

In vivo Study of Aqueous Extract of *Zingiber officinale* in Modulating DMBA Induced Genotoxicity in Albino Rats

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ABSTRACT

Objective: To study the anti-genotoxic properties of aqueous Ginger extract on Dimethyl benz (a) anthracene induced genotoxicity in rat bone marrow cells.

Methods: Group I (Normal control) animals were administered with distilled water (DW). Group III and IV animals were administered with 250mg/kg and 500 mg/kg aqueous extract of *Zingiber officinale* (ZE) orally, for seven consecutive days. After twenty four hours of the last dose of test drug, Group II, III and IV rats were injected with Dimethyl benz (a) anthracene (DMBA) 30 mg/kg b.w. intraperitoneally. Group V and VI animals were kept with standard diet and 250mg/kg and 500mg/kg aqueous extract of *Zingiber officinale* (ZE) alone respectively. After twenty four hours of DMBA treatment, to arrest metaphase inject Colchicine (2 mM) 3 mg/kg body weight intraperitoneally before the animals were sacrificed.

Results: Scoring of micronuclei in polychromatic (P) and normochromatic (N) erythrocytes followed by chromosomal aberrations were performed in bone marrow cells of animal. Both 250mg/kg and 500mg/kg body weight of Ginger extract produced a significant inhibition of DMBA induced genotoxicity like ratio of polychromatic to normochromatic (P/N) and frequency of micronuclei. It decreased the total chromosomal aberrations significantly, the DMBA induced chromosomal breaks, gaps, rings, deletions and other abnormalities in bone marrow cells were inhibited. Other biochemical parameters like plasma MDA, SOD and liver MDA, SOD and GSH were towards normal in DMBA injected rats in a significant extent ($p < 0.001$).

Conclusion: Aqueous extract of *Zingiber officinale* has anti-genotoxic property against DMBA induced genotoxicity.

Keywords: Chromosomal aberrations, DMBA, Genotoxicity,

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Zingiber officinale.**INTRODUCTION**

Carcinogen induced mutation plays a crucial role in the pathogenesis of several diseases including cancer.^{1,2} DMBA, is a potent carcinogen which on metabolism form diol epoxides and other reactive oxygen species (ROS).³ These toxic reactive oxygen species can induce chromosomal abnormalities and increase number of micronuclei through oxidative base damage and breakdown of DNA strand, leads to mutagenesis and carcinogenesis.⁴ Over the past decade medicinal plants product has gained importance that the capacity to synthesize a wide variety of phytochemicals that have actions against reactive oxygen species (free radical scavenging properties).^{5,6} Different food additives like spices and condiments have been used to enhance the taste and flavor of our preparation since ancient times. People in many parts of the world often consume several kinds of spices such as coriander, caraway, chili, pepper, black pepper, garlic, onion, cinnamon, etc. Members of Zingiberaceae (ginger species) have been shown to have antioxidant, anti-inflammatory, and anticancer activities.^{7,8} The important active components of the ginger rhizome are thought to be volatile oils and pungent principles like phenolic compounds such as gingerols [6-gingerol], shogaols, zingerone, and gingerols.^{8,9} Several authors have demonstrated that the ginger endowed with strong in-vitro and in-vivo anti-oxidant properties.^{10,11} The anti-genotoxic action of ginger has been found as one of the possible mechanism of oxygen free radical scavenging followed by decreased production of reactive oxygen species.^{4,6} The protective role of ginger powder on DMBA induced carcinogenesis has been evaluated in an *In vitro* study to

explaining its anti-genotoxic potential.^{8,11,12} Nevertheless, of the large number of Zingiberaceae spp. that have been used for culinary and/or medicinal purposes, only a few members have been studied for their potential anticancer activity. In this study, the edible plant ginger's (*Zingiber officinale*) aqueous extracts were prepared and to evaluate the anti-genotoxic potential of on 7, 12-Dimethyl Benz (a) anthracene (DMBA) induced genotoxicity in male wistar rats.

MATERIAL AND METHODS

Male Wistar rats of 6-8 weeks old were obtained from the National Institute of Nutrition, Hyderabad. The animals were housed individually in separate cages in standard laboratory condition (temperature 22-25°C, relative humidity 65 ± 5% and 12 h light/dark cycle). They were fed with standard diet (NIN formula) and water *ad libitum*. Prior to the experiment, they were acclimatized to the laboratory conditions for 1 week.⁶ This study protocol was approved by Institutional Animal Ethics Committee (Regd. No. 472/2005/CPCSEA) and the experiment was conducted as per OECD guidelines.

