loss of E-cadherin and Nm23-H1 proteins are associated with invasive cell types. It is also noticed that the presence of HPV alone is not sufficient for malignant progression, rather additional events are also required. **Objectives:** In the present study, the expression profiles of Nm23-H1 and E-cadherin proteins in HPV positive carcinoma of uterine cervix and nasopharyngeal carcinoma was evaluated to find out the impact of HPV infection on adhesion proteins. **Methods:** The expression pattern of E-cadherin and Nm23-H1 was assessed by Immunohistochemistry and the presence of HPV was assessed by PCR. Fifty-five cases of cervical tumors, Ten benign cervical lesions, One hundred and three NPC tumors and Twenty six benign lesions of nasopharynx were collected for the study. **Results:** A significant down regulation of E-cadherin in NPC and cervical carcinoma and its histological subsets was observed ($p = 0.009$). Expression of both proteins ranged from mild to moderate cytoplasmic expression in these tissues. HPV infection was associated with a down regulation of E-cadherin and Nm23-H1 in cervix cancer lesions, while no such down regulation was noticed in NPC lesions. Advanced disease stages of both cervical carcinoma and NPC showed a marked down regulation of E-cadherin and Nm23-H1 protein. Down regulation of the Nm23-H1 protein was also evident in NPC ($r = -0.616, p= 0.000$) and cervical carcinomas ($r= -0.366, p=0.006$) with relation to cervical lymph node status, which suggests the potential use of Nm23-H1 as a biologic marker for nodal metastasis in both the epithelial tumors. **Conclusion:** The results thus indicate that the role of the adhesion proteins and the interaction of HPV in human neoplasia is most likely complex and is tissue specific and that different regulatory mechanisms may act in different tumors.

**Transcriptional Regulation, Signal Transduction, Cancer Genomics & Proteomics**

**(Friday)**

F-1

**Modulatory Influence of Brassica compestris and Mentha piperita on DMBA Induced Skin Papillomagensis in Swiss Albino Mice**

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Epidemiologic studies have provided initial leads for the identification of numerous naturally occurring chemopreventive agents in the dietary components, effective against multi-stage carcinogenesis. *Mentha piperita* is shown to have antioxidant ant antiperoxidant properties. *Brassica compestris* contains indole-3-carbinol and sulforaphane (glucosinolates) which are potent stimulators of natural detoxifying enzymes in the body. The present study evaluates the modulatory influence of the ethanolic extracts of Mentha piperita and Brassica compestris on skin papillomas in female Swiss albino mice. Random-bred female Swiss albino mice (8 weeks old), weighing 24±2 gm were used. 7,12- Dimethyl benz(a) anthracene and croton oil procured from Sigma Chemicals Co, USA. Two stage protocol consisting of initiation with a single topical application of a carcinogen, DMBA (100µg/50µl of acetone) followed by thrice weekly treatment till 16 weeks with a promoter (croton oil- 1% in acetone) were employed in Swiss albino mice. The animals were divided into 3 groups (Gr I, II & III). Gr I animals were administered orally Mentha and Brassica suspension (400mg/kg body weight respectively), Gr II were given Brassica suspension (800mg/kg body weight) and Gr III were given the Mentha suspension (800mg/kg body weight) respectively at pre, peri and post initiational stages. In DMBA treated animals (control group), increased frequency of bone marrow micronuclei was accompanied by enhanced lipid
peroxidation and antioxidant depletion. Gr II and Gr III showed a greater inhibition (83%) of DMBA induced bone marrow micronuclei compared to control group while Gr I showed insignificant inhibition. Lipid peroxidation was significantly reduced in Gr II and Gr III compared with control group (p<0.01 and p<0.01 respectively). The levels of GSH in liver and the activities of hepatic GPx and GST were significantly increased in Gr II and Gr III as compared to the control (p<0.01 and p<0.001 respectively). Gr I significantly enhanced the activity of GST compared with the control (p<0.01) but the lipid peroxidation was also significantly increased as evidenced by the high levels of TBA-reactive substances (TBARS). Both GPx and GST play a pivotal role in the biotransformation of carcinogens into non-reactive metabolites that can readily be excreted. Thus the present investigation is suggestive of the chemopreventive activity of Brassica compestris suspension and Mentha piperita suspension, when administered alone, in Swiss albino mice against 7, 12-dimethyl benz(a) anthracene and croton oil. The chemoprevention may be due to modulation of detoxifying enzyme systems and pharmacological properties of Brassica compestris and Mentha piperita. However, no significant chemopreventive activity was observed when Brassica compestris and Mentha piperita were used in combination. This may be due to antagonistic interaction between Brassica and Mentha resulting in the activation of carcinogenesis.

F-2
Increased Expression of Matrix Metalloproteinase-2, Matrix Metalloproteinase-9 and their Inhibitors in Invasive Retinoblastomas
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Introduction: Matrix metalloproteinases (MMPs) are known to play a central role in the cell migration during cancer metastasis. Tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity by controlling the breakdown of extracellular matrix components. Objectives: To study the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 in retinoblastoma. Methods: Sixty tumors and a human retinoblastoma cell line (Y79) were evaluated by immunohistochemistry for MMPs and TIMPs. Growth fraction of the tumors was studied by Ki-67 labeling index. Statistical analysis done using non-parametric testing. Results: Among the 30 non-invasive tumors, MMP-2 and MMP-9 negative in 24 tumors each. TIMP-1 and TIMP-2 negative in 28 and 19 tumors respectively. Among the 30 invasive tumors, MMP-2 was positive in 23 tumors, MMP-9 in 19 tumors, TIMP-1 in 23 and TIMP-2 in 21 tumors. Ki-67 > 50% was seen in 26 invasive tumors as compared to 17 non-invasive tumors. The Y79 cell line was also seen to express the four markers. There was statistically significant increase in the expressions of both MMPs and TIMPs (P<0.0001) in the invasive tumors as compared to the non-invasive tumors. There were moderate correlations between the expressions of Ki-67 and TIMP-1 (r= 0.355; P< 0.01), Ki-67 and MMP2 (r= 0.306; P<0.01) and between TIMP-1 and MMP-9 (r= 0.314; P<0.028). Conclusion: The expressions of MMP-2, MMP-9, TIMP-1 and TIMP-2 was higher in the invasive tumors. Both MMPs and TIMPs have a key role in extracellular matrix invasion in retinoblastoma, largely through their elaboration by tumor cells.

F-3
Therapeutic efficacy of Pomegranate (Punica granatum Linn.)
Ajaikumar KB*, Fijesh PV, Simona PS and Jose Padikkala

