm head morphology of mouse

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Abstract

2- methyl furan is a dietary furan present in several vegetarian and non-vegetarian foods. The experiments were designed to evaluate the mutagenic potential of 2-methyl furan in inducing point mutations by scoring various types of sperm head abnormalities since sperm head morphology is controlled by both autosomal and sex chromosomal genes.

8-10 week old Swiss albino mice were orally administered with 3 doses (1,000,2,000and 4,000 ppm)of 2-methyl furan. Control animals received the same volume of sterile double distilled water. Animals of both series were maintained under identical conditions.

Treated animals were dissected after 24hr, and 1,2,3,4 and 5 weeks at weekly intervals following administration to cover one spermatogenetic cycle. Slides were prepared from epidydimis by the standard technique(Warbeck and Bruse,1978).1000 sperms were scored from the slides for assessing various sperm head abnormalities, which could be classified, into amorphous,hookless,double headed,double tailed, banana shaped, micro and mega head types. The results showed that amorphous and hook less were predominant compared toother types. However not much of variation could be seen in sperm head abnormalities in theexperimental set compared to the controls.

Application of appropriate statistical tests showed that the values are insignificant. This emphasises that 2-methyl furan lacks the ability to induce point mutations in autosomal and sex chromosomal genes which control the morphology of sperm in mice.

INTRODUCTION

Food, air and water constitute the most minimal and essential requirements of all living organisms.With an increasing knowledge in the fields of science and technology many compounds have been introduced to serve the human requirements for a comfortable life. Nearly one thousand new ones are added annually to this ever increasing list (1). They include drugs, chemicals, cosmetics, food additives, pesticides, insecticides, fungicides and other environmental pollutants. These add a new burden to the already existing imbalance in the ecosystem. This list is increasing every day. In order to meet the food requirements of an ever increasing population, the farmer uses many fertilizers to get higher and better yields. These contaminate air, water and food. Moreover they get accumulated in the body tissues.An accumulation of these or their biodegraded products in tissues may induce serious genetic damages to the precious genetic apparatus. Further such a hazard may get transmitted to future generations also.

It is known that diet plays an important role in the etiology of some human cancers (2-6).It is reported that diet is responsible for causing 1-5% of human cancers (5,6,and 7-9).

In view of this there is a dire need to evaluate the genetic damage induced by various components of our daily foods. In the context of this information, the present study has great relevance and significance to safeguard the genetic health of the teeming millions.

2-Methyl furan is present in both vegetarian and non-vegetarian foods like milk, meat, poultry products, canned beef, coffee, tea, cocoa, various fruits and nuts(10). 2-Methyl furan caused centrilobular necrosis of the liver and bronchial injury in male rats.Stich et al (1981) reported the mutagenic action of dietary furans employing Chinese hamster ovary cells in culture. They found them to be toxic and induced high frequency of chromatid breaks and exchanges in the absence of s9 mixture.

Materials and methods:

Among the available battery of tests in vivo cytogenetic protocols are regarded as reliable assays(1 to 6).Experiments were conducted in both single and cumulative dose series .In single dose series 8-10 week old Swiss albino mice were orally administered with 3 doses (1,000,2,000and 4,000 ppm) of 2-Methyl furan only once. Where as in cumulative dose series they were orally fed for three days at 24 hr. Intervals. Control animals received the same volume of sterile double distilled water. Animals of both series were maintained under identical conditions.

Treated animals were dissected after 24hr, and 1,2,3,4 and 5weeks at weekly intervals following administration to cover one spermatogenetic cycle. Slides were prepared from epidydimis by the standard technique(11).1000 sperms were scored from the slides for assessing various sperm head abnormalities, which could be classified,into amorphous, hookless, double headed, double tailed, banana shaped, micro and mega head types.The data presented in table was analysed statistically by the 2x2 contingency test of Pillai and Sinha (12)to see the statistical significance of the quantitative data on sperm head abnormalities induced by 2-Methylfuran.

Results:

The quantitative data on sperm head abnormalities scored and computed from control and treated animals at various stages of meiotic cycle after single and cumulative dose treatments with 2-Methylfuran are tabulated in table. Among the sperm head abnormalities only hookless and amorphous type of sperms were recorded in control as well as in treated animals in both series of experiments(Figure-I & Figure-II). Since they were also observed in controls, the significance was calculated over the respective control values.

TABLE
QUANTITAIVE DATA ON SPERM HEAD ABNORMALITIES INDUCED BY 2-METHYLFURAN IN MICE*

Period	Dose in ppm	Single Dose Series			X2 Values	No of normal sperms	Cumulative Dose Series		X2 Values
		No of Normal sperms	Abnormal Sperms				Abnormal Sperms		
			No	Percentage			No	Percentage	
	Control	997	3	0.3	□□	997	3	0.3	□□
	1000	996	4	0.4	0.14	996	4	0.4	0.14
	2000	994	6	0.6	1	995	5	0.5	0.5
24 hr	4000	993	7	0.7	1.6	993	7	0.7	1.6
	Control	998	2	0.2	□□	998	2	0.2	□□
	1000	996	4	0.4	0.67	996	4	0.4	0.67
	2000	994	6	0.6	2.67	994	6	0.6	2.67
Iwk	4000	993	7	0.7	2.79	992	8	0.8	3.61
	Control	997	3	0.3	□□	998	2	0.2	□□
	1000	996	4	0.4	0.14	996	4	0.4	0.67
	2000	996	4	0.4	0.14	996	4	0.4	0.67
II wk	4000	994	6	0.6	1	994	6	0.6	2.67
	Control	998	2	0.2	□□	997	3	0.3	□□
	1000	997	3	0.3	0.2	995	5	0.5	0.5
	2000	994	6	0.6	2	994	6	0.6	1
IIIwk	4000	994	6	0.6	2	993	7	0.6	1.6
	Control	996	4	0.4	□□	997	3	0.3	□□
	1000	995	5	0.5	0.11	996	4	0.4	0.14
	2000	994	6	0.6	0.4	996	4	0.4	0.14
Ivwk	4000	994	6	0.6	0.4	995	5	0.5	0.5
	Control	997	3	0.3	□□	997	3	0.3	□□
	1000	997	3	0.3	0.14	996	4	0.6	0.14
Vwk	2000	996	4	0.4	0.14	995	5	0.5	0.5
	4000	994	6	0.6	1	994	6	0.4	1

(

***Computed from 1000 Sperms for each dose and Period**

X2 Value significant at 5% level (Expected Value : 3.84)

PHOTOS

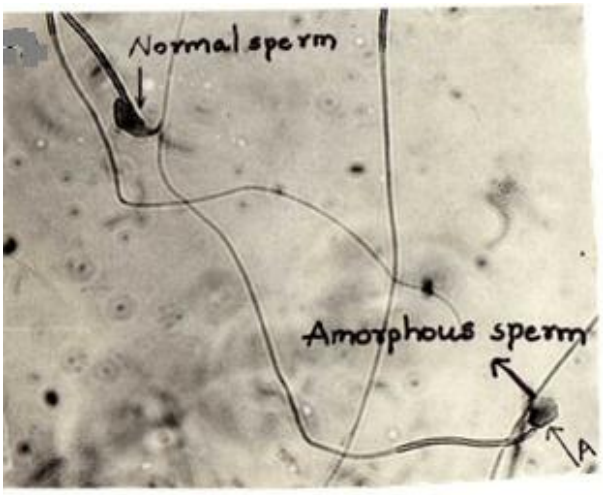

Figure-1

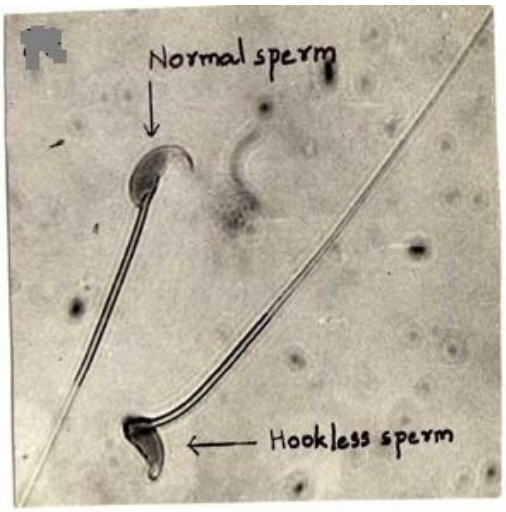

Figure-2

Discussion:

A close look at Table reveals that none of the quantitative data on the sperm head abnormality assay following single and cumulative dose administration of 2-Methylfuran was significant .An absence of cognisable difference between two sets of data indicates that 2-Methylfuran does not induce any point mutations. In the context of the observations of Cohen et al (13), that "negative chromosome findings with drugs that enjoy wide spread use should be published"(pp2425-2426). Thus the present results have got great relevance and significance.

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Phytochemical analysis of *Bacopa monnieri*

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ABSTRACT

Bacopa monnieri, an important medicinal plant belonging to the family of Scrophulariaceae, which occurs naturally in India and has a long history of being used in the traditional Ayurvedic medicine in the treatment of a number of disorders, particularly those involving anxiety, intellect and poor memory. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration was extracted with ethanol solvent for the purpose of preliminary screening .The different qualitative chemical tests were performed on the extract to detect the various phyto constituents or antioxidants present in them. The photochemical screening reveals the presence of many therapeutically important compounds such as glycosides, alkaloids, saponins, phenols, proteins and carbohydrates. Since the phenolic compounds have remarkable antioxidant activities, our present work aims at evaluating the antioxidant activities by the three in vitro models such as DPPH free radical scavenging and Superoxide Anion Radical scavenging Activity. From the present investigative phytochemical analysis of Bacopa monnieri plant extract it is revealed that the antioxidant activity of the plant material is due to the presence of phenolic compounds.

Key words: Bacopa monnieri, glycosides, alkaloids, saponins, phenols.

Introduction

Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz et al., 2004). Traditional medicinal plants are often cheaper, locally available and easily consumable, raw or as simple medicinal preparations. Nowadays, traditional medicinal practices form an integral part of complementary or alternative medicine. A member of the Scrophulariaccae family, this small, creeping herb is much-branched, with small leaves and light purple flowers. Bacopa's soft, sessile leaves are succulent, reniform and spatulate, measuring about 2.5 mm in length. The plant's stem has soft, ascending branches, and is about 10-30 cm long and 1-2 mm thick. Brahmi's flowers are blue or white in color, and grow on peduncles that are usually longer than the leaves. The fruits are ovoid, with acute capsules included in a persistent calyx.

Chemical composition

The active constituents of Brahmi include alkaloids, saponins, and sterols. Many of these principles, including the alkaloids brahmine and herpestin, and the sapponins d-mannitol and harsaponin, acid A, and monnierin, were isolated in India long ago. Other active constituents have since been identified, including betulic acid, stigmastarol, beta-sitosterol, as well as numerous bacosides and bacopasaponins. The constituents believed to be responsible for Brahmi's cognitive effects are bacosides A and B. (Chakravarty et al., 2003)

Material and Methods

Collection of the plant material

Bacopa monnieri (BM) plant was collected from Tirumala hills and indentified by a botanist, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher no. 1688).

Preparation of Plant Extract

The whole plant was shade-dried and powdered. The plant material was percolated with circulating 95% ethanol (200 ml) for three rounds. The residue was extracted twice using the same procedure. The extract was filtered and concentrated under reduced pressure in the Buchi rotavapour yielding a greenish-black sticky residue. Finally the extract was freeze - dried and was used for further studies.

Determination of in vitro antioxidant activity

Determination of Antioxidant Activity by DPPH Radical Scavenging Method

The extract solution for the DPPH test (Fargare, 1995) was prepared by re-dissolving 0.2 g of each dried extract in 10 ml methanol. The concentration of DPPH solution was 0.025 g in 1000 ml of methanol. Two ml of the DPPH solution was mixed with 10, 20 and 40 µl of the plant extract/ethanol solution and transferred to a cuvette. The reaction solution was monitored at 515 nm, after an incubation period of 30 minutes at room temperature, using a UV-Visible spectrophotometer. The inhibition percentage of the absorbance of DPPH solution was calculated using the following equation:

Inhibition%= (Abst=0 min -Abst=30 min)/ Abst=0 min ×100

Where Abst=0 min was the absorbance of DPPH at zero time and Abst=30 min was the absorbance of DPPH after 30 minutes of incubation. Ascorbic acid (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of plant extract solution to

the Ascorbic acid equivalent. IC50 is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Determination of Superoxide Anion Radical Scavenging Activity

Superoxide Anion Radical scavenging Activity was measured (Duan, 2007) with some modifications. The various fractions of plants extracts were mixed with 3 ml of reaction buffer solution (pH 7.4)containing 1.3 μM riboflavin, 0.02 M methionine and 5.1 μM NBT. The reaction solution was illuminated by exposure to 30W fluorescent lamps for 20 minutes and the absorbance was measured at 560 nm using UV-VIS spectrophotometer. Ascorbic acid (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of plant extract solution to the Ascorbic acid equivalent. The reaction mixture without any sample was used as negative control. The Superoxide anion radical scavenging activity (%) was calculated as:

$$\frac{Ao - As}{Ao} \times 100$$

Where, Ao = absorbance of positive control

As = absorbance of sample

PHYTOCHEMICAL STUDY

Preliminary phytochemical screening was performed [Harborne1988]. The presenceof phytoconstituentssuch as Tannins,Saponins, Phenolic, Terpenoids, Steroids, Phytosterol Anthraquinone, Glycosides Flavonoid'swere confirmed by the following procedure.

1. TEST FOR TANNINS

About 2ml of filtered extract was taken in a test tube and 2ml of ferric chloride added. The presence of blue-black coloured precipitate indicates the presence of tannins.

2. TEST FOR SAPONINS

To 0.5 ml of extract was added 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for stable persistent forth.

3. TEST FOR TERPENOIDS (salkowski test)

To 0.5 ml each of the extract was added 2ml of chloroform. Concentrated Sulphuric acid 3ml was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

4. TEST FOR CARDIAC GLYCOSIDES

To 2ml of extract 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.A violet ring may appear below the brown ring,

while in theacetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

5. TEST FOR ANTHRAQUINONE

0.5ml of the extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette in to another test tube observed for colour changes.

6. TEST FOR FLAVONOIDS

Dilute ammonia 5ml was added to the extract. Concentration sulphuric acid 1ml was added. A yellow colouration that disappears on standing indicates the presence.

7. TEST FOR STEROIDS

To 1ml extract 10ml of chloroform was added.10ml of concentrated sulphuric acid was added carefully to form coloured layer. Upper layer turns red.sulpuric acid layer forms yellow with green fluorescence, indicates the presence of steroids.

