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Fagonia arabica Extract Ameliorate the Mutagenic and Biochemical Effects of Antitumor Drug Mitomycin C in Male Mice.

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ABSTRACT

In the present study potential protective effect of *Fagonia arabic* extract against mutagenic and biochemical alterations induced by antitumor drug mitomycin-C (MMC) in mice will be investigated. Animals were divided into 10 groups (5 animals each) as follow: 1- Control (physiological saline). 2- MMC (0.5 mg/kg injected ip). 3- low dose of fagonia extract (0.5 mg/kg bw). 4-high dose of fagonia extract (5mg/kg bw). 5- MMC + low dose of fagonia extract (0.5 mg/kg bw) for 1 week. 6-MMC + high dose of fagonia extract for 1 week. 7- MMC + low dose of fagonia extract for 2 weeks. 8- MMC + high dose of fagonia extract for 2 weeks. 9- MMC + low dose of the extract for 4 weeks. 10- MMC + high dose of fagonia extract for 4 weeks. The results revealed thatMMC treatmentwas found to induce a significant (P <0.05) increase in MN frequency, chromosomal aberrations in bone marrow and spermatocyte cells of mice as well in the frequency of DNA damage and DNA fragmentation. However, the treatment with fagonia ameliorate these parameters .Fagonia significantly prevented MMC-induced elevation of AST,ALT,TB, and MMC-induced decrease in total protein and MDA in mice. The present study therefore demonstrate that *F. arabica* have two pronounced effects, on the MMC- induced genotoxicity and biochemical alterations, i.e. improving the native antioxidative defense system of the cells and, the antioxidant property by decreasing the lipid peroxidation and scavenging the free radicals.

Keywords: *F. arabica,* mitomycin-C, antitumor drug, mutagenic, biochemical, effects, mice**.**

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INTRODUCTION

Mitomycin-C (MMC), known as mutamycin or mitomycin is an antineoplastic antibiotic isolated from the soil bacterium Streptomyces caespitosus. It is a cell cycle-specific alkylating agent [1]. MMC is a growth inhibitor and potent antitumor agent against various types of cancers [2]. MMCis metabolically activated in vivo and acts as an alkylating agent having a clastogenic action in the G, and S-phase of the cell cycle. It has mutagenic and cytotoxic effects on testis which can be seen as a killing of differentiating spermatogonia in mice [3] and chromosomal aberrations in spermatocytes or spermatid micronuclei produced by the treatment of mice and rats [4].

MMCbinds covalently to DNA and produces both monoadducts and DNA-DNA and DNA protein crosslinks [5]. Its mechanism of action is S-phase dependent and it can induce chromosomal damages that will lead to a reduction of the rate of DNA replication and a dose-dependent delay in cell cycle progression in cultured human colon cancer cells [6]. Since the MMC-induced DNA damage usually occurs in the S-phase [7], one might suspect that a smaller contribution of different cell generations to genotoxic damage is accompanied by a parallel decrease of the MN frequency.

A statistically significant increase of MN frequency was observed after a treatment of fish cell line (RTG-2) with MMC, at all concentrations used (0.25, 0.5 and 1 µg/ml) depending on thelength of exposure period. Concentration and length of exposure period are inversely related. At the shortest exposure period, there was a maximum induction effect for all three concentrations tested and significant increases of MN frequencies are produced compared to the negative control. Induction of MN decreases as exposure period increases, which is also interrelated with a decrease in the G0/G1 percentage. At the longest exposure period, the highest concentration (1 µg/ml) does not provoke a significant increase in MN frequency, may be as a consequence of its high data dispersion [8,9].

Although hepatotoxicity is rare in patients treated with MMC, drug-induced alterations in liver function [10] and hepatic SOS in the setting of high-dose therapy with hematopoietic cell transplantation have been reported [11]. Hepatic dysfunction under chemotherapy mainly consists of abnormal biologic liver function tests indicating chronic cholestasis with elevation in the level of bilirubin, alkaline phosphatase (AP), and gamma glutamyl transferase (yGT) with or without abnormal levels of AST and/or ALT [12].