Ajaikumar KB*, Fijesh PV, Simona PS and Jose Padikkala

Introduction: Matrix metalloproteinases (MMPs) are known to play a central role in the cell migration during cancer metastasis. Tissue inhibitors of metalloproteinases (TIMPs) regulate MMP activity by controlling the breakdown of extracellular matrix components. Objectives: To study the expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 in retinoblastoma. Methods: Sixty tumors and a human retinoblastoma cell line (Y79) were evaluated by immunohistochemistry for MMPs and TIMPs. Growth fraction of the tumors was studied by Ki-67 labeling index. Statistical analysis done using non-parametric testing. Results: Among the 30 non-invasive tumors, MMP-2 and MMP-9 negative in 24 tumors each. TIMP-1 and TIMP-2 negative in 28 and 19 tumors respectively. Among the 30 invasive tumors, MMP-2 was positive in 23 tumors, MMP-9 in 19 tumors, TIMP-1 in 23 and TIMP-2 in 21 tumors. Ki-67 > 50% was seen in 26 invasive tumors as compared to 17 non-invasive tumors. The Y79 cell line was also seen to express the four markers. There was statistically significant increase in the expressions of both MMPs and TIMPs (P<0.0001) in the invasive tumors as compared to the non-invasive tumors. There were moderate correlations between the expressions of Ki-67 and TIMP-1 (r= 0.355; P< 0.01), Ki-67 and MMP2 (r= 0.306; P<0.01) and between TIMP-1 and MMP-9 (r= 0.314; P<0.028). Conclusion: The expressions of MMP-2, MMP-9, TIMP-1 and TIMP-2 was higher in the invasive tumors. Both MMPs and TIMPs have a key role in extracellular matrix invasion in retinoblastoma, largely through their elaboration by tumor cells.
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**Introduction:** _Punica granatum_ Linn. (Punicaceae) commonly called as 'Pomegranate' is a large deciduous shrub or small tree used medicinally in Europe, IndoChina, South Africa etc for the treatment of various diseases such as ulcer, hepatic damage, ascites, snakebite etc. Due to this high traditional medicinal use we selected this plant for the present study. **Objective:** To study the pharmacological activities of Pomegranate fruit rind and fruit juice. **Methods:** The present study evaluated the antioxidant, antiinflammatory (Carageenan, Dextran and Formalin induced), Hepatoprotective (Paracetamol and CCl4), Antiulcer (Ethanol and Aspirin induced), Antitumor (DLA induced solid tumor and EAC induced Ascites tumor), Radioprotective and Chemoprotective activities of fruit juice and 70% methanolic extract of fruit rind, separately. **Results:** The present study reports for the first time the antioxidant, anti-inflammatory, antiulcer, hepatoprotective, radioprotective, chemoprotective and antitumour activities of _P. granatum_ fruit rind and fruit juice. The fruit juice and 70% methanolic extract of fruit rind showed potent in vitro superoxide, nitric oxide and hydroxyl radical scavenging activities and anti-inflammatory activities against carageenan, formalin and dextran induced inflammatory models in Swiss albino mice. The administration of fruit juice (2.5ml/kg b. wt and 5.0ml/kg b. wt) and the methanolic extract of fruit rind (250mg/kg b. wt and 500mg/kg b. wt) in rats significantly inhibited the chemically induced gastric and hepatic damage in a dose dependent manner. The administration of fruit juice (20ml/kg b. wt and 40ml/kg b. wt) and the methanolic extract (50mg/kg b. wt and 100mg/kg b. wt) inhibited solid tumor and ascites tumor in Swiss albino mice. Fruit juice and methanolic extract of fruit rind exhibited significant radioprotective and chemoprotective activities. The toxicity studies revealed that both fruit juice and fruit rind are non-toxic. The phytochemical analysis of the fruit juice and fruit rind showed the presence of alkaloids and anthocyanidines. **Conclusion:** In conclusion, the present study revealed the protective effect of Pomegranate fruit against various diseases.

F-4

**Expression and Cellular Localization of NF- B Superfamily Members in Oral Carcinogenesis**

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**Introduction:** Oral cancer is one of the most common cancers in India and South East Asian region consisting up to 50% of all malignant tumors. HPV infection, alcohol and tobacco abuse are the major etiological factors for oral carcinogenesis. HPV and tobacco carcinogens are known to modulate the expression and activation of transcription factors such as NF-κB and AP-1. However, their role in the process of oral carcinogenesis is yet to be confirmed. **Objectives:** To know the transactivation and expression pattern of NF-κB superfamily members in different stages of oral precancer and cancer. **Methods:** Fifty fresh oral tissue biopsies were collected which included 3 normal controls, 16 precancerous and 31 cancerous cases prior to chemotherapy / radiotherapy from the collaborating hospitals and stored at -70°C. HPV diagnosis of samples was done by consensus and type-specific PCR primers. The transactivation status of NF-κB in oral biopsies was determined by Electrophoretic Mobility Shift Assay. Western blots and Immunohistochemical analysis were performed to know the expression levels and localization of different NF-κB proteins components. **Results:** Fifteen percent of malignant oral biopsies have shown presence of HPV primarily of high risk type 16 and 18. Oral cancer biopsies have shown constitutive activation of NF-κB with preferential homodimerisation of NF-κB p50 subunits. However, the homodimerization pattern of p50/p50 was found to be independent of HPV infection in oral squamous cell carcinomas. At
protein level, both by western blotting and immunohistochemistry an over-expression of p50 as well as c-Rel was observed in cancer tissues while the p65 level remained similar in normal oral and in different grades of oral lesions including cancer. **Conclusion:** NF-kB p50 and c-Rel may play an important role in the constitutive activation of NF-kB during oral carcinogenesis.

F-5
Noscapine and Polycystic Ovary Syndrome: Towards a New Development

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Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in women of reproductive age. PCOS accounts for 75% of women with anovulatory infertility. The late consequences, such as risk of endometrial cancer, cardiovascular disease, and infertility warrant an early and effective diagnosis of the syndrome. The underlying cause of PCOS is an abnormality of ovarian androgen, which is the common final manifestation of the failure of Hypothalamus-Pituitary-Ovarian axis. We present the development of a rat model of PCOS using anti progesterone RU486 and evaluate the efficacy of noscapine for its treatment. RU486 administration induced ovulation blockade, persistent vaginal cornification, uterine ballooning and alterations in serum levels of LH, FSH, progesterone, estradiol and testosterone mimicking a classical PCOS in women. Administration of noscapine corrected these derangements at dose of 120 mg/Kg of body weight. Moreover the serum level of LH, FSH, testosterone, estradiol and progesterone reverted to the basal level on noscapine administration. Ovary showed follicles in different stages of development with no cystic manifestation. It also restored the reproductive potential when compared with the PCO induced rat. Radiolabelling, Scintigraphic and SPECT studies showed prominent localization of ⁹⁹ᵐTc-Noscapine in rabbit's ovary, implicating ovary as target organ in its corrective effect. Protein profiling studies indicated restoration of overexpressed proteins to normal levels in noscapine administered rat ovaries as compared to induced polycystic ovaries. These results represent the first identification of noscapine as a participant in the correction of polycystic ovary disease (PCO). Thus the correction of PCO by administration of Noscapine is a novel and significant observation, and opens new avenues in PCO treatment.

F-6
Silibinin Inhibits TNF -Induced NF- B Activation via IKK -I B  Pathway in Human Colon Carcinoma Cells: Possible Implications in Colorectal Cancer Prevention and Intervention

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Colorectal cancer is the second most common invasive malignancy and cause of cancer-related deaths in the United States; however, there have been limited successes so far in its prevention and intervention. In this regard, transcription factor NF-kB has been implicated in the growth and survival of several human malignancies including colorectal cancer, suggesting that the agents that inhibit NF-kB activation could be effective in colorectal cancer therapy. Employing various human colorectal carcinoma cell lines, here we assessed the effect of
silibinin, the major active constituent in a widely consumed hepatoprotective dietary supplement milk thistle extract, on TNF alpha-induced NF-kB activation and upstream effectors in this pathway. Colorectal carcinoma cell lines were treated with silibinin followed by TNF alpha, and nuclear extracts were analyzed for NF-kB DNA binding activity by gel shift assay. Western immunoblotting was done for protein levels of p65, p50, and both phospho- and total IkB alpha in nuclear or cytosolic fraction, and in-beads kinase assay was done for IKK alpha activity. Treatment of HT29, LoVo and SW40 human colorectal carcinoma cells with silibinin resulted in a strong inhibition of TNF alpha-induced NF-kB activation, in a dose-dependent manner. Consistent with this, nuclear levels of p65 and p50 sub-units of NF-kB were also reduced concomitant with an increase in their cytosolic levels, following silibinin treatment of the cells. Additional studies showed that silibinin pre-treatment was more effective in inhibiting TNF alpha-caused NF-kB activation as compared to its post-treatment. In the studies assessing upstream molecular mechanism of this effect, silibinin treatment resulted in a significant increase in the level of I B with a concomitant decrease in phospho-IkB alpha. Kinase assays revealed that silibinin inhibits IKK alpha kinase activity, suggesting this to be a molecular target of silibinin effect in inhibiting NF-kB activation. Together, these results indicate that silibinin could be a useful agent for colorectal cancer prevention and intervention by inhibiting NF-kB-mediated cell survival signaling.

