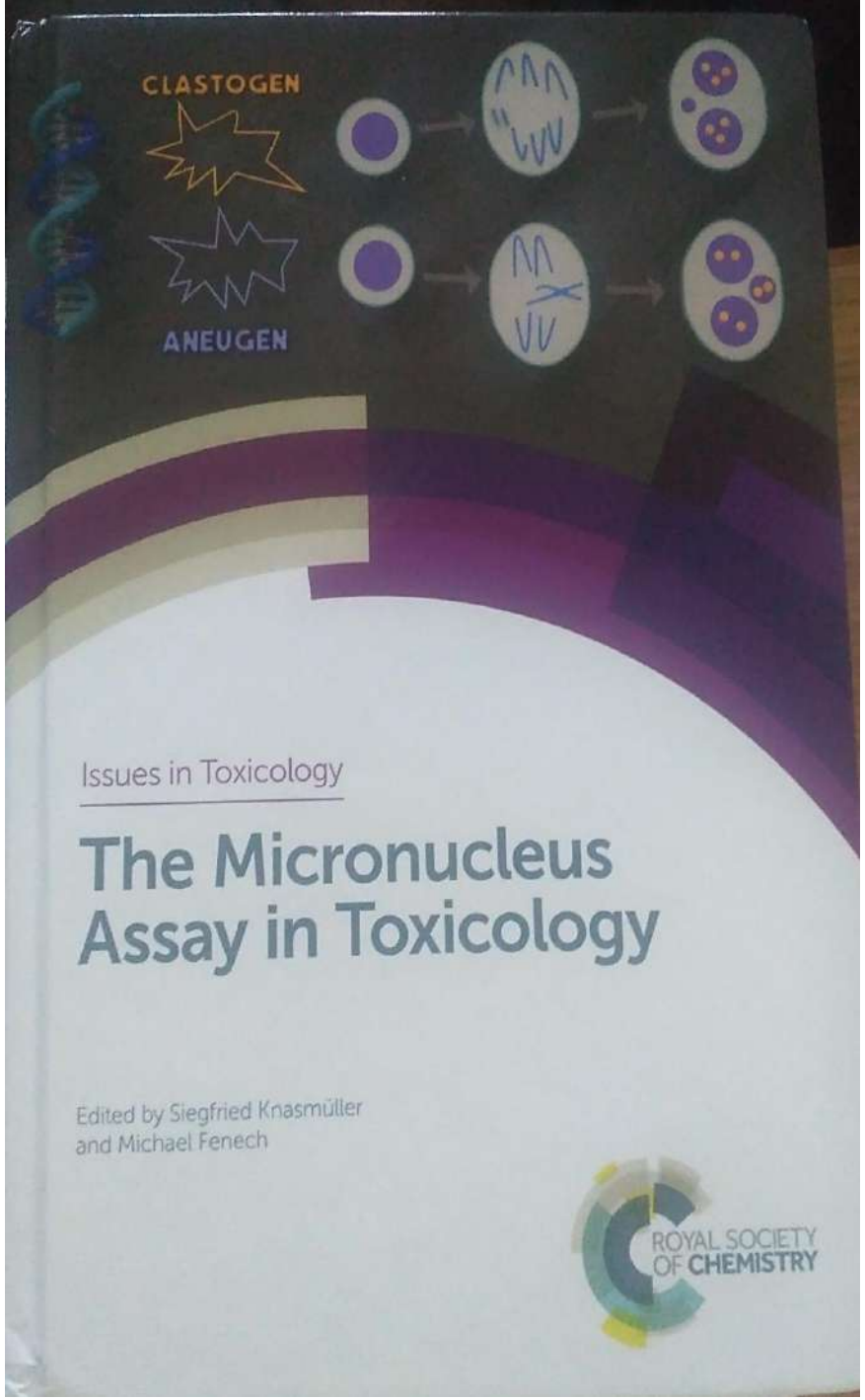

Issues in Toxicology

The Micronucleus Assay in Toxicology

Edited by Siegfried Knasmüller and Michael Fenech

ROYAL SOCIETY OF CHEMISTRY

CHAPTER 16

Micronucleus Assays in Amphibians

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16.1 Introduction

Genotoxic agents alter or damage genetic material, which can affect both somatic as well as germ cells of organisms. While genetic damage at the germ cell level has effects across generations, genetic changes in somatic cells can have significant population level effects.[1] Man-made chemical compounds available in international markets, including their mixtures, number in excess of 80 000 different kinds[2] and aquatic ecosystems acts as the major sink for these materials. Contamination of the aquatic environment will not only affect aquatic biodiversity, but also the organisms, including humans, living in the terrestrial habitat through contaminated food and water. Many of these chemical contaminants are potent genotoxins and need monitoring and management.