F. arabica is a green shrub of around 3 feet, belongs to Zygophyllaceae family, which is widely distributed in sub-Saharan countries, Afghanistan and Indian sub-continent. It is also commonly known as 'Dhamasa' in local language. It has been used traditionally for its anti-inflammatory activity and considerable analgesic and antipyretic effects [13].(Chopra et al.,1982). It is reputed to be a medicinal plant in scientific and folkloric literature and its medicinal values are well documented [13,14]. This herb has been found to be effective in many hematological, neurological, endocrinological and inflammatory diseases [15,16]. An aqueous decoction of the aerial parts of the genus Fagonia is a popular remedy in the indigenous system of medicine for cancer in its early stages and for the treatment of various other diseases of digestive and blood vascular system [17]. The medicinal properties of the plant were attributed due to its variety of active phytochemical constituents. The entire plants of various Fagonia species were investigated mainly for the presence of two major types of phytochemical compounds, i.e. flavonol glycosides and terpenoid glycosides.

Fagonia has been shown a strong free radical scavenging properties [18]. Compounds isolated from Fagonia also demonstrate cytotoxic activity towards erythrocytes, leukocytes and human breast cancer cell lines [15,19]. As well as, Fagonia has been shown to have anti-inflammatory properties including platelet aggregation, down-regulation of COX-2 and up-regulation ofVEGF [18]. In addition, the study of [20] for evaluating methanolic extracts of Fagonia for its anticancerous potential by utilizing cytotoxicity, antitumor and DNA damage assays revealed a strong anti-cancerous potential of this plant.These findings demonstrate that an aqueous extract of Fagonia can induce cell cycle arrest and apoptosis via p53-dependent and independent mechanisms, with activation of the DNA damage response.

Despite of the proven efficacy of F. arabica on various disorders no attempt has been made to study its protective effect on the mutagenicity (that include chromosomal aberration in both somatic and germ cells, micronuclei frequency, DNA damage) and some biochemical alterations induced by MMC in mice. Therefore the present study was designed to investigate the potential protective effect of F. Arabica extract on

clastogenic and biochemical effects of MMC on male mice using cytogenetic analysis, micronucleus and comet assays, DNA fragmentation and biochemical analysis.

MATERIALS AND METHODS

Preparation of extract

Whole plant of *F. arabica* was rinsed with distilled water and dried under shade at room temperature (25–30 °C). After drying, the whole plant materials (4kg) were subjected to size reduction to a coarse powder by using dry grinder. The powder was packed into a Soxhlet apparatus and defatted with petroleum ether (60–80 °C). The marc was dried and extracted with ethanol at 80 °C for 24 h. The Fagonia ethanol extract (FEE) was concentrated to dryness under reduced pressure in a rotary evaporator and stored in airtight containers in refrigerator below 4 °C. The percentage yield was found to 11.3% (w/w) [21].

Animals and treatment

Fifty adult Swiss albino male mice (10–12 weeks), weighing 30±2 g, were obtained from the animal house of the National Research Center, Dokki, Cairo, Egypt, and kept for 1 week for acclimatization prior to starting the study. Feed and water were supplied ad libitum. MMC was purchased from Sigma Chemical Company (Cas no. 50-07-7, Sigma, St Louis, MO, USA). Animals weredivided into 10 groups (5 animals each) as follow: 1- Control (physiological saline). 2- MMC (0.5mg/kg injected ip). 3- low dose of fagonia extract (0.5 mg/kg bw). 4-high dose of fagonia extract (5mg/kg bw). 5- MMC + low dose of fagonia extract (0.5 mg/kg bw) for 1 week. 6-MMC + high dose of fagonia extract for 1 week. 7- MMC + low dose of fagonia extract for 2 weeks. 8- MMC + high dose of fagonia extract for 2 weeks. 9- MMC + low dose of the extract for 4 weeks. 10- MMC + high dose of fagonia extract for 4 weeks. Mice were sacrificed and subjected to comet assay, DNA fragmentation, micronucleus test, chromosomal aberration analysis in both somatic (bone marrow cells) and germ cells (spermatocyte cells). For biochemical analysis, ALT, AST, TP and MDA levels were measured as liver function indicators.