8. TEST FOR PHYTOSTEROL

1ml of extract was dissolved in 10ml of chloroform and 10ml concentrated sulphuric acid along the side of the test tube. Brown ring indicates presence of phytosterol.

9. TEST FOR PHENOLIC

2ml of extract 1ml ferric chloride was added, a blue or green colour indicates presence of phenolic.

STATISTICAL ANALYSIS

Values of the measured parameters were expressed as mean ± SEM. One way-ANOVA (F value) was used to test the significance of difference among more than two arithmetic means, followed by Post – hoc test (Scheffe multiple comparison) to test the difference between each two means. The significance was considered at p values <0.05. All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for windows, version 11.5.

RESULT:

Determination of Antioxidant Activity by DPPH Radical Scavenging Method

DPPH Radical scavenging activity was determined of ethanolic extract. The experiments were performed in triplicates and mean values of Antioxidant activity of each of the fraction of the plant extract were determined. The extract of Bacopa monnieri (whole plant) tested for

antioxidant activity using DPPH radical scavenging was determined, the ethanolic extract showed the maximum antioxidant activity with IC50 values of 49.12µg/ml as shown in and

Table 1. Superoxide anion radical scavenging was determined for ethanolic extract. The experiments were performed in triplicates and mean values of antioxidant activity of each of the fraction of the plant extract were determined. The values of antioxidant activity determination by Superoxide Anion Radical Scavenging method follow the same order as that of DPPH assay. Bacopa monnieri (whole plant) tested for antioxidant activity using Superoxide Anion radical scavenging method, the extract showed the maximum antioxidant activity with 65.68 % and 62.34 % inhibition as shown in Table 1.

Table:1 Antioxidant Activity by DPPH Radical Scavenging Method and Superoxide Anion Radical Scavenging Method

Method	Method (IC50); µg/ ml /%
Plant DPPH-Radical Scavenging	49.12
Superoxide Anion Radical Scavenging Method (%)	62.4%

Table:2 Preliminary phytochemical screening of callus extraction of *Bacopa monnieri.*

In the present study preliminary phytochemical tests on the ethanolic extract revealed the presence or absence ofTannins, Saponins Phenolic, Terpenoids, Steroids, Phytosterol,Anthraquinone, Glycosides Flavonoid's(given tab-1)

Sl No.	Phytochemical(Test)	Ethanol	Aqueous
1.	Tannins	+	+
2.	Saponins	+	+
3.	Terpenoids	+	+
4.	Glycosides	_	+
5.	Anthroquione	_	_
6.	Flavanoids	+	_
7.	Steroids	+	+
8.	Phytosterol	+	+
9.	Phenolic	_	_

DISCUSSION

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants (Cheeseman, 1993). The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids (Young, 2001).Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets causing oxidative stress.Cells are protected against oxidative stress by an interacting network of antioxidants (Sies, 1997). Antioxidants were believed to be a cure for many disorders. The investigations on Bacopa monnieri plant extract were found to yield substantial positive data pointing towards the evidence of antioxidant activity. The data obtained from DPPH Radical Scavenging and Superoxide Anion Radical Scavenging assay clearly suggested that the antioxidant activity of Bacopa monnieri. It can also be noted that the extract of Bacopa monnieri was found to scavenge the free radicals such as peroxides, superoxides and hydroxyl radicals. From the various test it was concluded that Bacopa monnieri is found to possess greater antioxidant potential. Antioxidants are intimately involved in the prevention of cellular damage -- the common pathway for cancer, aging, and a variety of diseases.

The phytochemical screening of ethanolic extract of Bacopa monnieri showed the presence of many bioactive chemicalconstituents including Tannins, Saponins, Terpenoids, Steroids, Phytosterol, Anthraquinone, Glycosides Flavonoid's etc which are considered to be responsible for antioxidant activity. Thus, the saponins in the extract may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage.Therefore Bacopa monnieri was considered to possess antioxidant activity and therefore it can be used as an antioxidant along with the other suggested and proven to have therapeutic remedies.

CONCLUSIONS

Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract, and cancer. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Synthetic antioxidants are recently reported to be dangerous to human health.These experimental evidences of Bacopa monnieri suggest that because of its

antioxidant activity, it may be useful in the treatment of human pathologies in which free radical production plays a key role. The antioxidant property may be attributed to its phytochemical constituents.

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Oxidative stress induced renal damage: A pharmacological appraisal of Annona squamosa leaves extract under STZ induced diabetic rats

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Abstract

Annona squamosa is a multipurpose tree with edible fruits and is a source of medicinal value. The leaves of *Annona squamosa* were proved to have antioxidant activity. Oxidative stress is a major factor in the pathogenesis of diabetic complications. In the recent time plant sources are explored widely for medicinal value. The present study was made to investigate the protective effect of ethanol extract of *Annona squamosa* leaves on altered blood glucose levels and endogenous antioxidant enzymes in Streptozotocin induced diabetic rats ((STZ 50mg/kg body weight). Elevated blood glucose levels and down regulation of antioxidant enzymes activity including superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx) were observed in diabetic group when compared to control. However, blood glucose levels declined and SOD, CAT, GPx activities invigorated with *Annona squamosa* leaves extract (300mg/kg body weight) treatment to diabetic rats.Hence, I concluded that ethanol extract of *Annona squamosa* leaves potentially reduce the diabetic complication by reducing the glucose levels and improve the endogenous antioxidants activity.

Keywords:*Annona squamosa*, Antioxidant enzymes, Diabetes, Oxidative stress

Introduction

The alarming scalationintheglobalincidenceandprevalenceof diabetesmellitusoverthepast20years,toitscurrentestimates ofalmost300million,isprojectedto further increaseto440million individualsbytheyear2030 (Shaw*et al.*,2010).Type 2 diabetes is aconsequence of an imbalance between insulin responsiveness andinsulin production. One of the first clinical signs is glucose intolerance,due to insulin resistance of peripheral organs, including liver, fat andmuscle, and is initially compensated by increased pancreatic β-cellinsulin secretion.

Hyperglycemia-inducedimpairmentsinredoxbalanceareconsidereda keytriggerofdiabeticcomplications,throughup regulated generationofreactiveoxygenspecies(ROS) (Sourris *et al.*, 2012; Haidara*et al.*, 2006), togetherwithan impairedabilityoftheendogenousantioxidantdefensesystemto removethem (Wold*et al.*,2005) which lead to damage of cellular organelles andenzymes, increased lipid peroxidation, and development of insulinresistance (Maritim *et al.*, 2003). These consequences of oxidative stress can promote the development of complications of diabetesmellitus.The chronic hyperglycemia of diabetes is associatedwith damage, dysfunction and failure of various organs, e.g.kidneys, retina, heart, liver, peripheral and central nervous system,over the long term (Fajans, Cloutier,

&Crowther, 1997).High intracellular glucose concentration has been suggested to be a prerequisite for the development of functionaland structural changes in the kidney typical of diabetic nephropathy.

Herbal medicines have been used for many years by differentcultures around the world, both for the prevention and managementof diabetes (Marles and Farnsworth, 1995). *Annona squamosa (A. squamosa),* commonly known as custard apple, is cultivated throughout India, mainly for its edible fruit. The plant is attributed with medicinal properties, which include antifertility and antitumor activities in rats(Asolkar *et al.,* 1992). The young leaves of *A. squamosa* along with five grains of black pepper are used extensively for its antidiabetic activity by tribal men (1985). Some other medicinal values includinganti-inflammatory, antioxidative and antimicrobial potential (Yang*et al.,* 2008; Baskar *et al.,* 2007).Ethanol extract of the leaves and stem is reported to have anti-cancer activity (Bhakuni*et al.,* 1969). In the Ayurvedic system of medicine, herbal extracts but not purified compounds have been used from centuries because many constituents with more than one mechanism of action are considered to be beneficial.

No detailed study has been carried out on the effect of ethanolic extract of *A.squamosa* leaves on nephro protective activity in STZ induced diabetic rats .Hence, the present study was planned to evaluate the nephro protective effect of ethanolic extract of *A.squamosa* leaves on blood glucose levels and antioxidant activity in diabetic rats.

2. Material and Methods

2.1**Chemicals**

All chemicals were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pitrsburg, PA, USA), and Merck (Mumbai, India).

2.2**Preparation of ginger ethanol extract**

Annona leaves were collected from Seshachalam hills, Tirupati A.P. India. Four kilogram of fresh leaves were cleaned, washed under running tap water, cut into small pieces, air dried and powdered. 200g of this powder were macerated in 1000 ml of 99.9% ethanol for 48 hours at room temperature and were then filtered**.** The filtrate was concentrated to dryness under reduced pressure in a rotary evaporator (Model: HS-2005V).

2.3**Animals**

Thirty Wister strain male albino rats, weighing 250 ± 20 g, were obtained from the Indian Institute of Science, Bangalore, India. The animals were housed in plastic cages and kept in the laboratory under constant temperature (27 ± 2^0C) with 12 hrs dark and 12 hrs light for throughout treatment period. The rats were received a standard pellet diet (Sai Durga feed, Bangalore, India) and water *ad libitum.* The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee resolution number (10 /i/a/CPCSEA/IAEC/SVU/KSR-GVS/dt 15/11/2010).

Induction of diabetes:

After fasting for 15 hrs, rats were injected intraperitonially with a single dose of Streptozotocin of 50mg/kg body weight obtained from Sigma Chemical Co., St. Louis, MO, USA, freshly dissolved in citrate buffer (pH 4.5). After injection, they had free access to food and water and were given 15% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was identified by moderate polydipsia and marked polyurea. From the second day onwards fasting blood samples were collected from the rats by tail vein and the blood glucose was measured by Accuecheck Sensor comfort glucometer (Manufacturer-Roche Germany) to know the induction of diabetes.

2.4**Experimental Design**

The rats were divided into five groups each of six rats and the treatment was given everyday via orogastric tube for seven weeks.

Group I, Normal Control (NC): rats received 0.9% saline and feed with normal diet

Group II, Annona leaves extract (At): rats received 300mg/kg of ethanol extract of Annona leaves extract orally for one month treatment

Group III, Diabetic control (DC):Streptozotocin50mg/kg b.w isgiven intraperitonially for the induction of diabetes to this group

Group IV, Diabetic + Glibenclamide treatment (Di + Glb): diabetic rats treatedwith glibenclamide (20 mg/kg body weight orally).

Group V, Diabetic + Annona treatment (Di+ At): Diabetic rats received Annona ethanolic extract (300 mg/kg) for a period of 30 days.

After completion of one month treatment, the animals were sacrificed by cervical dislocation. Blood was collected from heart puncture and kidney tissue was used for the histopathological studies and biochemical assays.

Estimation of blood glucose levels

Estimation of blood glucose was carried out by using accuchek glucometer.

Estimation of antioxidant enzymes

SOD activity was assayed by the method of Misra and Fridovich (1972)at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer.Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrineby 50%, which is equal to 1 U per milligram of protein. CAT activity wasdetermined at room temperature by using the modified version of Aebi (1984)and absorbance of the sample was measured at 240 nm for 1 min in a UV-spectrophotometer.Activity of GPx was determined by the method of Flohe and Gunzler(1984) in the presence of NADPH and absorbance was measured at 340 nm usingcumene hydrogen peroxide. GR enzyme activity was determined according to themethod of Carlberg and Mannervik (1985).

Histopathology

Kidney tissues were isolated from the animal and washed with ice-cold saline. They were then fixed in 10% formalin solution. Sections of 3 µm thickness were stained with haematoxylin and eosin (H & E) for histopathological examination in the light microscope.

Statistical analysis

Statistical analysis of data was represented as mean ± SD. All the statistical analyses were carried out by SPSS software. Dunnett's multiple comparison tests and one-way analysis of variance (ANOVA) were used to assess the differences, $p<0.001$was considered as highly statistically significant.

Results

Blood glucose levels

The blood glucoselevels in STZ-injected diabetic rats were drasticallyincreased from the baseline (Table 1). This increaseof blood glucose was almost three-fold higher evenafter 30 days compared to the control rats. However,it was found that the elevated blood glucose levels

indiabetic rats dropped significantly ($P < 0.001$) after30-day Annona leaves extract administration. Glibenclamide, whichhas been used as a standard diabetic drug to comparethe beneficial effects of Annona leaves extract, showed significant($P < 0.001$) decrease in blood glucose levelsand this was almost equal to the normal control rats.

Table 1 : Effects of Annona leaves ethanolic extract and glibenclamide treatments on blood glucose level in streptozotocin- induced diabetic rats. All the values are mean ± SD of six individual observations

Groups	Blood glucose levels	
	Initial	Final
Group I (NC)	209 ± 5.19	229 ± 5.20
Group II (At)	226** ± 3.46	199** ± 3.63
Group III (DC)	216 ± 4.86	164** ± 1.29
Group IV (Di +Glb)	226** ± 3.26	205* ± 4.30
Group V (Di+ At)	215 ± 3.26	192** ± 3.43

Values in the parentheses denote per cent change over normal control Values are significant compared to normal control at *** P<0.05

Antioxidant enzymes

Fig.2represents the activity of oxidative stress marker enzymesin the different experimentalgroups. There was significant depletion in the SOD, CAT and GPx activities in the kidney tissues of diabetic rats ($p< 0.001$) .However Annona treatment significantly reversesthe SOD, CAT and GPx back to normal levels ($p < 0.001$).

Histopathology of kidney

Fig. 3 illustrates the pathological changes in thekidney of a diabetic rat including severe tubular degeneration,degeneration of glomeruli, focal necrosisof tubules, cystic dilatation of tubules and fatty infiltration.The above mentioned pathological symptomswere reduced in ginger-treated

diabetic rats. The histologicalpicture of Annona leaves treated diabetic rats showedrestoration of glomeruli, tubules and renal cells.

Discussion:

Diabetes mellitus is a life-threatening metabolic disorder and itis estimated that its annual incidence rate will continue to increasein the future, worldwide. Hyperglycaemia, the primary clinicalmanifestation of diabetes mellitus, is associated with the developmentof micro- and macro-vascular diabetic complications. Traditionalplant remedies have been used for centuries in thetreatment of diabetes (Kesari, Gupta, & Watal, 2005), but only afew have been scientifically evaluated. Therefore, we have investigatedthe effect of Annona leaves ethanolic extract on blood glucose levels and biomarkers of oxidative stress in kidney tissues of STZ induced diabetic rats.

