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Original Article

Antimutagenic and chemopreventive potentialities of fenugreek (*Trigonella foenum-graecum*) seed extract

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Key Words
Ames test; Anticarcinogenicity; Antimutagenicity; Chromosomal aberration; Micronuclei; Papillomagenesis

Abstract
Phytochemicals present in plant foods have been linked to reduce several chronic diseases including cancer. They have gained popularity as safer and cheaper alternative to conventional drugs. We studied the antimutagenic and anticarcinogenic activity of *Trigonella foenum graecum* (L.) seed (TFGS) extract using *Salmonella* microsomal assay and two stage skin papilloma model in mice, respectively. Different concentrations (2.5, 5, 10 and 20 g/100 ml of distilled water) of the aqueous seed extract of fenugreek were screened against the potent mutagens to evaluate its antimutagenicity using *Salmonella* assay. Besides, the above mentioned antimutagenicity method, the anticarcinogenicity study was carried out at chromosomal aberration and micronuclei level in Swiss Albino mice by mouse two stage papillomagenesis. The dose level of the study was 400 mg/kg body weight of the seed extract. Significant reduction in revertant colony count in the seed extract treated group compared to positive control group was obtained. At 20 g/100 ml concentration of TFGS, a strong inhibition rate was observed in both TA98 and TA100 strains of *Salmonella* against different mutagens. A clear dose dependent fall on chromosomal aberration in bone marrow cells of mice and micronuclei count was recorded in the treated group. Animals treated with TFGS extract at 400 mg/kg/day throughout all the stages of papillomagenesis showed a significant reduction in the total chromosomal aberrations and micronuclei score.

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INTRODUCTION

Cancer is a group of complex genetic diseases of uncontrolled cell division caused by both internal and environmental factors. Numerous lifestyle factors have been implicated in carcinogenesis process [1]. There is a close relation between carcinogenicity and mutagenicity and ninety percent of the carcinogens tested were found to be mutagenic in the Ames assay [2].

Chemoprevention is the attempt to use natural and synthetic compounds to intervene in the early stages of cancer, before invasive disease begins [3]. Chemoprevention of cancer through natural plant phytochemicals is gaining popularity for its relatively harmless action [4]. Phytochemicals are available in spices, vegetables and fruits. Briefly, they are plant chemicals or more appropriately defined as bioactive non-nutrient plant compounds that have been linked to reduce the risks of major chronic diseases and cancers [5]. Phytochemicals can have complementary and overlapping mechanism of action which includes gene expression modulation during cell proliferation, regulation of oncogenes and tumor suppressor genes, induction of cell-cycle arrest and apoptosis, modulation of enzyme activities in detoxification, stimulation of the immune system, control of hormone metabolism as well as antibacterial and antiviral effects [6, 7]. Spices play an integral role in determining the balance between pro- and anti-cancer factors that regulates the risk and tumor behavior [8]. Thus, incorporation of these plant products especially spices in our regular diet may prove to be beneficial for our health [9]. Though, the effects of spices in the context of total diet still remain to be evaluated.

One such spice, *Trigonella foenum graecum*, commonly called fenugreek, is a leguminous plant native to many Asian, Middle Eastern, and European countries [10]. Fenugreek belongs to the subfamily Papilionacae of the family Leguminosae (bean family, Fabaceae). The plant is an aromatic herbaceous annual, widely cultivated in Mediterranean countries and Asia. Fenugreek is a self-pollinated crop. It has a wide range of medicinal applications [11]. Fenugreek seeds and leaves have been used extensively in various medicinal preparations [12-14]. The seeds are very nourishing and are given to convalescents and also to encourage weight gain, especially in anorexia nervosa. Seeds, cooked or sprouted and eaten raw [15, 16], are a good source of many essential elements such as iron, phosphorus and sulphur [17]. An essential oil obtained from the seed is used in food flavoring in imitation maple syrup, vanilla compositions, liquorice, pickles *etc*.