Assessment of DNA damage

Comet assay

Comet assay was performed under alkaline conditions [22] with slight modification. Slides were scored using Comet Score Software (Tritek Corp., USA) and 100 cells were analyzed per sample. The parameters used to assess DNA damage were tail length (migration of DNA from nucleus) and the percentage of DNA damage frequency.

DNA fragmentation

Peripheral blood leukocytic cells were harvested by centrifugation and lysed by 600 ml lysing buffer (50 mMNaCl, 1mM Na2 EDTA, 05% SDS, pH 8.3) (Sigma). The cell suspension was shaken gently and kept overnight at 37˚C. DNA was extracted using the method of [23]. The DNA samples were mixed with 6x loading buffer and analyzed on a 2% agarose gel stained with 1 mg/ml ethidium bromide [24].

Cytogenetic analysis

Micronucleus assay

Mice were sacrificed and both femurs of mice were removed and aspirated with fetal calf serum. The bone marrow smears were made, fixed and stained with Giemsa. 2000 polychromatic erythrocytes (PCE) were scored per animal [25].

Chromosomal aberrations analysis

Mice were sacrificed 24 h after administration of the last treatment for chromosome aberration analysis. Cytogenetic analysis was performed on tibia bone marrow cells according to the recommendations of [26], with slight modifications. Experimental animals were injected (i.p.) with colchicines (4 mg/kg) 1.5 h

before sacrifice. Both tibia were dissected out and cleaned of any adhering muscle. Bone marrow cells were collected from both tibias by flushing in KCl (0.075 M, at 37 °C, 5 mL) and incubated at 37 °C for 25 min. Material was centrifuged at 2000 rpm for 10 min, fixed in methanol: acetic acid (Carnoy's fixative, 3:1 v/v). Centrifugation and fixation (in the cold) were repeated five times at least at intervals of 20 min. The material was resuspended in a little volume of fixative, dropped onto chilled slides, flame-dried and stained in 5% Sorenson buffered Giemsa (pH: 6.8). At least 75 good metaphases containing 40 chromosomes were examined per animal to score different types of aberrations. For spermatocyte cells, chromosomal preparations were made according to the air- drying method [27]. Mice were injected (i.p.) with colchicines (0.1%) 2 h before killing by cervical dislocation. The testes were transferred to 2.5 ml of a 2.2% citrate solution in Petri dishes and the tunica removed. The contents of the tubules were gently teased out with curved forceps. The cell suspension produced was aspirated well and centrifuged at 1000 rpm for 10 min the supernatant was discarded, and the pellet was resuspended in 2 ml of hypotonic solution (1% sodium citrate) at 37 C. After 12 min, the suspension was centrifuged for 10 min at 1000 rpm. Then the supernatant was removed. The cells were fixed 3 times with cold fixative solution (3:1 of methanol and glacial acetic acid). Slides were stained with Giemsa in phosphate buffer (pH 6.8) for 8 min. Fifty primary spermatocytes/mouse at diakinesis-metaphase I were scored. Abnormalities recorded included univalents (x–y univalent and autosomal univalent), chains, rings, N±1 and polyploidy.

Biochemical analysis

Measurement of lipid peroxidation (MDA)

Malondialdehyde (MDA), the end product of lipid peroxidation, was measured using thiobarbituric acid reactive substance (TBARS) assay with some modification according to [28].

Determination of AST, ALT and TP

Determination of Glutamic–oxaloacetic transaminase (GOT or AST) and Glutamic–pyruvic transaminase (GPT or ALT) activity: AST and ALT (GOT and GPT) was determined according to the method of [29] using kits at wave length 490-520 nm. Determination of total protein (TP) was done according to [30], using protein kits at wave length of 545 nm using spectrophotometer in mice liver.

Statistical analysis

Data are analyzed using one way analysis of variance (ANOVA) with SAAS program. Data are expressed as means ±standard deviation and standard error. The difference between the means was considered significant at $p < 0.05$. DNA damage frequency was expressed as a percentage.