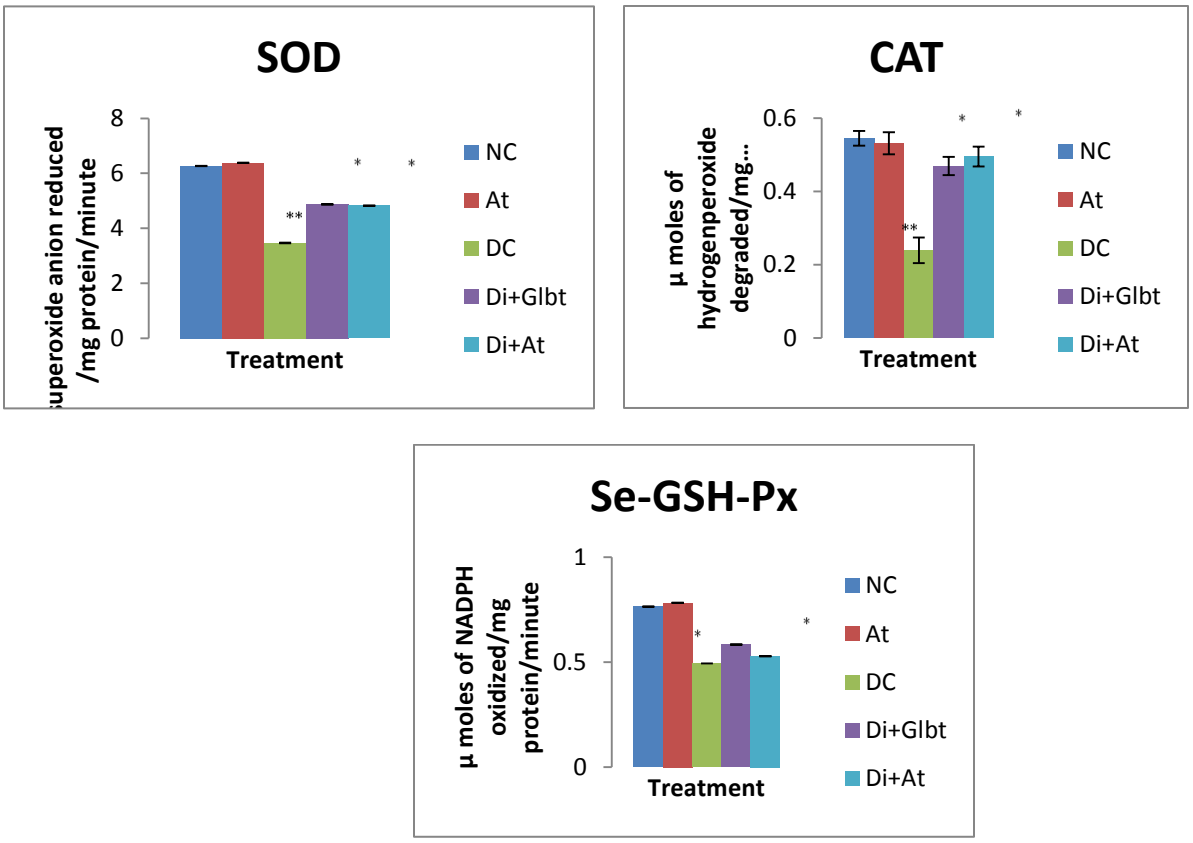
Streptozotocin injection resulted in diabetes mellitus, whichmay be due to destruction of beta cells of Islets of Langerhans asproposed by others (Kavalali *et al.*, 2002). Diabetes arises from irreversibledestruction of pancreatic beta cells, causing degranulationand reduction of insulin secretion (Zhang and Tan, 2000).STZ destroy the β-cell in the pancreas and increasethe overproduction of glucose and gluconeogenesis.Gluconeogenesis and overproduction of the glucose isthe prime factor of the hyperglycemia (Latner 1958). In our results, blood glucose levels were increased in diabetic rats this may be due to decreased insulin levels and initiation of gluconeogenesis in the liver resulting in diminish of pancreatic β-cell by STZ induced intoxication. However, *Annona* leaves extract recovered the blood glucose was observed in diabetic rats. It has been reported that using medicinal plant extract to treat STZ-induced diabetic rats results in activation of *ß*-cells and insulinogenic effects(Padmini and Chakrabarti, 1982).

In the current study, SOD activity was measured in kidney tissue of all the experimental groups such as Normal control, *Annona* treated, diabetic, Glibenclaimade treated diabetic and diabetic rats with *Annona* treatment.

Oxidative stress is the imbalance between production and removalof reactive oxygen species. Increased oxidative stress, whichcontributes substantially to the pathogenesis of diabetic complications,is the consequences of either enhanced ROS production orattenuated ROS scavenging capacity. Several reports have shownthe alterations in the anti-oxidant enzymes during diabetic condition(Preet *et al.*, 2005). SODhas been postulated as one of the most importantenzymes in the enzymatic antioxidant defensesystem which catalyses the dismutation

ofsuperoxide radicals to produce H_2O_2 and molecularoxygen hence diminishing the toxic effects causedby these radicals (Baynes, 1995).

Fig:1. Effect of NC,At, DC(STZ),Di+Glbt and Di+At on SOD, CAT in Se GPx activities in kidney tissue of male albino rats. The Values marked with *(P<0.05) **(P<0.01).



In our result, reduced activity of SOD in kidney tissue was observed in diabetic rats. Similar consequence were also ensued in Shanmugam *et al* (2011) studies which have been demonstrated that SOD activity was decreased as a result of hyperglycemia provoked free radicals may cause to inactivate the enzymes in diabetic kidney. SOD activity increased with treatment of ethanolic extract of Annona leaves to diabetic rats. It has been reported that Annona leaves extract have shown free radical scavenge activity that could be cause to enhance the SOD activity in diabetic condition.

Catalase (CAT) is a hemeprotein which catalyses the reduction ofhydrogen peroxides and protects the tissues fromhighly reactive hydroxyl radicals. The superoxideanion has been known to inactivate CAT, which isinvolved in the detoxification of hydrogenperoxide.Reduced activity

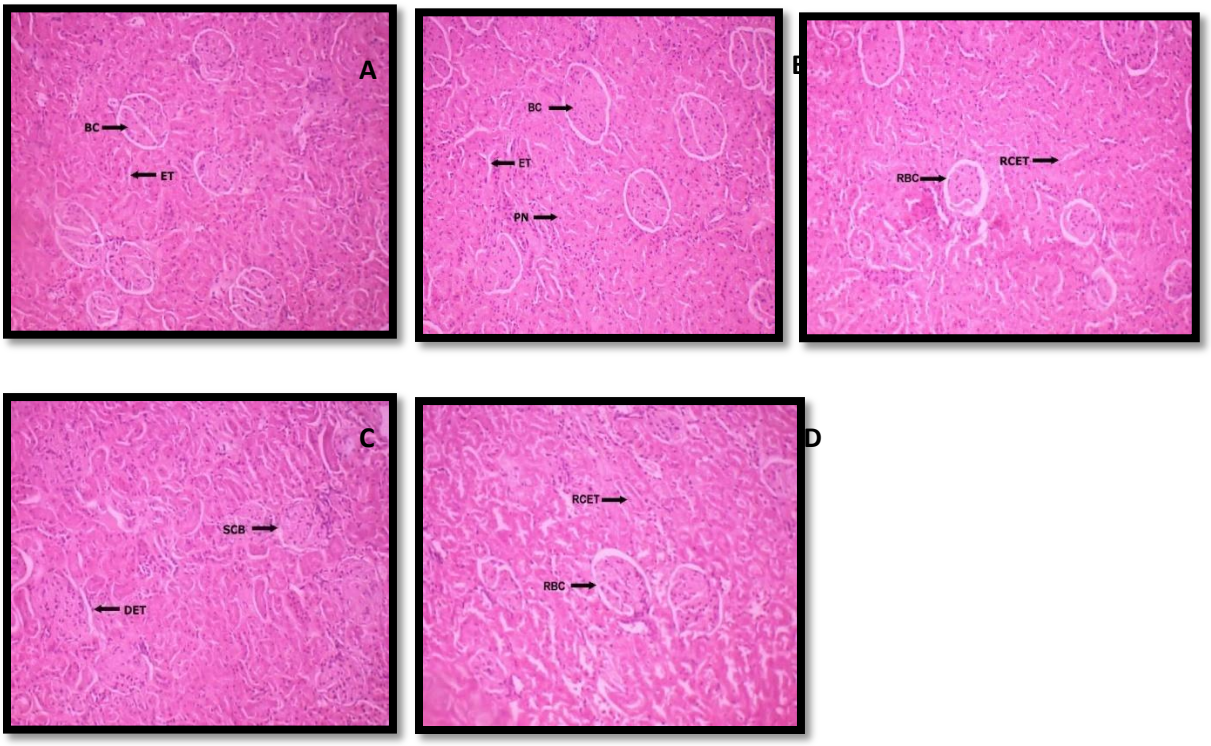
of CAT in kidney tissues have been observed in diabetic rats and this activity may result in a number of deleterious effects due to accumulation of hydrogen peroxide (Satheesh *et al.,* 2004; Huang *et* al., 2007).The activity of CAT in the kidney of diabetic rats was found to be decreased there by leading to a possible sustained elevation of intracellur hydrogen peroxide levels due to metabolic imbalances during diabetes accompanied by inhibition or down regulation of antioxidant enzymes as CAT may be responsible for oxidative stress in the disease state of hyperglycemia.

Basha and Subramanian (2011) have been reported that *Annona* increases plasma antioxidant capacity and decreases free radical toxicity in diabetic rats.Ghadir *et al.,* (2011) *Annona* scavenges the free radicals involved in the process of oxidative stress thus enhancing the concentration of antioxidant enzymes. In the present study similar consequences may occurred CAT activity was significant increase with Annona leaves extract treated to diabetic rats

GPx plays a primary role in minimizingoxidative damage. It has been proposed that GPx isresponsible for the detoxification of H_2O_2 in lowconcentration whereas Catalase comes into playwhen GPx pathway is reaching saturation with thesubstrate. GPx catalyze the reduction of hydrogenperoxide and hydro peroxides to non-toxicmetabolites. Depletion of GSH will render the enzyme GPx inactivation and or less active (Mahendran and Shyamala Devi, 2001). The reason for the reduced activity of GPx might lie in the decreased mitochondrial pool size of GSH because of entry of cytosolic GSH in to mitochondria is impaired indeed it has been reported that CAT and GPx can be inactivated by oxidative attacks (Fernadez *et al.,* 199l;Kerem and Korean, 2003).

Glutathione peroxidase (GPx), an important antioxidant enzyme,was significantly decreased in diabetic kidney tissues,which indicates impaired scavenging of H_2O_2 and lipidhydroperoxides. This result is consistent with the studies of Friesen, Buchau, Schott-Ohly, Lggssiar, and Gleichmann (2004). The decreasedGPx activity represents a compensatory mechanism to degrade H_2O_2. However, GPx activity significantly upregulated with Annona treatment in diabetic treated group. The increased GPx activity in *Annona* treated rats showing the antioxidant property of *Annona* in removing the hydro peroxides from the kidney tissue. The Phenolic compounds mentioned may involve in the antioxidant defense mechanism and protect the tissues against the free radicals (Manju and Nalini, 2005).

4 Impact of ginger on kidney tissue during diabetic condition.



A) Photomicrograph of normal control (NC) kidney, showing Normal rat kidney architecture shows glomeruli (GM) and proximal convoluted tubules. BC-Bowman's capsule; ET - Renal tubule; PN-Pycnotic nuclei
B) Plant extract control rat kidney architecture shows mild glomerular (GM) inflammation with lymphocytes and mild degenerative changes in renal tubules. BC-Bowman's capsule; ET -Renal tubule; PN-Pycnotic nuclei
C) STZ Induced rat kidney shows Glomeruli architecture is damaged within folding of the Bowman's capsules and mild necrotic changes in renal parenchyma. SCB-structural changes in bowman's capsule; DET-Degenerative changes in renal tubules; PN-Pycnotic nuclei
D) Glebenclamide treated STZ Induced rat kidney shows Intact Glomerules architecture of cell is normal and mild inflammatory changes. RCET-Regenerative changes in elongated renal tubules; RBC-Regenerative bowmans capsule; PN-Pycnotic nuclei
E) Plant extrct treated STZ Induced rat kidney shows normal, healthy and functional Glomeruli and regenerative renal tubules with minimal inflammation is seen.No evidence of any necrotic changes.RCET-Regenerative changes in elongated renal tubules; RBC-Regenerative bowmans capsule
F) The histopathological studies of kidney revealed severe tubulardegeneration, degeneration of glomeruli, focal necrosis of tubules,cystic dilatation of tubules and fatty infiltration in diabetic controlrats, which might be associated with increased diuresis and renalhypertrophy in diabetic rats. The collecting tubules showed thatdilation may be under the pressure of increased urine flow. Theseobserved pathological changes were decreased in diabetic ratswhen they were treated with Annona. The glomeruli appear to

be restored,tubules also appeared to be regenerated and less fatty infiltrationwas observed in diabetic rats treated with Annona , whichmay be due to the protective effect of Annona (Fig.4). Thus, in additionto the blood glucose-lowering effect, histopathological observationsalso support the notion that Annona at 1% and 2% producedsignificant increments of antioxidant enzymes and protected therenal tissues in diabetic rats.

G) In conclusion, our study suggests that Annona mayhave beneficial effects in diabetes that hold the hope of a new generationof antidiabetogenic drugs. However, comprehensive chemicaland pharmacological research is required to find out the exactmechanism of Annona for its antidiabetogenic effect and to identifythe active constituent(s) responsible for this effect.

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Sub-cloning and expression of nucleocapsid protein in *E.coliDH5α*

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Abstract

Sub cloning is a technique used to move a particular gene of interest from a parent vector to a destination vector in order to further study its functionality. N protein which encapsulates the negative strand RNA genome and in association with the L protein P protein and subunits of the viral RNA polymerase it forms the ribonucleoprotein core of the virus. The N protein plays several important roles during the virus transcription and replication cycles. Restriction enzymes are used to excise the gene of interest (N protein) from the clone it is purified and isolated plasmid DNA which contain N protein this is digested with BamHI to check the orientation and further cleaved restriction enzyme with EcoRI for 12 hrs to release the N gene and DNA analysed on agarose gel. Ligation is carried out along with a vector (PBR_{322}), ligation product is transformed into competent *E.coli* DH5α. The transformation results the white coloured colonies are indicative of positive clones while the blue colonies are indicates negative. The positive clones were induced by 1M IPTG and protein expression was checked by SDS-PAGE. DNA polymerase and primers are used to amplify specific target Sequence during multiple cycles of invitro DNA synthesis.