Research has shown that the seeds can inhibit cancer of the liver, lower blood cholesterol levels and also have an antidiabetic effect [11, 18] . Pretreatment of the seed extract have shown to restore activities of the overall antioxidant defense system in mice [19]. It posses cytotoxicity against several cancer cell lines inducing cell death [20] and inhibition of epidermal ornithine decarboxylase (ODC), cyclooxygenase (COX) and lipooxygenase activities, edema and hyperplasia [21]. Beneficial effects of fenugreek can be attributed to its bioactive molecules. One such component diosgenin is able to effectively treat inflammation and proliferation principally through inhibition of cell cycle signaling and induction of apoptosis in number of human cell lines [22]. Raju and Bird [23] reported that diosgenin significantly lowered the expression of 3-hydroxy-3-methyl coenzyme A (HMG-CoA) reductase at both mRNA and protein levels, suggesting the involvement of the cholesterol biosynthetic pathway in diosgenin's efficacy as an anti-cancer agent.

In the present study attempt has been made to evaluate the antimutagenic activity of *T. foenum graecum* (L.) against various mutagens in the microbial system (*Salmonella typhimurium*) and chemomodulatory potential of the same against 7,12-dimethylbenz-[α]-anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced mouse skin papilloma-genesis. The antimutagenicity was determined by revertant colonies formed in *Salmonella* auxotrophs (strains TA98 and TA100) and the current focus of chemomodulation was on intermediate biomarkers capable of detecting early changes that can be correlated with blocking of carcinogenic progression (chromosomal aberrations and micronuclei).

MATERIALS AND METHODS

Animals

Random bred male Swiss albino mice (8-9 weeks old) were obtained from the University animal house facility.

Bacterial strains

The tester strains *S. typhimurium viz* TA98 (MTCC 1251) and TA100 (MTCC 1252) were obtained from microbial type culture collection and gene bank, IMTEC (Institute of Microbial Technology), Chandigarh (India).

Preparation of culture

Overnight cultures of *S. typhimurium* strains TA98 and TA100 were grown at 37°C in Oxoid nutrient broth supplememted with 80 μg/ml ampicillin. 1 ml of this overnight culture was used to inoculate 50 ml of the fresh broth which was then incubated at 37°C for 2 h until an OD_{600} reaches 0.4 [24].

Preparation of metabolic activation (S9) mix

The S9 mix was prepared according to the recipe recommended by Maron and Ames [25] and Mortelmans and Zeiger [26]. S9, a cell free fraction was prepared by homogenization and centrifugation of rat liver pretreated with polychlorinated biphenyls (PCBs; Aroclor 1254) at 9000g for 10 min, and added when metabolic activation is required.

Seeds of *Trigonella foenum graecum* (L.) (TFGS)

The TFGS were procured from local market and identified from Department of Botany, University of Rajasthan, Jaipur, India, as RUBL 20658. The extract of TFGS was prepared using Soxhlet apparatus with methanol for 36 h at 70°C. The apparatus consisted of a condenser, an extractor and a thimble. The plant material was kept in the extractor and 500 ml of methanol in the thimble. The vessel was heated from below and the steam generated was used to extract the plant material via condenser, extractor and collected in the thimble. Finally, the extract was collected, filtered and evaporated to obtain the powdered form. Then different doses of the seed extract were prepared in double distilled water [27].

Chemicals

DMBA, TPA, sodium azide (NaN_3), 2-Aminofluorene (2-AF), benzo[α]pyrene (B[a]P) were procured from Sigma Chemical Co., St. Louis, MO, USA.

Parameters measured

Mutagenecity study of the TFGS extract by plate incorporation method [25, 28] :

Sterile top agar was made in separate test tubes (about 2 ml/tube) and maintained at 43-45°C in water bath. Tubes were prepared in triplets for each concentration

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of test material by adding 0.1 ml of bacterial culture, 0.1 ml of seed extract, 0.5 ml of metabolic activation (S9) mix (for 2-AF and B[a]P) or 0.5 ml phosphate buffer (for NaN_3), 0.2 ml Histidin/Biotine solution and 0.1 ml of mutagens NaN_3 (20 µg/plate), 2-AF (5 µg/plate), B[a]P (10 µg/plate, 5 µg/plate). Water or dimethyl sulphoxide (DMSO) was taken in case of negative control.

