SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 2, pp. 381-387, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

INHIBITORY EFFECT OF *DILLENIA PENTAGYNA* STEM BARK EXTRACT ON CISPLATIN AND BENZO[A]PYRENE-INDUCED MUTAGENICITY

Gabriel Rosangkima and *Surya Bali Prasad
Cell and Tumor Biology Lab., Department of Zoology
North-Eastern Hill University, Shillong- 793022, India

ABSTRACT

Modulatory effect of methanol extract of stem bark of *Dillenia pentagyna* (DPE) was evaluated against mutagenicity induced by cisplatin (CIS) and benzo[a]pyrene (B[a]P) in the mouse using bone marrow chromosomal aberration, micronucleus and sperm abnormality as mutagenicity parameters. In one group of experiment, animals received a single dose (20mg/kg body weight) of DPE through intraperitoneal injection (i.p) followed by cisplatin or benzo[a]pyrene treatment. In the other groups, three doses of DPE (20, 50 and 100mg/kg b. wt/day) were given through diet for seven consecutive days prior to a single treatment with cisplatin or benzo[a]pyrene. The animals from different treatment groups were used for the study of chromosomal aberration (CA), micronucleus (MN) and sperm abnormality assays. The result of present study shows that a single treatment with DPE did not show significant changes in the incidence of chromosomal aberration, micronucleus and sperm abnormality induced by CIS and B[a]P. However, pretreatment with DPE for seven consecutive days dose dependently reduced CIS and B[a]P-induced mutagenicity suggesting the protective role of *D. pentagyna* on CIS and B[a]P mutagenic potentials.

Keywords: *Dillenia pentagyna* – antimutagenicity - benzo[a]pyrene – cisplatin.

INTRODUCTION

Cis-diamminedichloroplatinum-II, commonly known as cisplatin (CIS) is widely used as a chemotherapeutic agent alone or in combination with other agents against a variety of cancers (Carter, 1984). However, its therapeutic efficacy has been limited due to the side effect and also its mutagenic potential (Krakoff, 1979; Khynriam and Prasad, 2001; Overbeck *et al*., 1996). An increased carcinogenic risk with the development of secondary tumors in patients/animals treated with cisplatin has also been reported (Cross *et al*., 1996; Greene, 1992). It has been shown to cause genotoxic effects in cultured mammalian cells (Zwelling *et al*., 1979) and bone marrow cells (Giri *et al*., 1998).

Benzo[a]pyrene (CAS Reg. No. 50-32-8), also known as 1,4-benzo[a]pyrene (B[a]P), is a polycyclic aromatic hydrocarbon (PAH) generated from the combustion of fossil fuels and tobacco and is both inhaled and consumed (Phillips, 1983). It has shown various toxicological effects, such as haematological effects, reproductive and developmental toxicity and immunotoxicity. It is the carcinogenic and genotoxic potential of these compounds that has attracted most attention.

A large number of dietary agents have the inhibitory potentials against genotoxicity and carcinogenicity (Ames, 1983; Ferguson, 1994). Phenolic compounds, fibre, chlorophyll, b-carotene, and vitamins such as C and E, a component of fresh fruits and vegetables were suggested to have antimutagenic and/or anticarcinogenic properties (Stavric, 1994; Ho, 1992; Kuo *et al*., 1992), and a negative association between the incidence of cancer and consumption of diet rich in fibres, fresh vegetables, vitamins and minerals was also reported (Archer, 1988; Stainmetz and Potter, 1991). Some of the food ingredients including vitamins, flavonoids and organosulphur compounds possess antimutagenic and anticarcinogenic activities (Stavric, 1994), and extracts of certain plants were reported to have the ability to inhibit the mutagenic activity of well established genotoxins (Ito *et al*., 1986; Khanduja and Majid, 1993; Abraham *et al*., 1986; Mejia *et al*., 1999).

