Journal of Pharmaceutical and Biological Sciences
ISSN: 2320-1924; CODEN: JPBSEV

Available online at: http://www.jpabs.org/
Original Article

Studies on genotoxic, geno-protective and cyto-protective effects of *Garcinia gummi-gutta* extract against mitomycin C

Vishma B. L., Prashantha Naik*, Sujayraj R. S

Dept. of Post-Graduate Studies and Research in Biosciences, Mangalore University, Mangalagangothri – 574 199, Karnataka, India

Received: 01-01-2017 / Revised Accepted: 24-01-2017 / Published: 26-02-2017

ABSTRACT

Garcinia gummi-gutta, a tropical plant whose fruit rind is used as an herbal medicine for certain illness. However, reports on its genotoxicity and geno-protective effects are not available. Hydromethanolic rind extract of *G. gummi-gutta* fruit was prepared by maceration method and tested against mitomycin C, an anticancer drug. Experiments were conducted on Swiss albino mice to assess the possible dose-dependent effect of the extract (100, 200 and 300 mg/kg BW i.p). The selected doses of the extract were orally administered, and bone marrow micronucleus assay was performed. For the protective effect, the extract was orally gavaged 1 hr prior to mitomycin C (i.p. treatment -5mg/kg). Assay was performed for total antioxidant activity of the extract. Ascorbic acid was taken as the reference standard for both the assays. The extract did not induce the formation of micronucleated cells. However, the higher dose induced a significant decrease in the P/N ratio ($p<0.05$). Induction of micronucleated-cells and reduction in P/N ratio at a highly significant level ($p<0.001$) by mitomycin C confirmed its genotoxic and cytotoxic potency. The co-administration of the extract resulted in a dose-dependent protective effect against MMC-induced genotoxicity as indicated by reduction in the incidence of MN-cells. The extract also imparted the recovery of P/N ratio; 200mg/kg BW was found to be optimum dose. The extract exhibited the antioxidant activity (EC_{50} 36.76±2.53) nearly 1/3rd of that of the ascorbic acid (EC_{50} 13.65±1.26). Thus, the extract has substantial ameliorating effects against mitomycin C-induced geno- and cyto-toxicities, which can be attributed to its free-radical scavenging property.

Keywords: Mitomycin C; *Garcinia gummi-gutta*; genotoxicity; cytotoxicity; antioxidant; genoprotection; cytoprotection

INTRODUCTION

Mitomycin C (MMC), a family of aziridine containing compound derived from *Streptomyces caespitosus* is used as a chemotherapeutic agent for adenocarcinoma, oesophageal carcinoma, anal, breast, gastric, cervical, pancreatic cancers, and bladder tumors[1]. It also finds its utility in glaucoma surgery [2]. Despite its potential therapeutic property with high commercial demand, it has a major limitation in terms of severe side effects. One such adverse effect is genotoxicity and cytotoxicity in non-target cells/tissues, and bone marrow is a main affected tissue. The genotoxic effect of MMC is well evident from various in vitro [3] and *in vivo* [4, 5] studies. Considering the potential genotoxic effect of MMC, there are many studies for exploration of agents which could mitigate the effect [4-6]. The drug in addition to its basic mechanism of inducing mutation by DNA cross linking, it is a potential inducer of oxidative stress leading to free radical-mediated toxicities [7]. In this context, certain plant products/extracts enriched with free radical scavengers are viewed as a potential remedy for minimization of adverse effects of mutagenic drugs [8].