Antimutagenic effect of TFGS extract

Reverse mutation assay using direct acting mutagen NaN_3, and promutagens 2-AF and B[a]P. The Ames assay was performed with *S. typhimurium* strains TA98 and TA100. The *Salmonella*/microsome reversion assay was conducted using the plate incorporation procedure described by Ames *et al* [28] and revised by Maron and Ames [25]. Based on the results of mutagenicity testing fenugreek aqueous seed extract were tested for its antimutagenic properties at four different concentrations *viz* 2.5, 5, 10 and 20 g/100 ml of distilled water against mutagenic activity of NaN_3, 2-AF and B[a]P by plate incorporation method as discussed above.

Anticarcinogenic effect (cytogenetic study) of TFGS extract

Dose tolerance study of the seed extract

Mice for tolerance study were divided into seven groups; group I served as control and groups II to VII as treatment groups with 9 animals in each group. Animals of the treatment groups were given 25, 50, 100, 200, 400, 800 mg/kg/day of TFGS in double distilled water by oral gavages for 7 consecutive days as per the previous studies carried out in our lab. All these animals were observed regularly till 7 days and no toxic effect were observed in terms of sickness, mortality, morbidity and behavior in animals treated with different doses of TFGS extract. This suggests that extracts of TFGS can be tolerated by mice up to 800 mg/kg/day. Experiments were designed to test the modulatory influence of TFGS seed extracts on mouse hepatic lipid peroxidation (LPO) level and reduced glutathione (GSH) content. Depending upon the level of GSH and decrease in LPO content 400 mg/kg b.wt of TFGS was selected [29].

Anticarcinogenicity study using mouse skin papilloma model

Animals were assorted into control (group I) and experimental groups (groups II-V) (Fig.1). The animals were marked and their body weights were taken. The hair on the dorsal region of the body (back) was removed three days before the commencement of the experiment and only those animals in the resting phase of the hair cycle were selected for the experiments. Mice were shaved before each treatment to allow a better distribution of the chemical.

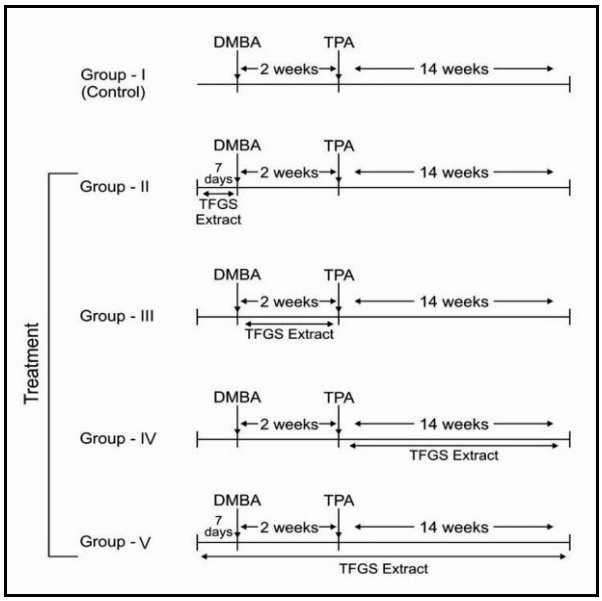

Figure 1. Experimental design

Two stage skin carcinogenesis model was used as reported earlier in our lab [30]. For the induction of tumors/papillomas, the two-stage protocol consisting of initiation with a single topical application of the carcinogen DMBA, followed by three times a week treatment with the promoter TPA, was standardized. Experiments were designed to see the effect of TFGS extract on DMBA/TPA induced skin papillomagenesis.

In control group freshly prepared DMBA solution (100 µl/50 µl acetone/animal) was topically applied on the shaven area of the skin by a micropipette. After an interval of two weeks, 0.2 ml (200 µl) of TPA (2 µg in 200 µl acetone) solution was applied on the initiated area and treatment was continued three times a week for 14 weeks. Preparation of DMBA solution and its application on the skin was done in subdued light to avoid its photoreaction. Whereas in the treatment groups all the animals were given TFGS extract (400 mg/kg b.wt/day) by oral gavage continuously at pre-initiation, post-initiation, promotion and throughout treatment intervals.

Cytogenetic study

Cytogenetic damage in the bone marrow cells were studied by chromosomal aberrations and micronuclei (MN) induction [31].