Drugs and chemicals

Rhizome of *Zingiber officinale* were purchased from local market and authenticated by Prof. M.K. Mishra, Department of Botany, Berhampur University.

DMBA (Sigma, USA) Colchicine, May-Grunwald stain, Giemsa stain, fetal bovine serum were purchased from Himedia Laboratories, Mumbai, India. All the chemicals were of analytical grade.

Preparation of the plant extract

Ginger rhizomes were purchased from local market. The aqueous extracts of *Zingiber officinale* were prepared according to the method of Hossain *et al* (1992). One kg of fresh Zinger rhizome was washed, chopped, dried, powdered and then soaked in 2 lit of water for 3hrs then heated at 60-65⁰C for 30 minutes, the extract was collected and the processes were repeated three to four times with the residual. The collected extract was pooled and passed through fine cloth. The filtrates were evaporated in air. The final greenish brown semisolid yield was 5.7 % (w/w). The extract was stored at 0-4⁰C until use.¹² Ginger extracts rich in gingerols, shogaols, and bother substances (gingerdiols, diarylheptanoids, zingiberene, arcurcumene, beta-bisabolene, neral, geranial, D-camphor, beta-phellandrene, geranial, linalool, E-alpha-farnesene, beta-eudesmol) have recently gained significant interest for their capacity to interfere with cancer at the initiation, progress and treatment phases. The gingerols (such as 6-, 8-, and 10-gingerols), a series of phenolic compounds present in ginger root (ginger containing 1.0–3.0% gingerols); have been shown to have chemo preventative effects that are related to their antioxidative and anti-inflammatory activities.^{4,7,13}

Experimental protocol

Thirty-six male wistar albino rats were divided into 6 groups (n=6). All are fed with Standard diet and water *ad libitum*.

Group I (control): Distilled water.

Group II (Disease control): 7, 12-Dimethyl benz (a) anthracene (30 mg/kg ip).

Group III and IV (Test drug): 250mg/kg and 500mg/kg aqueous extract of *Zingiber officinale* (7 days)^{13,14} + 7, 12-Dimethyl benz (a) anthracene (30mg/kg i.p.) one hour after the last dose to induce genotoxicity.^{15,16}

Group V and VI (Test drug): 250mg/kg and 500mg/kg aqueous extract of *Zingiber officinale* alone for 7 days).

(After 7 days, after treatment of test drug there was no more change in their body weight.)

Twenty four hours after the 7, 12-Dimethyl benz(a) anthracene injection, all the animals was injected with 2 mM of colchicine 0.5 ml/100 gm body weight (i.p.).^{6,7} After two hours, the animals were anaesthetized with (pentobarbitone 45 mg/kg ip) and blood samples were collected for estimation of superoxide dismutase (SOD) and malondione aldehyde (MDA). Then animals were sacrificed by cervical dislocation and bone marrow of femurs was collected for the assessment of bone marrow micronucleus frequency¹⁶ and chromosomal aberrations.¹⁷ Livers were also excised for estimation of SOD, MDA and GSH.^{18,19}

For Bone marrow micronucleus assay the method developed by Schmid (1975) was followed.¹⁷ Quickly, the bone marrow from femur was collected in a tube containing 1 ml of fetal bovine serum and centrifuged at 3000 rpm for 5 min.⁷ The collected pellet was re-suspended with few drops of fresh fetal bovine serum and vortexed. One drop of the suspension was smeared on coded glass slides, air dried, fixed in methanol, stained with May-Gruenwald stain followed by Giemsa. After drying, the polychromatic erythrocytes (2000 PCEs) were screened for micronuclei (MNPCEs) and normochromatic erythrocyte (NCEs) to calculate PCE/NCE ratio.⁶

The method of Adler and Savage was adopted for the chromosomal aberration test. Quickly after sacrificing the rats, the femoral bone marrow was flushed in to tube containing 6 ml of hypotonic KCl (0.075M) The marrow suspension was incubated at 37⁰C for 15-20 min and centrifuged at 1000 rpm for 10 min. The pellet was mixed with the methanol: acetic acid fixative (3:1). The

suspension was allowed to stand for 30 min and then centrifuged. The pellet was mixed thoroughly with fresh fixative and 2-3 drops of the suspension was dropped on a clean glass slides. The slides were flame-dried and stained with 10% Giemsa at pH 6.8 for 15-20 min. Coded slides were screened for chromosome abnormalities. One hundred well-spread metaphase plates per animal were scored for structural chromosomal aberrations and recorded. Concurrently, total chromosomal abnormalities (excluding gaps), number of abnormal metaphase plates per one hundred metaphase plates was counted per rat, and mitotic index was calculated.^{20,21}