Garcinia gummi-gutta, (L.) Roxb., a tropical species of the family Clusiaceae is known by common names such as Brindle berry, Malabar tamarind and other regional names like *Kodampuli*, *Panpuli*, so on. The fruit, particularly rind part of this plant has traditional medicinal value; to treat bowel complaints, intestinal parasites, rheumatism and obesity (as a weight loss supplement [9]. Various solvent extracts of different parts of the plant have been studied for pharmacological activities, including antimicrobial [10], anticancer [9] and anti-obesity [11]. The phytochemical

***Corresponding Author Address:** Prashantha Naik, Dept. of Post-Graduate Studies and Research in Biosciences, Mangalore University, Mangalagangothri – 574 199, Karnataka, India; E-mail: pnmangaloreuniversity@gmail.com*

screening of the rind extracts revealed the presence of various constituents possessing free-radical scavenging activities[12]. One of its constituents, (-)-hydroxycitric acid has been studied for mutagenicity by using Ames test and micronucleus assay in mouse[13, 14]. Our literature survey indicates that the fruit rind extract of the plant has not been studied for its possible genotoxicity and genoprotective effect against any of the toxic agents. In this context, an in vivo study was carried out to evaluate the ameliorating effect of the hydro-methanolic extract of *G. gummi-gutta* fruit rind against MMC-induced genotoxicity and cytotoxicity, using Swiss albino as the experimental model. Ratio between polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) (P/N ratio) was taken as an additional parameter to evaluate the cytotoxicity/bone marrow depression.

MATERIALS AND METHODS

Chemicals and reagents: Mitomycin C ($C_{15}H_{18}N_4O_5$; CAS RN 50-07-7), manufactured and marketed by Kyowa, Biochem Pharmaceutical Industries Limited (Mumbai, India) was used as the positive agent. L-ascorbic acid, Giemsa and May-Grunwald's stain powder, reagents of analytical grade for antioxidant activity were obtained from Merck India (Delhi, India) and SRL India (Mumbai, India).

Collection of the plant: *Garcinia gummi-gutta* (local name: *Panpuli*) was collected during the monsoon, from Maragodu village, Madikeri taluk of Kodagu district, Karnataka, India. The taxonomic identification of the plant was authenticated with the help of faculty in the Department of Applied Botany, Mangalore University. A specimen plant is preserved as herbarium and voucher No. was given for future reference. The plant name has been checked with the www.theplantlist.org, and Indian Biodiversity Portal: http://indiabiodiversity.org.

Preparation of the extract: Fruits were collected during the rainy season and rind was separated. The collected rind was shade dried in dust-free condition. Fruit rind was selected for the present study considering that it is used for various consumption and medicinal purposes. Maceration method was employed to prepare the hydro-methanolic extract (distilled water and methanol in 1:1 ratio) of the fruit rind. The shade-dried fruit rind was taken and powdered using a mixer. A known quantity (25 gm) of the rind powder was taken in a conical flask and subjected to extraction with the solvent (200 mL) in a mechanical shaker maintained in an air-conditioned room (28ºC) at a constant stirring rate (200 rpm). The stirring was continued for 24 hr duration, and then filtered using Whatman No.1 paper. The filtrate obtained was subjected to maceration for two more times for complete extraction. The extract was concentrated in a rotary evaporator and freeze dried by lyophilization. The extract so obtained was preserved at 4ºC until further use.

Experimental model: Swiss albino mice (*Mus musculus*; 2n = 40; 8–10 weeks old of the body weight 28±2 g) bred and maintained in the animal house of the Dept. of Biosciences, Mangalore University were used. The care and handling of the animals for experiments are in accordance with the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India. The animals were maintained under absolute hygienic condition in an air-conditioned room at a temperature of 24ºC (±2ºC) with 12 hr light/dark cycle and 50±5% humidity. They were fed with standard mice pellets (Lipton, India) and water ad libitum. The present study was carried out with the prior approval from the Institutional Animal Ethics Committee (Ref. 104 No.MU/AZ/99/2013-14/IAEC, dated 02.04.2013).

