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Protective Effect of *Jasminum grandiflorum* Linn. On DMBA-induced Chromosomal Aberrations in Bone Marrow of Wistar Rats

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Abstract: The present study has investigated the protective effects of *Jasminum grandiflorum* flowers and leaves in 7,12-dimethylbenz(a)anthracene (DMBA) induced chromosomal abnormalities in bone marrow of female wistar rats. Micronuclei quantification and detection of chromosomal aberrations were performed to detect and quantify the genotoxic action of DMBA. Oral pretreatment of *J. grandiflorum* flower and leaf extracts to DMBA treated rats significantly reduced the frequency of micronucleated polychromatic erythrocytes (MnPCEs) the in rat bone marrow. Also, the plant extracts significantly decreased the percentage of aberrant cells; the number of chromatic and chromosomal breaks in DMBA treated rats. Present results thus demonstrate that the plant products of *J. grandiflorum* (flowers and leaves) have potent protective effects in DMBA-induced chromosomal aberrations in female wistar rats.

Key words: DMBA, chromosomal aberrations, micronuclei, *Jasminum grandiflorum*

INTRODUCTION

Several studies have consistently shown that the development of cancer is related to genetic damage rate in somatic cells. Agents that can alter an organism's genome by causing toxic effect on cellular genetic materials are referred to as mutagenic. Such substances increase the error rate in the reduplication of the genome and induce mutations by damaging the organism's DNA (Schmid, 1973; Agarwal *et al.*, 1994). An abnormality in chromosomal structure (translocation, deletion, duplication) and chromosomal number (aneuploidy and polyploidy) due to exposure to chemical or physical carcinogen and/or mutagen is referred to as chromosomal aberrations. Cytogenetic studies have shown chromosomal aberrations in cell populations of both human and experimental cancer (Agarwal *et al.*, 1994; Gaizev *et al.*, 1996). An increase in chromosomal breakage and chromosome loss is associated with increased risk of cancer and in the progression of neoplastic transformation (Guerin *et al.*, 1978). Bone marrow micronucleus test and detection of chromosomal

aberrations have been widely used as a tool to indicate carcinogen-induced DNA damage as well as to assess the antimutagenic effect of natural and synthetic chemopreventive agents (Hagmar *et al.*, 2001; Bhuvaneshwari *et al.*, 2004).

Carcinogen induced mutations in somatic cells have been implicated in cell death and pathogenesis of several diseases including cancer. The mutagenic and carcinogenic action of mutagenic substances involves overproduction of DNA-attacking reactive oxygen species (Ames *et al.*, 1973). 7,12-dimethylbenz (a) anthracene (DMBA), the polycyclic aromatic hydrocarbon, is an immunosuppressor and potent organ-specific carcinogen. It has been reported that DMBA exposure result in a marked increase in tumor burden and tumor volume in rodent models and pronounced mutagenic response in several *in vivo* and *in vitro* mutation assay systems (Chang *et al.*, 1996). Studies have demonstrated the DNA damaging and mutagenic effects of DMBA in experimental models (Chandra *et al.*, 2003). Both *in vivo* and *in vitro* studies have demonstrated polyploidy and sister chromatic exchanges in DMBA-

induced genotoxicity (Guerin *et al.*, 1978; Bhuvaneswari *et al.*, 2004). It has been suggested that the N-ras mutation is an earliest event in DMBA-induced leukemogenesis (Osaka *et al.*, 1996).