RESULTS AND DISCUSSION

In the present study, F. arabica was investigated for its potential antigenotoxic and chemopreventive effects in MMC treated Swiss albino male mice by genetic (Mn, and chromosomal analysis in both bone marrow and spermatocyte cells), DNA damage analysis (comet assay and DNA fragmentation) and biochemical analysis for liverfunction.

DNA damage analysis

Comet assay

The comet assay (mean comet tail length) results (Table 1) showed a significant increase in comet tail length and DNA damaged cells percentage (23.7%) in MMC treated group up to 3 fold higher than control (7.3%), with high incidence of class 3 (tail length > 2X the diameter of nucleus) of comet, whereas the treatment with Fagonia improved these results in a dose and time dependent manner specially with high dose of fagonia for 4 weeks treatment (12 % damaged cells frequency). That coincide with [20], who found anti DNA damage potential of methanolic extracts of fagonia.

DNA fragmentation in peripheral leukocyticcells

The optical density of DNA extracted from peripheral leukocytic cells of mice treated with MMC showed fragmented DNA laddering or apoptotic bands with higher intensity of DNA fragmentation (38.92 ± 0.48) than in control (7.25± 0.62). DNA fragmentation were also observed in all groups with higher intensity of fragmentation in the group of 1 week treatmentand lowering gradually specially with high dose of fagonia for 4 weeks treatment in a time and dose dependent manner (Table). That confirm with the results of [31], who found that MMC–DNA damaging histograms occurs after long exposure periods. As well [9], who found that the random amplified polymorphism of DNA (RAPD) showed clear variation between treated and non-treated animals against MMC treatment.

Antimutagenic effect of fagonia on MMC treated mice

The results of the present study revealed that the treatment with MMC resulted in significant increase in the frequency of MN with 5 fold compared to control (Table 4). However, fagonia extract treatment showed a significant decrease in MN frequency in male mice compared to MMC treated groups speciallywith the high dose of fagonia treated for 4 weeks group (9.10±1.02) in a dose and time dependent manner.

In accordance with our results, MMC was found to induce a significant (P <0.05) increase in MN frequency in bone marrow cells of mice as recorded [32,9]. Since the MMC-induced DNA damage usually occurs in the S-phase [7], one might suspect that a smaller contribution of different cell generations to genotoxic damage is accompanied by a parallel decrease of the MN frequency and that in contract to our findings. Induction of MN decreases as exposure period increases, which is also interrelated with a decrease in the G0/G1 percentage [8].

MMC caused a highly significant increase in chromosome aberration in both bone marrow (Table2) and spermatocyte (Table 3) cells. The MMC treatment caused high percentage of aberrant cells due to its accumulation effect and that in accordance with [9], who found that Treatment with MMC to male mouse induced structural and numericalchromosomal aberrations. Break is the main type of chromosomal aberrations (7.80±0.37) in bone marrow cells (i.e. loss of the DNA content). Since DNA is considered as constant genetic component of every cell in all organs, the decrease of DNA content (Table 4) may be due to the genotoxicity of MMC. X-Y (11.6%) and autosomal univalents (10.4%) are the main types of chromosomal aberrations in spermatocyte cells treated by MMC. However, the fagonia treatment in combination with MMC significantly decrease all types of chromosomal aberrations in both bone marrow andspermatocyte cells.

The mutagenic and cytotoxic effects of MMC on testis which can be seen as a killing of differentiating spermatogonia in mice [3] and chromosomal aberrations in spermatocytes or spermatid micronuclei produced by the treatment of mice and rats [4] which showing a clastogenic action in the G, and S-phase of the cell cycle confirm our results.

Effect of fagonia and /or MMC on liver function (ALT, AST and TP) and lipid peroxidation (MDA) levels in mice

ALT and AST are important indicators of liver damage in clinic finding. Significant increase in ALT (98.80±0.37) and AST (150.20±0.37) were detected in MMC treated group (Table 5) compared to control. The increased ALT and AST values agreed with the results obtained by [12], who stated that changes in these enzymes level might differ depending on exposure time and dose. However, [21] found that administration of Fagonia improves the activity of ALT and AST in mice which coincide with our findings.