Dose and treatment schedule:

Treatment schedule: Animals were divided into six groups, each bearing six animals (3 males + 3 females) and treated as follows:

Group 1: Solvent control: 0.2 mL of distilled water (i.p. administration)
Group 2: Positive control: MMC – 5 mg/kg b. wt. (i. p. administration)
Group 3: Extract controls: Extract 100 (Group 3a), 200 (Group 3b) and 300 mg/kg BW (Group 3c) – oral gavage.
Group 4: MMC-5 mg/kg (i.p) + Extract – 100 mg/kg (oral gavage)
Group 5: MMC-5 mg/kg (i.p) + Extract – 200 mg/kg (oral gavage)
Group 6: MMC-5 mg/kg (i.p) + Extract – 300 mg kg (oral gavage)
Group 7: AA-100 mg/kg (oral gavage)

The required concentration of MMC was dissolved in distilled water and administered by i.p. injection in 0.2 ml volume. For the protective effect, the selected doses of the extract were suspended in distilled water and administered to animals in 0.3ml volume by oral gavage one hour prior to the MMC injection.

Micronucleus test: Animals were sacrificed 30 h after the treatment, and bone marrow preparations were made by employing the method of Schmid[15], modified by Rao et al.[16]. The air-

dried slides were fixed in methanol and stained with May–Grunwald–Giemsa. A total of 2,000 polychromatic erythrocytes (PCE) and corresponding normochromatic erythrocytes (NCE) were screened from each animal to determine the frequency of MN-cells and P/N (PCE/NCE) ratio according to the scoring criteria prescribed by MacGregor et al.[17]. Recovery effects of the extract/ascorbic acid against MMC-induced genotoxicity (micronucleated cells) and cytotoxicity (P/N ratio) were determined by using the formula:

% Recovery = (C – E) ÷ C x 100; where C is the value obtained for MMC, and E is the values obtained for the extract/ascorbic acid.

Determination of total antioxidant activity (TAA): Assay for total antioxidant activity was performed by following the method of Prieto et al.[18]. It is based on the principle of reduction of molybdenum (Mo) (VI) to Mo (V) giving rise to phosphomolybdenum complex. L-ascorbic acid was taken as the standard. The absorbance was measured at 695 nm against the blank using UV– visible spectrophotometer. The total antioxidant activity was expressed as percentage activity by using the formula:

% of total antioxidant activity = A(s) - A(b)/A(b) x 100,

Where A(s) is the absorbance of the sample and A(b) is the absorbance of the blank. Calibration curve was constructed taking % of inhibition on Y axis against a concentration gradient. EC_{50} values were determined and expressed as µg/mL.

Statistical analysis: The statistical analysis of the results obtained for MN test was performed with the help of GraphPadInStat 3 package (GraphPad Software Inc, USA). One-way ANOVA test was applied to compare the differences between the groups. Differences were considered to be statistically significant if $p<0.05$.

RESULTS

Micronucleus (MN) test: Results obtained for the MN test are presented in the table No. 3.1 (Fig.3.1a and 3.1b). The frequencies of MN-cells in the extract treated groups were almost similar to that of the solvent control; so there was no significant genotoxicity at any of the doses. The extract at the high dose (300mg/kg BW) reduced the P/N ratio significantly compared to the control ($p<0.05$). MMC has induced the formation of high frequency of MN-cells and reduction in P/N ratio, at a significant level compared to the control ($p<0.001$). The extract has decreased the frequency of MN-cells in a dose-dependent manner (100mg/kg BW – $p<0.05$; 200mg/kg BW – $p<0.01$; 300mg/kg BW – $p<0.001$) when compared with the MMC alone treated group. The lower two doses of the extract imparted the dose-dependent reduction in P/N ratio (100mg/kg BW – $p<0.05$; 200mg/kg BW – $p<0.01$). The magnitude of the cytoprotective effect by higher dose of the extract was found to be almost similar as that of the median dose ($p<0.01$). The co-administration of L-ascorbic acid resulted in decrease in the incidence of MN-cells and enhancement of P/N ratio when compared with the MMC alone treated group at a highly significant level ($p<0.001$).