F-7  
Black Tea Extract As Effective as Green Tea Polyphenols, in Prevention of BP Induced Lung Carcinogenesis

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Introduction: Lung cancer is one of the leading causes of cancer deaths worldwide and the number of cases continues to increase. Green Tea, rich in antioxidant flavanols is considered to have chemopreventive and anticarcinogenic properties but efficacy of black tea and their compounds is yet to be fully explored. Objectives: The present study was designed to evaluate the effect of black tea extract (Theaflavins) in comparison to the active green tea compound, on Benzo(a)pyrene (BP) induced lung carcinogenesis in strain A mice. Methods: Treatment was initiated at the post initiation phase. TF was administered by ip injection and the same amounts of green tea polyphenol EGCG were used as positive control. Identification of apoptotic and proliferative cells was carried out by TUNEL method and BrdU incorporation. Expression of Caspase-3 and COX-II was done by Western blotting and expression of Bcl-2 and C-Myc protein localized by immunohistochemistry. Results: Immunohistochemical localization in the precancerous lung lesions have shown a reduction in proliferating epithelial cells and increased number of apoptotic cells in the bronchiole region. This is also reflected in the downregulation of Cox-II, the molecular markers associated with proliferation and upregulation of Caspase-3. Suppression of Bcl-2 and C-Myc proteins expression were observed after treatment, which accounts for a significant reduction in incidences and delayed onset of hyperplasia, dysplasia and carcinoma in situ in BP treated lung. Conclusion: Treatments with black tea extract down regulate the expression of the marker proteins studied, which is as effective as green tea compounds. These findings were also supported by expression of the same proteins on NCI-H460 a human non-small cell lung cancer cell line.

F-8  
Effect of Melatonin at Different Time Points on DNA Synthesis of HepG2 Cells in Culture: An Implication for Cancer Chronotherapy
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Circadian rhythms are known to be exhibited by all peripheral tissues and mammalian cells in culture. Recently chronotherapy of cancer has attracted attention, as the efficacy of most drugs including antineoplastics varies according to the time of administration. Melatonin, a pineal gland hormone holds the unique position of being the only known chronobiotic regulator of neoplastic cell growth. Accordingly, we have investigated the effect of melatonin on DNA synthesis in HepG2 cells for 24 h period. HepG2 cells were treated with 8 mM melatonin and [3 H] thymidine (0.1 mCi/ml) was added at 6 time points for 24 h period (00:00; 04:00; 08:00; 12:00; 16:00; 20:00; 24:00). DMSO treated HepG2 cells served as controls. DNA synthesis was measured by the method of Yusof and Edwards (Carcinogenesis, 11, 761, 1990). DNA synthesis in HepG2 cell lines varied over 24 h period. Maximum DNA synthesis in HepG2 control was at 23:31 h. Melatonin inhibited the DNA synthesis maximally at this time point (0:59 h). Our results support the fact that cancer cells are more susceptible to anticancer agents when they are exposed at a time point when DNA synthesis is maximum. This study would form the basis for cancer chronotherapy, which aims in drug administration at appropriate timing according to the circadian rhythms of cancer cell susceptibility which will avoid adverse effects of chemotherapy.

F-9
TGFβ Estimation: Impact of Platelet Derived Fraction on Prognostication of Breast Carcinoma

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Cellular growth & differentiation, an orderly process is controlled by a number of physiological networks including growth factors - the most widely studied. TGFβs is a stable, multifunctional cytokine axis shows differential expression during breast tumorigenesis. High TGFβs is present in platelets which get released on degranulation. Most of circulating TGFβs is thus platelet derived and results into false elevation. Estimation of 'tumor derived' TGFβs thus is crucial in their evaluation as prognosticators in human breast carcinoma. An attempt here evaluates the correction of platelet derived TGFβs by 'normalization' with PF-4 (a known marker of platelet degranulation) in a series of 107 previously untreated breast cancer patients by comparison with clinico-pathologic criteria including survival.

F-10
Differential Expression of Sperm Protein 17 in Human Esophageal Squamous Cell Carcinoma

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Introduction: Carcinoma of the esophagus is the eighth most common cancer worldwide and the fifth leading cause of cancer death in the world with a low five-year survival rate. Identification of genes that are
differentially expressed in tumor cells is important for understanding the molecular basis of cancer. Gene expression profiling of esophageal cancer using differential display led to the identification of an over-expressed cDNA fragment encoding Sperm Protein 17(Sp17). Sp17 is an antigenic protein highly expressed in spermatozoa whose known function is to bind sperm to zona pellucida. Recently, however, Sp17 expression was demonstrated in multiple myeloma and ovarian cancer, suggesting that it may be a novel cancer-testis antigen. Aim: The aim of the study was to analyze the expression of Sp17 in human esophageal squamous cell carcinoma (ESCC) and normal tissues and to determine the levels of circulating anti-Sp17 antibodies in ESCC patient sera in comparison to healthy subjects. Methods and Results: Sp17 transcript was detected in ESCCs but no expression was detected in normal tissues. To evaluate protein expression and localization immunohistochemical studies were done using mouse monoclonal antibodies. ESCCs showed positive nuclear and cytoplasmic staining while no expression was detected in normal tissues. Since Sp17 is immunogenic, the aberrant expression of this cancer testis antigen in esophageal tissues may lead to generation of antibodies. The expression levels of circulating anti-Sp17 antibodies were determined in sera collected from esophageal cancer patients and healthy controls using Enzyme Linked Immunosorbent Assay (ELISA). High levels of circulating antibodies were observed in ESCC patient's sera as compared to healthy subjects. Conclusion: Sp17, a highly immunogenic protein in-vivo might serve as a candidate molecular marker for diagnosis of ESCC, however further investigation of its role in esophageal tumorigenesis is warranted.

F-11
Clinical Usefulness of Alterations in Sialic Acid, Sialyltransferase and Sialoproteins in Breast Cancer


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Sialic acid, the end moieties of the carbohydrate chains are biologically important and essential for functions of glycoconjugates and are reported to be altered in cancer patients. Two hundred and twenty five breast cancer (BC) patients, 100 patients with benign breast disease (BBD) and 100 healthy females (controls) were enrolled for the study. Eight hundred and twenty four follow-up samples of 225 breast carcinoma patients were also evaluated. The association of sialic acid forms, sialyltransferase and α-2-6 sialoprotein levels with presence and extent as well as prognosis of breast carcinoma was studied. Serum sialic acid forms and sialyltransferase revealed significantly elevated levels among untreated breast cancer patients as compared to the controls, patients with BBD as well as cancer patients in remission. Non-responders showed comparable levels of the markers with those found in breast cancer patients at the time of diagnosis. Higher levels of sialic acid forms at diagnosis were associated with poor prognosis. A positive correlation between serum levels of different forms of sialic acids and extent of malignant disease was observed. The changes in serum proteins with terminal α-2-6 sialic acid correlated well with alterations in the levels of sialic acid forms and sialyltransferase. Malignant tissues showed elevated levels of sialic acid and sialyltransferase as compared to surrounding normal tissues. The results suggested potential utility of these markers in evaluation of clinical outcome.

F-12
Effect of Naturally Occurring Sulphur Compounds and Isothiocyanate on the Cell Mediated Immune Responses of Metastatic Tumour Bearing Animals
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Introduction: Although various immune responses can be generated to tumour cells, the response frequently is not sufficient to prevent tumour growth. One approach to cancer treatment is to augment or supplement these natural defense mechanisms. Modulation or activation of immune system is important in cancer therapy. Objectives: The objectives of the present study was to analyze the immunostimulatory activities of naturally occurring organosulphur compounds such as DAS and DADS in metastatic tumour bearing C57BL/6 mice. Methods: Effect of DADS and DAS on NK cell activity, Antibody-dependent cellular cytotoxicity (ADCC), Antibody dependent complement mediated cytotoxicity (ACC) and their effect on anti and pro-inflammatory cytokines such as IL-2, TNF-α, IL-1β, IL-6 and GM-CSF were evaluated in metastatic tumour bearing C57BL/6 mice. Results: NK cell mediated cytolysis was enhanced maximally by the treatment of DAS (47%) and DADS (65.4%) on 4th day after tumour inoculation. Maximum NK cell activity in control tumour bearing animals was observed only on 15th day after tumour inoculation. Antibody-dependent cellular cytotoxicity (ADCC) was also enhanced by the treatment of DADS (50.3%) and DAS (34.3%), compared to metastatic tumour bearing control animals (21%). Antibody dependent cellular cytotoxicity (ADCC) was significantly increased by the treatment of DADS (31.3%). Treatment with DADS resulted in a significant upregulation of the serum concentration of IL-2 (47.1 pg/ml) compared to control animals (6.69 pg/ml). The serum concentration of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and GM-CSF, which were highly elevated in control animals were effectively reduced by the treatment of DADS. Conclusion: The above study revealed immunostimulatory effects of DADS and DAS on metastatic tumour bearing animals, and these activities may be due to the stimulation of cell mediated immune responses.