Total protein was found to decrease significantly (Table 5) with MMC treatment however fagonia treatment had a potential effect on that effect. That in accordance with [33] who found that MMC treatment causes cross-linking of DNA and inhibits RNA as well as protein synthesis through upregulating the expression of IL-8 and MCP-1 mRNA and protein secretion by the activation of mitogen-activated protein kinases (MAPKs).

Table (5) shows the effect of MMC on lipid peroxidation (MDA level) and their protection with F. arabica. The formation of malondialdehyde (MDA) was elevated significantly ($p < 0.05$) in MMC treated group (36.22 ± 0.36) when compared with control mice and their level was significantly depleted ($p < 0.05$) as

compared with MMC group when treated with F. arabica specially with 4 weeks period treatment (14.36± 0.26 and 10.18 ± 0.48 for low and high dose of fagonia, respectively).

The hepatoprotective effect of Fagonia extract may be attributed due to the reduced lipid peroxidation and improved defense of the hepatocytes against the reactive oxygen species. Therefore the study scientifically supports the usage of Fagonia for treatment of liver disorders and as a tonic.

According to WHO report [34], traditional medicine is very popular in all developing countries, and its use is rapidly increasing in industrialized countries. Medicinal plants are widely used in house hold remedies and by practitioners of traditional system of medicine in developing countries such as China, Pakistan, Nepal and India. At the same time interest in traditional and complementary and alternative medicine in industrialized countries has grown rapidly e.g., in European counties, Japan and USA [35].

Table 1: Visual score of DNA damage in blood samples of male mice exposed to MMC and/or *F. arabica* extract for several time intervals using comet assay.

Treatment	No. of animals	No. of cells			Class of comet				DNA damaged cells (%)
		Analyzed (*)	Total comets		0	1	2	3	
			Individuals	Total					
Control	3	300	(7+8+7)	22	278	18	4	0	7.3
MMC	3	300	(23+24+24)	71	229	23	21	27	23.7
Fagonia 0.5mg/kg(FLD)	3	300	(8+7+7)	22	278	18	4	0	7.3
Fagonia 5mg/kg (FHD)	3	300	(7+7+7)	21	279	18	3	0	7.0
MMC+FLD (1 wk)	3	300	(19+21+21)	61	239	21	17	23	20.3
MMC+FHD (1wk)	3	300	(18+20+21)	59	241	19	19	21	19.7
MMC+FLD (2 wks)	3	300	(18+20+18)	56	244	17	20	20	18.7
MMC+FHD (2 wks)	3	300	(18+16+16)	50	250	18	16	16	16.7
MMC+FLD (4 wks)	3	300	(15+16+14)	45	255	15	16	14	15.0
MMC+FHD (4wks)	3	300	(11+13+12)	36	264	12	13	11	12.0

¥: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of analyzed cells were 100 per animal; F. arabica: Fagoniaarabica.MMC: mitomycin-C; FLD: Fagonia low dose (0.5 mg/kg bw); FHD: Fagonia high dose (5mg/kg bw).

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Table 2: Effect of MMC and/or *F. arabica* extract on chromosomal aberrations frequency in bone marrow cells of mice.