Antioxidant activity: Table 3.2 presents the results obtained in the total antioxidant activity assay. The extract exhibited a concentration-dependent increase in the antioxidant activity in terms of reduction of molybdenum (Mo) (VI) to Mo (V), with EC_{50} value 36.76±2.53. L-ascorbic acid showed the antioxidant activity with EC_{50} value13.65±1.26.

DISCUSSION

Natural products, particularly derived from plants are considered to be potential agents for therapy as well as a remedy for adverse effects caused by various toxic agents. MMC, despite its utility as a broad-spectrum anticancer drug, it has adverse effects, particularly DNA damage and cytotoxicity on non-target cells/tissues. In the present study, hydro-methanolic rind extract of *G. gummi-gutta* fruit was studied for the possible genotoxic effect, and subsequently geno- and cyto-protective effects against MMC.

Results obtained from bone marrow micronucleus test confirmed the genotoxic potency of the drug. MMC at a single dose of 5mg/kg BW at 30 hr time interval induced the formation of micronucleated cells (MN-PCE and MN-NCE) at a highly significant level compared with the solvent control ($p<0.001$). The observed results are in parallel with the previous reports which also demonstrated the MMC-induced genotoxicity in terms of MN-cells [6, 19] and chromosomal aberrations [20, 21] in Swiss albino mouse.

The extract up to the maximum dose selected in the present study did not induce the formation of MN-cells at a significant level. Thus, the extract does not contain any constituent responsible for genotoxicity in terms of MN formation. In earlier studies, an isolate of the rind extract of the plant, (-)-hydroxycitric acid (the molecule with medicinal value for obesity) has been assessed for mutagenicity, and was found to be negative in five strains of *Salmonella typhimurium* up to

5000mcg/plate[13, 14]. However, the same compound was demonstrated to induce the formation of micronucleated cells by (-)-(-)-hydroxycitric acid in mouse [14]. The report on mutagenicity of (-)-hydroxycitric acid in contradict to our result is that the selected dose of the crude extract may be having (-)-hydroxycitric acid, below the threshold dose for induction of micronucleus.

In the present study, it was observed a significant decrease in P/N ratio at 300mg/kg BW of the extract compared with the control group ($p<0.05$), indicating that the extract has some cytotoxic (mitotic depression) effect in the bone marrow at its higher dose. The exact reason for the same is not known; however, it can be speculated that the extract with a particular constituent may possess some cytotoxic/antiproliferative effect at a higher dose. Further studies may be taken up to explore the constituent(s) possessing antiproliferative activity. However, garcinol, one of the constituents present in the plant whose cytotoxic activity against the cancer cell lines has been reported [11, 22]. The observed cytotoxic effect, i.e., reduction in P/N ratio may be due to garcinol present in the extract as one of the constituents.

Concerning to the protective effect against MMC-induced geno- and cyto-toxicities, we obtained positive results at a substantial level. The co-administration of the extract with MMC resulted in a dose-dependent significant reduction in the frequency of MN-cells (MN-PCE and MN-NCE), with the statistical significance $p<0.05$, 0.01 and 0.001 (Table 3.1; Fig. 3.1a). The observed genoprotective effect of the extract may be attributed to its antioxidant content. MMC, in addition to its basic mechanism of DNA damage through alkylation is also known for free-radical mediated genotoxicity as revealed by in vitro [23] and in vivo studies [24]. An earlier report indicates that various solvent extracts of *G. gummi-gutta* fruit rind is composed of triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrate and vitamin C[12]. Among these, flavonoids and vitamin C are well known for their potential antioxidant activities. So much so, the geno-protective effect of flavonoids [5, 25] and L-ascorbic acid acid [6] against MMC has been demonstrated in mouse.