In eukaryotes, the micronucleus (MN) assay is a relatively simple and reliable method that can be used to detect genotoxins in a cost and time effective manner. MN are acentric chromosomal fragments or whole

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NROR-01

Radiofrequency Radiation Exposure-Induced DNA Damage and Cell Cycle Arrest in Germ Cells of Male Swiss Albino Mice

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Mobile phone technology uses radiofrequency radiations (RFR) 900 1800 MHz for signal transmission. The widespread use of mobile phones with almost 7 billion subscribers globally has not only increased electro-pollution of the environment but also raised a concern about their possible health effects. Genotoxic potentials of RFR on different cell types have been well reported. However, their adverse effects with respect to DNA damage in male reproductive cells have not been investigated thoroughly. Therefore, in the present study, we aimed to investigate the effect of 35 days of whole body RFR exposure (6 h/day) on germ cells of male Swiss albino mice. Enumeration of germ cells by flow cytometer using propidium iodide (PI) showed arrest in pre-meiotic phase of spermatogenesis, leading to slow rate of spermatogonial to spermatocyte transformation. RFR-induced DNA breaks and excessive ROS production in testicular cells are apparent from high damage index in comet assay and high frequency of cells with low mitochondrial membrane potential in JC-1 assay. Furthermore, increased lipid peroxidation, decreased superoxide dismutase activity and reduced glutathione concentration in testes was an indicative of oxidative stress. Also, the defective reproductive phenotypes, including reduced sperm count, increased sperm malformation and histological abnormality of testes, were observed. These results show that RFR exposure causes DNA damage and cell cycle arrest in germ cells by mitochondrial-mediated oxidative stress which may be associated with elicit male germ cell toxicity.

NROR-02

Effect of Radiomimetic Bleomycin Sulphate on Mitosis in two Varieties of *Trigonella foenum-graecum* L. (Methi)

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Bleomycin sulphate is a radiomimetic anticancer drug. It is a product of the bacterium *Streptomyc verticillus*. It causes DNA breaks. The presence of this drug in the environment can be detrimental human health as well being of food crops. The effect of this antibiotic drug was investigated on tw varieties of *Trigonella foenum graecum* L. In this work, an attempt has been made to find out the mo resistant and the most sensitive variety among these two varieties of fenugreek. Seeds of two differe varieties of *T. foenum graecum* L. were collected. *T. foenum graecum* L. var. Hisar Suvarna and va GM 1. Mitotic studies were conducted on both the varieties in normal conditions by squash techniqu seeds were treated with five different concentrations of radiomimetic bleomycin sulphate. Mitot bnormalities of treated seeds were studied and recorded for conclusions. The variety GM 1 was t sistant variety towards different concentrations of bleomycin sulphate and the variety Hisar Suvar

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Department of Zoology, Sikkim University, Gangtok
ABSTRACT # OYS – 19

Ultrastructural changes in the junctional complex and reduced expression of junctional proteins are associated with oral precancerous and cancerous conditions

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Abstract: Oral cancer is considered as the 6th most common cancer in the world increasing at an alarming rate. High mortality rate, delayed clinical detection and poor prognosis make the disease management difficult. Therefore, developing a reliable diagnostic tool for early detection and prognosis of the disease is mandatory. Disruption of cell-cell junctional complex is a hallmark of cancer cell invasion and metastasis. Tight junctional (TJ) and adherens junctional (AJ) proteins play a vital role in maintenance of tissue architecture but are often dysregulated in different cancer. However, their role in precancerous and oral cancer condition is less understood. Present study investigates the expression of TJ protein, zona occludens-1 (ZO-1) and AJ protein, e-cadherin (E-cad) in oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC) tissues. Ultrastructural changes in the junctional complex were studied by transmission electron microscopy and expressions of proteins were examined by immunohistochemistry. Relationship between protein-protein, protein-clinicopathological features and protein-ploidy level was checked by Pearson's correlation test. Further, survival curve was estimated by the Kaplan-Meier method. Disrupted junctional complex and significantly decreased immunoexpression of proteins was observed in OED and OSCC. ZO-1 was associated with tumor-node-metastasis in OSCC and E-cad with histological grades, tumor-node-metastasis, and ploidy in OSCC. High ZO-1 and E-cad expressing patients survived longer than their low expressed counterparts. Our result demonstrates a relationship between junctional complex disruption and reduced protein expression in OED and OSCC, indicating their role in tumor progression. Therefore, their diagnostic value is equally important for early diagnosis even in the precancerous lesions.

Keywords: E-cadherin, zona occludens-1, oral epithelial dysplasia, oral squamous carcinoma

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Department of Zoology, Sikkim University, Gangtok
ABSTRACT # OYS – 07

Extracts prepared from the leaves of *Tagetes erecta* exhibits anticancer activity in Ehrlich ascites carcinoma (EAC) cells and *in vivo* tumor model

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Abstract: Search for new and effective novel phytochemicals with lower toxicity and side effects are an important part of cancer research treatment strategy improving patient care. *Tagetes erecta* is a common plant with a wide range of ethno-medicinal value. However, its anticancer potential has not been evaluated with very limited reports available. The present study was undertaken to elucidate the anticancer potential of aqueous extract of *Tagetes erecta* (AETE) against Ehrlich ascites carcinoma (EAC) cells and *in vivo* tumor model. Cytotoxicity in EAC cell line was evaluated by trypan blue dye, MTT and LDH release assays. Apoptosis study was done by using flow cytometer. For evaluation of anticancer potential of AETE, mice were randomly divided into four groups *viz.* Group I (normal control), Group II (positive control) and Group III and Group IV treated with 200 mg/kg. bw and 400 mg/kg. bw of AETE respectively. The tumor weight (TW), tumor volume (TV) and body weight (BW) of tumor-induced mice was measured. Histology in liver and tumor tissue was done to assess the structural changes. Biochemical estimation of liver and kidney enzyme levels were done in AETE treated and untreated mice to evaluate the side effects. AETE induced a dose and time-dependent effect in EAC cell line. The flow cytometric analysis revealed the increase in percentage of apoptotic cells from 8.93% to 44.97% ($p<0.001$) following AETE exposure (0.2 mg/ml). AETE exposure showed a significant reduction of TV and TW and increase in life span. AETE exposure (400 mg/kg. bw) did not alter the liver and kidney enzyme levels in the blood plasma indicating absence of any side effects. Further, GC-MS analysis revealed the presence of various bioactive compounds in the leaves of *Tagetes erecta* that might play role in anticancer potential of *Tagetes erecta*. We conclude that *Tagetes erecta* contains bioactive compounds of anticancer potential that may be used in the development of novel anticancer drugs.