MDA in plasma and liver was estimated by the method of Dhale *et al*, 1962²². The method of P. Kakar *et al*, 1984²³ Sedlak and Lindsay 1968²⁴ was followed for estimation of SOD and liver GSH respectively.^{18,19}

Statistical analysis

The data were expressed as mean \pm SEM. Statistical comparison were performed by One Way ANOVA followed by Duncann's multiple range test (DMRT). Results with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of *Zingiber officinale* extract on bone marrow micronucleus assay

The frequency of micronuclei, P/N ratio and % PCE are expressed in table-I. Animals was treated with DMBA alone produced a significant increase ($p < 0.05$) in micro nucleated cells in polychromatic erythrocytes and reduced P/N ratio significantly as compared to the control group animals. There was decrease in MNPCE and increase in P/N ratio with pre-treatment of 250mg/kg and 500mg/kg ginger extract to DMBA injected rats to a significant extent ($p < 0.05$). Also ginger

extract alone in both the doses did not alter the MNPCE, P/N ratio and % PCE as compared to the control group.

Effect of *Zingiber officinale* extract on bone marrow chromosomal aberration assay

The results of chromosomal analysis in bone marrow cell at metaphase stage are depicted in Table-II. The mitotic index of DMBA treated rats (gr-II) was reduced significantly along with a significant increase in chromosomal breaks, rings, other chromosomal abnormalities (deletion, fragmentation, minutes etc.) and total chromosomal abnormalities as compared to the control rats. Both the 250mg/kg and 500 mg/kg ginger extract treated rats with DMBA treatment showed a significant ($p < 0.05$) difference in mitotic index and all the abnormal chromosomal patterns in comparison to the DMBA treated rats (gr.-II). No significant difference was observed between control rats and rats fed with 250mg/kg and 500mg/kg ginger extract alone with respect to chromosomal aberrations.

Effect of *Zingiber officinale* extract on biochemical parameters

The effect of ginger on various biochemical changes in Plasma and liver of different treatment groups (Table III). DMBA alone increased MDA level and decreased SOD in Plasma and liver significantly ($p < 0.05$) as compared to the control animals. Treatment with 250mg/kg and 500 mg/kg ginger extract plus DMBA significantly altered the above said enzymatic changes with significant increase in liver GSH in comparison to the DMBA control group ($p < 0.05$). No significant difference was observed between two doses of ginger extracts alone as compare to the control animals.

DISCUSSION

The present study evaluates anti-genotoxic effect of ginger extract in wistar rats. Compounds have antioxidant properties are known to produce anti-genotoxic effect by reducing toxic free radicals. In a number of studies, emphasis has been laid down on use of certain dietary constituents for prevention of many drug and chemical induced genotoxicity in different phytoconstituents like Aloe Vera, Turmeric, Adhatoda vasaca, lipoic acid, garlic, curcumin, ginger, piperin, carotenoids and vit-C and vit-E.^{7,24,27} With such a background this study has been carried out to evaluate any anti-mutagenic activity against 7, 12-Dimethylbenz (a) anthracene (DMBA) induced genotoxicity.

DMBA present in the environment as a product of incomplete combustion of complex hydrocarbons is an indirect carcinogen. It forms DNA attacking toxic reactive oxygen free radical which causes chromosomal aberrations.²⁸ Both *In vivo* and *In vitro* studies have demonstrated polyploidy and sister chromatid exchanges in DMBA induced genotoxicity. An increase in micronucleus frequency and chromosomal aberrations in bone marrow of DMBA painted rodents have been reported.^{29,30} In many research studies DMBA has been proved to be a mutagenic and carcinogenic due to generation of excess toxic reactive oxygen free radical and induce lipid peroxidation.⁷

Single intraperitoneal injection of DMBA (30 mg/kg B.W.) showed a significant increase in frequency of MNPCs and decrease in P/N ratio as well as % PCEs in comparison to standard diet treatment group (Table-I). These findings corroborate with that of Pachaiappan and Shanmugam *et al*, 2009.³¹