The ratio between PCE and NCE (P/N) in healthy condition (control group) is almost near to 1.0. A significant reduction in the P/N ratio compared to the control group is an indication of cytotoxicity/mitotic depression in bone marrow [26]. The co-administration of the extract resulted in a slight recovery of the P/N ratio, which otherwise was drastically decreased in MMC-treated group ($p<0.001$). However, the observed effect was not completely dose-dependent such that only lower ($p<0.05$) and median dose ($p<0.01$) enhanced the P/N ratio at the significant levels. The higher dose imparted almost the same level of P/N ratio as that of the median dose ($p<0.01$). It may be due to the optimum dose effect; i.e., the quantity of a substance which produces the desired effect without any unfavourable effects. Thus, 200 mg/kg BW of the extract can be considered as the optimum dose for cyto-protective effect. There are many studies on decrease in cytotoxicity in terms of recovery of P/N ration induced by certain extracts and isolates against MMC [6, 27]. It has been advocated that micronucleus formation due to chromosomal and/or spindle damage is directly correlated with the cytotoxicity in terms of reduction in P/N ratio in bone marrow [23].

One of the mechanisms of geno- and cyto-protective effect of the extract against MMC is substantiated by conducting the total antioxidant activity assay. Results indicate that with an increase in the concentration of the extract, there was enhancement in the total antioxidant activity in terms of reduction of Mo (VI) to Mo (V). The EC_{50} value of the extract was found to be 32.76± 2.53, while for that of L-ascorbic acid was 13.65±1.26. In comparison, the extract possesses nearly 1/3rd of the antioxidant potency of vitamin C. Thus, one of the possible mechanisms for the protective effects is scavenging of MMC-generated free radicals (hydroxy and oxy radicals) by antioxidants present in the extract. Earlier studies on certain extracts rich in antioxidants have also demonstrated the geno- and cyto-protective effects against MMC [27, 28]. Although there was no complete prevention of geno- and cyto-toxicities induced by MMC, the extract could reduce the genotoxicity 36.32% and cytotoxicity 25.8%. However, in comparison, the efficacy of the protective effects of the extract was found to be much lower than that of the AA. Vitamin C in its pure form can act as a potent reducing agent and impart the ameliorating effect to a greater extent than that of the crude extract, which is composed of many constituents. Some of these constituents, including secondary metabolites may interfere with the antioxidants and/or dilute the antioxidant molecules.

CONCLUSION

Hydromethanolic rind extract of *G. gummi-gutta* exhibited protective effects against MMC induced geno- and cyto-toxicities in bone marrow at a substantial level. The ameliorating effects may be due to the inhibition of free-radical mediated toxicities of MMC. The higher dose of the extract was found to induce the cytotoxicity.

Acknowledgements: The authors are thankful to Mangalore University's DST-PURSE (Department of Science & Technology - Promotion of University Research and Scientific Excellence) for providing the short term fellowship to one of the authors (Vishma B.L.) to carry out the present work.

Table 3.1. Effect of hydromethanolic rind extract of *G. gummi-gutta* (HREGG) on MMC-induced micronucleated cells and PN ratio:

Treatment Dose (mg/kg BW)	**[A] Total MN-cells %±SD**	**% Inhibition**	**P/N ratio ±SD**	**% Recovery**
Dist. water	0.20±0.06	--	1.02±0.13	--
E(100)	0.18±0.04	--	0.98±0.12	--
E(200)	0.20±0.07	--	0.94±0.09	--
E(300)	0.22±0.07	--	0.89±0.07^{x}	--
AA(100)	0.18±0.05	--	1.08±0.08	--
MMC(5)	6.47±0.71^{y}	--	0.62±0.09^{y}	--
E(100)+ MMC(5)	5.67±0.8 3^{a}	12.36	0.68±0.08^{a}	9.7
E(200)+ MMC(5)	4.68±0.72^{b}	27.67	0.76±0.07^{b}	22.6
E(300)+ MMC(5)	4.12±0.52^{c}	36.32	0.78±0.09^{b}	25.8
AA(100) + MMC(5)	3.18±0.48^{c}	50.85	0.86±0.12^{c}	38.71