Dillenia pentagyna Roxb. (Dilleneaceae) is a deciduous tree, distributed in Indo-Malaysian areas extending to tropical Australia and throughout India particularly in sub-tropical Himalayas. It is found in most places of Mizoram state in India. Our preliminary investigation through literature review and personal interview with local herbal practitioners revealed that the stem bark of this plant has been used by the people of Mizoram for the treatment of gastric cancers, diarrhea and other human ailments. The stem bark and fruit has also been used by some of the Indian ethnic communities as cure for blood dysentery, stomach pain and fistula (Pal and Jain, 2000). We have noticed the promising antitumor activity of methanol extract of stem bark of this plant against murine ascites Dalton's lymphoma (Rosangkima and Prasad,

*Corresponding author email: sbpnehu@hotmail.com

2004). We have also reported the inhibitory effect of this plant extract in the level of sialic acid and lipid peroxidation in the tissues of Dalton's lymphoma-bearing mice (Rosangkima and Prasad, 2007a), and a significant inhibitory effect in the level of total reduced glutathione and glutathione reductase activity in Dalton's lymphoma cells was also noted (Rosangkima and Prasad, 2007b). All these findings, thus, generated an interest to investigate the antimutagenic activity of this plant in murine model. Therefore, the present investigation was undertaken to evaluate the antimutagenic potential of *Dillenia pentagyna* against CIS and B[a]P induced mutagenicity in mice.

MATERIALS AND METHODS

Chemicals

Colchicine, cisplatin, corn oil, benzo[a]pyrene and Giemsa stain were obtained from Sigma Chemicals Co. Ltd., USA. All other chemicals of analytical grade were purchased from SRL Co. Mumbai, India.

Animals

Inbred Swiss albino mice colony is being maintained under laboratory conditions keeping 5-6 animals in a propylene cage at 23-25^{0}C. The animals were fed with commercially available food pellets and water *ad libitum*. For some experiment in involving micronucleus assay, tumor-bearing mice were used. For this Dalton's lymphoma cells (1x 10^7 cells) in PBS , 0.25 ml vol. were transplanted. Tumor transplanted animals generally survived for 19-21 days.

Plant material and preparation of test sample

The stem bark of *D. pentagyna* was collected from Kawlkulh village, Mizoram state, India, in July 2006. The methanol extract of stem bark of *D. pentagyna* (DPE) was prepared as described previously (Rosangkima and Prasad, 2004). For the treatment through intraperitoneal (i.p) injection, the plant extract was dissolved in 0.05% NaOH solution. For the treatment through the diet, plant extract was dissolved in 50% alcohol (14, 35, and 70mg/100 ml) and mixed thoroughly with the feed powder in the ratio of 1:2 (vol:wt) which was then dried in an oven at 35^{o}C to 40^{o}C. Since the daily intake of feed per animal was approximately 7.5g, different doses of extract treatment come to 20, 50 and 100mg/kg b. wt/day.

Antimutagenic activity

In the antimutagenic activity studies, experimental animals were divided into 3 groups. Group I (CIS/B[a]P control) animals received a single dose of mutagen (CIS/B[a]P). Group II animals received a single i.p injection of DPE (20mg/kg b. wt) and CIS/B[a]P. Group III animals were given DPE pretreatment through the diet at the dose of 20, 50 and 100mg/kg b. wt/day for 7 consecutive days prior to a single dose of mutagen (CIS/B[a]P). CIS (8mg/kg b. wt) and B[a]P (125mg/kg b. wt) were administered through i.p. injection and gavages respectively.

Chromosomal analysis

Mice were sacrificed by cervical dislocation 24 h after mutagen treatment. The animals were subjected to mitotic arrest by injecting colchicine (i.p 4mg/kg b. wt.) 2 h prior to sacrifice. Bone marrow cells were collected from humerous and femur by flushing in PBS. The cells were washed with PBS and collected by centrifugation (1000 rpm, for 5 min at 4°C). The cell pellet was subjected to treatment with hypotonic solution (0.075M KCl) for 25 min at 37^{o}C. The cells were separated and fixed in acetic acid: methanol (1:3; v/v), repeated again with a 30 min interval. Two drops of cell suspension were dropped on a clean and chilled slide, subjected to flame drying and stained for 5 to 7 min with working Giemsa (1ml of stock giemsa + 0.25ml methanol + 2.8ml Sorensen's buffer, pH 6.8), washed and mounted in DPX. Five hundred good metaphase spreads were examined per animal under 100x oil immersion. The observed chromosomal aberrations (CA) included chromatid break (CB), chromosomal fragment (CF), exchange (Exch) and sister chromatid union (SCU).