Key words:*E.coli DH5α* , N protein, BamHI, $PBR_{322,}$ and RNA polymerase

INTRODUCTION

The N protein is a structural subunit of nucleocapsid and is the most abundant protein found during infection. It is the key protein which encapsulates the negative strand RNA genome and in association with the L protein, P protein and subunits of the viral RNA polymerase it forms the ribo nucleoprotein core of the virusN protein is a highly charged, basic protein that can self associate to form dimers (**He et al., 2004; Surjit et al., 2004**). N protein is thought to participate in the replication and transcription of viral RNA and to interfere with cell-cycle processes of host cells (**Parker & Masters, 1990).**

The three-dimensional structure of the N-terminal portionof the protein is similar to those of other RNA-binding ***o***ne of the most abundant viral proteins producedinside a coronavirus-infected cell is the nucleocapsid(N) protein, a multifunctional phosphoprotein (**Calvo et al.,2005; Chen et al., 2005; Jayaram et al., 2005**). During the replicative mode of the transcribing complex, the nascent RNA is encapsulated by denovo synthesized N protein, resulting in the synthesis of the full length plus strand N-RNA complex. This in turn serves as a template to synthesize and replicate the minus strand N-RNA complex for its external morphogenesis and budding as viral particles.

In molecular biology, subcloning is a technique used to move a particular gene of interest from

a parent vector to a destination vector in order to further study its functionality. Subcloning is not to be confused with molecular cloning, a related technique. Restriction enzymes are used to excise the gene of interest (the insert) from the parent (mycoplasma). The idea behind using the same restriction enzymes is to create complementarysticky ends, which will facilitate ligation later on. The enzyme used to ligate DNA fragments is T4 DNA ligase, which originates from the T4 bacteriophage. CIAP is also added to prevent self-ligation of the destination vector insert should become successfully incorporated into the parent plasmid.The plasmid is often transformed into a bacterium like *E.coli*. Ideally when the bacterium divides the plasmid should also be replicated. In order to ensure growth of only transformed bacteria (which carry the desired plasmids to be harvested) ampicillin is used in the destination vector for selection. The full length N gene encoding the nucleocapsid protein was sub cloned into the bacterial expression vector to facilitate the over expression of the protein in *E.coli*. The full length N gene was excised out of parent plasmid and blunt end ligated in to the vector, recombinant plasmid harboring the N gene was transformed in to DH5α cells. Colonies harboring the gene in the proper orientation were subsequently implicated in expression of the recombinant N protein.

MATERIALS AND METHODS

All the recombinant DNA protocols for the isolation of plasmid, ligation, restriction analysis, transformation, screening for plasmids or inserts, agarose gel and acrylamide gel electrophoresis etc., were performed as described in molecular cloning **(Sambrook et.al., 1989)** and the promega applications guide.

Isolation of plasmid DNA from clone

Grow the clone culture in LB broth at 37°c for 12-24 hrs. Centrifuge 1.5ml of culture for 10mins at 4,000 rpm. Add 1ml of Distilled water to the pellet, resuspend it and centrifuge this at 4000rpm for 5mins. Re suspend the pellet in 100ul of solution-I. Add 200ul of Solution-II and mix by gentle inversion. Incubate this at room temperature for 10mins. Add 300ul of solution-III Mix it immediately and Incubate on ice for 15 mins Centrifuge this at 14,000rpm for 15mins. Transfer the supernatant to a fresh tube and add equal volume of Phenol: Chloroform (1:1) Example: For 500µl of supernatant add 250µl of Phenol and 250µl of Chloroform Add 500ul of

70% Ethanol to the pellet and centrifuge this at 14,000rpm for 5 mins. Discard the supernatant and resuspend the pellet in 30ul of nuclease free water.

Agarose gel Electrophoresis

Cast a gel of 1% by dissolving 0.2gms Agarose in 20ml of 1X TAE, add 2µl of ethidium bromide for visualization of DNA bands load the DNA sample i.e. 10µl of sample+5µl of gel loading dye in to the wells, allow it to electrophores for some time and visualize the bands under gel document system.

Purification of DNA

Add 1ul RNase to the DNA mixture and incubate the sample at 37˚C for one hour, add volume of 3M Sodium acetate (pH-5.2) and 2.5 vol absolute alcohol (90%) keep it on ice for 10mins. Spin at 12,000rpm for 15-20 minutes and collect the pellet and wash it with 150µl of 70% ethyl alcohol. Keep it at room temp for 15-20minutes Airs dry the pellet and dissolves it in appropriate volume of Nuclease free water, then separate the DNA fragments on agarose gel

Qualitative and quantitative estimation of DNA

Spectrophotometry follows the Principle of Beer-Lambert's law: Take 2 ml distilled water in a cuvette and calibrate the spectrophotometer at 260nm as well as 280nm, it acts as a blank. Add 10 µl of each DNA sample to 1990µl distilled water and mix well note the OD at 260nmand ODat 280nm values on spectrophotometer. Calculate the OD_{260}/OD_{280} ratio

Concentration = OD260 X 50ug/ml X Dilution factor

Competent cells preparation

Day: 1

Prepare the LB medium and autoclaved. Inoculate a single colony of E.coli DH5α Cells into 10ml LB medium, grow overnight with shaking at 37ºC.

Day: 2

Prepare 100mM Calcium chloride solution and Chill this solution for further use. Inoculate 10ml of freshly prepared LB medium with 1ml of Overnight grown culture. Grow this at 37ºC shaking at 150 rpm to an Optical Density of 0.375 at 590 nm, pour the culture into pre

chilled centrifuge tubes and chill this on ice for 10 mins and centrifuge at 3200rpm for 7 mins and discard the supernatant and resuspend the pellet gently by swirling in 5ml of Chilled Calcium chloride solution. Centrifuge this at 3200rpm for 5 mins. Collect the pellet and resuspend it in 2.5ml of calcium chloride Solution. Leave the resuspended cells on ice for 30mins and centrifuge this at 3200rpm for 5min discard the supernatant and resuspend the pellet in 200µl of calcium chloride solution, dispense the cells into pre chilled sterile 1.5ml tubes and Freeze immediately and transfer this to -20ºc for storage.

Transformation

To 200µl of competent cells add 200ng of plasmid DNA gently mix this and place it on ice for 30 mins. Heat shock in a 42ºC water bath for 90 sec followed by rapid chilling on ice for 2 min. 800µl of sterile LB broth was added to the transformation mix and the cells were allowed to grow for 45 min at 37°c with vigorous shaking. About 200µl of transformation mixture was plated on to a LB-amp (100µg/ml). Incubate these plates at 37°C for 12-16 hrs in incubator until the transformed colonies appeared on the plate. After the incubation period check for the presence of blue and white colonies. White colonies indicate the positive clones carrying the insert in them.

Mini preparation of plasmid DNA from transformed cells:

Single transformed colony were incubated in a 3ml of LB broth with Ampicillin (100 ug/ml) and incubate overnight at 37°c, the cells were harvested by centrifugation pellet was resuspended in 100ul of solution-I and incubate at room temperature for 5 min. To this 200ul of freshly prepared solution-II and 150ul of solution-III was added and the tubes were shaken till a curdy white precipitate was observed. The tubes were stored on ice for 10 min. The precipitate was removed by centrifugation and collect supernatant extracted with equal volumes of phenol: chloroform: isoamylalcohol after centrifugation upper aqueous phase was extracted with equal volumes of chloroform and collect the aqueous phase and the DNA was precipated by adding 2.5M ammonium acetate, 2 volumes of chilled ethanol and incubated at -20°c for 1½ hours. The DNA pellet obtained after centrifugation was washed with 70% ethanol and the pellet was resuspended in 40µl of TE buffer (pH 8.0). Separate the plasmid DNA on the agarose gel and if any contamination is observed purification of DNA is to be done and contamination free plasmid is separated on the agarose gel.

Digestion of plasmid DNA

The plasmid DNA was setup for restriction digestion overnight at 37°c with Plasmid DNA (2.5µl), Bam HI (2µl), assay buffer(2µl) and sterile distilled(13.5µl) water and subsequently analyzed on the 1% agarose gels.

Restriction digestion to release the N gene:

Restriction digestion with Eco R I was setup using Restricted DNA from Bam HI (2.5µl), Eco R I (2 µl), assay buffer (2 µl) for 12 hours. The restricted DNA was subsequently analyzed on the 1% agarose gel.

Gel elution of the n gene insert from agarose gel:

Slice the gel piece containing the DNA into small pieces add 2.5ml of sodium iodide solution and incubate at 55°c for 10 min in a water bath and add30µl of silica glass beads solution mix it and leave it at room temperature for 10 min and Centrifuge add 400µl of sterile distilled water to pellet. Separate the DNA fragments on agarose gel.

Ligation:

Recovered DNA (1µl), 1X ligase assay buffer (2µl), T_4 DNA ligase (1µl) and sterile distilled H_2O (16µl) incubate at 16ºC overnight. Separate the ligated DNA on the agarose gel and visualize on UV transilluminater.

Competent cells preparation:

Mentioned as above

Transformation:

Aliquot100µl of competent cells into 1.5ml sterile eppendorffs add 7µl of ligation mix to the tubes gently mix this and place it on ice for 15 mins. Heat shock in a 42ºC water bath for 2mins and place back on ice for approximately 10 mins add 1ml of LB medium and incubate this at 37ºc with shaking for one hour to allow the expression of antibiotic resistance gene. Prepare a LB agar plate and spread 100µl of ampicillin, 40µl of X-gal and 4 µl of IPTG onto a plate let this

dry for some time, to this add 100µl of transformed culture and spread it, other plate without ampicillin is control. Spread100µl of competent cell onto the agar incubate these plates at 37°C for 24hrs in incubator. After the incubation period check for the presence of blue and white colonies. White colonies indicate the positive clones carrying the insert in them. The white colonies are taken and are grown in the LB broth and the protein optimization is performed.

Expression optimization

Transfer transformed colonies to 3ml of freshly prepared LB broth with 3 µl of antibiotic ampicillin and grow this at 37ºC for 3hrs, 1ml of this culture acts as un induced control. The remaining 2ml of culture add 2µl of IPTG and grow this at different temperatures such as 37ºC, 30 ºC, 25 ºC. Pellet the culture and check for expression of protein by loading it onto denaturing gel i.e., SDS-PAGE.

SDS-PAGE

Assemble two glass plates (one notched) with two side spacers, clamps, grease, etc. Stand assembly upright using clamps as supports, on glass plate pour some pre-heated 1% agarose onto glass plate, place assembly in pool of agarose this seals the bottom of the assembly prepare Separating gel, ensuring no air bubbles form pour into glass plate assembly carefully. Prepare stacking gel, then pour onto top of set resolving gel, insert comb, allow to set, Remove comb, fill with electrophoresis buffer. Assemble top tank onto glass plate assembly. Fill with 1X electrophoresis buffer. Load the samples into the wells created by the comb. Allow the samples to electrophorese at 100Volts. After Electrophoresis remove the gel by separating the glass plates and transfer the gel to Staining solution. Let it stand for 2-3hrs after this transfer the stained gel to destaining solution to remove the excess of stain observe the gel for the formation of sharp thick bands

Results and discussion

In association with the L&P proteins and subunits of the RNA polymerase, the N protein forms the ribonucleo protein core of the virus. This RNP core is implicated in both transcription of the viral RNA as well as replication of the RNA genome. N protein is composed of three distinct regions containing RNA-binding motif(s), and appropriate signals for modulating cell signaling (**Jaehwan et al., 2005**)

The basic and foremost step involved is the isolation of plasmid DNA from the clone. **The isolated DNA is depicted in the figure given below:**

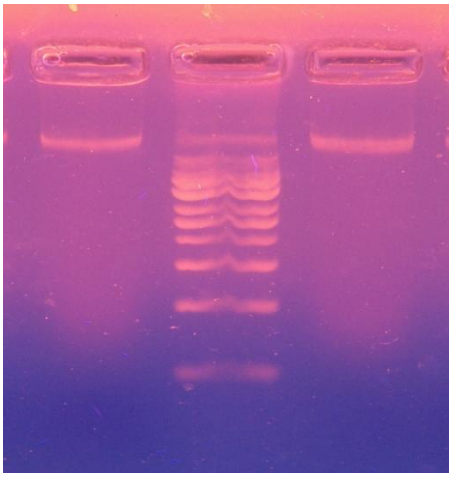


Figure: isolated DNA from clone

M = 1 Kb Marker

The Qualitative and quantitative estimation of the isolated DNA was done by Spectrophotometry. The Absorbance was measured at 260nm and further at 280nm for the quality and the following expression was used to calculate the quantity of DNA:

Concentration = OD260 X 50ug/ml X Dilution factor

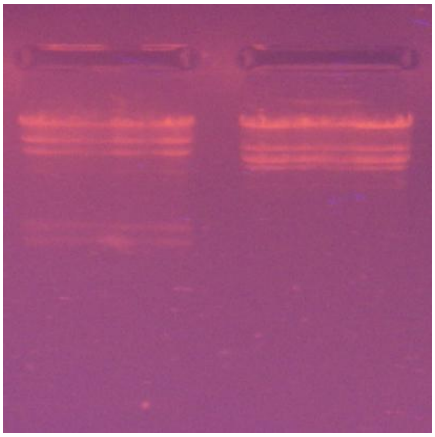
= 0.236 X 50 X 2000/10

= 2360

Hence for 1µl the concentration is approximately. The quality of DNA is calculated by taking the ratio absorbance at 260 and 280 pure DNA is in the range of 1.8-2.0. Hence the DNA isolated is pure and free of any protein and RNA contamination.

Plasmid (PBR_{322}) containing the full length N gene was digested with Bam HI to check the orientation of the N gene. This was an essential step to identify the appropriate expression vector into which the gene could be sub cloned. Bam HI was chosen as it has unique sites within the N gene and on the plasmid carrying it. Restriction digestion with EcoRI for 12 hours was done to release the N gene and the DNA sample was analysed on 1 % agarose gel. Gel elution of

the N gene insert from agarose gel was done by using silica glass bead method. In order to obtain the fragment corresponding to the N gene insert, double digestion was carried out.