In chromosomal aberration test, DMBA even reduced mitotic index, increased frequency of chromosomal break,

ring and other multiple aberrations like minutes and fragments to a highly significant extent (Table-II). A similar observation was recorded by Kuppusamy *et al*, 2008.⁵ On biochemical parameters, DMBA decreased plasma SOD, liver SOD, liver GSH but increased the lipid peroxidation marker that is plasma MDA and liver MDA to a highly significant extent.(Table-III). Our results thus suggest that the observed increase in the frequency of MnPCEs and chromosomal aberrations in DMBA treated rats are due to excessive generation of reactive oxygen free radicals. Pachaiappan *et al*, 2009 and Subramanian *et al*, 2008 also observed similar biochemical changes following DMBA injection.³⁰⁻³²

Administration of 250 mg/kg and 500 mg/kg ZE for 7 days prior to DMBA injection, significantly reversed the frequency of MNPCs, the alteration in %PCEs and P/N ratio on bone marrow micronucleus assay. (Table-I) Also this dose of ZE modified the DMBA induced changes in mitotic index, incidence chromosomal break, ring and other multiple aberrations to a significant extent as compared to that of DMBA treated group alone (Table-II). All these above parameters of the chromosomal aberration test and micronucleus test following ZE treatment were comparable to that of standard group alone. These were shown in figure 1. A similar observation was found in the study of K. Nirmala *et al*, 2007 who have reported the *In vivo* antimutagenic potential of Ginger on formation and excretion of urinary mutagens in rats using an *In vitro* assay method.²⁰

With respect to biochemical estimations, pretreatment with 250 and 500mg/kg ZE restored the DMBA induced decrease in plasma and liver SOD, liver GSH significant but result due to 500mg/kg is nearer to normal level (comparable to control animal) (Table-III). Again DMBA induced rise in lipid peroxidants, measured

from plasma and liver was reduced to near normal by 500mg/kg ZE pretreatment (Table-III). Our observations corroborate with that of Sekiwa *et al*, 2000 and Zancan *et al*, 2002.³²⁻³⁴ Sradhasini Rout *et al.*, 2014 reported that 10% ginger powder mixed diet given for 30 days showed anti genotoxicity, in contrast to that in present study aqueous extract of *Zingiber officinale* produce significant anti-genotoxicity with shorter duration of treatment for 7 days.²

The strong antioxidant activity of ginger is well documented in earlier studies.⁶⁻¹² Considering the observations in our study, the anti-genotoxic activity exerted by Ginger could be explained through its anti oxidant activity against DMBA induced oxidative stress and subsequent nuclear damage in erythropoetic cells.

CONCLUSION

Thus in our *In vivo* study, Ginger possessed anti-genotoxic effect along with antioxidant activity in wistar albino rats The regular consumption could be therefore beneficial to counteract the adverse effects by enhancing antioxidant defense mechanism and neutralizing the toxic effect of reactive oxygen species generated by environmental genotoxicants. More research studies on other genotoxic models are suggested to establish the role of ginger against the genotoxicants.

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Table I. Effects of ginger (aq. extract) in the diet on DMBA-induced micronuclei in bone marrow cells

Group	Drug & dose	No. of MNPCE	%Of MNPCE	PCE/NCE	%Of PCE
I	DW	1.33 ^a ±0.5	0.07 ^a ±0.02	0.97 ^a ±0.03	49 ^a .12
II	DMBA (30mg/kg)	13.50 ^a ±1.06	0.66±0.05 ^b	0.41 ^b ±0.01 ^b	28.94
III	ZE 250 mg/kg +DMBA	4.17 ^b ±0.66	0.31 ^b ±0.03	0.71 ^b ±0.01	39.77
IV	ZE 500 mg/kg +DMBA	2.50 ^{b,c} ±0.43	0.12 ^{b,c} ±0.02	0.83 ^{b,c} ±0.02	45.17
V	ZE 250 mg/kg	1.11 ^c ±0.37	0.06 ^c ±0.04	1.0 ^c ±0.03	49.45
VI	ZE 500 mg/kg	1.17 ^c ±0.48	0.05 ^c ±0.02	1.06 ^c ±0.02	51.40

Values are expressed as mean ± SE (n=6). Data analyzed by one-way ANOVA with post hoc Duncan's multiple range test (DMRT). The values not showing common super script differ significantly (p < 0.05)*Percentage of polychromatic erythrocytes was calculated as follows: [PCEs / (PCEs + NCEs) × 100.