Keywords: *Tagetes erecta*; Cytotoxicity; MTT assay; Ehrlich ascites carcinoma; Solid tumor

Bisphenol A induces genotoxicity, cytotoxicity and germ cell toxicity in Swiss albino mice

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Bisphenol-A (BPA), a monomer of polycarbonate and epoxy resins is an important environmental contaminant. Due to its widespread exposure, it represents a major toxicological and public health concern. Present study aims to investigate the genotoxic and reproductive potential of BPA*in vivo*. Swiss albino mice were divided into three groups viz: vehicle control (propylene glycol), low dose (10mg/kg bw) and high dose (50mg/kg bw); treated for a period of 35 days. Genotoxicity was evaluated by studying the incidence of micronucleated polychromatic erythrocytes (MNPCEs) and comet assay in bone marrow cells. Germ cell transformation kinetics and mitochondrial membrane potential was studied in all groups employing flow cytometry. Additionally, reproductive toxicity was evaluated by recording the abnormal sperm heads and total sperms count. Both the treated doses of BPA induced statistically significant increase ($p<0.001$) of micronucleated polychromatic erythrocytes when compared to control group. Increase ($p<0.001$) in the ratio between polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) observed indicates the cytotoxic effects of BPA. Significant increase in damage index (DI) in bone marrow cells was noted in BPA exposed mice. Comet assay clearly demonstrated significant difference in all the parameters in the treated groups which indicates the genotoxic potential of BPA which supports our MNPCEs data. Dose dependent decrease in sperm count and increase in abnormal sperm head was observed in treated group ($p<0.001$) when compared to control. Flow cytometric estimation of germ cell subtypes in mice testis revealed significant decrease in spermatids. Decrease in spermatogonia to spermatid turn over (1C:4C) and primary spermatocyte to spermatid turnover (1C:2C) indicates the defects in spermatogenesis after BPA exposure. Depolarisation of mitochondrial membrane resulted in destabilization of cellular redox homeostasis in treated group. Histological evaluations such as germ cell depletion, atrophied seminiferous tubule and compact disorganized arrangement of germ cells were observed in treated groups. To conclude, BPA exposure induce genotoxicity as well as reproductive toxicity causing DNA damage in germ cells which alters cell cycle progression leading to low sperm count in mice.

OL8: Oral Presentation

Toxicity assessment of arecoline N- oxide: A metabolite of Areca nut alkaloid arecoline

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Arecoline, the major alkaloid of Areca nut has both pro fibrogenic and pro carcinogenic effects. Study of its metabolome in mice indicated the formation of arecoline N- oxide *in vivo*. Few studies indicate that arecoline N- oxide itself might be biologically active and play a role in Areca nut mediated pathogenesis of oral cancer.

In this study, we investigated the presence of arecoline N- oxide in commercially available Areca nut via GC-MS analysis. Cytotoxicity assessment of synthesized arecoline N- oxide was done in Ehrlich Ascites Carcinoma (EAC) cells, a mouse cancer cell line and mice test system. Oxidative stress induction in xenobiotic metabolizing tissues of mice post exposure was investigated *in vivo*.

GC-MS analysis of Areca nut extract indicated its absence in Areca nut, emphasizing on its formation involving biologically active enzymatic metabolism of arecoline. Arecoline N- oxide was found to be cytotoxic in both *in vitro* and *in vivo* test systems. Oxidative stress assessment indicated generation of oxidative stress in liver and kidney- increase in MDA level and decrease in SOD and GSH level in exposed tissue samples.

Conclusion: Arecoline N- oxide has toxic effects in both *in vivo* and *in vitro* test system.

P18: Poster Presentation

Evaluation pf cytotoxicity of arsenic and smokeless tobacco and their combined interaction in CHO cells

Sweety Nath Barbhuiya, Dharmeswar Barhoi and Sarbani Giri*

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Several reports are available regarding the toxicity induced by arsenic and smokeless tobacco alone but information about their combined interaction is lacking. Thus, the present study is aimed to evaluate the combined toxicity of arsenic and smokeless tobacco in Chinese hamster ovarian (CHO) cell line.