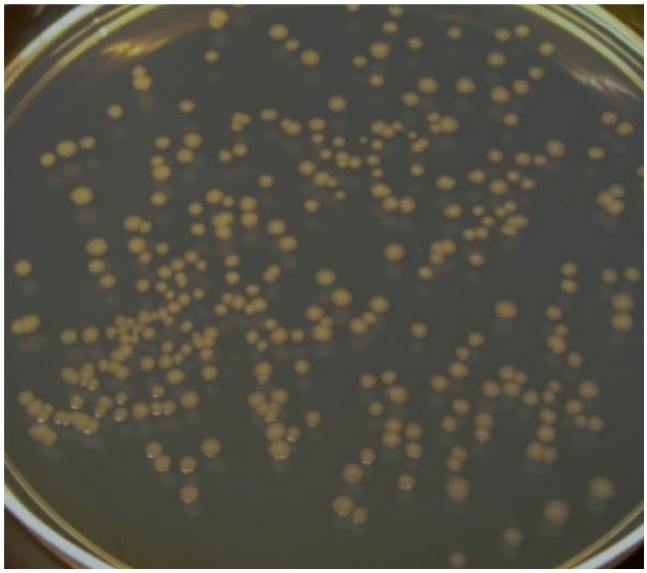


Double digestion with Restriction Enzymes: Bam HI.

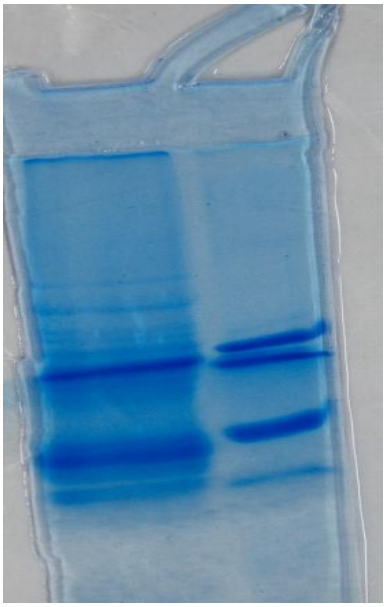
The ligation reaction was set up using a vector: Insert ratio of 1:3. This was incubated at 16°c for 16hrs. The ATP-dependent DNA ligases catalyze the joining of single-stranded breaks (nicks) in the phosphodiester backbone of double-stranded DNA in a three-step mechanism. The first step in the ligation reaction is the formation of a covalent enzyme-AMP complex. The co-factor ATP is cleaved to pyrophosphate and AMP, with the AMP being covalently joined to a highly conserved lysine residue in the active site of the ligase. The activated AMP residue is then transferred to the 5' phosphate of the nick, before the nick is sealed by phosphodiester-bond formation and AMP elimination. The reaction catalyzed by the eubacterial NAD^+-dependent eubacterial ligases is essentially identical but for the initial formation of the enzyme-AMP intermediate resulting in the breakdown of NAD^+ and release of nicotinamide mononucleotide (NMN) rather than pyrophosphate; although these two groups of enzymes belong to the same family of nucleotidyl transferases , they share almost no protein sequence similarity outside the catalytic core.

After the ligation reaction, the ligation product was transformed into competent *E.coli DH5α*. The transformation resulted in the production of colonies with two differently coloured colonies. The white coloured colonies are indicative of positive clones while the blue colonies are indicative of clones into which the plasmid wouldn't have transformed. The transformed

positive clones were induced by 1 M IPTG and protein expression was checked by 10% SDS-PAGE.



Transformed clones



M Protein

M= Protein marker :14.4 Kda – 76 Kda

10% SDS-PAGE gels showing the protein bands (induced)

Applications of DNA subcloning are expanding rapidly in all fields of biology and medicine. In medical genetics such applications range from the prenatal diagnosis of inherited human diseases to the characterization of oncogenes and their roles in carcinogenesis. Pharmaceutical applications include large-scale production from cloned human genes of biologic products with therapeutic value, such as polypeptide hormones, interleukins, and enzymes. Applications in public health and laboratory medicine include development of vaccines to prevent specific infections and probes to diagnose specific infections by nucleic acid hybridization or polymerase chain reaction (PCR).

SUMMARY AND CONCLUSSON

Subcloning is a technique used to move a particular gene of interest from a parent vector to a destination vector in order to further study its functionality. N protein which encapsulates the negative strand RNA genome and in association with the L protein, P protein and subunits of the viral RNA polymerase it forms the ribonucleoprotein core of the virus. The N protein plays several important roles during the virus transcription and replication cycles. Restriction enzymes are used to excise the gene of interest (the insert) from the parent (mycoplsma), the insert is purified with a common purification method is gel isolation. Isolation of plasmid DNA from the clone and it is purified. Plasmid containing the full length N gene was digested with Bam HI to check the orientation of the N gene. The digested product is further cleaved with Restriction enzyme with Eco RI for 12 hours was done to release the N gene and the DNA sample was analysed on 1 % agarose gel. Then the ligation reaction was set up using a vector: Insert ratio of 1:3. The ligation product was transformed into competent E.coli DH5α. The transformation results, the white coloured colonies are indicative of positive clones while the blue colonies are indicative of clones into which the plasmid wouldn't have transformed. The transformed positive clones were induced by 1 M IPTG and protein expression was checked by 10% SDS-PAGE. DNA polymerase and Primers are used to amplify specific target DNA sequences during multiple cycles of invitro DNA synthesis, making it possible to detect target DNA sequences.

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Curcuma longa in combination with *Trigonella foenum graecum* restored altered lipid metabolic profiles in alloxan induced type 1 diabetic rat kidneys

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ABSTRACT

Curcuma longa (CL) and *Trigonella foenum* graecum (TFG) are medicinal plants used in Ayurveda for treating various diseases; one of it is diabetes mellitus. The present study was undertaken to investigate the effects of ethanol extracts of CL and TFG on blood glucose levels, tissue lipid profile and lipid peroxidation in alloxan induced diabetes on treatment for 30 days. Albino rats each weighing 180 ± 30g were given a single dose of intra peritoneal injection of 120 mg of alloxan per kg body weight. The rats were divided into five groups; each group consists of six rats. 1st group treated as control, 2nd group (alloxan induced diabetic rats), 3rd group (Diabetic + CL), 4th group (Diabetic + TFG) and 5th group (Diabetic + CL + TFG). Ethanolic extracts of CL rhizome powder and TFG seed powder was administered orally 250 mg/kg b.w/day for 30 days. The impact of CL and TFG on blood glucose levels in experimental rats were studied and the levels of lipid peroxides (TBARS) and tissue lipids (cholesterol, Triglycerides) were also estimated in both control and experimental rats. Treatment withCL and TFG individually and combinedly resulted in a significant reduction of blood glucose levels. CL and TFG also resulted in a significant decrease in tissue lipids and lipid peroxidation. The analyzed results conclude that a combination of herbal plants exerted a significant anti hyperlipidemic and anti peroxidative effect in alloxan induced diabetic rats when compared to individual treatment.

Key words: *Curcuma longa*, *Trigonella foenum graecum*, antiperoxidation, lipids, alloxan induced diabetes.

Introduction: Diabetes mellitus is a major public health problem in the world. There was estimation that 143 million people worldwide are suffering in the form of diabetes and this number would increase by almost five times by 2030. Diabetes mellitus is syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy- was yielding fuels such as lipids and protein (Scheen*et al* 1990). Insulin dependent diabetes mellitus (IDDM) or type 1 diabetes is an auto immune disorder caused by auto aggressive T-lymphocytes that infiltrate the pancreas and destroy insulin producing β-cells. This leads to hypoinsulinemia and thus a hyperglycemic condition (Bach*et al* 1995), which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy and cardiac problem (Arky *et al*1982). Most of the metabolic complications associated with type 1 diabetes are due to insulin deficiency and related glucose under utilization of the insulin dependent tissue such as liver, and

glucose over utilization in insulin dependent tissue, such as Kidney (Sochor *et al* 1985).The insulin deficiency causes excessive break down of lipid in adipose depots, resulting in increased level of free fatty acids (FAA) (Gupta *et al* 1999). Significant changes in lipid metabolism and structure also occur in diabetes (Sochar *et al* 1985). In diabetic rats, increased lipidperoxidation was also associated with hyperlipidemia (Morel and Chisolm *et al*1989).

Liver and kidney participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids, and triglycerides. During diabetes, a profound alteration in the concentration and composition of lipids occurs. Several workers have shown the presence of lipid deposits in the kidney of diabetic human and experimental animals and they have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease (Guijarro *et al* 1995; Lee *et al* 1991). Base on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important. Many traditional plant treatments for diabetes are used throughout the world. Plant drugs (Baily and Day *et al* 1989), and herbal formulation (Mitra *et al* 1996; Annapurna *et al*,2001; Bhattacharya *et al.,* 1997)are frequently considered to be less toxic and more free from side effects than synthetic one. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels (Gia, 2003, Elder *et al* 2004). Various herbal formulations such as *Curcuma longa* and *Trigonella foenum graecum* are well known for their antidiabetic effects.

Curcuma longa is a rhizomatousherbaceousperennial plant of the ginger family, Zingiberaceae. Research on *Curcuma longa* has been focused on alzheimer's, arthritis, cancer and diabetes. In India, *Curcuma longa* has been in use as a culinary ingredient since 3000 BC. As a medicine it is used to treat a wide variety of ailments including stomach ache, skin problems, muscular problems and arthritis. Practitioners of traditional Indian medicine believe that curcumin powder is beneficial against many diseases including biliary disorders, diabetes, hepatic disorders, rheumatism, sinusitis, cancer and Alzheimer disease. Some of the compounds such as ascorbic acid, beta-carotene, caffeic acid, curcumin, eugenol, p-coumaric acid, p-methoxy-cinnamic acid, protocatechuic acid, syringic acid and vanillic acid, which perform antioxidant activity are present in *Curcuma longa* might be responsible in inducing antioxidant action on treatment of diabetes.

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family fabaceae. It has a wide range of medicinal applications, one of its constituent alkaloids, called 'trigonelline', has shown potential for use in cancer therapy. Many researches have shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels. The seed and leaves are anticholesterolemic, anti-inflammatory, antitumor, carminative, demulcent, deobstruent, emollient, expectorant, febrifuge, galactogogue, hypoglycaemic, laxative, parasiticide, restorative and uterine tonic. Fenugreek seeds are used as a traditional remedy for the treatment of diabetes (Basch *et al* ,2003). Some of the compounds such as Alanine, Ascorbic-Acid, Beta-Carotene, Beta-Sitosterol, Histidine, Isovitexin, which perform antioxidant activity are present in *Trigonella foenum graecum* and might be responsible in inducing antioxidant action on treatment to diabetic rats.

The present investigation was under taken to study the effect of CL and TFG constituents on hyperglycemic, oxidative stress, lipid peroxidation(LPO) and tissue lipid profile (TC, TG) in Alloxan induced diabetic rats.

Material and methods:

Animals: A total of 30 adult (3 months old) male Wistar stain rats weighing 180 ± 30 g obtained from animal house of Bangalore were used for this study. They were housed six per each polycarbonate cage under standard laboratory conditions at a room temperature of 24 ± 2^{0}C, humidity of 45-64% with 12h light/dark cycle. The animals were fed with standard diet obtained from Hindustan Lever Limited, Mumbai and tap water was given *adlibitum* until treatment or time of sacrifice. All institutional guide lines were adhered to in the care and treatment of the animals used in the present study.

Induction of Diabetes:

Alloxan is used to induce Insulin dependent diabetes mellitus (IDDM) (Type 1). Single injection of Alloxan at a dosage concentration of 120mg/Kg body weight is usually enough for induction. So, after fasting for 18h, rats were injected intraperitoneally with a single dose of 120mg/Kg body weight. Alloxan freshly dissolved in saline. After injection, they were allowed to have free access to food and water. 15% glucose solution was also provided overnight to counter hypoglycemic shock. Diabetes in rats was identified by moderate polydipsia and marked

polyuria. From the second day onwards fasting blood samples were collected from the rats by tail vein and the blood glucose was measured by Accu Chek Glucometer (Sensor Comfort) to confirm the induction of diabetes. If the blood glucose levels were more than 300mg/dl, insulin (IIU protamine Zinc insulin) is given to the diabetic rats and the rats are allowed to acclimatize for diabetic condition for one week. After one week the rats with hyperglycemia (blood glucose level 250 mg/dl) were selected and used for the end of experiment.

Preparation of plant extracts: The fine powder of *Curcuma longa* rhizomeand *Trigonella foenum graecum* seeds powder were purchased (AGMARK symbol) in Tirupati. The powder is extracted by cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the plant material and the extraction was continued. The process was repeated three times. The three extractions were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in rotary evaporator. The resulting ethanol extract was air-dried. Finally yellow and light yellow powdery, crude ethanol extract of *Trigonella foenum graecum* and *Curcuma longa* were obtained respectively. Without any further purification the plant crude ethanol extract was used in the study.

Experimental design: The rats were divided into five groups of six each. Overnight fasted animals were injected with Alloxan (120 mg/Kg b.w) dissolved in 5 units of saline) intraperitoneally (i.p). The experimental period was 30 days. Group I: Control rats, Group II: Diabetic rats, Group III: Diabetic rats were given *Curcuma longa* ethanol extract (250 mg/kg b.w/day), Group IV: Diabetic rats were given Trigonella ethanol extract (250 mg/kg b.w/day), Group: V Diabetic rats treated with both Curcuma and Trigonella ethanol extracts. At the end of 30 days the animals were deprived of food overnight and sacrificed cervical dislocation and the kidney tissues were excised at -80^0C**.**

1. Estimation of Blood glucose:

Blood glucose was measured by using Accu Chek glucometer (Sensor Comfort) on 0 day, 7^{th} day and 30^{th} day of all 5 group rats.

2. [Lipid Peroxidation (LPO)]:

This assay is used to determine MDA levels as described by (Ohkawa *et al.,* 1979). The Kidney tissues were homogenized (5% - w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM

EDTA, The homogenates were centrifuged at 10,000 rpm for 10 min at 0°C in cold centrifuge. The separated supernatant part was used for the estimation. 200 µl of the tissue extract was added to 50 µl of 8.1% sodium dodecyl sulphate (SDS), vortexed and incubated for 10 min at room temperature. 375 µl of 20% acetic acid (pH 3.5) and 375 µl of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 min. The samples were allowed cool at room temperature. A mixture of 1.25 ml of butanol: phyridine (15:1) was added, vortexed and centrifuged at 1000 rpm for 5 min. The colored layer (500 µl) was measured at 532 nm using 1, 1, 3, 3-tetraethoxypropane as a standard. The values were expressed in µ moles of malondialdehyde formed / gram wet weight of the tissue.