Table II. Effect of *Zingiber officinale* (aq. extract) on bone marrow chromosomal aberration assay

Group	Drug & dose	Mitotic index (%)	Chromosomal aberration per rat				Total aberrations per rat
			Gap	Break	Ring	Other aberration	
I	DW	4.18±0.40	0.83±0.31	1.50±0.43	0.33±0.21	0.83±0.31	2.67±0.85
II	DMBA 30mg/kg	1.07±0.19	12.17±0.9	14±1.07 ^b	5.17±0.79 ^b	12±0.93 ^b	31.17±1.62 ^b
II	ZE 250 mg/kg+DMBA	2.75 ^b ±0.2	5.83 ^b ±0.6	8.33 ^b ±0.81	2.83 ^b ±0.31	7.50 ^b ±0.67	18.67 ^b ±0.85
III	ZE 500mg/kg+DMBA	3.93 ^{b,c} ±0.3	3.00 ^{b,c} ±0.3	3.50 ^{b,c} ±0.43	1.67 ^{b,c} ±0.3	3.17 ^{b,c} ±0.48	8.33 ^{b,c} ±0.72
IV	ZE 250mg/kg	4.31 ^c ±0.38	0.92 ^c ±0.16	1.30 ^c ±0.07	0.41 ^c ±0.12	0.75 ^c ±0.53	2.37 ^c ±0.24
V	ZE 500mg/kg	4.50 ^c ±0.18	1.00 ^c ±0.26	1.00 ^c ±0.37	0.5 ^c ±0.22	0.67 ^c ±0.33	2.17 ^c ±0.84

Values are expressed as mean ± SEM. Data analyzed by one-way ANOVA with post hoc Duncan's multiple range test (DMRT). The values not showing common super script differ significantly (p < 0.05).

*Mitotic index has been calculated by analyzing 1000 cells/animal (for a total of 6000 cells/treatment) and percentage of the mitotic cells calculated for each treatment group.

**Frequency per 100 cells. Each chromosomal aberration has been counted by analyzing 100 cells/animal (6 animals/group, for a total of 600 cells/treatment) and mean ±SE were calculated per treatment group. Gaps were not included in total chromosomal aberrations.

Table III. Effect of *Zingiber officinale* (aq. extract) on biochemical parameters

Group n=6	Drug & dose	Plasma		Liver		
		SOD U/ml	MDA nmol/ml	SOD U/100mg	MDAnmol/100 mg	GSH μ mol/100mg
I	DW	32.3 \pm 0.87	3.25 \pm 0.26	23.33 \pm 1.37	6.14 \pm 0.36	5.83 \pm 0.84
II	DMBA (30mg/kg)	20.51 \pm 0.73	6.46 \pm 0.35 ^a	18.59 \pm 1.24 ^a	11.92 \pm 0.72 ^a	7.58 \pm 0.87 ^a
III	ZE250mg/kg + DMBA	28.61 ^{b,c} \pm 0.87	4.29 ^{b,c} \pm 0.45	21.91 ^{b,c} \pm 1.21	8.04 ^{b,c} \pm 0.34	12.08 ^{b,c} \pm 0.77
IV	ZE500mg/kg +DMBA	31.42 ^{b,c} \pm 0.95	4.08 ^{b,c} \pm 0.29	25.25 ^{b,c} \pm 1.21	6.46 ^{b,c} \pm 0.33	15.00 ^{b,c} \pm 0.92
V	ZE 250mg/kg	31.85 ^c \pm 0.80	3.19 ^c \pm 0.17	24.39 ^c \pm 0.68	5.73 ^c \pm 0.44	13.17 ^c \pm 0.34
VI	ZE 500mg/kg	31.9 ^c \pm 0.80	3.08 ^c \pm 0.19	26.19 ^c \pm 0.88	5.46 ^c \pm 0.24	14.17 ^c \pm 0.84

Values are expressed as mean \pm SE (n=6). Data analyzed by one-way ANOVA with post hoc Duncan's multiple range test (DMRT). The values not showing common super script differ significantly ($p < 0.05$).

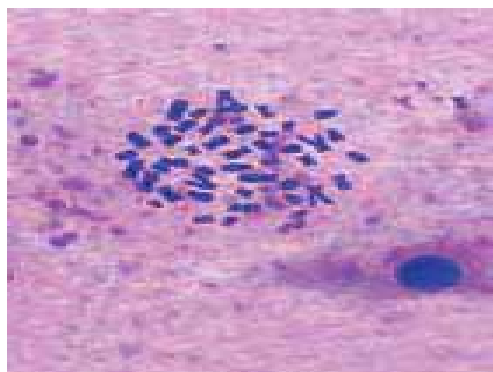
**A. Normal control****B. DMBA treated (abnormal chromosomes)****C. 250mg/kg****D. 500mg/kg**

Figure 1. Photomicrograph showing chromosomal structure and abnormal chromosomes in different groups