The combined interaction of arsenic and *sadagura* was determined by analyzing the combination index (CI) generated in combination index (CI)-isobologram method using a computer assisted program CompuSyn. CHO cells were treated with different concentrations of *sadagura* (1-10 mg/ml), sodium arsenite (1.3-41.6 µg/ml) and *sadagura* with lime (1-10 mg/ml) and MTT assay was done after 48 h and 72 h exposure. Further, CHO cells were exposed to different concentrations of combined doses based on individual Dm value in 1:1 ratio to evaluate the interactions between the chemicals.

The results of MTT assay clearly indicated that sodium arsenite is more toxic among the three chemicals tested. Combined treatment of arsenic and *sadagura* showed synergistic effect in CHO cells at lower concentrations of IC_{50}. However, higher combined doses of IC_{50} showed antagonistic effect.

Conclusion: In the present study, combined exposure of arsenic and *sadagura* showed synergistic effect in CHO cells, indicating increased toxicological implications.

OL4: Oral Presentation

Systemic effects in 4NQO induced model as an indicator for early diagnosis of pre cancerous lesions

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Health concerns of various types including lethality is the prime signature of tobacco consumption. Yet, the treatment workup and diagnosis gets concentrated towards the malignant growth with avoidance of other systemic effects. Therefore, it is highly warranted that during diagnosis of early precancerous stages, systemic effects to be considered that might play crucial role in disease pathology.

Present study employs 4 nitroquinoline –N-oxide (4NQO) induced Swiss albino mice as an oral precancerous model and to study the extent of its toxicological impacts especially in reproductive tissues. Toxicity was evaluated by witnessing the abnormal sperm head, total sperm count, biochemical assays and testis histology. Also, flow cytometric estimation of germ cell sub types and mitochondrial membrane potential assays to assess extent of apoptosis was performed.

Statistically significant decrease in the tissue index and increased percentage of sperm head defects were noted in 4NQO treated mice. Flow cytometric estimation of germ cell subtypes revealed a significant increase in spermatogonial populations and decrease in spermatids. Testicular cells stained with JC-1 dye showed an increased population of cells with depolarized mitochondrial membranes ($\Delta\psi m$) in treated mice compared to control. Furthermore, biochemical assays revealed excess free radical generation resulting in histological and morphological changes in testis and germ cell morphology.

Conclusion: 4NQO induced reproductive toxicity indicate its effects on non target tissues. However, reproductive damage could be used as an indicator to check the developing concurrent oral lesions, which otherwise is not considered. The association between precancerous lesions and concomitant reproductive toxicity could serve as a potent tool for predicting the two unrelated pathologies in context of each other.

P10: Poster Presentation

Role of Vitamin C, Natural Antioxidant, against Bisphenol A Induced Toxicity in Swiss Albino Mice

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Bisphenol-A (BPA), a monomer of polycarbonate and epoxy resins, produced in high volumes and are widely used in many consumer products. Due to its widespread exposure, it represents a major toxicological and public health concern. Present study aims to investigate if BPA induce oxidative stress on the testis of male mice and whether co-administration of vitamin C, an antioxidant, can prevent oxidative stress.

Animals were divided into, control (propylene glycol), BPA1-10mg/kg bw; BPA2-50mg/kg bw; vitamin C (25mg/kg bw) and BPA+vitamin C (50mg/kg bw + 25mg/kg bw) treated for a period of 35 days. Reproductive toxicity was evaluated by recording the abnormal sperm heads, total sperms count and biochemical assay and testis histology.

Decrease in sperm count and increase in abnormal sperm head was observed in BPA treated groups when compared to control. The malondialdehyde levels were significantly higher while glutathione and superoxide dismutase levels were lower in BPA treatment group compared to control. Furthermore, decrease in malondialdehyde levels whereas an increase in glutathione and superoxide dismutase levels was observed in BPA+ vitamin C group compared to BPA treated groups but it was not statistically significant. Histological evaluations of testis in mice in BPA treated groups revealed germ cell depletion, atrophied seminiferous tubule and dense disorganized arrangement of germ cells.

Conclusion: BPA cause oxidative damage by disturbing the balance system between reactive oxygen species and antioxidant defense system in mice testis leading to low sperm count and sperm morphology. In addition, vitamin C co-administration along with BPA does not have significant protective effects.

P18: Poster Presentation

Evaluation pf cytotoxicity of arsenic and smokeless tobacco and their combined interaction in CHO cells

Sweety Nath Barbhuiya, Dharmeswar Barhoi and Sarbani Giri*

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Conclusion: In the present study, combined exposure of arsenic and *sadagura* showed synergistic effect in CHO cells, indicating increased toxicological implications.

malondialdehyde (MDA) level, reduced glutathione (GSH) and Superoxide dismutase (SOD) activity was done spectrophotometrically after 30 days exposure. Moreover, changes in estrous cycle and ovarian, uterine histology were also observed. Results: Our findings clearly indicate that mice exposed to SG+L+SA group, has altered the estrous cycle completely, characterized by prolonged diestrous phase ($p<0.001$). Also reduction in body weight and tissue index of the reproductive organs was observed. The results of ovarian histopathologicaI study revealed decreased number of primordial follicles and enhanced atretic follicle number ($p<0.001$) in SG+L+SA treated group. Moreover, uterine sections also showed different types of histopathological changes including hyperplasia. Treatment of arsenic and smokeless tobacco resulted in oxidative stress which is evident from increased MDA level, decreased SOD activity and diminished reduced GSH level. Conclusion: From the study it can be concluded that generation of oxidative stress may be the possible mechanism behind the impaired follicular growth, atresia and disturbed estrous cycle.