3. Triglycerides :

Triglycerides were estimated by the method of (Natelson *et al*1971) with slight modifications as given below. Triglycerides were assayed by hydrolyzing them to glycerol and the liberated glycerol was determined.

Tissue homogenates were prepared in 1N H_2SO_4 and to it 4 ml of chloroform was added. 0.5 ml of tissue homogenate was taken. To it 0.5 ml of 1N H_2SO_4 and 4 ml of chloroform were added. The contents were centrifuged at 1000 rpm for 15 min 0.5 ml of chloroform layer was taken and to it 0.4 ml of methanol and 0.1 ml of alkaline barium solution were added and the contents were heated for 30 min at 80°C, the total volume was made up to 1 ml with 2N H_2SO_4 and centrifuged for 10 min at 1000 rpm. 0.5 ml of this supernatant was taken and to it 0.1 ml of sodium periodate was added and shaken well for 1 min, 0.1 ml of sodium arsenate and 5 ml of chromotrophic acid reagent was added and heated for 30 min and cooled. The samples were read at 575 nm in Spectrophotometer against the reagent blank. The results were finally expressed in mg of triglycerides / gram wet weight of the tissue.

4. Total Cholesterol: The total cholesterol content was estimated using Liebermann Burchard reaction as described by Natelson, (1971). The Kidney tissues were homogenized in isopropanol. The contents were centrifuged at l000 rpm for 15 min. 0.5 ml of supernatant was taken and to it 4 ml of cholesterol reagent was added. Then the contents were heated at 90°C for 15 min. After cooling, the samples were read at 560nm in spectrophotometer against the reagent blank. The results were finally expressed in mg of total cholesterol/gram wet weight of the tissue.

Enzyme units:

Enzyme activities were expressed in standard unit's i.e. μmoles of product formed or substrate cleaved/mg protein/hour.

Statistical analysis: The data obtained was expressed as Mean value with their SD. Readings of the five different groups were compared using one way ANOVA analysis with DUNNETTS multiple comparison test. Stastical analysis was performed using SPSS. Using M.S -Office, Excel software the data has been analyzed for the significance of the main effects and treatment s along with their interactions. The results were presented with the F – value. F- Value was found to be significant with $P<0.001$.

Results:

BLOOD GLUCOSE:

Glucose is the primarysource of energy for body's cells;fats and oils (i.e. lipids)being primarily a compact energy store. Insulin, a hormone produced by the pancreas, helps maintain normal blood sugar levels. Uncontrolled or high blood sugar levels can lead to health complications such as blindness, heart disease, kidney disease. Increase in concentration of glucose in the blood leads to a condition called "diabetic coma" or hyperglycaemia.

In the present study blood glucose levels were measured by using glucometer (Accu Chek) in all groups of control, diabetic, diabetic treated with *Curcuma*, diabetic treated with *Trigonella* and diabetic treated with both *Curcuma* + *Trigonella* on 0 day, 7^{th} day and 30^{th} day of the experimental period. Blood glucose levels were expressed in units of mg/dL In case of group II (diabetic rats) the blood glucose levels were significantly increased after induction of Alloxan. In the groups III, IV and V where the rats were subjected to CL, TFG and CL+TFG respectively have showed decreased blood glucose levels. However, the decrease is more significant in Group V where the rats were subjected to combined extracts of CL+TFG. The decreased levels of blood glucose in group V back to control levels after treatment with both *Curcuma* + *Trigonella* extract treatment. The various blood glucose values of alterations are as shown in Table 1.1.

Lipid peroxidation:

Peroxidation is important in food deterioration and in the oxidative modification of biological molecules particularly lipids. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant activity. In the present study the inhibition of lipid peroxidation induced by H_2O_2 by the seed extract was carried out in rat kidney.

Table 1 represents the concentration of TBARS, Cholesterol and triglycerides in kidney of control and experimental rats respectively. Kidney of diabetic rats showed significantly increased levels of TBARS, Cholesterol and Triglycerides, when compared with normal rats. In rats treated with *Curcuma* and *Trigonella* there was a significant decrease in the content ofTBARS, Cholesterol and Triglycerides in both the tissues, when compared with diabetic control rats. The combined treatment of CL+TFG was most effective in preventing alteration of lipid profile. The maximum prevention in the alteration of the lipid profile was observed in the combined treatment of CL and TFG by 30 days.

Table: 1.Showing Blood glucose levels in the control and experimental rats

Group/ Day	Group I	Group II	Group III	Group IV	GroupV
0 Day **Mean** **SD**	105± 6.3	107± 5.6	106± 6.4	102± 5.3	104± 9.4
7thDay **Mean** **SD**	106± 7.9	225± 20.2	179± 15.3	213± 16.2	225± 19.8
30th Day **Mean** **SD**	108± 7.2	290± 26.7	101± 10.9	102± 12.2	81± 6.3

Values are mean ± S.D. of 6 individual rats
Values in the parenthesis are % change from that of control
Values are significantly different from control at P < 0.0001

DISCUSSION:

Most of the body cells use the sugar called glucose as their major source of energy. Glucose molecules are broken down within cells in order to produce adenosine tri phosphate (ATP) molecules, energy-rich molecules that power numerous cellular processes. Glucose molecules are delivered to cells by the circulating blood and therefore, to ensure a constant supply of glucose to cells, it is essential that blood glucose levels be maintained at relatively constant levels.

Table: 2. Showing Lipid metabolic profile levels in Kidney tissues of control and experimental rats

Parameter / Groups	Group I	Group II	Group III	Group IV	Group V
Lipid peroxidation	38.16±7.497	51.81±9.16	35.29±3.148	33.18±3.20	31.02±6.585
Triglycerides	1.810±0.071	4.92±0.032	1.620±0.059	1.410±0.067	1.32±0.079
Total Cholesterol	63.86±1.559	89.02±2.149	59.13±1.636	58.32±2.185	53.01±1.442

Values are mean ± S.D. of 6 individual rats
VALUES in the parenthesis are % change from that of control
Values are significantly different from control at P < 0.0001

Blood glucose concentration is maintained within the normal range of 70 to 110 mg/dl. The levels of glucose in the blood are monitored by the cells in the pancreas. If the blood glucose level falls to dangerous levels (as in very heavy exercise or lack of food for extended periods), the Alpha cells of the pancreas release glucagon, a hormone which alerts the liver to increase blood glucose levels and converts stored glycogen into glucose (Glycogenesis). Thus glucose is released into the bloodstream, increasing blood sugar levels. There are several other causes for an increase in blood sugar levels. Among them diabetic stress due to the accumulation of reactive oxygen species is a major cause.

A significant increase in blood glucose levels found in alloxan treated rats could be due to the destruction of pancreatic beta-cells by alloxan induced oxidative stress. The elevations of glucose in alloxan treated rats (group II) were due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA (Omamto and Uchigata *et al* 1981). In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered; hence blood glucose levels were increased (Gottried and Rosenberg, 1973; Sochar *et al.*, 1985).

Blood glucose levels were decreased in group III, where diabetic rats subjected to *Curcuma*. Oral administration of *Curcuma longa* extract lowers blood glucose and attenuates alloxan-induced hyperlipidemia in diabetic rabbits. (Sarah Nwozo, *et al.*, 2009). *Curcuma longa* rhizomes have been reported to possess active constituents showing blood glucose lowering

activity in alloxan induced diabetic rats (Shnkar *et al.*, 1980). The administration of an aqueous extract of turmeric and abromine powder resulted in a significant reduction in blood glucose and an increase in total hemoglobin (Halim Eshrat Ali Hussain *et al* 2002).

Blood glucose levels were decreased in group IV, where diabetic rats subjected to *Trigonella*. Seeds of fenugreek have been showed multiple benefits in patients with diabetes such as reduction of blood sugar and its complications (Madar *et al.*, 1988; Preet *et al.*, 2006). Whether using the whole seeds or extracts showed that fenugreek seeds decreased fasting blood sugar levels in animals. The administration of *Trigonella foenum graecum* seed powder to diabetic animals have showed lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism close to normal values in animal model systems (Vats *et al.*, 2003; Raju *et al.*, 2001).

However decreased blood glucose levels were more significant in group V, which were administered combined plant extracts. Chattopadhyay 1999 reported that *Azadiracta indica* leaf extract was found to have the most potential activity as blood sugar lowering agent followed by *Catharanthus rooseusGymnemaq sylvestre* and *Oscimum sanctum.* Thus, the present study provides substantial evidence on the hypoglycemic action of CL, TFG ethanolic extracts in alloxan induced diabetic rats. The significant antihyperglycemic effect may be due to the potentiation of plasma insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells or its release from the bound form, as evidenced by the significant increase in the level of insulin by CL, TFG ethanol extracts in diabetic rats.

Diabetes is known to affect large number of metabolic pathways, including lipid metabolism, by altering the activities of various enzymes involved in these pathways. Alloxan, a beta cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Omamoto*et al*1981). Renal glucose uptake is markedly increased during diabetes and is inversely correlated with renal FFA uptake, which is reduced during diabetes (Meyer et al 1998). Thus the major assumption that kidney lipid deposits, which plays an important role in the pathogenesis of diabetic kidney disease (Guijarro *et al* 1995; Lee *et al* 1991), originate from increased levels of

serum lipids has to be further investigated with the possibility of an increase in renal lipid synthesis resulting in renal lipid accumulation.

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids (Loci*et al*1994). During diabetes, enhanced activity of this enzyme increases lipolysis and releases more free fatty acids in to the circulation (Agardh*et al*1999). Increased fatty acids concentration also increases the β-oxidation of fatty acids, producing more acetyl CoA and cholesterol during diabetes. In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats. The increased concentration of free fatty acids in kidney may be due to lipid breakdown and this may cause increased generation of NADPH, which results in the activation of NADPH dependent microsomal lipid peroxidation. The results show increased lipid peroxidation in the tissues (liver and kidney) of diabetic group. Previous studies have reported that there was an increased lipid peroxidation in liver, kidney and brain of diabetic rats (Latha *et al* 2003; Ananthan *et al* 2004). Administration of CL, TFG and CL+TFG reduced the lipid peroxidative markers in kidney tissue of diabetic rats. This indicates that CL, TFG inhibit oxidative damage due to the antiperoxidative effect of ingredients present in CL, TFG. Trigonella seed powder has shown many positive and encouraging results in the management of diabetes. (Sharma *et al*, 1990). Scavenging activity of *Curcumin* administration also reduces lipid peroxidation (Elder *et al* 2004). The use of CL+TFG in combination can be suggested as an effective alternative for the amelioration of diabetes.

Triglycerides:-

Accumulation of triglycerides is one of the risk factors in Coronary Heart Disease (CHD). The significant increase in the level of triglycerides in liver and kidney of diabetic control rats may be due to the lack of insulin. Since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis triglycerides (Frayn 1993). In the present study triglyceride content was decreased in diabetic rats treated with CL plant extract .The significant fall in serum triglyceride levels indicate antitriglyceride activity, antiglycemic, antilipidemic and

antioxidant properties of *Curcuma*. Triglyceride content was decreased in diabetic rats treated with combination of Curcumin from *Curcuma longa* (L), and partially purified product from *Abroma augusta* (L) in streptozotocin induced diabetes **(**Halim Eshrat and Ali Hussain *et al.,* 2002**).** Turmeric *(Curcuma longa*), as well as its active constituent Curcumin, inhibits lipid peroxidation. *Curcuma* and *Abroma augusta*also decreases serum cholesterol levels in hyperlipidaemic rats (Halim Eshrat and Ali Hussain *et al.,* 2002. The increase in kidney lipid level during diabetes appears to be due to increased glucose flux and reducing equivalents leading to enhanced over all biosynthetic pathways. Administrations of *Trigonella* in case of in group IV, the TG levels were reduced in kidney tissues of diabetic rats. This indicates that TFG inhibit oxidative damage due to the antiperoxidative effect of ingredients present in *Trigonella*.

The decreased total triglycerides were more pronounced in group V, whereas compared to individual plant extract treated. The decreased lipid peroxides and tissue lipids clearly showed the antihyperlipidemic and antiperoxidative effect of CL+TFG apart from its antidiabetic effect.

Cholesterol:-

Cholesterol is an amphipathic lipid and is present in tissues and in plasma lipoprotein either as free cholesterol with a long chain fatty acid as cholesterol ester.

The increased levels of plasma cholesterol, LDL cholesterol and/ or the decreased levels of HDL cholesterol are primary risk factors in the development of coronary heart diseases (Castelli *et al*1977; lipid research clinics programme II, 1984). In the current study total cholesterol levels were increased in diabetic rats (Group 2). The increase in total cholesterol was observed or reported in serum of STZ induced diabetic rats (Nawanjo*et al* 2005). Pari and Saravanan, (2000) reported that higher levels of cholesterol, triglycerides and free fatty acids inserum and tissues (Liver and Kidney) of alloxan diabetic rats. (Li *et al*2007) demonstrated that some lipid peroxidation products such as total cholesterol, MDA, HDL-Cholesterol etc in blood significantly diverges from their normal levels in diabetic rats. These results confirm that there is a strong correlation between oxidative stress and diabetes occurrence. The concentrations of lipids such as total cholesterol, LDL-C, HDL-C were significantly higher in diabetic rats than in the control group. Serum total cholesterol level increases significantly in diabetic group. These effects may be due to higher activity of cholesterol biosynthesis enzymes and or high levels of liposysis (Mallick *et al.,* 2006).

In CL plant extract treated diabetic rats (group III), the total cholesterol content in kidney tissue was decreased. This was due to the hypocholestemic effects of plant extract by the

inhibition of cellular cholesterol synthesis.Total cholesterol levels were decreased in group IV, where diabetic rats treated with *Trigonella.* Seeds of *Trigonella* have hypocholesterolemic and hyperinsulinomic effects on type-I and type-II diabetes mellitus patients and experimental diabetic animals (Puri *et al*2002; Stark and Madar *et al*1993; Duke*et al* 1992). The combined treatment of CL and TFG was found to be most effect in preventing the changes in total cholesterol levels after 30 days treatment in group V. The decreased total cholesterol levels were more pronounced in group V whereas compared to individual plant extract treatment. Lipid peroxides and Total cholesterol levels clearly showed the antihyperlipidemic and anticholesterolemic and antiperoxidative effect of CL+TFG apart from its antidiabetic effect.

The present study substantiates this new concept and suggests the effectiveness of combined therapy of CL and TFG on the control of glucose homeostasis and lipid metabolism during experimental diabetes and can be considered as a better alternative for further investigation for the amelioration of diabetes.