Micronucleus assay

Micronucleus (MN) was assayed following the method of Fenech *et al.* (2003). Briefly, 24 h after mutagen treatment, bone marrow cells from both the femurs were collected by flushing in PBS. The cell suspension was centrifuged at 1000 rpm for 5 minutes at 4^{o}C. The cell pellet was treated with a weak hypotonic solution (0.075M KCl:saline, 1:9 v/v) for 5 min. After centrifugation, the cells were fixed in fresh fixative (acetic acid:methanol, 1:3 v/v) for 15 min at room temperature and repeated twice. Two drops of cell suspension were dropped onto clean and wet chilled slide. The slides were air-dried and stained with May Grunwald-Giemsa stain. A total of 2500 polychromatic erythrocytes (PECs) were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MnPCEs). Only rounded bodies approximately one-fifth to one-sixteenth the size of the main nucleus, lying within three nuclear diameter distance from the main nucleus, and possessing a staining intensity similar to that of the main nucleus, were scored as micronucleus. All the slides were scored by the same observer.

Sperm abnormality assay

The male mice in different treatment groups (Group I, II and III) were sacrificed on the 10th day of mutagen treatment. The cauda epididymis were removed and placed in physiological saline. It was then minced into pieces and kept undisturbed for 20 min. The spermatozoa were spread on a clean slide, air-dried, fixed in absolute

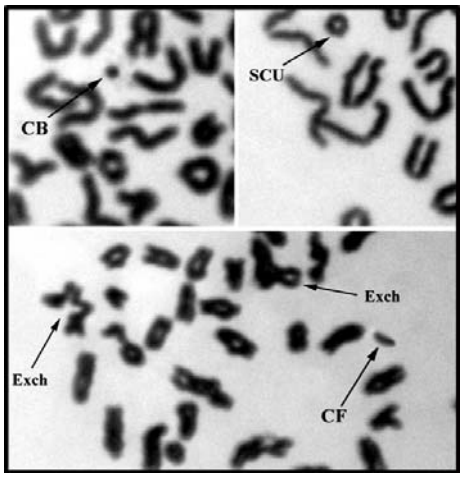

Fig. 1. Representative of different types of chromosomal aberrations (chromatid break - CB, chromosomal fragment - CF, exchanges - Exch and sister chromatid union - SCU) induced by CIS or B[a]P in the bone marrow cells of mice.

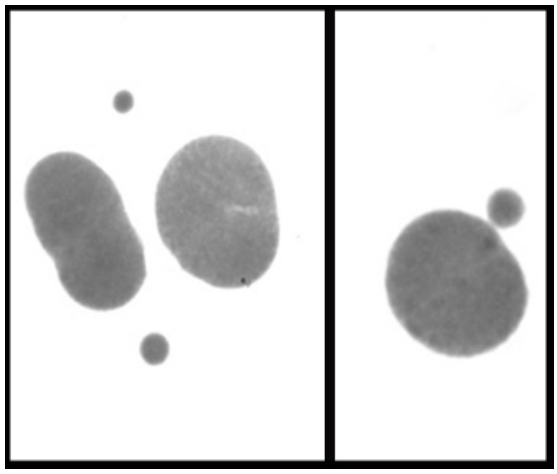

Fig. 2. Representative of micronuclei induced by CIS and B[a]P in the bone marrow cells of mice.

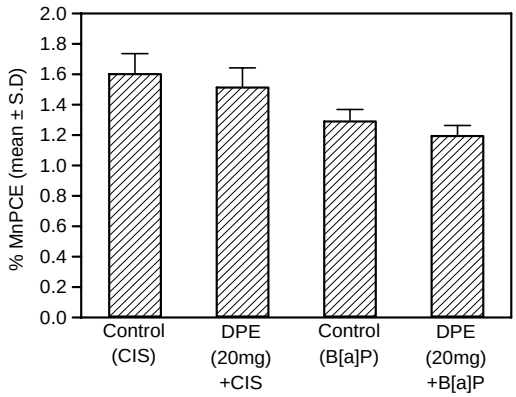

Fig. 3. Frequency of micronucleus induced by CIS and B[a]P in the bone marrow cells of mice after a single treatment with DPE (20 mg/kg b. wt.). Results were mean ± S.D. Student's t- test, n=6 as compared to the corresponding mutagen control. *P<0.05.