F-13
Effect of Biophytum sensitivum on B16F-10 Melanoma Cells Induced Metastasis

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Introduction: Metastasis remains to be the major cause of death in cancer patients. Although several drugs have been recommended for cancer therapy there are no drugs presently available which can specifically arrest the metastatic process. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones. The study of such medicines might offer a way to find novel medicines in oncology. Objectives: To evaluate the antimetastatic activity of Biophytum sensitivum on B16F-10 induced metastatic model in C57BL/6 mice. Methods: Metastasis was induced by injecting B16F-10 melanoma cells via, the lateral tail vein of animals. Biophytum methanolic extract was administered daily at a dose of 500 µg/dose/animal (10 doses). After 21 days animals were sacrificed and the number of tumor nodules were counted in the treated and untreated animals. Biochemical markers of metastasis such as lung hydroxyproline, uronic acid, serum sialic acid and gammaglutamyl transpeptidase were estimated. Results: Simultaneous administration of the extract produced a significant reduction (95%) in tumor nodule formation. Increased lung collagen hydroxyproline (23.71 µg/mg protein) and uronic acid (329.2 µg/100 mg tissue) content in the lungs of control
animals was significantly reduced in Biophytum treated animals. Similarly elevated serum sialic acid (118µg/ml) and GGT activity (117n mol p-nitroaniline/ml) in control animals was significantly reduced in animals treated with the extract. The lifespan of the Biophytum treated animals was also seen to be significantly increased.

**Conclusion:** The observed results show that *Biophytum sensitivum* could inhibit metastatic growth of B16F-10 melanoma cells in the mice model.

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**F-14**  
**Immunological Studies in Juvenile Laryngeal Papillomatosis**

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Juvenile Laryngeal papillomatosis is a relative common benign tumour of the childhood causing life threatening complications. Laryngeal papillomatosis is reported to be associated with the infection human papilloma virus (HPV) types 6 and 11. The preliminary work was done during the period 1995 to 1999. A study of 75 children suffering from juvenile laryngeal papilloma, both primary and with repeated recurrences were managed and treated in Government Children Hospital Egmore, Chennai. Samples were typed and found to be HPV type 6 and 11. No detailed study has been undertaken in India as to the typing of HPVs and biology of the viruses and their correlation with the aggressiveness of the disease. Immunological mechanisms, which effect the course of laryngeal papillomatamosis not well, understood. To elucidate the role of immunogenetic risk factors investigations can be done regarding Human Leucocyte Antigen (HLA) associations of juvenile laryngeal papilloma patients by Polymerase Chain Reaction (PCR) based methods. So it is proposed to study: 1. The types of HPV by PCR, 2. Correlation between types of HPV and their aggressiveness, 3. Cellular and humoral immune response, 4. HLA associations.

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**F-15**  
**Radioprotective and Anticlastogenic Activity of Phyllanthus amarus**

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**Introduction:** Drugs that are capable of protecting normal host tissues from the lethal effects of radiation without compromising its tumor reducing activity is of considerable interest in radiation medicine. Radiation also produces clastogenic changes, which can cause genotoxicity to normal cells. **Objectives:** To evaluate the radioprotective and anticlastogenic activity of an extract of the plant *Phyllanthus amarus* (*P. amarus*) in BALB/c mice. **Methods:** *P. amarus* extract (250 & 750 mg/Kg B.wt) was administered orally to mice continuously for 5 days prior to whole body radiation (6Gy) and for one month after irradiation. The animals were sacrificed at different time points and various hematological parameters such as total WBC count, bone marrow cellularity and a-esterase levels were analyzed. Antioxidant parameters viz. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione-S-transferase (GST), lipid peroxidation (LPO) and reduced glutathione (GSH) were assayed in blood and the liver. For anticlastogenic study BALB/c mice
were treated with *P. amarus* five days before radiation and irradiated at dose of 1.5Gy for micronuclei study and 3Gy for chromosomal aberration study. **Results:** Irradiation decreased the levels total WBC, bone marrow cellularity, \(\alpha\)-esterase, CAT, SOD, GPX, GSH and increased the LPO levels and GST. Administration of *P. amarus* was found to enhance the decreased levels of antioxidant enzymes and GSH and decreased the elevated lipid peroxidation levels. *P. amarus* treatment reduced the number of micronucleated polychromatic and normochromatic erythrocytes and decreased frequency of chromosomal gaps, breaks and other aberrations occurred in mouse chromosomes as a result of radiation. **Conclusion:** The results indicated the radioprotective as well as anticlastogenic activity of *P. amarus*.

**F-16**

**Immunomodulatory and Anticancer Effects of *Pleurotus ostreatus* Mycelia Derived Proteoglycans**

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**Introduction:** Both edible and medicinal Basidiomycetes have been evaluated for their nutritional and pharmacological properties. Many mushroom polysaccharides are reported as immunomodulatory and antitumour agents. Owing to the structural variability and diversity, these mushroom polysaccharides are capable of modulating immune system. *Pleurotus ostreatus* is one of the widely cultivated edible mushrooms and is well studied for its anticancer properties. **Objective:** Study of immunomodulatory and anticancer activities of neutral mushroom proteoglycans isolated from cultured mycelia of *Pleurotus ostreatus*. **Methods:** Mycelia of *Pleurotus ostreatus* were cultured in the conical flasks. Water-soluble fraction of alcohol-precipitated mycelia was passed through anion and cation exchange columns followed by gel filtration (sephadex G-100) column. Three neutral fractions were found and they were tested for in vitro and in vivo immunomodulatory and anticancer effects in mice model. **Results:** All the three proteoglycans stimulated mice thymocytes and splenocytes proliferation and elevated NK cell cytotoxicity. In vitro killing of S-180 and Dalton cells were also observed in presence of three fractions. Mushroom mycelia proteoglycans stimulated macrophage to produce NO. In vivo injection of proteoglycans to S-180 bearing mice decreased the number of cells, fluid volume and packed volume of ascites. **Conclusion:** Thus, the three neutral proteoglycans derived from the mushroom (*Pleurotus ostreatus*) mycelia could be used as immunomodulators and anticancer agents.

**F-17**

**Status of DNA methylation in liver and kidneys of rats during different stages of post natal development and as affected by 4-dimethylaminoazobenzene (4-DAB) administration**

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DNA methylation is involved in regulation of gene expression and differentiation in a cell but its role in early stages of dedifferentiation and initiation of carcinogenesis is lesser understood. To study DNA methylation in preneoplastic lesions, 3 different age groups of rats were taken (2, 14 and 90 days old) and 4-DAB (also called butter- yellow; a hepatocarcinogen) was administered for 7 days at a dose of
108 fJg /g body wt. DNA isolated from liver and kidneys of control and 4-DAB administrated rats was hydrolysed by the method which does not affect 5- methylcytosine content (Diala and Hoffman, 1982), and was quantitated by HPLC. There is age related demethylation of DNA in both the organs of control rats. However, there was no significant change in total DNA content /g tissue with age or 4 - DAB administration. Carcinogen administration was found to increase the percent 5- methyl cytosine content in livers of 9 and 21 days old rats whereas there was no change in 90 days old rats. No significant change was found in kidneys in any age group rats upon carcinogen administration. Results indicate that differential gene expression during post natal development might be influencing the initiation phase of carcinogenesis in its target organ liver. Also the methyl moieties of 4 - DAB might play some role in causing methylation in liver.