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A Review on Cord Stem cell banking ,a present day Life saving technology

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Abstract

Stem cells are immature cells that have the ability to become other kind of cells that form organs and tissues. They are powerful unique cells that are the building blocks of the body.Stem cell banking(CSB) is playing a vital role in present day medical technology. After the baby is born the umbilical cord has been clamped and cut , some blood remains in the blood vessels of placenta and attached portion of the umbilical cord. This cord blood is rich in powerful hematopoietic stem cells. As on today cord blood stem cells has been used in more than 3000 Transplants to generate healthy blood and immune system. Over the last 20 years cord blood stem cells used in the treatment of more than 80 serious diseases and disorders. In addition to saving lives through stem cell technology cord blood stem cells(CBSC)have properties that have been shown in the laboratory to help the body repair itself. Its estimated that one in three people in USA may benefit from the regenerative therapy in their life time. when stored properly new born stem cells will not expire, they remain safe and ready for the future use. CBSC therapy is being investigated in patients for regenerative medicine applications in clinical studies for conditions such as Autism, Pediatric stroke, Cerebral palsy, Traumatic brain injury. So let's save our child from blood cancers, tumors, immunodeficiency disorders using CSB . In India ,Life cell international is the first and largest stem cell bank and stem cell providers founded in Chennai, India (2004). Recently in 2013 Ishwarya Rai Bachan become a pattern of life cell and good will ambassador for stem cell banking and advocates stem cell banking as the best gift for a child for the life time.

Some ascepects of applied statistical methods in genetics and zoology

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Abstract

To developing quantitative process skills demanded in modern biology. In science courses and incorporating inter disciplinary(Maths, Biology) learning activities in genetics and zoology courses in this article we describe to developed two courses measure the learning with respect to biology and statistics in this paper we have easy to understanding maths and Statistical concepts and improve their ability to solve a biological problems. Many biological processes or described by mathematical equations, mathematical algorithms and statistical test to propose hypothesis regarding the relationships of our biodiversity

Under graduate students majoring in biology often questions why they are required to take statistics and mathematics. Maths and Statistics are incapable of applying the concepts to solve a biological and genetically analysis of data problems. To improve Mathematical skills among under graduate biology students. We describe the activities developed to attain assignment tools improved learning in biology and statistics the development of new ways in which the fields of maths and biology can enhance knowledge. To develop Statistical skills among biology students is just right for several reasons.

The Statistical unit addressed concepts relevant to analysis of genetics data such as the basic loss of probability, binomial, probability distribution, null hypothesis. We have to use in genetic data's expected genetic results out comes with the purpose of measuring.

The understanding of basic statisticalconcepts that students brought into the genetic courses.If we go with projects requiring the application of some kind of skills to answer questions. In statistical point of view that means we estimate the future results. And also we focus on the integration of statistical methods we have also used here.

In any data analysis if students have to improvedmostly in skills the required interpretation of graphs and correlation coefficients this out comes suggests the efforts of students very easy patterns from calculate correlations.

This paper presents the importance of understanding of ability to apply statistical concepts of correlation association between variables in biology.

A STUDY ON CAPSAICIN CONTENT AND SOME NUTRITIONAL COMPONENTS OF MALE STERILE HYBRIDS AND THEIR PARENTS OF CAPSICUM

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Abstract

Chillies are rich in biochemical compounds. They are used extensively as a spice in food industry and in medicine as a rubefacient and caraminative. The pungency of chillies is due to alkaloid capsaicin, which is a potent analgesic, anti-inflammatory and anti-obesity agent. During the present study 6 exotic and 5 indigenous pollinator parents are hybridized with a genetic male sterile line resulting in 11 hybrids exhibiting high degree of variation in their heterosis. The nutritional components sugars, proteins, ascorbic acid contents and the alkaloid capsaicin contents were estimated both in parents and their corresponding hybrids. A great variation in the contents of the nutritional components and the alkaloid capsaicin was observed. The heterosis observed for these components in hybrids was analysed.

FLUORIDE INDUCED BIOCHEMICAL CHANGES IN ALBINO MICE

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Abstract

Fluoride is an essential trace element for human beings and animals. As fluoride is found in small quantities in almost all foods, it enters the human body mainly through the oral route along with food and water. It can be rapidly absorbed by passive diffusion through stomach, small intestine, mouth, lungs and skin. Chronic exposure to fluoride above the permissible limits, it causes a disease called "Fluorosis". Fluorosis is an important clinical and public health problem in several parts of the world. There are several million people in India exposed to drinking water sources with high fluoride content. If fluoride is in excess of 1.5 mg / litre, consuming such water can cause dental fluorosis and if it is too much in excess, the body faces the problem of skeletal fluorosis. In the present investigation mice were treated with different doses of fluoride through drinking water and the biochemical parameters like total proteins, carbohydrates and glycogen contents were observed. The fall in glycogen concentration in treated animals were observed this may be due to inhibition of many enzymes concerned with glycolysis. Total Proteins and carbohydrate content showed decreased trend. During stress condition, organisms needed more energy to detoxify the toxicants and to overcome stress, chronic period of stress, proteins and carbohydrates are also a source of energy.

Key words: Fluoride, Carbohydrates, Proteins, Glycogen, Albino mice.

EFFECTS OF AFLOTOXICOSIS IN FRESH WATER FISH *CYPRINUS CARPIO*

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Abstract

Mycotoxins are potentially toxic molecules which are extremely difficult to remove when they enter the food chain. The major concern of mycotoxicosis is with their entry as feed contaminants of fish, fowl and other cattle. Fish has been cultured using various feed ingredients by many farmers, who purchace feed grains directly from the feed mills or from arable farmers to prepare compound diets for their fish, without paying considerable attention towards the quality of the feed, which are possibly contaminated with fugal species capable of producing mycotoxins. Mycotoxins are virtually ubiquitous in livestock diets and blood analysis reveals the harmful effect of mycotoxicosis in fish. Stunted growth, weight loss, blood profile and biochemical alterations were discovered in the serum of aflatoxin B_1 exposed common carp *Cyprinus carpio*over an experimental period of 30 days. The results shows -16.37% and -1.14% changes were recorded in the body weight and body length of the fishes respectively, denoting the considerable effect of aflotoxin B_1. Similarly, -26.0% change in hemoglobin level, -16.7% change in albumin, -24.2% change in total proteins, -22.5% change in globulins, -11.6% change in cholesterol, -4.3% change in creatinine and -26.7% change in Triglycerides were recorded. In contrast to all other parameters mentioned earlier, the total leukocyte counts (WBC) were significantly elevated (8.75% change) on exposure to aflatoxins. Since, aflotoxin induction causes severe sub-cellular, hepatic and muscular damage, which might lead to possible immunological changes observed. Under such conditions the animal needs activated immune mechanism which could be a possible reason for the elevated WBC count during aflotoxin pathogenesis which in-turn lead to functional and structural alterations at sub cellular level. The harmful effects of mycotoxins emphasizes the need for further research in the area to more thoroughly evaluate the risk to the health of fish and their implications on the health of consumers of this meat minimizing hazards to human health.

Key words:*Cyprinus carpio*, Aflotoxins, Aquaculture, Mycotoxins.

EFFECT OF CADMIUM ON ANTIOXIDANT METABOLIC MODULATIONSIN HEART AND MUSCLE OF FEMALE RABBITS

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Abstract

Disturbance in pro-oxidant/anti-oxidant systems and oxidative challenges of cadmium induced altering the antioxidant system in both heart and muscle tissue of female rabbits were studied. The cadmium induced oxidative stress leads to the tissue destruction by the elevated free radicals by suppression of antioxidant scavenger system. The imbalance between the free radicals and scavenger antioxidants leads to the tissue damage caused by the free radicals and peroxidants in rabbits. The present findings suggest that cadmium induction decreased Superoxide dismutase (SOD) activity is observed in both tissues like myocardial heart and muscle of rabbits over control rabbits. The decreased SOD activity on induction of cadmium envisages reduced neutralization of superoxide anions which might leads to cause an increase in superoxide radicals. The Glutathione-S-transferase (GST) activity was significantly inhibited in both heart and muscle on induction of cadmium. The rate of inhibition was maximum in heart myocardial tissue than in muscle when compared to control. Suppressed Reactive oxygen species (ROS) formation in both heart and muscle tissues by inhibiting Xanthine oxidase (XOD) activity on induction of cadmium. Decreased XOD can be envisaged that the XOD probably inter converting into xanthine dehydrogenase enzyme either by reversible sulphydryl oxidation or by irreversible photolytic modification. We concluded that the cadmium toxicity could have induced oxidative damage in both heart and muscle by enhancing peroxidation of membrane lipids by inducing inhibition of the antioxidant enzymes.

KEY WORDS : Cadmium, antioxidants, Reactive oxygen species (ROS)

CHLORPYRIFOS INDUCED TOXIC STRESS ON FREE AMINO ACIDS AND AAT, ALAT ENZYME ACTIVITY LEVELS IN ALBINO RAT

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Abstract

Every year nearly one billion pounds of pesticides, many of which are linked to cancer, birth defects, neurological disorders and environmental impacts. Currently one of the most important problems connected with the use of pesticides in most developing countries is the incidence of intoxication, death and residue problems resulting mainly from the misuse of certain pesticides. Pesticides play an eminent role in agriculture industry, so the pesticide pollution was considered as a major and serious problem of today's world. In the present investigation Albino rats were taken for the experiment and divided into 4 groups, each group having 6 animals. Group I was treated as control where as II, III and IV groups of animals were given single, double and multiple doses of chlorpyrifos with an interval of 48 hours. After stipulated time period of dosing, the animals were sacrificed by cervical dislocation. Liver and kidney tissues were isolated for biochemical studies. Total proteins were found decreased while the free amino acid level was elevated in different tissues under chlorpyrifos toxicity. Aminotransferase activities (AST & AlAT) were increased by chlorpyrifos administration. Maximum elevation of AST & AlAT activities were observed in multiple doses. The elevation in AST & AlAT activities suggests enhanced feeding of amino acids in to the energy cycle.

Insilico Molecular Modelling and Docking Studies on Therapeutic Target Non Structural Protein3 (NS3) of Dengue Virus1.

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Abstract

Dengue virus (DENV) is a family member of flaviviruses, tranfer their pathogenecity to humans by the vector mosquitoes and it is showing similar positive sense genomic RNA as such flavivirus, encodes single polyprotein in order to synthesize structural and non structural proteins. Non structural proteins of dengue virus play important role in replication and development of virus. Non Structural protein3 (NS3) has serine protease and RNA helicase activities. Ns3 combined with the NS2b activator to form the heterodimer for the cleavage at intermolecular sites in polyprotein to generate other non-structural proteins. In the present study we build three dimensional model structure of Non Structural protein 3(NS3) using modeller 9.10, molecular docking studies with common drugs available in market for dengue virus, yellow fever virus and westinile virus and its analogs to find lead molecules in Auto Dock Vina in PyRx. Docking studies with 440 analogues of mycophenolic acid from the ZINC database was identified top five lead molecules such as ZINC 78034162, 62001571, 62001658, 03813466 and 34527414. These compound structures have least binding energy through hydrogen bond interaction, bond lengths and bond angles with NS3 target protein comparatively by means of reference drug mycophenolic acid. In conclusion the compounds which are showing high binding affinity with NS3 target protein better than mycophenolic acid is suggesting therapeutic lead molecule for inhibition of NS3 therapeutic target of dengue viurs1.

Keywords: Dengue virus, Molecular docking, Yellow fever virus, Westinile virus

Screening of Marine Bioactive Compounds as Anti Lung Cancer Agents with Reference to Proline Rich Protein 11 (PRR11): An Approach using Molecular Modelling, Structure Based Virtual Screening.

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Abstract

Background: Lung cancer is one of the most common fatal diseases to cause death among cancers in human beings. Lung cancer cells stimulate rapid metabolic events and striking expression of some proteins are answerable for survival. PRR11 is a Cytosolic protein over expressed during cell division S phase and RNAi-mediated silencing of PRR11 caused S phase arrest, suppressed cellular proliferation and colony formation ability in lung cancer cells. DNA and protein level of PRR11 over expression observed in lung cancer indicates that it is a novel protein involved in cell cycle regulation; hence it considered as therapeutic target for lung cancer.

Materials and Methods: In the present study molecular modelling of PRR11 using modeller 9.10 and identification of lead molecules by structured based virtual screening was done in Auto Dock Vina with marine bioactive compounds. The drug property prediction was done using Molinspiration and Osiris servers.

Results: The docking results showed that the ZINC 28863059, 28863044, 28863051, 79642438, 27766155have high binding affinity and low binding energy with PRR11. Met1, Lys30, Ser40, Trp86, Cys90, Gln93 and Glu96 are key amino acids showing hydrogen bond interactions with lead molecules. Drug property prediction for the lead molecules revealed that, they follow all the drug properties and passed *insilico* adverse effect properties.

Conclusion: Identified lead molecules were may acts as inhibitor candidates from marine sources against PRR11 involved in lung cancer.

Key words Modelling, Virtual screening, Auto dock vina, Binding affinity, Marine bioactive compounds.

Effect of culture filtrate of *Trichoderma* species against important seed borne fungal pathogens of paddy

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Abstract

Culture filtrate of all the *Trichoderma* sps tested showed significant activity against test pathogens of paddy. The percent inhibition of the test pathogens in *T. viride* treatment varied between 13.7(*F. oxysporum*) to 82% (*P. oryzae*), In case of *T. harzianum*, it varied between 13 (*F. oxysporum*) to 82% (*C. lunata*). In case of *T. virens*, the inhibition percentage varied between 24.44 (*F. solani*) to 87.36% (*D. halodes*). Similarly in *T. koningii* treatment, the percent inhibition ranged between 69% (*T. padwickii*) to 100% inhibition (*P. oryzae* and *F. oxysporum*). Among the treatments total inhibition was observed only in case of*T. koningii* against *P. oryzae* and *F. oxysporum* and none of the other treatments recorded total inhibition. Among the culture filtrates of *Trichoderma* sps tested, it is observed that culture filtrates of *T. koningii* recorded significantly higher percentage of inhibition against all the test pathogens followed by *T. viride*, *T. virens* and *T. harzianum*. More than 80% inhibition of the test pathogens was observed in case of *T. koningii* treatment except *T. padwickii* and *D. tetramera*.