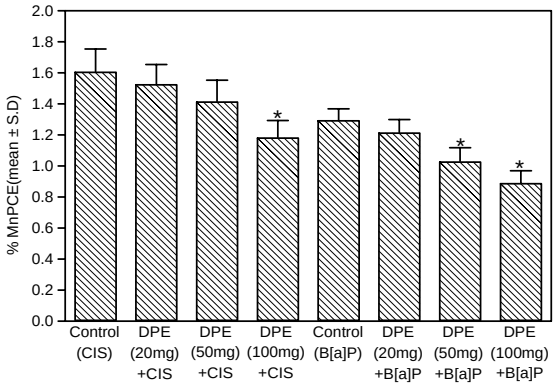

Fig. 4. Frequency of micronucleus induced by CIS and B[a]P in the bone marrow cells of mice after pretreatment with different doses of DPE through the diet. Results were mean ± S.D. Student's t- test, n=6 as compared to the corresponding mutagen control. *P<0.05.

methanol for 15 min and then stained with 1% aqueous eosin-Y on the following day. Five hundred sperms from each mouse were examined for the abnormalities in sperm head and tail shapes following the criteria as close as possible to those established by Wyrobeck and Bruce (1975).

Statistical analysis

Results were expressed as mean ± S.D of number of experiments. Significance was evaluated by Student's *t*-test. *p* values less than 0.05 were regarded as significant.

RESULTS

Chromosomal aberrations

CIS and B[a]P caused the development of various chromosomal aberrations in bone marrow cells of mice. Chromatid break, chromosomal fragment, exchange and sister chromatid union were observed after treatment with CIS and B[a]P (Fig. 1), with CB and Exch occurring more frequently (Table 1). Treatment with a single dose of DPE (24 h) did not show significant changes in the frequency of CA induced by CIS and B[a]P. However, pretreatment of animals with DPE (20, 50 and 100mg/kg b. wt./day) significantly decreased the frequency of CA induced by both CIS and B[a]P (Table 2). Out of three different doses used, 100mg/kg b. wt./day showed maximum inhibitory effect against CIS and B[a]P-induced chromosomal aberrations.

Micronucleus

The development of MN was observed in bone marrow cells of tumor-bearing mice after CIS and B[a]P treatment. Treatment with a single dose of DPE (20

Table 1. Changes in the frequency of chromosomal aberrations induced by CIS and BaP in bone marrow cells of mice following single co-treatment with DPE (20 mg/kg b. wt).

Treatment	Chromosomal aberrations					
	CB	CF	Exch	SCU	Total[a]	Total CA (mean±SD) Per 100 cells
Control (CIS)	425	55	242	59	909	181.8 ± 19.13
DPE+CIS	348	201	179	75	809	161.8 ± 12.27
Control (B[a]P)	47	2	-	-	49	9.8 ± 0.83
DPE+ B[a]P	38	4	-	-	42	8.6 ± 1.14

[a]Five hundred metaphase plates were scored per group (n = 5 animals) for chromosomal aberrations. Student's *t*-test, as compared to the respective control values, *p<0.05. CB = Chromatid break, CF = Chromosomal fragment, Exch = Exchange, SCU = Sister chromatid union.

Table 2. Effect of different doses of pretreatment with DPE on the frequency of chromosomal aberrations induced by CIS and BaP in bone marrow cells of tumor-bearing mice.

Mutagens[a]	DPE (mg/kg/day)[b]	Chromosomal aberrations					
		CB	CF	Exch	SCU	Total[c]	Total CA (mean±SD) Per 100 cells
CIS	20	398	78	218	87	781	156.2 ± 5.31*
	50	329	72	176	65	642	128.4 ± 13.22*
	100	292	57	156	55	560	112.0 ± 7.31*
B[a]P	20	31	2	-	-	33	6.6 ±1.14*
	50	25	1	-	-	26	5.2 ± 0.83*
	100	20	1	-	-	21	4.6 ± 0.54*

[a]Animals were administered with a single dose of CIS or BaP. [b]Animals were treated with DPE through the diet for 7 consecutive days prior to CIS or BaP treatment. [c]Five hundred metaphase plates were scored per group (n = 5 animals) for chromosomal aberrations. Student's *t*-test, as compared to the respective control values, *p<0.05. CB = Chromatid break, CF = Chromosomal fragment, Exch = Exchange, SCU = Sister chromatid union.

Table 3. Changes in the frequency of sperm abnormalities induced by CIS and BaP in mice following single co-treatment with DPE (20 mg/kg b. wt).