Medicinal plants exert their chemopreventive potential by interfering with covalent interaction of a carcinogen with DNA, modifying DNA repair process, antioxidant properties and preventing cellular proliferation. Profound evidence has revealed that medicinal plants can reduce genetic damages induced by mutagens and carcinogens (Premkumar *et al.*, 2004; Balasenthil *et al.*, 2000). Although the protective roles of some medicinal plants were reported in carcinogen-induced genotoxicity, several other medicinal plants remain to be investigated. *Jasminum grandiflorum* is a well-known glabrous twining shrub widely grown in gardens throughout India. *J. grandiflorum* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. *J. grandiflorum* flowers are traditionally used to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding (Joshi, 2000). We recently demonstrated the anticarcinogenic and antilipidperoxidative effects of *J. grandiflorum* in DMBA-induced mammary carcinogenesis (Kolanjiappan *et al.*, 2005). However, no reports are available on the protective action of *J. grandiflorum* in DMBA induced chromosomal aberrations in wistar rats. The present study was thus designed to evaluate the protective effect of *J. grandiflorum* flowers and leaves against DMBA-induced chromosomal aberrations in bone marrow of female wistar rats.

MATERIALS AND METHODS

Animals: Female Wistar albino rats, 7-8 weeks old, weighing 130-140 g were used for the study. The animals were obtained from central animal house, Rajah Muthiah Institute of Health Science, Annamalai University, Annamalainagar, India. The rats were housed in polypropylene cages and were maintained in controlled atmosphere (temperature of $22\pm 2^\circ\text{C}$ and 50-70% humidity) with a 12 h light: dark (LD) cycles in an experimental room. The local institutional animal ethics committee, Annamalai University, Annamalainagar, India, approved the experimental design.

Chemicals: The carcinogen, 7, 12-dimethylbenz (a) anthracene (DMBA), colchicine, Giemsa, May-Grunwald's stain were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

Preparation of the plant extract: *J. grandiflorum* flowers and leaves were obtained from the gardens around Chidambaram, Tamil Nadu, India, and identified by the Botanist, Department of Botany, Annamalai University. A voucher specimen was deposited, in the Department of Botany, Annamalai University.

The aqueous and ethanolic extracts of *J. grandiflorum* flowers and leaves were prepared according to the method of Hossain *et al.* (Hossain *et al.*, 1992). Five hundred gram of fresh flowers or leaves of *J. grandiflorum* were dried, powdered and then soaked in 1500 mL of 95% ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapour at $40-50^\circ\text{C}$ under reduced pressure. An 11% semisolid light yellow material of *J. grandiflorum* flowers and 12% semisolid light greenish yellow material of leaves were obtained.

Hundred gram of dried fine powder of *J. grandiflorum* leaves or flowers were suspended in 250 mL of water for 2 h and then heated at $60-65^\circ\text{C}$ for 30 min. The extract was collected and the processes were repeated three times with the residual powder and the collected extract was pooled and passed through fine cotton cloth. The filtrates were evaporated at $40-50^\circ\text{C}$ in a rotavapour. *J. grandiflorum* leaves yielded 13% greenish brown semisolid and flowers yielded 12.5% brownish semisolid. All the extracts were stored at $0-4^\circ\text{C}$ until used. A known amount of the residual extract ($300\text{ mg kg}^{-1}\text{ bw}$ (ethanolic), $500\text{ mg kg}^{-1}\text{ bw}$ (aqueous)) was suspended in distilled water and was orally administered to the animals by gastric incubations using force-feeding needle during the experimental period.

A total number of 60 animals were divided into 10 groups and each group contained 6 animals. Groups 2 to 5 animals were pretreated with aqueous extract of *J. grandiflorum* flowers ($500\text{ mg kg}^{-1}\text{ bw}$), ethanolic extract of *J. grandiflorum* flowers ($300\text{ mg kg}^{-1}\text{ bw}$), aqueous extract of *J. grandiflorum* leaves ($500\text{ mg kg}^{-1}\text{ bw}$) and ethanolic extract of *J. grandiflorum* leaves ($300\text{ mg kg}^{-1}\text{ bw}$), respectively for five days. At the end of the 5th day, groups 1 to 5 animals were intraperitoneally injected with DMBA ($30\text{ mg kg}^{-1}\text{ bw}$, single dose) after 2 h of administration of the plant extracts. Groups 6 to 9 animals were received aqueous extract of *J. grandiflorum* flowers ($500\text{ mg kg}^{-1}\text{ bw}$), ethanolic extract of *J. grandiflorum* flowers ($300\text{ mg kg}^{-1}\text{ bw}$), aqueous extract of *J. grandiflorum* leaves ($500\text{ mg kg}^{-1}\text{ bw}$) and ethanolic extract of *J. grandiflorum* leaves ($300\text{ mg kg}^{-1}\text{ bw}$) alone respectively and were not received DMBA. Groups 10