F-18
Antiangiogenic Activity of Boerhaavia diffusa In Vitro and in Animals

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Introduction: Angiogenesis is a key early event in tumor progression and metastasis. Angiogenesis and inflammation is well associated and inflammatory modulators are known to affect the process of tumour angiogenesis. Several studies have shown that many of the dietary/medicinal phytochemicals possess strong Anti-inflammatory and antiangiogenic activity thereby inhibiting tumour progression. Objectives: The aqueous-methanol extract of Boerhaavia diffusa was used to study its antiangiogenic activity by affecting the inflammatory modulators using in vivo as well as in vitro models. Methods: B16F10 melanoma cell were used to induce neo vessel formation on the ventral side of animals. Serum cytokine level was evaluated in both the control and extract treated (20mg/Kg) the angiogenesis induced animals by using respective ELISA kits. For in vitro studies rat aortic ring assay was performed in the presence or absence of extract. Results: Administration of extract (20mg/Kg) significantly inhibited the tumour directed capillary formation. Analysis of the serum cytokine profile showed a drastic increase of proinflammatory cytokines such as IL-1b, IL-6, TNF-a, GM-CSF and the endothelial proliferating agent VEGF in the angiogenesis induced control animals, which were significantly reduced by Boerhaavia treatment. Rat aortic ring assay revealed that extract inhibited the production of proangiogenic factors from B16F10 melanoma cells as the conditioned medium from the treated cells showed reduced capillary outgrowth. Conclusion: Boerhaavia treatment to the animals showed a strong antiangiogenic activity. Since the treatment regulated the level of proinflammatory and pro/anti angiogenic cytokines in the serum, the observed antiangiogenic activity is attributed, at least in part, to the regulation of the levels of these cytokines and growth factors in the circulatory fluids of the angiogenesis induced animal. Moreover the mRNA expression studies in the same cell line showed a reduced level of expression by Boerhaavia treatment further confirmed this interpretation.

F-19
Anti-Inflammatory and Antitumor Activities of Water Soluble Polysaccharides Isolated from a Macrofungus, Phellinus rimosus (Berk) Pilat

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**Introduction:** Inflammation, a fundamental protective response, can be one of the factors to accelerate the development of cancer. Epidemiological studies have shown that chronic intake of non-steroidal anti-inflammatory drugs (NSAID) reduces the incidence of colon, prostate, lung and breast cancers. Mushrooms are used in traditional Chinese medicine to treat several disease conditions. *Phellinus rimosus* is a wood rotting macro fungus, found growing on jackfruit tree trunks in Kerala. Recent investigations in our laboratory have shown that extracts of the basidiocarps of this mushroom possess significant biological activities. The major constituent of the extract is polysaccharide. **Objective:** To isolate the water-soluble polysaccharide from the basidiocarps of *P. rimosus* and to evaluate its anti-inflammatory and antitumour activities. **Methods:** The water-soluble polysaccharide from basidiocarps of *Phellinus rimosus* was isolated by the method described by Mizuno et al (2000). The anti-inflammatory activity of the polysaccharide was evaluated using carrageenan induced acute and formalin induced chronic inflammatory models in mice. Antitumor activity of the polysaccharide was determined by solid tumor model in mice using Daltons Lymphoma Ascites cells (DLA). **Results:** The water-soluble polysaccharide isolated from *P. rimosus* showed significant anti-inflammatory and antitumour activities in a dose dependent manner. The polysaccharide showed 72.94% and 61.9% inhibition in acute and chronic inflammation respectively at a concentration of 10 mg/kg body weight when administered orally. Tumor volume was reduced by 97.7% and tumor weight by 82.2% when the polysaccharide was administered orally at a concentration of 10mg/kg body weight. **Conclusion:** The results of the present investigation reveal that the water-soluble polysaccharide isolated from the basidiocarps of *Phellinus rimosus* possessed profound antitumor and anti-inflammatory properties.

F-20

**Transcription Factors Snail and Slug Downregulates Junction Components and Enhances Invasiveness of Epithelial Ovarian Cancer**

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**Introduction:** Epithelial Mesenchymal Transition (EMT) is vital for cell migration during development and is also implicated in tumor metastasis. During EMT, epithelial cells lose their polarity and cell-cell adhesion and acquire motile mesenchymal characteristics that enhances tumor invasiveness and metastasis. The Zinc finger transcription factors Snail and Slug have been reported to be involved in EMT by repressing different junction components. Here we hypothesized that pathological version of EMT is responsible for invasiveness of ovarian cancer, which is fascilated by Snail and Slug. **Objective:** 1. To examine the roles of transcription factors Snail and Slug in ovarian cancer. 2. To dissect out the individual roles of Snail and Slug during tumor progression. **Methods:** To prove our hypothesis, in vitro as well as in vivo experiments were conducted. For in vitro experiments, ovarian cancer cell line SKOV3 was taken as a model system. Stable and transient clones were isolated after transfection with an mSnail and mSlug. In selected clones, expression of junctions like adherens, tight and desmosome by RT-PCR and Western Blotting were also monitored. Similarly in the clones invasiveness and clonogenecity were examined by Matrigel assay and Soft agar assay. Tumorogenecity was confirmed in nude mice. **Results:** The ectopic expression of Snail or Slug resulted in EMT, enhanced motility, invasiveness and tumorogenecity in the cell line SKOV3. Snail suppress expression of adherent and tight junction components, while Slug suppresses expression of all three junction components viz adherens, tight and desmosome. The in vivo and in vitro experiment show increased invasiveness, clonogenecity and tumorogenecity property in clones. **Conclusion:** These results indicate that the Snail and Slug are involved in invasiveness of ovarian cancer. They may be putative targets in treatment of ovarian cancer.
F-21
Antioxidant, Anti-Inflammatory and Antitumor Activities of Cultured Mycelia of Morel Mushroom, *Morchella esculenta*

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**Introduction:** Molecular oxygen, while providing efficient energy production from ingested food, results in the free radical and peroxide by-products, which cause high intrinsic toxicity. Damage to DNA by oxygen free radicals is frequently postulated to cause initiation and progression of cancer. Chronic inflammatory diseases may promote the progression of neoplastic process. *Morchella esculenta* is an economically important excellently edible mushroom found growing in the Western Himalayan region. It is reported to be used in medicine and health care system by the traditional societies. Cultivation of this mushroom has not been successful till now and hence its mycelium is extensively used as a flavoring agent.

**Objective:** To evaluates the antioxidant, anti-inflammatory and antitumor activities of ethanolic extract of *Morchella esculenta* mycelium grown in submerged culture.

**Methods:** The antioxidant activity of 50% ethanolic extract was estimated by determining the superoxide, hydroxyl and nitric oxide radicals scavenging and inhibition of lipid peroxidation activities. Anti-inflammatory activity was determined by carrageenan induced acute and formalin induced chronic inflammatory models. Antitumor activity of the extract was determined by mouse solid tumor model induced by Daltons Lymphoma Ascites cells (DLA).

**Results:** The extract showed significant antioxidant, anti-inflammatory and antitumor activities in a dose dependent manner. Oral administration of 500mg/kg body weight of extract showed 66.6% and 64.2% inhibition of acute and chronic inflammation respectively. Administration of 1000mg/kg body weight of the extract orally showed 74.1% inhibition in tumor volume and 79.1% decrease in tumor weight 30 day after tumor cell implantation.

**Conclusion:** The present investigation thus reveal that the ethanolic extract of *Morchella esculenta* mycelium possessed profound antioxidant, anti-inflammatory and antitumor activities.

F-22
Chemopreventive Effects of Vanadium in Chemically Induced Rat Colon Carcinogenesis: A Focus on DNA Damage and DNA Protein-Cross Links by Comet Assay

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**Introduction:** The trace element vanadium inhibits cancer development in variety of experimental animal models. **Objectives:** The present study was designed to investigate the chemopreventive effects of vanadium on 1,2 dimethyl hydrazine induced genotoxicity in rat colon preneoplasia. **Methods:** Male Sprague-Dawley rats were divided into four groups. Group A were designed as normal controls. Group B received DMH once a week (20mg/kg wt) intraperitoneally for 12 weeks. Group C rats received the same treatment of DMH as in group B, along with 0.5-ppm vanadium as ammonium monovanadate ad libitum in drinking water throughout the experiment. Vanadium alone was given to group D rats without any DMH injection. **Results:** The 12 week treatment with DMH resulted in significantly higher levels of DNA damage in rat colon as measured by the comet assay (higher mean values for length to width ratios (L:W) of DNA mass (P<0.001) and mean frequencies of cells with comets (P<0.01). The vanadium co-treatment reduced DNA damage in colon cells by 45% (P<0.001 and P<0.02 for L:W and tailed cells respectively). The comet assay also showed statistically higher
mean base values of DNA-protein mass (P<0.01) and mean frequencies of tailed cells (P<0.001) in the carcinogen-induced group after treatment with proteinase K. Treatment with vanadium for the entire period caused a significant (P<0.02) reduction (48%) in DA protein cross-links in colon cells. **Conclusion:** The results demonstrate that the early protective effect of vanadium in chemically induced rat colon carcinogenesis may be mediated by a reduction of carcinogen-induced DNA damage.