Oral Presentation-17 **HH025**

Aqueous extract of *Moringa oleifera* inhibits tumor progression and improves survival of tumor bearing mice

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Modern cancer intervention includes surgery, chemotherapy, radiation therapy and targeted therapy which have adverse side effects. Thus, it is necessary to search a better treatment strategy minimizing side effect. The present study was aimed to evaluate the protective effect of aqueous extract of *Moringa oleifera* (AEMO) leaf against tumor progression *in vivo*. To evaluate the protective effect of AEMO against tumor progression, 16 animals were randomly divided into 4 groups' viz. Group 1 (control), Group 2 (tumor control, inoculated with EAC cells). Group 3 and Group 4 animals were inoculated with EAC along with AEMO treatment of 200 mg/kg and 400 mg/kg from the 12^{th} day of tumor inoculation till 50^{th} day. The body weight (BW) and tumor volume (TV) of experimental animals were measured in every alternative day and survival was monitored for entire life time. Development of solid tumor was assessed in both 30^{th} and 50^{th} day of treatment and maximum reduction of tumor weight was observed at 50^{th} day. At 50^{th} day, the measured tumor weight was 12.39 ± 1.25 ($p<0.001$) and 8.36 ± 0.46 ($p<0.001$) when treated with 200 mg/kg body wt. and 400 mg/kg body wt. of AEMO, respectively, as compared to tumor control (27.96 ± 1.57). A significant reduction of tumor volume was observed in AEMO treated animals. By the 50^{th} day of AEMO treatment, the decrease in tumor volume was 37.13% ($p<0.001$) and 78.74% ($p<0.001$) upon treatment with 200 mg/kg body wt. and 400 mg/kg body wt., respectively. Moreover, both the treatment with AEMO increased survival time of experimental animals as compared to tumor control. Conclusion: From our study, AEMO was found to reduce the tumor growth which could be attributed to the bioactive compounds present. Thus, *Moringa oleifera* can act as a good chemo preventive and therapeutic agent against cancer.

Joint detection of claudin-1 and JAM-A and increased salivary total sialic acid may serve as a therapeutic target in precancerous and oral cancer patients

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Concomitant changes in the expression of junctional proteins and disruption of cell-cell junction is a hallmark of cancer cell invasion and metastasis. In view to this, discovering a biomarker capable of early detection of cancer can play a crucial role to help cure the disease. Tight junction (TJ) complex proteins play vital role in maintenance of tissue architecture and are often reported to be dysregulated in different cancer. However, their role in precancerous and oral cancer condition is less understood. Expression level of two TJ proteins, claudin-1 and Junctional adhesion molecule (JAM-A) were examined in patients with precancerous condition i.e. erythroplakia (ERY) and cancerous condition i.e. oral squamous cell carcinoma (OSCC) by employing immunohistochemistry. Any relationship between TJ proteins and salivary total sialic acid (TSA) was checked since saliva is in direct contact with tumour cells.Additionally, flow cytometric estimation of ploidy and cell cycle was executed. Statistically significant increase in the expression level and delocalisation of TJ proteins from cell membrane to cytoplasm and nucleus were prominent on OSCC. Pearson'scorrelation test revealed a positive correlation between claudin-1 and JAM-A in ERY ($r=0.50$; $p=0.822$) and OSCC ($r=0.54$; $p=0.015$). However, no correlation was observed with any of the clinicopathological parameters except for age in claudin-1 of ERY. Significant increase in average TSA content was observed in ERY and OSCC when compared to control with 85% of the total OSCC subjects overexpressing JAM-A and 95% overexpressing claudin-1. Flow cytometric estimation of ploidy revealed highest frequency of aneuploid tumors in OSCC. To conclude, changes in the expression of TJ proteins and delocalization of claudin-1 and JAM-A in ERY and OSCC propose their potential role in tumor progression and cell signaling. Also, increased salivary TSA in combination with TJ proteins may be identified as an additional biomarker. Positive correlation observed between JAM-A and claudin-1 suggest joint detection of these proteins as a therapeutic target and a future diagnostic tool in precancerous and oral cancer patients.

Bisphenol A induces genotoxicity, cytotoxicity and germ cell toxicity in Swiss albino mice