served as control and all animals were provided standard pellets and water *ad libitum*. All the animals were sacrificed at the 6th day by cervical dislocation for the assessment of micronucleus and chromosomal aberrations.

Assessment of chromosomal aberrations in bone marrow was carried out according to the procedure of (Kilian *et al.*, 1977). The femur bones were removed from animals injected intraperitoneally with 0.1% colchicines, (1 mL/100 g body weight 90 min before sacrificing the animals. The bone marrow contents were flushed into 5 mL of physiological saline and centrifuged at 500 g for 5 min. The sediment obtained were resuspended in 6 mL of hypotonic KCl (0.075 M) and incubated at 37°C for 25 min. The pellets were then fixed using methanol: acetic acid (3:1) fixative and stained with Giemsa stain. One hundred well spread metaphase cells were scored for each animal and structural chromosomal aberrations were observed and recorded.

Bone marrow micronucleus test was carried out according to the method of Schmid (1975). The femur bones removed from the Wistar rats were cleaned and the content was flushed into tube containing 1 mL of calf serum and was centrifuged at 500 g for 10 min. The obtained pellet was suspended with few drops of fresh serum and slides were prepared and air-dried for 18 h.

After drying, the slides were stained with May-Grunwald stain followed by Giemsa stain. The frequency of MnPCEs in each group was calculated by scoring 2500 polychromatic erythrocytes (PCEs) per animal.

Statistical analysis: The values are expressed as mean±SD. The statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). p-values less than 0.05 were considered statically significant.

RESULTS

The frequency of MnPCEs and chromosomal aberrations in control and experimental animals in each group are given in Table 1 and 2, respectively. All groups (groups 2-5) treated with DMBA showed a high frequency of MnPCEs and chromosomal aberrations (structural aberrations: chromatic gap, chromosomal gap, chromatic break, chromosomal break, fragment, minute) as compared to control animals. However, rats treated with DMBA alone (group 1) showed highest frequency of MnPCEs and chromosomal aberrations as compared to control rats. The frequency of MnPCEs and chromosomal abnormalities were significantly reduced in DMBA treated animals pretreated with oral

Table 1: Effect of *J. grandiflorum* on DMBA-induced bone marrow micronuclei formation

Group	Parameters	MnPCEs/ 2500PCEs [#]	PCEs/NCEs	PCEs ⁺ (%)
1	DMBA	67.59±4.21 ^a	0.81±0.06 ^c	44.80
2	<i>J. g</i> Flower (Aqueous Ext) → DMBA	39.34±2.62 ^b	0.94±0.05 ^b	48.40
3	<i>J. g</i> Flower (Alcoholic Ext) → DMBA	26.32±2.83 ^c	0.91±0.06 ^c	47.50
4	<i>J. g</i> Leaf (Aqueous Ext) → DMBA	41.15±2.95 ^d	0.95±0.05 ^b	48.60
5	<i>J. g</i> Leaf (Alcoholic Ext) → DMBA	31.91±3.62 ^b	0.92±0.07 ^b	47.90
6	<i>J. g</i> Flower (Aqueous Ext)	6.86±0.92 ^e	1.04±0.06 ^{bc}	51.10
7	<i>J. g</i> Flower (Alcoholic Ext)	5.41±0.56 ^e	1.02±0.07 ^d	50.00
8	<i>J. g</i> Leaf (Aqueous Ext)	7.13±0.57 ^e	1.04±0.06 ^d	51.00
9	<i>J. g</i> Leaf (Alcoholic Ext)	5.93±0.52	1.01±0.08 ^d	49.90
10	Control	5.76 ±0.34 ^e	1.01±0.07 ^d	50.10