Treatment	Amorphous	Banana	Hookless	Microhead	Double tail	Mean % of abnormal sperms ± SD
Control (CIS)	132	28	83	11	6	8.66±0.89
DPE+CIS	123	23	80	9	3	7.93±0.38
Control (B[a]P)	77	19	18	14	2	4.33±0.35
DPE+B[a]P	70	18	22	13	4	4.23±0.19

A total number of 3000 sperms were observed in each treatment group. Results are expressed as Mean ± S.D. Student's t- test, n=6 as compared to the corresponding control. A single dose of DPE and CIS or B[a]P were administered on the same day.

mg/kg b. wt) did not show significant changes in the development of MN induced by CIS and B[a]P (Fig. 3), while pretreatment with DPE through the diet in a dose depend manner decreased the incidence of MN induced by both CIS and B[a]P (Fig. 4).

Sperm abnormality

Various form of sperm abnormalities were induced after CIS and B[a]P treatment (Table 3). A single treatment with DPE did not show significant changes in the frequency of sperm abnormality induced by CIS and B[a]P (Table 3). Amorphous, hookless, banana, microhead and doubled tail were among different types of abnormality observed in sperms(Fig. 5A-F). Out of different types of abnormal sperms analyzed, amorphous heads were noted to be more than the others. However, pretreatment with DPE at different doses (20, 50 and 100mg/kg b. wt/day) for 7 consecutive days prior to

Table 4. Effect of different doses of pretreatment with DPE on the frequency of sperm abnormalities induced by CIS and BaP in mice.

Mutagens	DPE (mg/kg)	Amorphous	Banana	Hookless	Micro-head	Double tail	Mean % of abnormal sperms ± SD
CIS	20	84	20	74	6	2	6.19±0.32*
	50	78	19	74	5	1	5.85±0.55*
	100	77	9	70	2	1	5.30±0.24*
B[a]P	20	72	15	13	8	1	3.62±0.18*
	50	66	7	10	3	1	2.89±0.15*
	100	59	11	10	5	0	2.83±0.31*

A total number of 3000 sperms were observed in each treatment group. Results are expressed as Mean ± S.D. Student's t- test, n=6 as compared to the corresponding control, *P≤0.05. DPE was administered through the diet for 7 consecutive days prior to mutagen(s).

mutagen(s) treatment significantly decreased the frequency of sperm abnormalities induced by both CIS and B[a]P (Table 4). Out of three different doses of DPE studied, maximum inhibition of sperm abnormality was observed with 100mg/kg b. wt/day (Table 4).

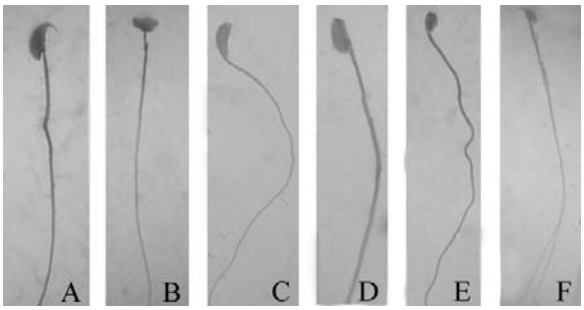

Fig. 5. Representative of normal (A) and different types of abnormal sperms (amorphous - B, banana - C, hookless - D, microhead - E and doubled tail - F) induced by CIS or B[a]P in mice.

DISCUSSION

The development of chromosomal aberration, micronucleus and sperm abnormality has been used as reliable biological indicators in the mutagenic bioassay of different drugs (Tandon and Sodhi, 1985; Giri *et al*., 2002). Micronucleus is chromatin masses that arise from chromosome fragments of intact whole chromosomes lagging behind at the anaphase (Czyzewska and Mazur, 1995). Micronucleus can be easily accredited in the cytoplasm of immature polychromatic erythrocytes (Schmid, 1976). Since blood cells originate in the bone marrow cells, the incidence of chromosomal aberration and micronucleus were analyzed in the bone marrow cells.