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Bisphenol-A (BPA), a monomer of polycarbonate and epoxy resins is an important environmental contaminant. Due to its widespread exposure, it represents a major toxicological and public health concern. Present study aims to investigate the genotoxic and reproductive potential of BPA*in vivo*. Swiss albino mice were divided into three groups viz: vehicle control (propylene glycol), low dose (10mg/kg bw) and high dose (50mg/kg bw); treated for a period of 35 days. Genotoxicity was evaluated by studying the incidence of micronucleated polychromatic erythrocytes (MNPCEs) and comet assay in bone marrow cells. Germ cell transformation kinetics and mitochondrial membrane potential was studied in all groups employing flow cytometry. Additionally, reproductive toxicity was evaluated by recording the abnormal sperm heads and total sperms count. Both the treated doses of BPA induced statistically significant increase ($p<0.001$) of micronucleated polychromatic erythrocytes when compared to control group. Increase ($p<0.001$) in the ratio between polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) observed indicates the cytotoxic effects of BPA. Significant increase in damage index (DI) in bone marrow cells was noted in BPA exposed mice. Comet assay clearly demonstrated significant difference in all the parameters in the treated groups which indicates the genotoxic potential of BPA which supports our MNPCEs data. Dose dependent decrease in sperm count and increase in abnormal sperm head was observed in treated group ($p<0.001$) when compared to control. Flow cytometric estimation of germ cell subtypes in mice testis revealed significant decrease in spermatids. Decrease in spermatogonia to spermatid turn over (1C:4C) and primary spermatocyte to spermatid turnover (1C:2C) indicates the defects in spermatogenesis after BPA exposure. Depolarisation of mitochondrial membrane resulted in destabilization of cellular redox homeostasis in treated group. Histological evaluations such as germ cell depletion, atrophied seminiferous tubule and compact disorganized arrangement of germ cells were observed in treated groups. To conclude, BPA exposure induce genotoxicity as well as reproductive toxicity causing DNA damage in germ cells which alters cell cycle progression leading to low sperm count in mice.

YSA-05

Arsenic and smokeless tobacco induced genotoxicity, sperm abnormality and oxidative stress depends upon the nutritional status of mice

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Background: Arsenic, a well-established carcinogen has been shown to cause various adverse long-term effects in humans. Recently, high concentrations of arsenic have been documented in Barak Valley, Assam. Indiscriminate smokeless tobacco consumption is a common practice in this region. Correlation between nutritional status and arsenic and smokeless tobacco induced health effects has not been taken up in humans or test systems.

Methods: Mice were divided into groups based on protein content in the diet: High protein (40%), optimum protein (20%) and low protein (5%). Simultaneous exposure to arsenic and smokeless tobacco orally were given to evaluate the extent of cytological and genotoxicological damage. Micronucleus assay and Comet assay of the femur bone marrow cells were conducted. Germ cell toxicity was evaluated by recording the sperm head abnormalities and total sperm count. Liver, kidney and intestine tissues were analyzed for various oxidative stress evaluations: lipid peroxidation (MDA), Glutathione (GSH) and superoxide dismutase (SOD). Histological examination of Liver and kidney was performed and was screened for various ultrastructural abnormalities.

Results: Diet protein was found to have positive influence in arsenic and sadagura induced genotoxicity. Notably, high protein diet groups had lower arsenic induced genotoxic, germ cell abnormalities and oxidative stress as compared to optimum protein and low protein diet counterparts.

Conclusion: Our study indicates that high protein diet may protect the body from long-term arsenic and smokeless tobacco induced toxicity in mice test system. This observation has implications and invites further studies especially epidemiological studies in human population exposed to arsenic in South Asian countries.

Acknowledgement: *The authors are thankful to UGC for research fellowship to SD, DST-FIST for infrastructure facilities and Department of Life Science and Bioinformatics, Assam University, Silchar for laboratory support.*

P-43

Frequency of Unique Smokeless tobacco consumption with lime in Southern Assam, India, its role in pH, anion and nicotine content and its impact on buccal epithelial cells by Cytome Assay

Sarbani Giri*, Samrat Das, **Sweety Nath Barbhuiya**, Salam Himika Devi, Doli Talukdar, Dharmeswar Barhoi, Puja Upadhaya, Neelam Pandey, Pimily Langthasa and Rudrarup Bhattacharjee

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Background: Smokeless tobacco consumption has become a matter of global public health concern. In India, the highest incidence of oral cancer is reported in the North Eastern region, particularly in Assam and which is attributed to indiscriminate consumption of smokeless tobacco. In Southern Assam, Smokeless tobacco locally known as "*Sadagura*" is the most prevalent life style habit.

Methods: A survey was conducted (*n*=5000) to estimate the prevalence of smokeless tobacco consumption in Southern Assam, India. Buccal micronucleus cytome assay in exfoliated buccal cells was carried out (*n*=75) to determine the extent of cytogenetic damage caused by *Sadagura* consumption. In order to quantify the amount of nicotine present in *Sadagura*, Gas chromatography - Mass spectrometry (GC-MS) was employed. In addition, levels of various anions *viz.* phosphate nitrate and chloride in *Sadagura* extract alone and with lime was determined.

Results: In Southern Assam, 61% of the total study population consumes different forms of smokeless tobacco daily, of which *Sadagura* constituted approximately 86%. A statistically significant increase in the cytome parameters of exfoliated buccal cells has been observed when compared to control population. In GC-MS study, the average concentration of nicotine in *Sadagura* was found to be 5380.5 ppm. Presence or absence of lime does not affect the nicotine content. However, a significant change in pH, phosphate and nitrate content was observed whereas the dry weight, chloride and nicotine content remain unaltered.

Conclusion: Our study is first to report the alarmingly high level of smokeless tobacco consumption in Southern Assam and highlight that lime enhance anionic composition and do not effect nicotine content which might affect the Buccal Cytome parameter leading to genotoxicity.