Key words: *Trichoderma*, Paddy, seed borne fungi

Antimicrobial activity of aqueous and solvent extract of Seeds of *Psoralea corylifolia* L. against some important biodegrading microorganisms of Maize and Sorghum

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Abstract

Antifungal activity of aqueous and solvent extract showed a significant activity against all the test microorganism. In aqueous extract, total inhibition of *F. graminearum* was observed at 15% conc. And similarly total inhibition of *F. equiseti* , *F. moniliforme, F. semitectum* and *F. solani* was observed at 40% conc and above. However total inhibition was not observed even at 50% conc in case of *F. lateritium*, *F. proliferatum* and *F. oxysporum*. Among the different species tested, *F. graminearum* was highly susceptible. Species of *Aspergillus* were fairly resistant. Total inhibition of *A. niger, A. flavipes, A. terreus* and *A. fumigatus* was observed at 45% concentration. While *A. tamarii* and *A. columnaris* were observed at 50% conc. Total inhibiton of *A. flavus, A. flavus oryzae* and *A. candidus* was not observed even at 50% concentration.

In solvent extract, total inhibition of *F. graminearum* was observed in Petroleum ether extract more than 85% inhibition of *F. monilforme, F. proliferatum, F. semitectum* and *F. solani* fungi was observed in Petroleum ether extract . Least inhibition (%) was observed in case of *F. lateritium.*The inhibition percentage of *Fusarium* species varied between 60 and 77 in case of benzene extract and maximum inhibition (77%) was observed in case of *F. semitectum* at 2% conc. Least percentage of inhibition was observed in case of *F. lateritium*. Chloroform extract recorded highest percent inhibition of 71.4% in case of *F. solani* at 2.0% conc. More than 50% inhibition of all the test *Fusarium* species were observed at 0.5% conc. *F. equiseti* was least susceptible (59.3%) even at 2% conc of the chloroform extract. In case of methanol extract the susceptibility of *Fusarium* species was much lower than that of the other extract except ethanol. The percent inhibition was lowest in case of *F. equiseti* (27.03%) and highest in case of *F. proliferatum* (79.53%) at 0.5% conc. Maximum inhibition of *F. solani* (80.2%). *F. proliferatum* (87.53%) and *F. moniliforme* (84.10%) was observed at 2% conc.

Key words:*Psoralea corylifolia* L, aqueous extract, solvent extract, *Fusarium, Aspergillus*.

Functions of Red Grape Extract on Nicotine Induced Oxidative Stress on Antioxidants Defence Mechanism in the Heart Tissue of Male Albino Rat With Reference to Aging

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Abstract

A grape (*Vitisvinifera*) is commercial juice products from Concord grapes have been applied in medical research studies, showing potential benefits against the diseases. Nicotine as most biologically active chemical in tobacco smoke. Nicotine has been reported to induce changes both *in vivo* and *in vitro*. Pathogen free, Wistar strain male albino rats were used in the present study, rats were divided into 4 groups of six in each group i) Normal Control (NC) (Control rats received 0.9% saline) ; ii) Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months); iii) Red Grape extract treated (RGEt) (red grape extract 50mg/kg body weight (after the standardization) via orogastric tube for a period of 2 months.); IV)) Nicotine + Red Grape (Nt+RGEt) (Rats were received the nicotine with a dose as mentioned for Group II through subcutaneous injection and , red grape extract as mentioned for Group III via orogastric tube for a period of 2 months). The animals were sacrificed after 24 hrs after the last treatment by cervical dislocation and isolated the heart tissuesuch as the activites of the levels of Superoxide dismutase(SOD), Catalase (CAT), Glutathione (GSH) and Glutathioneperoxidase (GPx), were decreased in nicotine treated rats in the heart tissueand increase was observed in the combination (Nt+RGEt),but at 50 mg/kg body weight found to be more effective. This results stating that red grape extract treated rats are beneficial, especially for the nicotine subjects to improve the antioxidants enzymes and thereby to improve the health status and life span.

Key words: Nicotine, Red Grape extract, SOD, CAT, GSH, GPx, Heart and Male albino rat.

ASSURING SAFETY AND QUALITY OF MILK AND DAIRY FOODS

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Abstract

The traditional dairy products provide an excellent opportunity for "value-added" dairy foods andrepresent an untapped potential for growth in domestic as well as international market. Despite theirincreasing popularity, economic significance and potential economic significance, traditional dairy foodsare largely produced by small –scale processors employing age- old methods of processing, handling,storage and distribution, which limit the quality and shelf life of the products. Milk and dairy foods aregood growth medium for many microorganisms. The quality and safety of milk and dairy products largelydepend on controlling entry and growth of microorganisms from cow to consumer.The main objective of this presentation is to review the role of microorganisms in safety and quality ofmilk and dairy products and discuss strategies for controlling the threat of pathogens and spoilageorganisms including microbial risk assessment and the Hazard Analysis and Critical Control Points(HACCP) program.

Key words ; safety, Quality, value added Dairy Foods, traditional dairy foods, processing.

Invivo anticholinergic effect of Withania somnifera in non-transgenic Alzheimer's disease animal model

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Abstract

Alzheimer's disease is the most common cause of dementia, worldwide about 27 million people suffered from dementia of which about 4 million resides in India.There is no cure for Alzheimer's disease, but drugs designed to slow disease progression are available. Some herbs may help to improve brain function, but scientific evidence to prove that they can treat Alzheimer's disease, is limited. *Withania somnifera* (Ashwagandha) is one such plant used to treat many diseases including Alzheimer's disease. Alzheimer's disease (AD) is a progressive neurodegenerative disorder with many cognitive and neuropsychiatric symptoms.As the dried roots of *Withania somnifera* are widely used in the treatment of many disorders, the current investigation aimed to assess the extraction and screening of active compounds in different extracts of *Withania somnifera*and cholinergic inhibitory effect of aqueous extract of *Withania somnifera* in scopolamine-induced alzheimer's disease in rat. The rats were randomly divided into 6 groups of 5 each: normal control rats treated with saline, scopolamine (2mg/Kg b.w. i.p.) – induced Alzheimer's rats treated with saline, scopolamine-induced Alzheimer's rats post-treated with aqueous extract of three different concentrations (100, 200 and 300mg/Kg b.w. oral) and scopolamine-induced Alzheimer's rat post-treated with donepezil, a reference control (5mg/Kg b.w. oral) for 10 successive days.Increased acetylcholinesterase activity and decreased Acetylcholine content in the tissues, cerebral cortex and hippocampus were recorded in scopolamine-induced Alzheimer's rat. Post-treatment with aqueous extract of *W. somnifera* extract caused inhibition in the levels of acetylcholinesterase and recovery in acetylcholine content in a dose dependent manner. Phytochemical screening of different extractions revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, glyosides and reducing sugars.

Key words: Ashwagandha, *Withania somnifera*, phytochemical screening,Alzheimer's disease, Cholinergic system, rat brain.

The Study of Neuro protective role of Chloroform and aqeous extracts of Bacopamonnieri on Catecholamine metabolism during Pentylenetetrazole - induced Epilepsy in different brain regions

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Abstract

Epilepsy is one of the most prevalent serious neurological disorders, affecting 0.5 to 1.0% of the world's population. Interestingly, the prevalence of epilepsy in developing countries is generally higher than in developed countries. In this modern era, epilepsy is the most frequent neurodegenerative disease after stroke. Bacopamonnieri (BM), belongs to Scrophulariaceae family and commonly known as Brahmi, has been used for a long time in Ayurvedic medicine as nerve tonic for promoting mental health and improving memory and has been tested for their neuroprotective and antiepileptic activity. Bacopa's soft, sessile leaves are succulent, Reni form and spantulate, measuring about 2.5 mm in length. Brahmi's leaves are used in tribal veterinary medicine, particularly in the treatment of epilepsy. The present study mainly aims to investigate the neuroprotective effect of Chloroform and Aqueous extracts of Bacopamonnieri on catecholamine metabolism in brain during Pentylenetetrazole (PTZ) induced epilepsy. The rats were divided into 4 groups 6 in each group. First group was treated with saline control, the second group with Pentylenetetrazole, the third and fourth groups were pretreated with Chloroform and Aqueous extract (AE) respectively before the Pentylenetetrazole injection. Different monoamine levels (NE, EP, DA and 5-HT) were estimated in different brain regions of rat such as cerebral cortex (CC) cerebellum (CB), pons medulla (PM) and hippocampus (HC). All the catecholamine were significantly increased in all the brain regions during PTZ-induced epilepsy. Pretreatment with Chloroform extract of BM decreased the monoamine contents in all the regions significantly. Similarly AE of BM decreased all the catecholamine levels and all the brain regions increased. The present study reveals that Chloroform extract of BM offers antiepileptic effect and neuroprotective against Pentylenetetrazole induced epilepsy.

Key words: Epilepsy, Bacopamonnieri ,Pentylenetetrazole

Therapeutic approach on Functional Characterization of 3-Deoxy Glucosone by Molecular Modeling and Virtual Screening.

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Abstract

3-Deoxyglucosone (3DG) significantly increased the risk of diabetic nephropathy. An increase in advanced glycation end products (AGEs) and oxidative stress have been associated with diabetic nephropathy and cardiomyopathy. The glycation (millard reaction) of proteins by glucose has been linked to the development of diabetic complications and other diseases. At this early glycation the glyoxal, methylglyoxal and 3-deoxyglucosone are formed. 3DG is known as a highly reactive intermediate of millard treaction and a precursor of AGEs. 3DG is accumulated in uremic serum and uremic erythrocytes. In uremic patients, 3DG levels significantly increased in plasma due to enhance in the synthesis of 3DG under glycemic conditions and it enhance the oxidative stress resulting in down regulation of antioxidant enzyme activity. In the present study we perform three dimensional structure of 3DG by using Modeller 9v10. The final model reliability was assessed by procheck through Ramachandran plot calculations, verify 3D and procheck programs. The virtual screening was performed with 500 similar structural compounds of meglumin against 3DG by using Autodock vina. In PyRx virtual screening, ZINC35052362, ZINC35052358, ZINC04566613, ZINC04566615, ZINC56960281, ZINC04566616, ZINC03644703 and ZINC56960285 shows highest binding affinity. Meglumin similar compounds have shown -6.0, -5.9, -5.9, -5-8, -5.8, -5.7, -5.6 and -5.6 k.cal/mol binding affinity with 3DG.our results demonstrated that meglumin similar compounds have shown significant binding affinity with 3DG when compared to meglumin. Based on our experiments we hypothesized that meglumin similar compounds may show significant activity in diabetes than meglumin.

Keywords: 3-deoxyglucosone, Diabetic nephropathy, molecular modeling, virtual screening, meglumin.

GINGER EXTRACT ATTENUATES ETHANOL- INDUCED OXIDATIVE STRESS IN RAT SMALL INTESTINE

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Abstract

Chronic alcohol consumption can exert deleterious effects on the structures and functions of all parts of the Gastro intestinal tract (GIT). This study evaluates the protective effect of ethanol extract of ginger on ethanol-induced oxidative stress in the small intestine of rats. Oxidative stress in the intestine tissue was evaluated by estimation of the activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidation (Se-GSH-Px). In the present study GST activity was significantly ($p<0.001$) increased whereas GR, Se-GSH-Px, SOD and CAT activities were significantly ($p<0.001$) decreased in the intestine of rats treated with ethanol alone (6g/kg). Ethanol extract of ginger (200mg/kg) administration exerts a significant ($p<0.001$) increase in GR, Se-GSH-Px, SOD and CAT activities, a marked reduction in the GST. However, ginger administration ameliorated the effects of ethanol, suggesting that ginger is a potential antioxidant against ethanol-induced oxidative stress.

Key words: Gastro Intestinal tract, oxidative stress, intestine, antioxidants

Thin layer chromatographic enzyme inhibition method for the detection of lead compound.

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ABSTRACT

Chromatographic technique for detection of lead compound by enzymatic method is necessary as it is a simple and low cost method. This method is an alternative to chemical method of detection. So many enzyme sources were tried for the detection of lead but chick brain was selected as better source for detection of lead. Detection of lead compound with least concentration was find out on thin layer chromatographic plates. Appearance of white inhibition spots on a pink background on TLC plate indicates the presence of lead compound. The lead compounds were separates from other compounds with the help of specific solvent system. This enzyme method is sensitive for the detection of lead compounds from water sample.

Key words: TLC, Lead, Enzyme source.

Alcohol induced Exaggeration in plasma lipid profile and cardiac biomarkers repealed by ginger treatment in myocardial infarction rats

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Abstract

The present study was aimed to investigate the cardio protective effect of ethanol extract of ginger (EG) on alcohol induced exaggeration in plasma lipid profiles and cardiac bio marker enzymes in myocardial infarction rats. Plasma lipid profiles such as Free fatty acids (FFAs), Triglycerides (TG), Total Cholesterol (TC), Phospholipids (PL), Low density lipoprotein (LDL) and cardiac marker enzymes like, Lactate dehydrogenase (LDH), Asperatate aminotransferase (AST), Creatine kinase -MB (CK-MB), Cardiac troponin –T(cTn-T), and troponin –I (cTn-I)levels were significantly ($p<0.001$) increased in plasma of alcohol treated (6g/kg) rats, except High density lipoprotein (HDL). However, with EG supplementation lipid profiles and cardiac biomarkers are significantly ($p <0.001$) decreased in alcoholic rats while HDL was significantly increased in plasma. The present findings revealed that EGcould potential prevent the ethanol toxicity on cardiac tissue with reference to lipid profile and cardiac biomarkers. The histopathological studies also supported that ginger protected the cardiac tissue from alcohol toxicity.

Key words: Ethanol, Ginger, CK-MB, Plasma lipid profile, Myocardial infarction

A Survey of Diabetic complications in Kandukur town: a Study with reference to Socio-Economic Status

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Abstract

In the present study, we undertake the complications of diabetic condition in kandukur town. The peoples who suffer from diabetic condition is having the age of 55 to 65 years and height of the diabetic patients are ranges from 5 to 5.5 feet. They are taking rice as the food. The diabetic patients are visiting MBBS doctor or diabetic specialist. The diabetic patients are spending more than 1500 Rs for month. Hence, from the above survey we conclude that the diabetic patients are from 5 to 5.5 feet and they are taking rice as food.

Keywords : Diabetes, food consumption, etc

A Project Report on the medicinal plants to cure diseases in Oguru and Kandukur in 2014

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Abstract

A survey was conducted on the usage of medicinal plants in Oguru and Kandukur places. The peoples in oguru and kandukur used many plants to cure many disease. Of them Neem, Phyllanthus, ginger, psidium, pomegranate etc.The plants like phyllanthus was used to cure liver related disorders. Ginger was used to cure kidney and diabetes. Psidium was used to cure kidney related disorders. Oimum was used to cure bacterial disease.

Keywords : Medicinal plants, diseases,

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