GROUP TASK

The following are the projects which will be given for group task during grant writing workshop. Each group consisting of 4 participants will be provided with one project. Each group has to complete the task in specified time.

Title of the project: "**MANEB PARAQUAT INDUCED ALTERATIONS IN OXIDATIVE STRESS IN POLYMORPHS AND ASSESMENT OF CORRELATION IN THE EXPRESSION OF TOXICANT RESPONSIVE GENES IN LIVER AND PLOYMORPHS IN THE EXPOSED RATS**"

Objectives

1. To assess the status of oxidative stress by measuring myeloperoxidase(MPO),catalase (CAT) and superoxide dismutase(SOD) activites and the levels of lipid hydroperoxides and glutathione in polymorphonuclear leukocytes of control and maneb+paraquat exposed rats.
2. To investigate the involvement of reactive nitrogen species in pesticides induced oxidative stress by estimating nitrite content in polymorphonuclear leuckocytes (PMN) of control and maneb + paraquat exposed rats.
3. To analyze the effect of maneb + paraquat on the expression of toxicant responsive genes-CYP1A1, CYP1A2, CYP2E1 and GSTA4-4 in liver and polymorphonuclear leukocytes (PMNs).

Design of the study

Male Wistar rats will be used as animal model for the evaluation of toxicity induced by maneb and paraquat. Rats will be divided into groups depending on the treatment i.e, control/vehicle treated, maneb + paraquat treated group. Maneb and paraquat will be administrated intra-peritoneally to the animals at the dose of 30mg/kg and 10mg/kg, respectively, twice a week for 1,3 and 6 weeks. Animals will be sacrificed after 1, 3 and 6 weeks under ether anesthesia. The polymorphs and liver will be used for RNA preparation for RT-PCR. Cell lysate will be utilized for enzyme and other biochemical estimations.

Total number of cases/samples/animals to be studied

At least ten animals per group will be utilized for each experiment and minimum five sets of experiments will be performed for each parameter. Number of animals used will be decided as per the requirement to generate statistically significant data.

Methodology:

1. Expression of CYPs and GST A4-4
2. Extraction of RNA and Reverse Transcription Polymerase Chain Reaction
3. Isolation of PMNs

4. **Enzymatic estimations**
    1. Myeloperoxidase
    2. Catalase
    3. Superoxide dismutase
    4. Glutathione-S-Transferase

5. **Biochemical Estimations:**
    - Lipid peroxidation
    - Glutathione levels
    - Nitrite content
    - Protein Estimation

2. Title of the project: **"Multi-centric study for the evaluation of lead poisoning in India: preparation of lead map of India using the state of art technology approved by centers for Disease Control (CDC) and Environmental Protection Agency (EPA)"**

Objectives

1. Screening for lead poisoning by evaluating blood lead levels using established international standard and protocols as per environmental protective agency(EPA)
2. Maintain a national database of lead poison levels in different segments of the population, and disseminate information pertaining to the current status of the problem to all concerned governmental agencies and other institutions.
3. Organize training programs for healthcare organizations on prevention and early detection of lead poisoning cases at our national referral center for lead poisoning in India (NRCLPI).
4. Make policy recommendation on abatement and prevention of lead poisoning to the government for national implementation.
5. Create awareness among public through the media, publications and outreach programs.

Detailed research plans

Our plan is to screen a minimum of 100 people under each of the following categories

1. People working in lead based industry
2. Family members of lead based industry
3. Consumers of products containing lead such as hair dye, food, unbranded medicines.

3. Title of the project: "**BONE BANK FOR ALLOGRAFTS FROM LIVING AND NON-LIVING DONORS"**

Objectives

1. To retrieve, process and store bone allografts from both non living and living donors in fresh frozen state in large quantities and obtain secondary sterilization with gamma irradiation, for undertaking complex orthopadeic reconstruction surgeries. Cadaveric bones will provide anatomic structural allografts, something surgeries. Cadaveric bones will provide anatomic structural allografts, something which femoral heads from living donors are incapable of providing**.**
2. To continue surveillance and study the long term follow up of the allograft bone which has already been transplanted taking into consideration factors like effect of resorption on integrity of graft, differences in clinical performance of bulk and morselized grafts, and causes or signs of failure such as fracture of allograft or non union of graft to host bone.