Acknowledgements: *The authors are grateful for FTYS to SG and UGC for research fellowship. The infrastructure support by DST-FIST to Department of Life Science and Bioinformatics is thankfully acknowledged.*

P-59

Protective role of selected medicinal plants from Southern Assam, India against genotoxic cytotoxic and antioxidant potential

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Background: Many plant species are yet to be explored for their role in protection against genotoxicity, cytotoxicity and antioxidant properties so that their anticarcinogenic potential can be evaluated. Selected plants *viz. Moringa oleifera* (MO), *Tagetes patula* (TP), *Ageratum conyzoides* (AC) and *Ocimum gratissimum* (OG) extract, locally available in the region of Southern Assam, North East India was evaluated for their role in cellular transformation.

Methods: Aqueous extract of selected plants were administered orally in mice test system at a dose 200mg per kg body weight for a period of 2 weeks. A single i.p dose of Mitomycin C (2mg/ kg body wt.) was given to all groups 24 hrs prior to sacrifice except control. Results were compared with standard chemotherapeutic drug Mitomycin C (MMC) and control (C) groups. Anti-genotoxic effects were evaluated using the frequency of micronucleated polychromatic erythrocytes (MNPCE) and results further validated by Comet assay. Cytotoxicity was assessed by determining the polychromatic to normochromatic erythrocytes ratio (PCE/NCE). Reduced glutathione (GSH) and Thiobarbituric acid reacting substance (TBARS) assay in liver tissues were carried out to assess the antioxidant potential of plants.

Results: Among the plant extract tested, AC found to show maximum protection against MMC-induced genotoxicity. Moreover, DNA damage analysis by comet assay also indicated the reduction of MMC mediated mutagenicity by AC. The protective roles were evaluated as AC ($p<0.05$)> OG ($p<0.05$) >TP ($p<0.01$) > MO ($p<0.001$). Biochemical assays indicate that the selected plants possess antioxidant potential by significant increase of GSH level ($p<0.001$) and reducing tissue malondialdehyde (MDA) level differently.

Conclusion: In the present study, our results reveal that the selected plants exhibit potential antioxidant activity. Moreover, reduction in the incidence of micronucleus/DNA damage in mouse bone marrow cells suggests that selected plants may have anti-carcinogenic property which requires further test *in vitro* followed by *in vivo*.

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P-72

Genotoxicity of Bisphenol A in developing chick embryos evidenced by Micronucleus test and Comet assay

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Background: Bisphenol-A (BPA) present in plastics, a monomer of polycarbonate and epoxy resins is an important environmental contaminant, known to cause significant health risk to various organisms including humans. Present study aims to investigate the potential embryotoxicity of BPA on developing chick embryos.

Methods: Genotoxic potential of BPA was assessed in peripheral blood by micronucleus test (MN) and validated using alkaline comet assay. LD_{50} of BPA for 24hr, 48hr and 72hr was determined in frertilized eggs of *Gallus gallus domesticus*. MN and comet assays were performed on 11th day old chick embryos.

Results: Data revealed significant increase in the frequency of micronucleated erythrocytes in the peripheral blood of chick embryos after 48hr and 72hr and not to 24hr exposure to BPA when compared to control. Intergroup comparison revealed a significant difference between 24 and 72hr exposures. The ratio between polychromatic erythrocytes and normochromatic erythrocytes (PCE/NCE) was found to decrease after 48hr and 72hrs of treatment. Significant increase in the number of erythroblast was observed at the highest dose. Intergroup analysis revealed a significant difference between 24hr, 48hr with 72hr. Comet assay, clearly demonstrated significant increase in Tail DNA, Olive tail moment and Damage Index (DI) in all the treated groups which indicates the genotoxic potential of BPA which was at per our MN data. Cells exposed to 72hr exposure were more prone to extensively high DNA damage class (>20µm).

Conclusion: We conclude that BPA at a lower dose increase genotoxicity and cytotoxicity which increase with duration of exposure.

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Chronic arsenic and smokeless tobacco exposure results in genotoxicity, oxidative stress and sperm abnormality while ascorbic acid supplementation induces protection in mice

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Background: Arsenic, a well established carcinogen and ground water contaminant is a serious public health problem especially in South East Asia region. Recently, high concentrations of arsenic have been reported in Barak Valley, Assam. Smokeless tobacco consumption is one of the most prevalent life style habits in this region. Smokeless tobacco locally known as "*Sadagura*" is consumed indiscriminately by population from Barak Valley. Very few works has been undertaken to assess the ill effects of arsenic in combination with smokeless tobacco.
Methods: Mice were divided into seven groups based on the treatment. Study animals were exposed to arsenic (0.2mg/kg body weight /day, 2mg/kg body weight/day) and smokeless tobacco (5g/kg body weight /day) both in combination as well as individually orally for 90 days. The results were compared with control and also with group receiving ascorbic acid supplementation (25mg/kg body weight/day). Genotoxicity assessment was done by performing Micronucleus assay and Comet assay of the femur bone marrow cells. Hepatic, renal and intestinal tissues were analyzed for various oxidative stress evaluations viz., lipid peroxidation (MDA), Glutathione (GSH) and superoxide dismutase (SOD). Histological examination of Liver and kidney were performed and was screened for various ultrastructural abnormalities. Germ cell toxicity was evaluated by recording the sperm head abnormalities and total sperm count.
Results: It was observed that chronic arsenic and smokeless tobacco exposure lead to increase in genotoxicity and sperm abnormalities which may be oxidative stress induced, while ascorbic supplementation resulted in decrease in cytological and genotoxicological damage.
Conclusion: Our study indicates that chronic exposure (90 days) of arsenic and smokeless tobacco have the potential to cause genotoxicity, oxidative stress and impairment of sperms in mice test system. Our results also highlights to the fact that ascorbic acid supplementation has the prospective to mitigate the ill effects of arsenic and *sadagura*. This observation has implications and invites further studies especially epidemiological studies in human population exposed to high arsenic especially in South East Asian countries.