Treatment groups	Gap	Structural aberrations					Total structural aberrations	Numerical aberration		Total numerical aberrations
		Break	Freagmens	Delations	Endomitosis	Centromeric attenuation		Hypolploidy	Hyper-polidy	
Control	0.60±024[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.20±0.20[a]	0.40±0.24[a]	1.20±0.58[a]	0.20±0.20[a]	0.00±0.00[a]	0.20±0.20[a]
MMC	2.60±0.40[i]	7.80±0.37[i]	3.80±0.37[i]	4.00±0.31[i]	3.60±0.24[i]	5.20±0.37[i]	27.00±0.31[i]	6.40±0.81[g]	7.60±0..67[g]	14.0±1.41[g]
Fagonia 0.5mg/kg(FLD)	0.80±0.20[c]	0.60±-0.24[c]	1.00±0.00[c]	1.20±0.20[c]	0.80±0.20[c]	1.00±0.00[c]	5.40±0.40[c]	3.00±0.54[d]	4.20±0.73[d]	7.20±0.73[d]
Fagonia 5mg/kg (FHD)	0.40±0.24[b]	0.80±0.20[b]	0.80±0.20[b]	0.40±0.24[b]	0.20±0.20[b]	0.20±0.20[b]	2.80±0.20[b]	2.40±0.40[c]	2.80±0.37[c]	5.2±0.20[c]
FLD+MMC(1Wk)	2.60±0.24[h]	6.00±0.44[h]	4.0±0.44[h]	4.00±0.44[h]	3.20±0.20[h]	2.40±0.24[h]	22.20±0.37[h]	6.20±0.37[f]	6.20±5.58[f]	12.4±0.9[f]
FHD+MMC(1Wk)	2.60±0.24[g]	4.20±0.20[g]	3.00±0.00[g]	3.20±0.20[g]	3.00±0.00[g]	3.20±0.20[g]	19.20±0.58[g]	4.80±0.37[d]	5.60±0.40[d]	10.4±0.70[d]
FLD+MMC(2Wks)	2.60±0.24[g]	4.20±0.20[g]	3.00±0.00[g]	3.00±0.00[g]	3.00±0.31[g]	2.80±0.20[g]	18.60±0.40[g]	4.60±0.24[d]	5.20±0.20[d]	9.8±0.2[d]
FHD+MMC(2Wks)	1.60±0.24[f]	3.20±0.20[f]	2.40±0.24[f]	2.80±0.37[f]	2.20±0.20[f]	2.40±0.24[f]	14.60±0.92[f]	3.60±0.24[d]	4.80±0.37[d]	8.4±0.5[d]
FLD+MMC(4Wks)	1.40±0.24[e]	2.80±0.20[e]	1.40±0.24[e]	2.00±0.31[e]	2.00±0.31[e]	2.20±0.20[e]	11.80±0.58[e]	3.00±0.31[c]	3.60±0.40[c]	6.6±0.6[c]
FHD+MMC(4Wks)	1.00±0.00[d]	1.80±0.20[d]	1.20±0.20[d]	1.20±0.20[d]	1.00±0.00[d]	1.00±0.00[d]	7.20±0.20[d]	2.00±0.31[b]	2.20±0.37[b]	4.2±0.5 [b]

MMC: mitomycin-C; FLD: Fagonia low dose (0.5 mg/kg bw); FHD: Fagonia high dose (5mg/kg bw).

Table 3: Effect of MMC and/or *F. arabica* extract on chromosomal aberrations frequency in spermatocyte cells in mice.

Treat	Number of examined cells	Structural aberrations										Numerical aberrations				Total aberrations	
		X-y univalent		Autosomal univalent		Chain		Ring		Total aberrations		N-1		N+1			
		No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Control	250	1	0.4	0.	0.0	0	0.0	0.0	0.0	1	0.4	0	0.0	1	0.4	1	0.4
MMC	250	29	11.6	26	10.4	14	5.6	10	4.0	79	31.6	15	6.0	14	5.6	29	11.6
Fagonia 0.5mg/kg(FLD)	250	2	0.8	1	0.4	1	0.4	1	0.4	5	2.01	1	0.4	2	0.8	3	1.2
Fagonia 5mg/kg (FHD)	250	1	0.4	2	0.8	2	0.8	1	0.4	6	2.4	2	0.8	2	0,8	4	1.6
FLD+MMC(1Wk)	250	25	10.0	18	7.2	10	4.0	19	7.6	72	28,8	13	5.2	11	4.4	24	9.6
FHD+MMC(1Wk)	250	20	8.0	10	4.0	13	5.2	15	6.4	59	23.6	10	4.0	9	3.6	19	7.6
FLD+MMC(2Wks)	250	17	6.8	12	4.8	6	2.4	10	4.0	45	18	9	3.6	6	2.4	15	6
FHD+MMC(2Wks)	250	15	6	9	3.6	4	1.6	8	3.2	36	14.4	7	2.8	4	1.6	11	4.4
FLD+MMC(4Wks)	250	9	3.6	6	2.4	4	1.6	7	2.8	26	10.4	5	2.0	3	1.2	8	3.2
FHD+MMC(4Wks)	250	4	1.6	2	0.8	2	0.8	6	2.4	14	5.6	2	0.8	1	0.4	3	1.2