[A] From 2000 PCE and corresponding NCE/animal; 6 animals/group
HREGG - Hydromethanolic rind extract of *G. gummi-gutta*; MMC - Mitomycin C;
E -Extract; MN-PCE - Micronucleated polychromatic erythrocytes;
MN-NCE - Micronucleated normochromatic erythrocytes;
$^{x}p< 0.05$ - Extract *Vs* Control; $^{y}p< 0.001$ - MMC *Vs* Control
$^{a}p< 0.05$; $^{b}p< 0.01$; $^{c}p< 0.001$ - Extract *Vs* MMC

Table 3.2: Total antioxidant activity of hydromethanolic rind extract of *G. gummi-gutta* (HREGG) and L-ascorbic acid:

Conc. (µg)	HREGG		L-Ascorbic acid	
	% of inhibition (mean ± SD)	EC_{50} (µg/mL) (mean ± SD)	% of inhibition (mean ± SD)	EC_{50} (µg/mL) (mean ± SD)
5	12.28±1.26		25.28±2.71	
10	28.73±2.42		35.81±3.22	
25	37.62±2.87	36.76±2.53	76.32±3.87	13.65±1.26
50	65.81±3.65		93.56±4.67	
75	84.70±4.21		96.54±3.95	
100	91.69±3.98		98.28±4.52	
150	97.81±4.33		99.21±4.27	

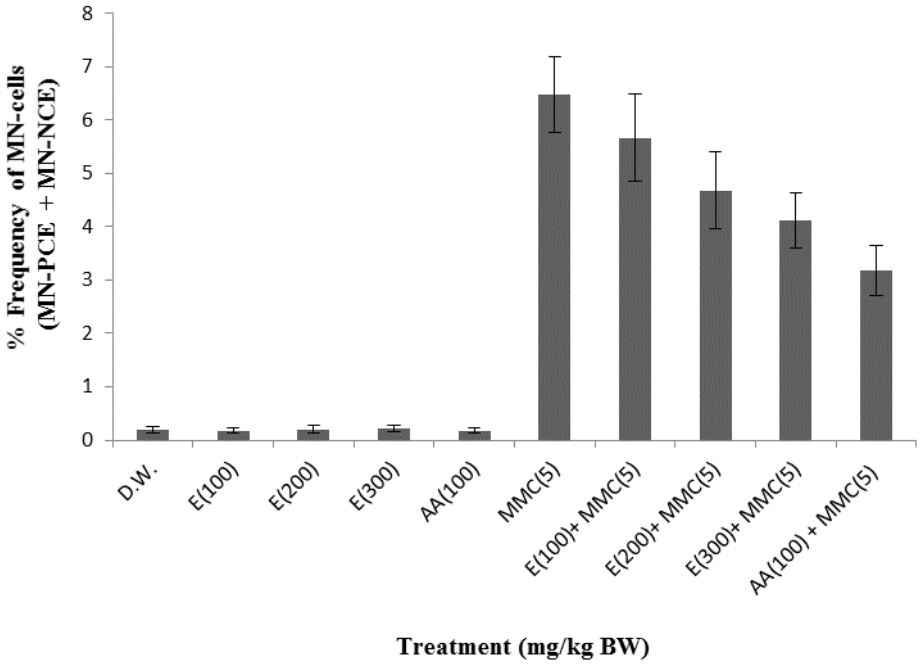

Fig 3.1a: Dose-dependent Effect of *G.gummi-gutta* fruit rind extract against MMC-induced formation of micronucleated cells in comparison with the control and reference standard