The therapeutic efficacy of CIS has been limited by its dose dependent side effects (Krakoff, 1979) and its genotoxic properties have also been reported (Overbeck *et al*., 1996; Cross *et al*., 1996; Giri *et al*., 1998). B[a]P can bind to the aryl hydrocarbon receptor (AHR), which then induces the expression of many genes, including members of the cytochrome P450 family of enzymes. B[a]P is then metabolized to an array of reactive species that form covalent bonds with nucleic acids and proteins within target cells, generate reactive oxygen species (Xie and Herschman, 1995; Balinsky and Jaiswal, 1993), and cause genetic mutations and cancer (Conney, 1982; Shields *et al*., 1993). It is clear that B[a]P also induced mutagenicity in the strain YG1024 (Watanabe *et al*., 1990). Carcinogens bind to the cell macromolecules resulting in mutagenic events leading to cell transformation and neoplastic changes. Some phytochemicals prevent these changes from occurring either by directly binding to the carcinogens/their metabolites or by metabolising and eliminating toxic xenobiotics. The results of present studies show that CIS and B[a]P treatment of mice causes significant elevation of chromosomal aberration, micronucleus and sperm abnormality depicting their mutagenic and genotoxic potentials (Table 1 and 3, Fig. 3). A single treatment with DPE did not show significant changes in the frequency of chromosomal aberration, micronucleus and sperm abnormality induced by CIS and B[a]P, while pretreatment for 7 consecutive days dose dependently decreased the frequency of chromosomal aberration, micronucleus and sperm abnormality. Since the micronuclei in young erythrocytes arise mainly from chromosomal fragments, the observed decrease in the incidence of micronuclei can be considered to indicate an inhibitory effect of DPE on the *in vivo* chromosomal damage induced by CIS and B[a]P.

In conclusion, the result of present study showing significant reduction in CIS and B[a]P induced mutagenicity in presence of DPE suggests the protective role of this plant on CIS and B[a]P mutagenic/genotoxic potentials. Further, based on our earlier findings on changes in reduced glutathione etc, it is proposed that *D. pentagyna* may exert its antimutagenic potential by inducing some of the antioxidant enzymes that detoxify

mutagens, or by acting as a free radical scavenger. However, other contributory steps may also be involved in its antimutagenic potential. Therefore, further investigation is needed in this direction.

ACKNOWLEDGMENT

The financial assistance rendered by University Grants Commission, Department of Science and Technology, New Delhi, and North-Eastern Hill University is gratefully acknowledged.

REFERENCES

Abraham, SK., Mahajan, S. and Kesavan, PC. 1986. Inhibitory effect of dietary vegetables on the in vivo clastogenicity of cyclophosphamide. Mutation Research. 172: 51-54.

Ames, BN. 1983. Dietary carcinogens and anticarcinogens. Science. 221: 201-204.

Archer, VE. 1988. Cooking methods, carcinogens and diet-cancer studies. Nutrition and Cancer. 11: 75-79.

Belinsky, M. and Jaiswal, AK. 1993. NAD (P) H: quinone oxidoreductase 1 (DT diaphorase) expression in normal and tumor tissues. Cancer Metastasis Review. 12: 102-117.

Carter, SK. 1984. Cisplatin - past, present and future. In: Platinum co-ordination complexes in cancer chemotherapy. Eds. Haker, M.P. Douple, E.B. and Krakoff, I.H. p. 359-376. Martinus, Nijhpff publishing, Boston.

Conney, AH. 1982. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. Cancer Research. 42: 4875-4817.

Cross, HJ., Tilby, M., Chipman, JK., Ferry, DR. and Gescher, A. 1996. Effects of quercetin on the genotoxic potential of cisplatin. International Journal of Cancer. 66: 404-408.

Czyzewska, A. and Mazur, L. 1995. Suppressing effect of WR-2721 on micronuclei induced by cyclophosphamide in mice. Teratogenesis, Carcinogenesis and Mutagenesis. 15: 109-114.

Fenech, M., Chang, WP., Kirsch, VM., Holland, N., Bonassi, S. and Zeiger, E. 2003. Human micro-nucleus project (HUMN project): Detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutation Research. 534: 65-75.

Ferguson, LR. 1994. Antimutagens and cancer chemopreventive agents in diet. Mutation Research. 307: 395-410.

Giri, A., Khynriam, D. and Prasad, SB. 1998. Vitamin C mediated protection on cisplatin-induced mutagenicity in mice. Mutation Research. 421: 139-148.

Giri, S., Prasad, SB., Giri, A. and Sharma, GD. 2002. Genotoxic effects of malathion: an organophosphorus insecticide, using three mammalian bioassays in vivo. Mutation Research. 514: 223-231.

Greene, MH. 1992. Is cisplatin a human carcinogen? Journal of National Cancer Institute. 84: 306-312.

Ho, C. 1992. Phenolic compounds in food. In: Phenolic Compounds in Food and their Effects on Health I. Analysis, Occurrence and Chemistry. Eds. Ho, C., Lee, CY. and Huang, M. p. 1-7. American Chemical Society, Washington, USA.