**F-23**

**A Novel Anti-Cancer Lead Molecule from Curcuma Longa Induces Apoptosis In Human Laryngocarcinoma (HEp-2) Cells**


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**Introduction:** Natural products are the most consistently successful source of drug leads and they continue to provide structural diversity. A wide variety of natural products have been recognised to have the ability to induce apoptosis in various tumour cells of human origin and many of these substances are from herbal source. However, a large number of medicinal plants remain to be investigated further for their possible pharmacological value and also there is a need to develop new drugs to treat the increasing number of patients with different cancers. **Objectives:** To extract the active lead from *Curcuma longa* and validate its biological activity using *in vitro* bio screening in HEp-2 cells and also to understand the signalling pathway leading to apoptosis. **Methodology:** Active ingredient from *Curcuma longa* was pulled out by sequential solvent extraction and *in vitro* bioscreening using [*H]-Thymidine incorporation assay. Characterisation of the lead molecule was done by NMR and Mass spectrometry. Apoptosis was confirmed by flow cytometry analysis. RT-PCR, nitric oxide production and colorimetric assay were performed to detect different molecular targets. **Results and Conclusion:** Bioassay guided purification has enabled us to obtain novel lead molecule with proven anti-cancer activity from *Curcuma longa*. Flow cytometry analysis has confirmed the induction of apoptosis by this molecule in HEp-2 cells. Activation of caspase -3 was also confirmed using colorimetric assay. In order to prove the possible mechanism of induction of apoptosis by the lead molecule, RT-PCR analysis and nitric oxide (NO) assay has clearly demonstrated the activation of interferon (IFNγ) followed by elevation of iNOS leading to production of nitric oxide (NO) in HEp-2 cells. NO in turn activated caspase -3 to induce apoptosis. Elaborate studies with this compound with respect to its efficacy to induce apoptosis in different cancer cell lines and understanding the mechanism of action may provide valuable information for its possible application in cancer therapy.

**F-24**

**Curcumin Induces Apoptosis through the Impairment of Ubiquitin Proteasome Pathway**

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**Introduction** and **Objective:** Curcumin has long been used as a popular dietary spice and herbal medicine in several southeastern countries. Recent evidence suggests that the curcumin has chemopreventive and anti-tumor activities because of its ability to induce apoptosis. However, the molecular mechanisms through which curcumin induces apoptosis are not fully understood. **Methods:**
Mouse neuro 2a and HeLa cell lines were used for all studies in culture. Co-immunoprecipitation, immunoblotting and immunofluorescence staining were done using antibodies against various ubiquitin proteasome system components to delineate its pathway of action. Mitochondrial membrane potential was measured using confocal microscopy. **Results:** Here, we show that the curcumin-induced apoptosis is mediated through the impairment of ubiquitin-proteasome system. Exposure of curcumin to the mouse neuro2a cells causes dose-dependent decrease in proteasome activity and increase in ubiquitinated proteins. Curcumin exposure also decreases the turnover of the destabilized enhanced green fluorescence protein, a model substrate for proteasome and cellular p53 protein. Like other proteasome inhibitors, curcumin targets proliferative cells more efficiently than differentiated cells and induces apoptosis via mitochondrial pathways. Addition of curcumin to the neuro2a cells induces rapid decrease in mitochondrial membrane potential and release of cytochrome c into cytosol followed by activation of caspase-9 and caspase-3. **Conclusion:** Altogether, our result concludes that the curcumin-induced apoptosis is mediated through the impairment of UPP. Proteasome inhibitors are recently considered to be one of the promising groups of anticancer agent. Since, curcumin inhibits proteasome function and pharmacologically has been found to be safe, it has enormous potential in the prevention and therapy of cancer.

**F-25**
**Bitter Fraction of *Swertia chirata* can Prevent Carcinogenic Risk Due to DMBA Exposure**

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**Introduction:** Prevention of cancer can be achieved either by avoidance of risk factors or by increasing the availability of protective factors that can minimize the chances of developing cancer. In recent times the importance of cancer protective factors are receiving much attention as because some but not all of the risk factors for cancer can be avoided. **Objectives:** The present study was an attempt to assess the cancer protective potential of the plant *Swertia chirata* that is used in Ayurveda as liver tonic. **Methods:** DMBA induced mouse skin carcinogenesis model was used for demonstrating the ant carcinogenic effect of purified extract of *Swertia chirata* (amarogentin). Liver enzymes (GST, GPx, SOD, CAT) were determined biochemically, immunohistochemical procedures were followed for detection of proliferating and apoptotic cells in skin lesion and expression of COX 2 and Caspase 3 proteins were analysed by Western blot. **Results:** It was observed that amarogentin could protect from DMBA induced skin carcinogenesis as revealed by reduction in incidence of skin papilloma. Liver Phase II detoxification enzymes were activated following treatment. The plant extract produced a reduction in proliferating epithelial cells and increased the number of apoptotic cells in the precancerous skin lesions. This is also reflected in the expression of the molecular markers associated with proliferation and apoptosis viz. downregulation of Cox-II and upregulation of Caspase-3. **Conclusion:** Relative Risk or Relative Protection and Attributable Risk, which regulate development of tumor can be assessed by determining the influence of test agent on these two cellular processes associated with the progression of carcinogenesis.

**F-26**
**Tea Polyphenol Epigallocatechin 3-gallate Reverses the Anti-apoptotic Effects of Lowgrade Repetitive Stress through Inhibition of Akt and NF-kB Survival Pathways**

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**Introduction:** Exposures to low grade oxidative stress has become a common phenomenon, in these days of increasing environmental stress. Therefore, it becomes extremely relevant to investigate the factors determining the anti-apoptotic influence of repetitive stress and to document whether repetitive or chronic use of naturally occurring compounds influence the anti-apoptotic outcome of repetitive stress. Upregulation of antioxidant defense by means of activation of catalase, superoxide dismutase, glutathionperoxidase has been identified as an underlying cause of the apoptosis inhibitory effects exerted by repetitive stress. Activation of MAPK family enzymes p42/44, JNK, p38MAPK has been implicated in both cell survival and death. p42/44 exert control over cell proliferation and survival, JNK associated with apoptotic death, p38MAPK has been shown to influence both apoptosis and survival. Akt (protein kinase B-PKB) is another serine/threonine kinase which controls vital role in cell survival. Akt/PKB activation through phosphorylation on serine473 and threonine308 inhibits apoptosis by activating pro-apoptotic proteins BAD, FKHR or by activating cell proliferating gene NFkB. Overactivation of the Akt elevates the propensity of malignancy and has been identified as prime target of chemotherapy. Green or black tea polyphenols have recently been shown to possess growth inhibitory and pro-apoptotic effects especially in cancer cells. The tea catechin Epigallocatechin-3-gallate (EGCG) constitutes approximately 60% of the catechins in tea. The plasma/serum levels of tea polyphenols resulting from regular intake of tea are in the low micromolar range, peaking close to 1 mM. In this study.