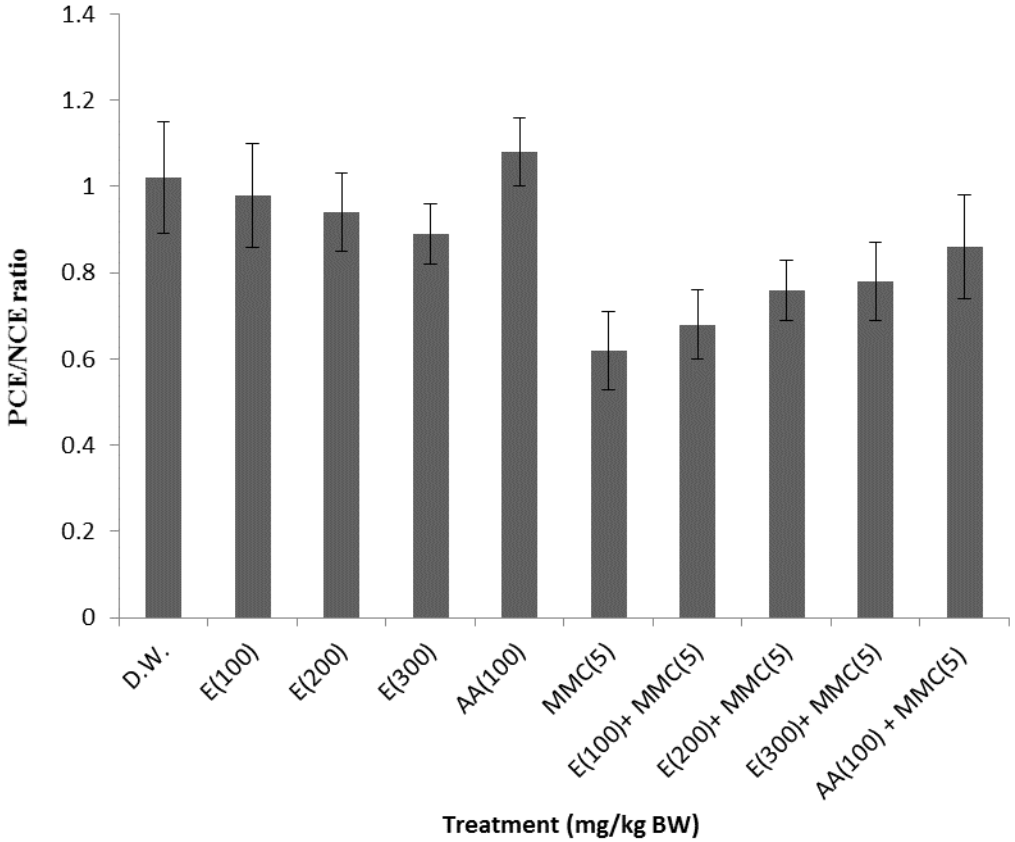

Fig 3.1b: Dose-dependent Effect of *G.gummi-gutta* fruit rind extract against MMC-induced reduction in P/N ratio in comparison with the control and reference standard