Keywords:Arsenic; Sodium arsenite; Smokeless tobacco; *Sadagura*; Micronucleus Assay; Comet Assay; Sperm head abnormality; Lipid peroxidation; Reduced glutathione; Superoxide dismutase.

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NROR-01

Radiofrequency Radiation Exposure-Induced DNA Damage and Cell Cycle Arrest in Germ Cells of Male Swiss Albino Mice

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Mobile phone technology uses radiofrequency radiations (RFR) 900 1800 MHz for signal transmission. The widespread use of mobile phones with almost 7 billion subscribers globally has not only increased electro-pollution of the environment but also raised a concern about their possible health effects. Genotoxic potentials of RFR on different cell types have been well reported. However, their adverse effects with respect to DNA damage in male reproductive cells have not been investigated thoroughly. Therefore, in the present study, we aimed to investigate the effect of 35 days of whole body RFR exposure (6 h/day) on germ cells of male Swiss albino mice. Enumeration of germ cells by flow cytometer using propidium iodide (PI) showed arrest in pre-meiotic phase of spermatogenesis, leading to slow rate of spermatogonial to spermatocyte transformation. RFR-induced DNA breaks and excessive ROS production in testicular cells are apparent from high damage index in comet assay and high frequency of cells with low mitochondrial membrane potential in JC-1 assay. Furthermore, increased lipid peroxidation, decreased superoxide dismutase activity and reduced glutathione concentration in testes was an indicative of oxidative stress. Also, the defective reproductive phenotypes, including reduced sperm count, increased sperm malformation and histological abnormality of testes, were observed. These results show that RFR exposure causes DNA damage and cell cycle arrest in germ cells by mitochondrial-mediated oxidative stress which may be associated with elicit male germ cell toxicity.

NROR-02

Effect of Radiomimetic Bleomycin Sulphate on Mitosis in two Varieties of *Trigonella foenum-graecum* L. (Methi)

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Bleomycin sulphate is a radiomimetic anticancer drug. It is a product of the bacterium *Streptomyc verticillus*. It causes DNA breaks. The presence of this drug in the environment can be detrimental human health as well being of food crops. The effect of this antibiotic drug was investigated on tw varieties of *Trigonella foenum graecum* L. In this work, an attempt has been made to find out the mo resistant and the most sensitive variety among these two varieties of fenugreek. Seeds of two differe varieties of *T. foenum graecum* L. were collected. *T. foenum graecum* L. var. Hisar Suvarna and va GM 1. Mitotic studies were conducted on both the varieties in normal conditions by squash techniqu seeds were treated with five different concentrations of radiomimetic bleomycin sulphate. Mitot bnormalities of treated seeds were studied and recorded for conclusions. The variety GM 1 was t sistant variety towards different concentrations of bleomycin sulphate and the variety Hisar Suvar

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Cholesterol and Parkinson's disease: An update

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Abstract

Several endogenous molecules including cholesterol have been implicated in the pathogenesis of Parkinson's disease (PD). Epidemiological and studies in animal models have provided a strong link between elevated cholesterol (hypercholesterolemia) and incidence of similar parkinsonian pathologies starting from behavioural abnormalities to biochemical lesions in brain. Moreover, the oxidation product of cholesterol, oxysterols are reported to trigger neurochemical alterations similar to that observed in PD. Recent studies highlighted the involvement of oxidative stress, mitochondrial dysfunction, and neuroinflammation as the mechanisms underlying neurotoxicity in PD induced by hypercholesterolemia or oxysterols. The chapter described all the evident mechanisms triggered by hypercholesterolemia and oxysterol in brain with special emphasis on their contribution towards pathogenesis of PD.

Keywords: Hypercholesterolemia; Oxysterol; Neurodegeneration; Oxidative stress; Mitochondrial dysfunction; Neuroinflammation

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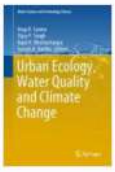

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Hydro-physico-chemical Grouping of Cachar Paper Mill Effluents in Assam Using Multivariate Statistical Model

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Sangeeta Dey, Manabendra Dutta Choudhury, Suchismita Das

Chapter
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Abstract

Effluents from the paper mill are highly toxic and are one of the major sources of aquatic pollution. Their toxic nature is derived from the presence of several naturally occurring and xenobiotic compounds, which are formed and released during various stages of papermaking. In the present study, mill effluents are collected from three different stations around the Cachar paper mill (CPM) in Assam and samples are analyzed for 17 parameters such as appearance, color, odour, conductivity, total dissolved solids, pH, total alkalinity, hardness (total and Ca), chloride, nitrite, BOD and COD along with the heavy metals As, Cu, Cd, Cr, Fe, Pb and Zn. The results are compared with water quality standards prescribed by World Health Organization. Multivariate statistical analyses are performed on data sets obtained to gain a better