MMC: mitomycin-C; FLD: Fagonia low dose (0.5 mg/kg bw); FHD: Fagonia high dose (5mg/kg bw).

Table 4: Effect of MMC and/or *F. arabica* extract on micronuclei frequency and DNA fragmentation in mice.

Treatment	Micronuclei M±SD	DNA Fragmentation M±SE
Control	6.80±10.83[a]	7.25±0.62 [a]
MMC	30.20±1.30[h]	38.92±0.48[h]
Fagonia (FLD)	7.60±0.54[a]	8.04±0.47[a]
Fagonia (FHD)	8.10±1.02[b]	10.78±0.19[b]
FLD+MMC(1Wk)	28.40±1.67[g]	34.82±0.39[g]
FHD+MMC(1Wk)	23.00±1.22[f]	29.86±0.42[f]
FLD+MMC(2Wks)	18.20±0.83[e]	24.80±0.37[e]
FHD+MMC(2Wks)	15.30±0.64[d]	20.10±0.10[d]
FLD+MMC(4Wks)	11.8±1.30[c]	16.14±0.67[c]
FHD+MMC(4Wks)	9.10±1.02[b]	11.04±058[b]

MMC: mitomycin-C; FLD: Fagonia low dose (0.5 mg/kg bw); FHD: Fagonia high dose (5mg/kg bw).

Table 5: Effect of fagonia and /or MMC on liver function (ALT, ASTand TP) and MDA levels in mice.

Treatment	ALT	AST	T.P	(MDA) M+SE
Control (-Ve)	27.20±0.58[a]	49.30±0.30[a]	9.04±0.51[f]	7.84±0.40[a]
MMC(+Ve)	98.80±0.37[i]	150.20±0.37[i]	2.88±0.44[a]	36.22±0.35[i]
Fagonia (LD)	31.20±0.58[b]	53.60±0.6[b]	9.20±0.37 F	8.90±0.45[a]
Fagonia (HD)	29.40±0.50 b	50.60±0.60 a	9.98±0.56[f]	11.46±0.34[c]
FLD+MMC(1Wk)	96.80±0.37[h]	148.20±0.37[h]	5.82±0.18[b]	33.25±0.33[h]
FHD+MMC(1Wk)	93.60±0.67[g]	144.00±0.83[g]	6.26±0.19[b]	27.08±0.49[g]
FLD+MMC(2Wks)	90.90±0.45[f]	137.80±0.80[f]	7.05±0.35[c]	22.12±0.44[f]
FHD+MMC(2Wks)	87.70±0.43[e]	132.60±0.74[e]	7.84±0.41[c]	19.34±0.27[e]
FLD+MMC(4Wks)	78.00±1.04[d]	127.20±0.91[d]	8.70±0.20[d]	14.36±0.26[d]
FHD+MMC(4Wks)	67.40±1.02[c]	102.100±0.95[c]	8.85±0.47[d]	10.18±0.48[b]

ALT: alanine transaminase; AST: aspartate aminotransferase; TP: total protein; MDA: lipid peroxidation. MMC: mitomycin-C; FLD: Fagonia low dose (0.5 mg/kg bw); FHD: Fagonia high dose (5mg/kg bw).

CONCLUSION

The present study therefore demonstrate that *F. arabica* have two pronounced effects, on the MMC induced genotoxicity and biochemical alterations, i.e. improving the native antioxidative defense system of the cells and, the antioxidant property by decreasing the lipid peroxidation and scavenging the free radicals.

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