Chromosomal aberrations

Animals were administered intraperitoneally with 0.025% colchicine to arrest the dividing cells at metaphase. The animals were sacrificed after 75 min by cervical dislocation, the bone marrow cells from femur were flushed with 0.56% KCl and incubated at 37°C for 20 min. Suspension was centrifuged, the pellet was kept in acetic acid:methanol (1:3) for 1 h and

centrifuged again. Then, pellet was resuspended in acetic acid:methanol (1:3), incubated for 10 min and centrifuged. This cycle was repeated thrice. The final cell suspension was dropped on pre-chilled slides, flame dried and stained with 4% Giemsa (Sigma Chemical Co., St. Louis, MO, USA). Well spread metaphases were examined to score the aberrations. A total of 400 metaphase plates were scored per animal. Different types of aberration like chromatid breaks, chromosome breaks, centric rings, dicentrics, acentric fragents and exchanges were scored.

Micronuclei assay

To test the mutagenicity mouse bone marrow micronuclei assay was performed as per protocol suggested by Schmid [32]. For this, the animals were not pretreated with colchicine and the slides were prepared, femurs flushed out in 0.84% sodium chloride solution (NaCl/physiological saline). One or two drops of fetal calf serum (FCS) were added and mixed. Smear was made on a pre-chilled clean slide and left for drying and stained with May-Grunwald and Giemsa stain (Sigma Chemical Co., St. Louis, MO, USA).

The slides were observed using a light microscope. Micronuclei appeared in normochromatic erythrocyte (NCE) and polychromatic erythrocyte (PCE). NCE and PCE can be distinguished by their differential staining property. Using May-Grunwald and Giemsa stain, the MN in NCE appears dark pink, while in PCE it stains light blue. The PCE are also slightly larger than the NCE. A total of 1000 cells were scored per animal [33].

Statistical analysis

All the experiments were carried out thrice and the results are expressed as mean ± standard deviation; statistical evaluations were performed by using one way ANOVA.

RESULTS

Mutagenecity study of the seed extract by plate incorporation method

All the strains of *S. typhimurium* (TA98 and TA100) exposed to different concentrations of TFGS extracts did not show two-fold or greater increase in the mean number of revertants as compared to the negative control group. All strains used in the study exhibited marked increase (> 10-fold) in the number of revertants when treated with positive control agents. The results confirmed the sensitivity of the tester strains to mutagens and thus the validity of the assay. The results indicated that the mean number of histidine revertants in the treatment groups were comparable to the mean number of revertants in the negative control group in TA98 and TA100 both in the absence and presence of metabolic activation. Dry extract of TFGS up to 20 g/100 ml of distilled water both in the presence and absence of metabolic activation was found to be non-mutagenic to *S. typhimurium* test strains (Tables 1 and 2).

Antimutagenic activity of TFGS extract

This test was performed to determine the inhibition of mutagenic activity of the direct acting mutagens NaN_3 (TA100), 2-AF, B[a]P (TA98, TA100). The extract was tested in the dose range of 2.5-20 g/100 ml of water concentration. DMSO was used as solvent control. The level of antimutagenicity was calculated in terms of percent inhibition using following formula**:**

Inhibition (%) = (1-T/M) x 100

-T; number of His+ revertants in the presence of seed extracts

-M; number of His+ revertants in the absence of seed extracts

The antimutagenic effect was considered moderate (M) when inhibition was 25-40%, strong (S) when above 40%, and weak (W) when less than 25%.

A dose related increase in the percentage inhibition was recorded with TA98 and TA100 against the mutagens. At 20 g/100 ml concentration a strong inhibition rate was observed in both TA98 and TA100 against the mutagens. Maximum inhibition was observed against NaN_3 in TA100 at 20 g/100 ml concentration (Tables 3 and 4).