**Objective:** We try to identify the key upstream elements that promote survival in repetitively stressed cells and unravel the inter-relationships between the important regulators of survival. Furthermore, we aim at identifying a natural product, which through continuous exposure at low levels may negate the anti-apoptotic/pro-survival effects of repetitive stress. **Method:** For development of repetitive oxidative stress model V79 fibroblasts were exposed to 30 mM H2O2 at 37 °C for 30 min in culture 5 days a week for a period of 4 weeks. The inhibitors SB-203580 (5 mM) or LY-294002 (5 mM) or EGCG (4.5 mM) were added in the culture medium of both control or repetitively stressed cells. Untreated controls were subcultured normally and received no other treatment. After 4 weeks of repetitive stress, UVC radiation of 1 J/m2/s for 5 sec or 7.5 mM H2O2 for 15 minutes were used to induce acute stress in previously treated or untreated cells. Activation of most of the proteins was observed by western blotting and, bands were identified by ECL. Caspase-3 activity was monitored fluorometrically by using its substrate AcDEVD. NFkB activity was measured by Mercury Pathway profiling SEAP system kit from Clontech. DNA fragmentation was quantitated from DNA of sub G0/G1 phase by FACs analysis. **Results:** Akt/Protein kinase B (PKB) became gradually phosphorylated at Serine436 and Threonine 308 during this period of repetitive stress. Exposure of the cells to LY294002 (5 mM), a phosphoinositide 3-kinase (PI-3-kinase) inhibitor and 4.5 mM Epigallocatechin 3-gallate (EGCG), a tea polyphenol almost completely blocked Akt activation by repetitive stress. Exposure to 5 mM SB203580, a p38 Mitogen Activated Protein Kinase (p38MAPK) inhibitor during the repetitive stress period resulted in moderate inhibition of Akt phosphorylation. p38MAPK also became increasingly phosphorylated during the repetitive stress period and the phosphorylation could be prevented by exposure to SB203580. However, p38MAPK phosphorylation remained largely unaffected by the presence of either EGCG or LY294002. Transcriptional activity driven by Nuclear Factor kappa B (NFkB) was significantly (4 fold) enhanced by repetitive oxidative stress. This increase was largely abolished by simultaneous exposure to either SB203580 or EGCG. The repetitively stressed cells demonstrated a significant resistance to apoptosis by subsequent acute stress in the form of ultraviolet radiation (UVR) at 5J/m2 or H2O2 (7.5 mM). The opposition to apoptosis conferred by repetitive stress was drastically reduced (>80%) by constant exposure to EGCG during the stress period. Similar exposure to SB203580 also resulted in considerable abrogation (>70%) of the anti-apoptotic influence of repetitive stress while the presence of LY294002 brought about a moderate reversal (about 50%). **Conclusion:** Our data indicate that activation of Akt and NFkB pro-survival pathways by repetitive low-grade stress results in a radical inhibition of the normal apoptotic response after subsequent acute stress. The tea polyphenol EGCG impedes the activation of both Akt and NFkB by repetitive stress and as a result aids in the preservation of the normal apoptotic response during subsequent acute stress.
F-27
Chemopreventive Effect of Diphenylmethyl Selenocyanate against Benzo (a) Pyrene Induced Lung Carcinogenesis in Strain a Mice

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\textbf{Introduction: }Lung cancer has become the most common cause of cancer deaths due to increased tobacco habit and environmental pollutants especially automobile exhausts. Chemoprevention is recognized as a viable means to reduce cancer death in humans. Experimental evidences revealed that organoselenium compounds is an important chemopreventive agent, which possess antioxidative, antimitagenic and anticarcinogenic properties. Therefore chemopreventive intervention using synthetic organoselenium compounds may be a practical and cost effective approach for reducing lung cancer risk. Benzo (a) Pyrene (BP), one of the major polycyclic aromatic hydrocarbons present in tobacco smoke and in automobile exhausts is a risk factor for bladder, skin and lung cancer. \textbf{Objective: }In this study we evaluated the chemopreventive potential of diphenylmethyl selenocyanate against BP induced lung carcinogenesis in strain A mice. \textbf{Methods: }BP was injected subcutaneously at the subcapular region at a dose of 0.2mg/mouse in 1% aqueous gelatin as a suspension in strain A litter within 24 - 48hrs. after birth and the Se-compound was given orally after 5\textsuperscript{th} weeks of BP treatment at a dose of 3mg./Kg. b. w. upto 22\textsuperscript{nd} weeks. Progressive cellular and histological changes initiated by BP eventually resulting in formation of lung tumours were followed to identify the precancerous lesions, the targets for chemoprevention. \textbf{Results: }Hyperplasia and severe dysplasia were evident in carcinogen control group after 8\textsuperscript{th} and 22\textsuperscript{nd} weeks respectively, were effectively reduced after oral administration with diphenylmethyl selenocyanate. The Se compound can also significantly (p<0.01) reduce the hepatic microsomal lipid peroxidation and induce hepatic GST activity when measured after 8\textsuperscript{th} and 22\textsuperscript{nd} weeks of BP exposure. \textbf{Conclusion: }The result indicates that diphenylmethyl selenocyanate have the potential to modulate the lung carcinogenesis in mice exposed to BP an act as effective chemopreventive agent.

F-28
Management of Cancer Patients with Ayurvedic Therapy HUMA

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\textbf{Introduction: }The burden of cancer is increasing worldwide. According to WHO cancer patients in the developing world will double to 10 million new cases annually by 2015. Disturbingly, most of them will have limited access to the radiation therapy that could save or prolong their lives. Many patients with cancer are compelled to try various complementary and alternative medicines for cancer treatment and palliation. HUMA a poly-herbal Ayurvedic Rasayana formulated by Late Dr. S M Atiq in early 80's have gained tremendous popularity as an alternative cancer therapy (ACT) among patients in recent years. \textbf{Objectives: }To assess the effectiveness and side effectiveness and side effects of HUMA therapy in cancer patients with advanced disease. \textbf{Methods: }We retrieved and studied records of 200 cancer patients who tried HUMA. Out of which 47 (23.5%) were oral, 56 (28%) GI tract, 32 (16%)
haematological, 21 cervical (10.5%), and 46 patients had other types of cancer. Metastasis was present in 175 (87.5%), and 46 patients had other types of cancer. Metastasis was present in 175 (87.5%) patients. Eighty-seven (43.5%) patients had tried conventional therapy earlier. All patients trying HUMA therapy also received conventional life support care but without radio or chemotherapy. **Results:** Marked responses viz. decrease in pain, drying of pleural effusion & ascites and regression of tumor was observed in 73 (36.5%) of patients. Complete regression of tumors/lesion along with relapse free survival benefit with this ACT was observed in few oral and rectal tumor patients. No adverse side effects were observed in any patients. **Conclusion:** HUMA is tried primarily for palliation, however, marked remission of cancer observed in some patients deserves scientific attention.

F-29
**Prooxidant Activity of Resveratrol in the Presence of Copper Ions: Implications For Anticancer Properties**

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Several plant derived polyphenolic compounds are considered to possess anticancer and apoptosis inducing properties. Such compounds are recognized as naturally occurring antioxidants but also exhibit prooxidant properties under appropriate conditions particularly in the presence of transition metal ions such as copper. Over the last several years we have shown that various classes of plant polyphenols including flavonoids, curcuminoids, catechins and stilbenes are capable of catalyzing oxidative DNA cleavage in the presence of copper ions. Evidence in the literature suggests that the antioxidant properties of plant polyphenols may not fully account for their chemopreventive effects. On the basis of our own observations and those of others we have proposed a mechanism of DNA fragmentation and cytotoxic action by plant polyphenolics that involves mobilization of endogenous copper and the consequent prooxidant action. Resveratrol (3,4',5-trihydroxy stilbene) is a polyphenolic compound present in dietary material such as peanuts, grapes and red wine. It has been shown to be chemopreventive against various stages of chemically induced carcinogenesis. In our laboratory we have shown that resveratrol catalyzes the reduction of Cu(II) leading to oxidative DNA cleavage. In the present report we have studied the structure activity relationship between resveratrol and its structural analogs piceatannol (3,3',4,5' tetrahydroxy stilbene) and trans stilbene which does not contain any hydroxyl group. Piceatannol was found to be the most effective in the DNA cleavage reaction as well as a reducer of Cu(II) followed by resveratrol and trans stilbene which does not show any activity. The results indicate that the number and position of hydroxyl groups is important for the prooxidant action of resveratrol and piceatannol in the presence of copper ions. Further, we have also characterized the mutations induced by resveratrol-Cu(II) using plasmid Bluescript SK(+). Our studies indirectly support our hypothesis that prooxidant action of plant polyphenols may be an important mechanism of their anticancer properties.