[#]Values are expressed as mean±SD (n = 6; 2500 PCEs were scored per animal). Values not sharing a common superscript significantly differ at p<0.05. (DMRT) ⁺Percentage of polychromatic erythrocytes was calculated as follows: [PCEs/(PCEs+NCEs)] ×100

Table 2: Mitotic index and frequencies of chromosomal abnormalities in experimental and control animals

Group	Parameters	Mitotic index (%) ^A	Chromosomal aberrations ^B rat ⁻¹					Total aberrations rat ⁻¹	Abnormal metaphase rat ⁻¹
			G *	B'	B''	F	M		
1	DMBA	1.76±0.06 ^a	12.92±0.18	7.54±0.24	2.45±0.192	7.10±0.76	2.36±0.15	18.63±1.54 ^a	15.15±1.85 ^a
2	<i>J. g</i> Flower (Aqueous Ext) → DMBA	2.31±0.83 ^b	7.51±0.08	4.93±0.17	1.17±0.06	5.16±0.50	1.17±0.06	12.12±1.62 ^b	10.31±1.61 ^b
3	<i>J. g</i> Flower (Alcoholic Ext) → DMBA	2.48±0.91 ^b	5.17±0.07	3.48±0.10	1.16±0.05	3.33±0.54	1.01±0.06	9.21±1.01 ^b	6.27±1.59 ^b
4	<i>J. g</i> Leaf (Aqueous Ext) → DMBA	2.43±0.76 ^b	6.93±0.07	5.14±0.07	1.00±0.05	5.60±0.52	1.50±0.08	11.26±1.81 ^b	9.35±1.65 ^b
5	<i>J. g</i> Leaf (Alcoholic Ext) → DMBA	2.12±0.92 ^b	5.16±0.06	3.61±0.08	1.16±0.06	3.16±0.51	1.33±0.07	9.14±1.47 ^b	6.92±1.07 ^b
6	<i>J. g</i> Flower (Aqueous Ext)	4.09±1.02 ^c	0.46±0.01	1.33±0.01	0.33±0.01	1.83±0.05	0.33±0.01	2.16±0.46 ^c	1.42±0.06 ^c
7	<i>J. g</i> Flower (Alcoholic Ext)	4.27±1.04 ^c	0.44±0.03	1.16±0.01	0.16±0.01	1.65±0.04	0.31±0.01	2.43±0.35 ^c	1.46±0.67 ^c
8	<i>J. g</i> Leaf (Aqueous Ext)	4.32±1.07 ^c	0.46±0.01	1.33±0.02	0.33±0.02	1.83±0.05	0.28±0.02	2.25±0.37 ^c	1.57±0.09 ^c
9	<i>J. g</i> Leaf (Alcoholic Ext)	4.14±1.03 ^c	0.43±0.02	1.16±0.02	0.16±0.01	1.67±0.05	0.33±0.02	2.43±0.26 ^c	1.57±0.08 ^c
10	Control	4.72±1.07 ^c	0.48±0.02	1.16±0.01	0	1.33±0.02	0.32±0.02	2.57±0.64 ^c	1.62±0.10 ^c

Values are expressed as mean±SD; n = 6. Values not sharing a common superscript significantly differ at p<0.05. (DMRT) G- Gap, B'- Chromatic Break, B''- Isochromatid Break, F- Fragment, M-minute. A-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. B- 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±SD were calculated per treatment group. *- Gaps were not included in total chromosomal aberrations

administration of *J. grandiflorum* flower and leaf extracts. Of these extracts, the ethanolic extract of *J. grandiflorum* flowers showed better effect in DMBA induced genotoxicity. Oral administration of *J. grandiflorum* plant extracts alone displayed no significant differences in MnPCEs and chromosomal abnormalities as compared to control rats.