Anticarcinogenicity study using mouse skin papilloma model

Cytogenetic study

Chromosomal aberrations and micronuclei were studied in all the groups of the animals and the following observations were made:

Table 1. Mutagenic activity of TFGS in TA98

TFGS aqueous extract concentration	Number of His+ revertants/plate of TA98 using TFGS extract
20 g/100 ml	132 ± 23.1
10 g/100 ml	57 ± 51
5 g/100 ml	33 ± 25.6
2.5 g/100 ml	59 ± 79.5
Negative control	313 ± 57.4

Table 2. Mutagenic activity of TFGS in TA100

TFGS aqueous extract concentration	Number of His+ revertants/plate of TA100 using TFGS extract
20 g/100 ml	115± 21.9
10 g/100 ml	131 ± 31.8
5 g/100 ml	159 ± 33.6
2.5 g/100 ml	45 ± 25.4
Negative control	234

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Table 3. Antimutagenic activity of TFGS against B[a]P and 2-AF using TA98

TFGS aqueous extract concentration	Number of His+ revertants/plate of TA98 using TFGS extract			
	2-AF		B[a]P	
	S9 metabolic activator		S9metabolic activator	
	Number	Inhibition rate	Number	Inhibition rate
20 g/100 ml	470 ± 25.4**	49.7%	510 ± 26.4**	47.4%
10 g/100 ml	630 ± 36.5**	32.6%	625 ± 12.8**	35.5%
5 g/100 ml	720 ± 26.4**	22.1%	766 ± 31.1*	21.1%
2.5 g/100 ml	795 ± 27.4*	14.9%	840 ± 13*	13.4%
Positive control	935 ± 27.6	-	970 ± 36.2	-
Negative control	264 ± 19.2	-	350 ± 9.6	-

Experiments performed in triplicates and expressed as mean ± standard deviation. Negative control; in which both mutagen and TFGS extract was absent, only DMSO with S9 or water without S9 was used. Positive control; when only mutagen is added not the TFGS. All data was statistically analyzed by one way ANOVA; *****P < 0.05, ******P < 0.001.

Table 4. Antimutagenic activity of TFGS against NaN_3, B[a]P and 2-AF using TA100

TFGS aqueous extract concentration	Number of His+ revertants/plate of TA100 using TFGS extract					
	NaN_3		2-AF		B[a]P	
	Sodium-phosphate buffer		S9 metabolic activator		S9 metabolic activator	
	Number	Inhibition rate	Number	Inhibition rate	Number	Inhibition rate
20 g/100 ml	170 ± 9.6**	66.6%	320 ± 27.5**	57.3%	330 ± 12.9**	57.6%
10 g/100 ml	260 ± 25.6*	49%	385 ± 14.4**	48.6%	465 ± 35.4**	40.3%
5 g/100 ml	375 ± 9*	26.4%	434 ± 39.6**	42.1%	510 ± 17.4**	34.6%
2.5 g/100 ml	390 ± 13*	23.5%	497 ± 17.6**	33.7%	590 ± 12.2**	24.3%
Positive control	510 ± 60.2	-	750 ± 40.6	-	780 ± 29.2	-
Negative control	205 ± 38.6	-	190 ± 20.2	-	240 ± 12.6	-

Experiments performed in triplicates and expressed as mean ± standard deviation. Negative control; in which both mutagen and TFGS extract was absent, only DMSO with S9 or water without S9 was used. Positive control; when only mutagen is added not the TFGS. All data was statistically analyzed by one way ANOVA; ******P < 0.001.

Animals of the group treated with a single topical application of DMBA and two weeks later promoted by TPA (three times a week) showed a significant increase in total chromosomal aberrations (157.84 ± 1.5) in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments with respect to untreated animals (1.45 ± 0.97) (Fig.2). Animals of this group also showed a significant increase in the number of micronuclei (23.09 ± 0.74) (P < 0.001) as compared to untreated normal group (0.35 ± 0.07) (Fig.2).

Group II, in which animals treated with TFGS extract (400 mg/kg/day) at pre-initiation stages of papilloma-genesis, showed a significant reduction in the total chromosomal aberrations (63.74 ± 0.40) (P < 0.001) in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragents as compared to control (164.84 ± 1.5) (Fig.2). A significant reduction was also noticed in the number of micronuclei (7.39 ± 0.67) as compared with control (23.09 ± 0.74) (Fig.3).