F-30
**Anticancer Potential of Novel Synthetic Nucleoside Analogs**

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HPV is a DNA tumor virus and the causal agent of cervical cancer, the most common cancer in Indian women. HPV 16 is the most prevalent type and in India, more than 90% of the HPV infections are of HPV type 16. Two novel synthetic nucleoside analogs, viz. 9-(1'-â-D-arabinofuranosyl)-4-nitro-1,3-dideazapurine (1) and 5'-deoxy-5'-(mercapto-propionic acid)-adenosine (2) have been screened for their anticancer potential on HPV (Human Papillomavirus) 16 and 18 - positive cervical cancer cell lines, SiHa and HeLa, respectively. After a 24-hour treatment, compound 1 caused apoptosis in SiHa and necrosis in HeLa cells at 200µg/mL whereas compound 2 showed no effect on the morphology of these cells. Further studies were carried out to observe the effect of these nucleoside analogs on the binding efficiency of the transcription factor AP-1 to HPV-specific gene sequence, using Electrophoretic Mobility Shift Assay (EMSA). Compound 2 exhibited a downregulation of AP-1 binding to the HPV- specific gene sequence. Since AP-1 is an indispensable key regulator of epithelial cell-specific transcriptional activity of various HPV types, downregulation of its binding to the HPV genome may lead to interference with the expression of HPV genes, particularly the E6 and E7 genes which are responsible for tumorigenic transformation of cervical epithelial cells. However, compound 1 did not exhibit any effect on AP-1 binding. Western Blotting experiments were carried out to check the expression pattern of two of the constituent proteins of AP-1, c-fos and fra-1, in SiHa cells treated with compounds 1 and 2. Compound 1 was found to cause a down regulation in the expression of c-fos, which became almost nil in cells treated with 200µg/mL concentration. At the same time, fra-1 expression was found to gradually increase in cells treated with increasing concentrations of this nucleoside analog. Compound 2 downregulated c-fos expression at 10µg/mL concentration while fra-1 expression was found to be slightly upregulated with its increasing concentrations. Earlier workers have shown that as tumorigenic cervical epithelial cells traverse towards normalcy, c-fos expression is downregulated and fra-1 expression increases2. Hence, both these nucleoside analogs may be developed as anti-HPV molecules. A decrease in the expression of c-fos has also been shown to be associated with the onset of senescence in cells and an inhibition of DNA synthesis. Growth factors have been shown to cause induction of c-fos gene and protein expression. Since senescence and apoptosis have been proposed as possible mechanisms behind the antioncogenic properties of anticancer drugs, the compounds 1 and 2 may, in general, be considered as potential cancer chemotherapeutic agents.
assay, RNA extraction and amplification by RT-PCR, Western blotting for Protein level analysis. **Results and Conclusion:** Apoptosis or programmed cell death is a genetically regulated process occurring naturally in a response to a variety of signals. Many anticancer agents exert their cytotoxicity through DNA damage and induction of apoptosis. We found that the lead molecule obtained from *Andrographis paniculata* leaf, was able to induce apoptosis in human HeLa cells. In this work the extraction, purification and elucidation of active molecular lead based on the bioactivity directed based screening is described. We also explored the mechanisms through which this molecule induces apoptosis. This molecule activated p53 and subsequently resulted in the up-regulation of pro-apoptotic protein Bax followed by cytochrome c release from mitochondria leading to caspase-3 activation and then cells undergo apoptosis. DNA fragmentation assay further confirmed the apoptosis of the cells. This compound also suppressed Bcl-2 expression in HeLa cells. This work is an example of a natural product with interesting anti-proliferative activity, in which the basic skeleton can be further, used as a template for the production of New Chemical Entity.

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**Antitumour and Immunomodulatory Activity of Andrographis paniculata Nees**

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**Introduction:** Several immunomodulators are now being used in cancer therapy. Use of plants as a source of immunomodulators is still in a developing stage. **Objectives:** Methanolic extract of *Andrographis paniculata* (*A. paniculata*) was studied for its antitumour and immunomodulatory activity using animal models. **Methods:** Cytotoxicity of the extract was determined by trypan blue exclusion method and by MTT assay. Solid tumors were injected by injecting DLA cells (1x10⁶ cells/animal, s.c) on the hind limb. For ascites tumors animals were injected with 1x10⁶ EAC cells/animal to the peritoneal cavity. Animals were treated with methanolic extract of *A. paniculata* (10mg/dose/animal, i.p) for 10 consecutive days inorder to do immunomodulatory study. The levels of total WBC, bone marrow cellularity, α-esterase activity, the antibody titer and the number of plaque forming cells (PFC) were determined in normal as well as in tumor bearing animals. **Results:** Methanolic extract of Andrographis paniculata was found to be toxic towards both DLA cells EAC cells at a concentration of 500 µg /ml. The extract was also found to produce cytotoxicity towards L929, B16 F-10, and Vero cells at a concentration of 250µg/ml. Administration of the extract could inhibit the solid tumor development in mice induced with DLA cells and increased life span of mice bearing ascites tumor by 34.6%. *Andrographis paniculata* extract treatment was found to increase total WBC count. Bone marrow cellularity and α-esterase positive cells were also enhanced by the extract administration. Administration of the extract enhanced the antibody titre and PFC. **Conclusion:** The observed antitumour activity of *Andrographis paniculata* may be related to its immunomodulatory activity.

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**Cancer Chemopreventive Potential of a Novel Organoselenocyanate Against DMBA-PMA Induced Skin Carcinogenesis in Mice**

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Introduction: Selenium is an essential dietary trace element required for mammals including humans to fight against various diseases including cancer. Extensive work is in progress to obtain suitable selenium compounds useful for prevention of cancer. It has been found that organoselenocyanates are far superior to other selenium compounds in terms of their chemopreventive efficacy. Objectives: In continuation of our anticarcinogenesis-drug development programme we have synthesized and evaluated the cancer chemopreventive potential of a novel organoselenocyanate 2-(2-Selenocyanato-ethyl)-benzo[de]isoquinoline-1,3-dione{Nap-(CH2)2SeCN} against DMBA-PMA induced skin carcinogenesis in Swiss albino mice. Methods: The test compound was administered orally in pre and pre+post treatment schedules at the dose of 3mg/Kg bw. Lipid peroxidation, activities of Phase II enzymes and GSH level in liver and skin tissues were estimated biochemically. Results: The compound was found to be non-toxic at the dose tested for the experiment. Significant (p<0.01) downregulation of lipid peroxidation and upregulation of Phase II detoxifying enzymes (GST, SOD, CAT) along with GSH level were observed following treatment, estimated on the 12th week of the first DMBA application, in comparison to the carcinogen control. Significant reduction in number of papilloma formed per mouse was noted after treatment (from 3.8 per mouse in carcinogen control to 2.3 per mouse). Papilloma incidence was also inhibited by 32-47%. Conclusion: The chemopreventive potential of the 2-(2-Selenocyanato-ethyl)-benzo[de]isoquinoline-1,3-dione{Nap-(CH2)2SeCN}seems clear at this stage of investigation.

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Green Medicine in Preventive Oncology

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Cancer is a major health problem worldwide which is likely to assume alarming proportions in the next two decades. Conventional therapies cause serious side effects and, at best, merely extend the patient's lifespan by a few years. In the last few decades, there has been probably an upsurge in the use of green medicine (herbal-based drugs) because of the miraculous success of them and no side effects like that of synthetic medicines. Keeping this into view research is being carried out to evaluate the potential anticancer activities of various vegetables, fruits and others plants. A general screening of papers published during last 2-4 years revealed that in addition to earlier reports, recently more than 65 plants have been found to possess anticancer activity on different cell lines and forms of cancer. It is further interesting to note that not only medicinal herbs or trees, some fruits and vegetables also have potential anticancer property. In this paper such reports have been summarized for the purpose of dissemination of information as well as to encourage further research and developing useful drugs and formulations in preventive oncology. Vegetables, which are reported to be anticancerous are: Onion (Allium cepa Linn.), Bitter gourd (Momordica charantia Linn.), Potato extract, Garlic (Allium sativum Linn.), Broccoli (Brassica oleracea Linn.) etc. Coffee (caffeine), green tea and cocoa (Theobroma cacao Linn.) are also anticarcinogen. Fruits, which have been found as anticancerous, include: Cashew kernel oil, Soursop (Annona muricata Linn.), Actinidia chinensis Planch. (Kiwi fruit), Citrus sinensis (Linn.) etc. The herbs and trees that were investigated by various researchers on different cancer cell lines and shown positive Results are about 45 including already reported ones like Withania somnifera Dunal, Emblica officinalis Gaertn., Psoralea corylifolia Linn., Curcuma longa Linn., Catharanthus roseus G. Don, Taxus brevifolia Linn., etc. Some weeds found to be anticancerous are: Ageratum conyzoides Linn., Blumea lanceolaria, Achyranthes bidentata, Croton oblongifolius Roxb., Tribulus terrestris Linn. etc.