Ito, Y., Maeda, S. and Sugiyama, T. 1986. Suppression of 7,12-dimethylbenz [a] anthracene-induced chromosome aberrations in rat bone marrow cells by vegetable juices. Mutation Research. 172: 55-60.

Khanduja, KL. and Majid, S. 1993. Ellagic acid inhibits DNA binding of benzo[a]pyrene activated by different modes. Journal of Clinical Biochemistry and Nutrition. 15: 1-9.

Khynriam, D. and Prasad, SB. 2001. Hematotoxicity and blood glutathione levels after cisplatin treatment of tumor-bearing mice. Cell Biology and Toxicology. 17: 357-370.

Krakoff, IH. 1979. Nephrotoxicity of cis-dichloro-diammineplatinum. Cancer Treatment Report. 63: 1523-1525.

Kuo, M., Lee, K. and Lin, J. 1992. Genotoxicities of nitropyrenes and their modulation by apigenin, tannic acid, ellagic acid and indole-3-carbinol in the Salmonella and CHO systems. Mutation Research. 270: 87-95.

Mejıa de, EG., Castano-Tostado, E. and Loarca-Pina, G. 1999. Antimutagenic effects of natural phenolic compounds in beans. Mutation Research. 441: 1-9.

Overbeck, TL., Knight, JM. and Beck, DJ. 1996. A comparison of the genotoxic effects of carboplatin and cisplatin in Escherichia coli. Mutation Research. 362: 249-259.

Pal, DC. and Jain, SK. 1998. 2000 Prescriptions. In: Tribal Medicine. p. 45-282. Naya Prokash, Calcutta 700 006.

Phillips, DH. 1983. Fifty years of benzo[a]pyrene. Nature. 303: 302-308.

Rosangkima, G. and Prasad, SB. 2004. Antitumour activity of some plants from Meghalaya and Mizoram against murine ascites Dalton's lymphoma. Indian Journal of Experimental Biology. 42: 981-988.

Rosangkima, G. and Prasad, SB. 2007a. Effect of Dillenia pentagyna extract on the level of sialic acid and lipid peroxidation in Dalton's lymphoma-bearing mice. Pharmacologyonline. 1: 436-450.

Rosangkima, G. and Prasad, SB. 2007b. Changes in endogenous glutathione level associated with the antitumor activity of the stem bark extract of *Dillenia pentagyna* against murine ascites Dalton's lymphoma. Pharmacologyonline (In press).

Schmid, W. 1976. The micronucleus test for cytogenetic analysis. In: Chemical Mutagens: Principles and Methods for their Detection, Vol. 4. Ed. Hollaender, A. p. 31-53. Plenum press, New York, USA.

Shields, PG., Bowman, ED., Harrington, AM., Doan, VT. and Weston, A. 1993. Polycyclic aromatic hydrocarbon–DNA adducts in human lung and cancer susceptibility genes. Cancer Research. 53: 3486-3492.

Stavric, B. 1994. Antimutagens and anticarcinogens in foods. Food Chemistry and Toxicology. 32: 79-90.

Steinmetz, KA. and Potter, JD. 1991. Vegetables, fruits and cancer: I. Epidemiology. Cancer Causes and Control. 2: 325-357.

Tandon, P. and Sodhi, A. 1985. *Cis*-dichloro-diammineplatinum(II) induced aberrations in mouse bone marrow chromosomes. Mutation Research. 156: 187-193.

Watanabe, M., Ishidate Jr, M. and Nohmi, T. 1990. Sensitive method for detection of mutagenic nitroarenes and aromatic amines: new derivatives of Salmonella typhimurium tester strains possessing elevated O-acetyl transferase levels. Mutation Research. 234: 337-348.

Wyrobeck, AJ. and Bruce, WR. 1975. Chemical induction of sperm abnormalities in mice. Proceedings of National Academy of Science, USA. 72: 4425-4429.

Xie, W. and Herschman, HR. 1995. v-src induces prostaglandin synthase 2 gene expression by activation of the c-Jun N-terminal kinase and the c-Jun transcription factor. Journal of Biological Chemistry. 270**:** 27622-27628.

Zwelling, LA., Bradley, MO., Sharkey, NA., Anderson, T. and Kohn, KW. 1979. Mutagenicity, cytotoxicity and DNA crosslinking in V79 Chinese hamster cells treated with cis- and trans-Pt (II) diamminedichloride. Mutation Research. 67: 271-280.