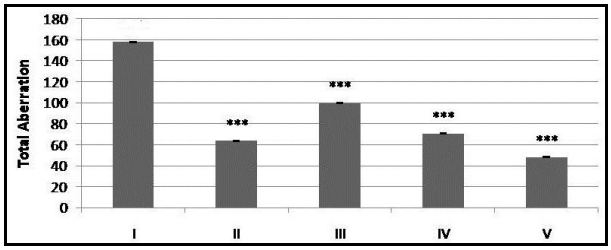

Figure 2. Total Aberrations recorded after initiation by DMBA followed by TPA with or without TFGS (400 mg/kg b.wt./day) treatment at various stages of carcinogenesis. Group 1, control – 16 weeks duration; group II, TFGS for 7 days before the application of DMBA (pre-initiation treatment); group III, TFGS for 14 days after the application of DMBA (post-initiation treatment); group IV, TFGS from the application of TPA (promotional treatment); group V, TFGS for 7 days before the application of DMBA and throughout the experiment; *******P < 0.001 compared to control (group I).

Maximum reduction in total chromosomal aberrations and micronuclei had occurred in Group V, in which animals were treated with TFGS throughout the experiment (Fig.4); the total chromosomal aberrations of this group was 48.35 ± 0.53 (P < 0.001). Animals of this group also showed a significant decrease in the number of micronuclei (5 ± 0.77) (P < 0.001) as compared to control.

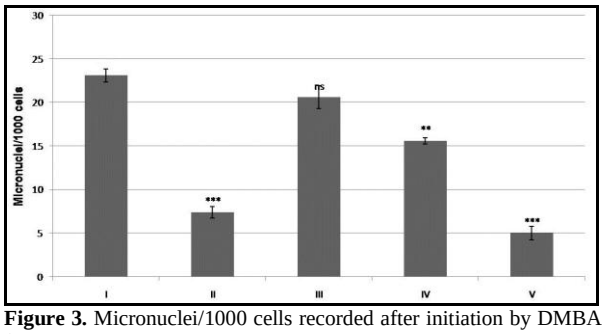

Figure 3. Micronuclei/1000 cells recorded after initiation by DMBA followed by TPA with or without TFGS (400 mg/kg b.wt./day) treatment at various stages of carcinogenesis. Group 1, control – 16 weeks duration; group II, TFGS for 7 days before the application of DMBA (pre-initiation treatment); group III, TFGS for 14 days after the application of DMBA (post-initiation treatment); group IV, TFGS from the application of TPA (promotional treatment); group V, TFGS for 7 days before the application of DMBA and throughout the experiment; $^{ns}P > 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control (group I).

DISCUSSION

Herbs and spices are known to exhibit an array of biochemical and pharmacological activities including antioxidant and anti-inflammatory properties that are believed to contribute to their antimutagenic and anticarcinogenic activities. In the present study a significant dose-dependent antimutagenic effect of four different concentrations of TFGS aqueous extract were demonstrated at defined concentrations of NaN_3 (20 µg/plate) 2-AF (5 µg/plate) and B[a]P (10 µg/plate, 5 µg/plate) in two strains of *S. typhimurium* (TA98 and TA100).

The mutagenicity of NaN_3, 2-AF and B[a]P were defined as 100% or 0% inhibition. NaN_3 causes cytotoxicity in several animal and plant test systems, by inhibiting the protein synthesis [34]. Following metabolic activation, 2-AF and its derivatives react with cellular DNA to form covalent adducts [35]. B[a]P is a procarcinogen; this molecule intercalates in DNA, covalently bonding to the nucleophilic guanine nucleotides at the N2 position [36]. The mutagenicity of all three mutagens is related to reactive oxygen and free radical generation [37].

Gene mutations are readily measured in bacteria and other cell systems when they cause a change in the growth requirements of the cell. The *S. typhimurium*/ microsome assay (*Salmonella* test; Ames test) is a widely accepted short-term bacterial assay for identifying substances that can produce genetic damage leading to gene mutations as well as the effectiveness of antimutagens which has the capacity to normalize the damage by inhibiting the mutagens. In the present study percent inhibition of mutagenesis was observed maximum against NaN_3 as 66.6% in TA100 at highest concentration of TFGS (20 g/100 ml distilled water), followed by B[a]P and 2-AF inhibition. In TA98 the levels of inhibitions were comparatively less as 49.7% and 47.4% against 2-AF and B[a]P, respectively, at the similar concentration.