3. To use or transplant massive cortico cancellous or bulk allografts such as proximal or distal femoral allograft,acetabular or hemi pelvis graft in case of bone tumor surgery or revision arthroplasties of hips and knees, and study the performance of these grafts.

4. To study the pattern of systemic release of proinflammatory cytokines before and after the allograft transplant.

5. To assess the role of the proinflammatory cytokines in the pathogenesis of transplant-related complications and determine the relevance of measuring or monitoring cytokine levels after skeletal allograft transplantation.

Methodology

Selection of Non Living Donor

Retrieval of allograft

Assessment of immune response

4. Title of the project: "**To elucidate the Role of CRTAase Induced Epigenetic Modulation Via Acetylation By The Novel Mechanism In The Gene Expression Profile Of Lung Carcinogenesis Using Polyphenols And their Synthetic Analogue**"

Objectives

1. To establish the human non small cell lung cancer A549 cell line culture
2. To establish transacetylation activity of calreticulin using histone protein as target
3. To perform the qualitative micro array profiling
4. To quantify the transcription product of above mentioned genes by Real Time-PCR
5. To transfect the cells with calreticulin R(CAL R) gene
6. To induce Hyperacetylation by treating the CRTAase transfected cells with various polyphenolic acetates
7. To study histone hyperacetylation induced modulation of gene expression of various genes by RT-PCR which are responsible for Tumor suppression, cell cycle arrest and apoptosis.
8. To detect the level of NF-KB regulated cytokines (TNF- α, IL-6, IL-8)- by ELISA kits

Work plan

Quantification of genes through Real-Time PCR

By studying the extent of apoptosis by FITC kits using Flow cytometer (FACS) and Fluorescent Microscope.

By cell cycle arrest analysis using flow cytometer

Establishment of TRANS-ACETYLASE ACTIVITY OF CALRETICULIN

Establishment of the TRANSACETYLASE ACTIVITY OF THE CALRETICULIN using extracted histones protein as the target of acetylation.

Confirmation of histone acetylation by western blotting

To study the effect of histone hyperacetylation

Demethylating agents will also be used.

5. Title of the project: **Fungal infections to HIV positive patients in Manipur State: A phenotypic and molecular study of etiologic agents, antifungal susceptibility pattern, and therapeutic management**.

Objectives:

1. To isolate the etiologic agents of fungal infections in HIV positive patients, to characterize their species spectrum, using phenotypic and molecular methods and to correlate their association with CD4 counts and progression of disease
2. To determine the susceptibility pattern of the etiologic agents isolated against antifungal agents such as amphotericin B, 5-flurocytosine,fluconazole,itraconazole and voriconazole, caspofugin, micafungin and anidulafungin, using CLSI microbroth dilution method and by the E-test and to utilize the results for more efficacious chemotherapy of patients afflicted with fungal infections. The study will also include clinical follow up and evaluation of the patients receiving antifungal therapy.
3. To study the molecular epidemiology of *C.gattii* and *C. neoformans*, the etiologic agents of cryptococcosis, by multilocus sequence typing, using 5 etiologic agents of cryptococcosis, by multilocus sequence typing, using 5 housekeeping genes. An attempt will be made to study the genetic diversity between northeastern and northwestern isolates.

Work plan

Patients and specimen collection

Group I

Group-II

Laboratory investigation

Microscopy and culture

Antigen detection

Antibody detection antifungal susceptibility testing

PCR amplification and direct DNA sequencing of fungal pathogens

Multilocus sequence typing of *Cryptococcus gattii* and *C. neoformans*