DISCUSSION

The aim of the present study was to demonstrate the protective effects of *J. grandiflorum* flowers and leaves in DMBA induced bone marrow chromosomal abnormalities in female wistar rats. Previous studies from our laboratory demonstrated that *J. grandiflorum* flowers significantly reduced the incidence of tumor formation and tumor volume as well as reduced the levels of lipid peroxides and improved the antioxidant defense mechanism in DMBA induced mammary carcinogenesis (Kolanjiappan *et al.*, 2005). The rodent bone marrow and peripheral blood micronucleus test have been used as tool to evaluate the mutagenicity of environmental mutagens and carcinogens (Hagmar *et al.*, 2001; Hayashi *et al.*, 2000). The purpose of the micronucleus assay is to identify a mutagenic substance that causes cytogenetic damages, which results in the formation of micronucleus containing lagging chromosome fragments or whole chromosomes. Measurement of micronuclei in polychromatic erythrocytes is suitable due to the reasons that their macronucleus is extruded in the processes of their formation from erythroblasts (Gaizev *et al.*, 1996; Hayashi *et al.*, 2000). Chromosomal aberrations are well detected in the cells in the metaphase of mitosis, due to the fact that the chromosomes are compact in shape and their structure is easy to examine at this stage (Hagmar *et al.*, 2001).

DMBA is present in the environment as a product of incomplete combustion of complex hydrocarbons. DMBA, being an indirect carcinogen, requires further metabolic activation to become an ultimate carcinogen. The thiol epoxides and other toxic reactive oxygen species formed during metabolic activation of DMBA can cause chromosomal damage by binding with adenine residues of DNA. It has been suggested that DMBA is highly mutagenic to lac I in mammary tissues and that adducts with both G:C and A:T base pairs participate in forming mutations in DMBA-treated Big Blue rats (Manjanatha *et al.*, 1998). Ha-ras mutations occur in the very early pre-dysplastic phase and amplification and over expression of C-erbB and its product, the epidermal growth factor receptors occur in the late phases of DMBA

treatment in DMBA induced hamster buccal pouch carcinogenesis (Husain *et al.*, 1989). Quintanilla *et al.* (1991) demonstrated that DMBA caused predominantly A to T transversions in Ha-ras codon 61 in mouse skin and hamster buccal pouch tumors, which is consistent with the ability of DMBA to form bulky adducts with adenosine. An increase in micronucleus frequency and chromosomal aberrations in bone marrow of DMBA painted or injected rodents have been reported (Guerin *et al.*, 1978; Bhuvaneswari *et al.*, 2004).

Substances, which reduce genetic instability in somatic cells, are said to be antimutagenicity agents. Medicinal plants with bioactive chemopreventive principles can minimize the deleterious effects of mutagens and carcinogens. The protective role of *J. grandiflorum* on micronuclei frequency and chromosomal aberrations induced by DMBA was investigated in Wistar rats. DMBA elevated MnPCEs frequency in the bone marrow of DMBA injected animals, was suppressed by *J. grandiflorum* flower and leaf extracts. Oral administration of *J. grandiflorum* flower and leaf extracts significantly decreased the percentage of aberrant cells and the number of chromatic as well as chromosomal breaks in DMBA-injected animals. Although the exact mechanism of protective role of *J. grandiflorum* is unclear, the possible mechanisms include an enzymatic induction and increase enzymatic degradation of DMBA by liver, modification of biodistribution of DMBA modification of erythropoiesis and/or enhance the antioxidant defense mechanisms to neutralize the toxic effects of reactive oxygen species generated by DMBA. The present study thus demonstrates the protective role of *J. grandiflorum* in DMBA-induced chromosomal aberrations in female Wistar rats.

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