In this study, we have investigated the anticarcinogenic potential of TFGS during DMBA induced genotoxicity. The cytogenetic parameters used for the study of chemoprevention in mammalian system were chromosomal aberrations and micronuclei frequency; both of which are relatively rapid and sensitive indicators of genetic damage. Chromosomal aberrations and micronuclei appear as a hallmark of neoplastic transformation after exposure to genotoxic agents such as DMBA and the degree of damage is correlated with tumor progression [38]. In control group where only DMBA is used along with TPA the number of total chromosomal aberration and micronuclei was 157.84 ± 1.5 ($P < 0.001$) and 23.09 ± 0.74 ($P < 0.001$) per 1000 cells studied, respectively. The result is well expected as polyploidy and sister chromtid exchanges are the end results of DMBA induced oxidation of DNA bases as well as deoxyribose sugars through its diol-epoxide metabolites [39-41]. The genome is particularly vulnerable to oxidative damage when the antioxidant defences are insufficient. Dietary intake of chemopreventive agents has been suggested as an effective strategy for minimizing the toxic effects of mutagens, genotoxins and carcinogens [42].

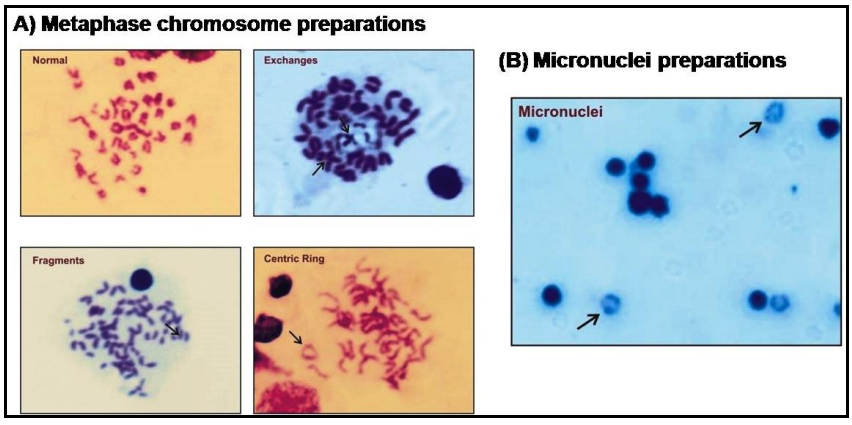

Figure 4.
(A) Metaphase chromosome preparations;
(B) Micronuclei preparations from bone marrow of Swiss albino mice (x1000)

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Among the bioactive compounds isolated from fenugreek seeds are fibers, polysaccharides, protodioscin, diosgenin, yamogenin, gitogenin, trigogenin, trigonellin and others [43, 44]. The phytochemical tests along with estimation of total phenolic compounds carried out in our lab showed positive results for carbohydrates, reducing sugars, secondary metabolites such as tannins, saponins, terpenes, flavanoids, phenols and proanthocyanidins in aqueous extract of TFGS. They play an important role in exhibiting the inhibitory activity against the chemical mutagens and are responsible for antioxidant and antimutagenic property.

Moreover fenugreek has already shown free radical scavenging property during DMBA induced genotoxicity by bringing back the status of thiobarbituric acid reactive substances and antioxidant enzymes to nearly normal range in liver in our previous study [45].

It has been reported that supplementation of diet with fenugreek seeds lowered lipid peroxidation [46-49]. In the present evaluation it has been observed that maximum reduction in the number of aberrations at the chromosome level was recorded in throughout treatment group followed by pre-initiation probably by elevating the antioxidant defense of the body. Similar reduction in micronuclei number was also noticed with maximum decrease at throughout treatment group. When the seed extract was applied from 7 days before the application of DMBA till the end it exhibited an overall improvement in the antioxidant defense and combated the damage to the spindle apparatus. Sakr *et al* [50] also reported ameliorative activity of fenugreek seed extract against adriamycin-induced cytotoxicity in albino rats.

Human body has an array of antioxidant defence mechanisms to combat the harmful effects of reactive oxygen species generated by genotoxic agents [51]. ROS mediated oxidative stress has been implicated in the pathogenesis of several diseases including oral cancer and as intracellular modifier of DNA [52]. Our result implies that TFGS has maintained the status of detoxifying agents during mutagenicity and carcinogenicity by enhancing the antioxidant defence mechanism which neutralizes the toxic effects of reactive oxygen species generated by mutagens and carcinogens. This promising role of TFGS adds another anticancer agent in the armamentarium of naturally occurring agents.

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