REFERENCES

[1] Crooke ST, Bradner WT. Mitomycin C: a review. Cancer Treat Rev 1976; 3:121–39.
[2] Maquet JA et al. Protocol for mitomycin C use in glaucoma surgery. Acta Ophthalmol Scand 2005; 83(2):196-00.
[3] Milic M, Kopjar N. Evaluation of in vitro genotoxic activity of bleomycin and mitomycin C in human lymphocytes using the alkaline comet assay. Arh Hig Rada Toksikol 2004; 55(4):249-59.
[4] Stagos D et al. Effects of plant phenolics and grape extracts from Greek varieties of *Vitis vinifera* on Mitomycin C and topoisomerase I-induced nicking of DNA. Int J Mol Med 2005; 15(6):1013-22.
[5] Mazumdar M et al. Role of quercetin on mitomycin C induced genotoxicity analysis of micronucleus and chromosome aberrations in vivo. Muta Res 2011; 721(2):147-52.
[6] Vijayalaxmi KK, Venu R. In vivo anticlastogenic effects of L-ascorbic acid in mice. Mutat Res 1999; 438(1):47-1.
[7] Rjiba-Touati K et al. Effect of recombinant human erythropoietin on mitomycin C-induced oxidative stress and genotoxicity in rat kidney and heart tissues. Hum Exp Toxicol 2016; 35(1):53-2.
[8] Roncada T et al. Possible modulating actions of plant extracts on the chromosome breaking activity of MMC and Ara-C in human lymphocytes in vitro. Toxicol In Vitro 2004; 18(5):617-22.
[9] Semwal RB et al. A comprehensive scientific overview of *Garcinia cambogia*. Fitoterapia 2015; 102: 134-48.
[10] Varghese LS et al. Evaluation of antimicrobial activity of leaves of *Garcinia gummi gutta* - a plant of ethnomedicinal importance. Eur J Pharm Med Res 2016; 3(10): 275-79.
[11] Saadat N, Gupta SV. Potential role of garcinol as an anticancer agent. J Oncol 2012; (2012): 1-8.
[12] Sharmila KP et al. Phytochemical profile and in vitro antioxidant activity of *Garcinia gummi-gutta*. JBSO 2015; 3:7-12.
[13] Soni MG et al. Safety assessment of (-)-hydroxycitric acid and Super CitriMax, a novel calcium/potassium salt. Food Chem Toxicol 2004; 42(9):1513-29.
[14] Lee KH, Lee BM. Evaluation of genotoxicity of (-)-hydroxycitric acid (HCA-SX) isolated from *Garcinia cambogia*. J Toxicol Environ Health 2007; 70(5):388-92.
[15] Schmid W. Chemical mutagen testing on in vivo somatic mammalian cells. Agents Actions 1973; 3(2):77–5.
[16] Rao SKP et al. Bovine albumin as a substitute for fetal calf serum in the micronucleus test, In: International Symposium on Recent Trends in Medical Genetics, Madras, 1983, p. 28.
[17] MacGregor JT et al. Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutat Res 1987; 189:103–12.
[18] Prieto P et al. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999; 269:337–41.
[19] Grisolia CK. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. Mutat Res 2002; 518 (2):145–50.
[20] Maimon MC, Margery WS. Effects of mitomycin C on human chromosomes. J Cell Biol 1964; 23(2): 386–95.
[21] Shaw MW, Cohen MM, Chromosome exchanges in human leukocytes induced by mitomycin C. Genetics 1965; 51:181-90.
[22] Ranjbarnejad T et al. Garcinol exhibits anti-proliferative activities by targeting microsomal prostaglandin E synthase-1 in human colon cancer cells. Hum Exp Toxicol 2016 (accessed 27 December 2017, epub ahead of print).
[23] Dusre L et al. DNA damage, cytotoxicity and free radical formation by mitomycin C in human cells. Chem Biol Interact 1989; 71(1):63-8.
[24] Sulaiman GM. Role of caffeic acid phenethyl ester on mitomycin C induced clastogenesis: analysis of chromosome aberrations, micronucleus, mitotic index and adenosine deaminase activity in vivo. J Appl Genet 2012; 53(2):213-19.
[25] Siddique YH, Afzal M. Antigenotoxic effect of apigenin against mitomycin C induced genotoxic damage in mice bone marrow cells. Food Chem Toxicol 2009; 47(3):536-39.
[26] Suzuki Y et al. The micronucleus test and erythropoiesis. Effects of erythropoietin and a mutagen on the ratio of polychromatic to normochromatic erythrocytes (P/N ratio). Mutagenesis 1989; 4(6):420-24.
[27] Kumari S. Mitigating effect of Indian propolis against mitomycin C induced bone marrow toxicity. Cytotechnology 2016; 68(5):1789-800.
[28] Prasad S, Naik P, Vijayalaxmi KK. Efficiency of *Coleus aromaticus* extract in modifying cyclophosphamide and mitomycin-C induced clastogenicity in mouse bone marrow cells. Indian J Exp Biol 2002; 40(9):1020-25.