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Full Length Research Paper

Antimutagenic activity of aqueous extract of *Momordica charantia*

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Toxicological studies have undergone a significant evolution during the past decade, with inclusion and great emphasis on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. Present study was taken up to evaluate antimutagenicity of aqueous extract of *Momordica charantia (*MC*)* by bone marrow micronucleus assay (MNT) and chromosomal aberration test (CAT) in mice. Cyclophosphamide (100 mg/kg, i.p) was used as a genotoxic challenge and bone marrow of control and MC treated mice was collected after 24, 48 and 72 h, respectively. In MNT, the bone marrow smears were stained with May-Grunwald's followed by Giemsa stain. Polychromatic and Normochromatic erythrocytes were counted and P/N ratio was calculated. In CAT, colchicine, four mg/kg, i.p, was administered 90 min before sacrifice, bone marrow smears were prepared, stained with Giemsa stain and observed under 100X for different types of chromosomal aberrations. Mitotic index was calculated. The MC has significantly decreased the formation of micronuclei, increased the P/N ratio, inhibited the formation of chromosomal aberrations and increased the mitotic index. Hence, *Momordica charantia* has significant antimutagenic activity.

Key words: Antimutagenic activity, chromosomal aberration test, micronucleus assay, *Momordica charantia*.

INTRODUCTION

All chemicals that produce DNA damage leading to mutation or cancer are described as genotoxic. Toxicological studies have undergone a significant evolution during the past decade, with much greater emphasis being placed on chronic toxicity, carcinogenicity, teratogenicity and mutagenicity. The mutations in somatic cells are not only involved in the carcinogenesis process but also play a role in the pathogenesis of other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading causes of death in the human population (De Flora and Izzotti, 2007). Micronucleus test and chromosomal aberration test are used for studying antimutagenic activity of a drug. One of the best ways to minimize the effect of mutagens and carcinogens is to identify the anticlastogens /antimutagens (substances which suppress or inhibit the process of mutagenesis by acting directly on the mechanism of cell) and desmutagens (substances which some how destroy or inactivate, partially or fully the mutagens, thereby affecting less cell population) in our diets and increasing their use. Nature has bestowed us with medicinal plants. There is a need to explore them for use as antimutagenic and anticarcinogenic food or drug additives.

Momordica charantia, family of Cucurbitaceae, is commonly known as bitter gourd or bitter melon in English and karela in Hindi. Antidiabetic activity of *M.*

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Abbrevations: MN, Micronuclei; **PCE,** polychromatic erythrocytes; **NCE,** normochromatic erythrocytes; **MC,** *Momordica charantia*; **CAT,** chromosomal aberration test.

charantia is well-known. In diabetic patients Karela lowers blood sugar, delays complications such as nephropathy, neuropathy, gastroparesis and cataract, atherosclerosis (Grover and Yadav, 2004; Seham et al., 2006). It also has antiulcer activity (Gurbuz et al., 2000). Diabetes and ulcer are some of the stress induced diseases. Our earlier studies (Meera and Nagarjuna, 2009) indicated antistress and immunopotentiating activity of *M. charantia*. For using Karela or bittergourd as neutraceutical, it is essential to ensure that it is nongenotoxic.

Hence, an attempt is made to carry out *in vivo* mouse bone marrow micronucleus test and chromosomal aberration test to evaluate antimutagenic activity of *M. charantia* by moderating the genetic damage induced by cyclophosphamide.

MATERIALS AND METHODS

Preparation of extract

Fresh karela or fruits of *M. charantia* were procured from local market in July 2008. Fresh unripe fruits were sliced; pulp and seeds were removed and then mechanically squeezed. The juice obtained was dried in hot air oven below 60 C to get dried powder (Grover and Yadav, 2004). The powder obtained was passed through sieve no. 40. The drug solution (10 mg/ml) was made using water as a vehicle for administration to animals.

Experimental animals and treatment

Eight to ten weeks old Swiss albino mice of either sex, weighing 25 - 30 g maintained under standard environmental conditions (25 ± 2°C, relative humidity 45 ± 10%, light and dark cycle of 12 h) and fed with standard pellet diet and water *ad libitum*, were used for the present study. The experimental protocol, which is in accordance with the OECD (Organization of Environmental Carcinogen Detection) guidelines No.470 and WHO guidelines for mutagenicity studies in animals, (www.oecd.org) was approved by the Institutional Animal Ethics Committee before starting the experiments. The animals were divided into eight groups consisting of six animals each. Group one served as normal control, group two, three four was treated with clastogen, Cyclophosphamide 100 mg/kg, i.p. and bone marrow was collected after 24, 48 and 72 h of clastogen administration, respectively. Group five was treated with *M. charantia* (900 mg/kg, p.o.) for seven days. Groups six, seven, eight were treated with *M. charantia* for seven days followed by Cyclophosphamide as a challenge. The dose for mice was calculated based on LD_{50} (91.9 mg/100 gm) values of *M. charantia* (Meera and Nagarjuna, 2009). On seventh day, bone marrow was collected at 24, 48 and 72 h after clastogen administration, respectively.

Bone marrow micronucleus assay (Hayashi et al., 1994)

On seventh day, the animals were anesthetized and the bone marrow was aspirated from femur and tibia into one ml of 5% bovine albumin in phosphate buffered saline (pH 7.2). The cell suspension was centrifuged (1000 rpm for 5 min) and the smears were prepared from the pellet on chemically cleaned glass slides and stained with May-Grunwald's and followed by Giemsa stain. The smears were analyzed under oil immersion using Labomed-Model Digi 2 microscope (90 - 260 V) for the presence of Micronuclei (MN) in polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). P/N(Polychromatic erythrocyte/Normochromtic erythrocyte) ratio was determined by counting a total of about 500 erythrocytes per animal and 2000 erythrocytes were examined for the presence of micronuclei (Borroto et al., 2003).

Chromosomal aberrations test (Goncalves et al., 2008; Seetharama and Narayana, 2005)

On seventh day, each animal was injected with 0.04% colchicine in a dose of four mg/kg i.p, 90 min prior to death, for mitotic arrest. The bone marrow was aspirated from femur and tibia into suspending medium 0.075 M KCl, centrifuged and supernatant was discarded. The pellet was mixed with fixative (three: one, methanol: acetic acid) and then centrifuged. The preparation was given two changes of fixative and smears were prepared. The slides were flame-dried and stained with 10% Giemsa at pH 6.8 for 15 - 20 min. Smears were screened for different types of chromosomal abnormalities-rings, breaks, exchanges and minute.

Statistical analysis

The results were expressed as Mean ± SEM and analysis was carried out by one-way ANOVA. Post-hock analysis was done by Turkey's multiple comparison tests to estimate the significance of difference between various individual groups. $P < 0.001$ was considered significant.

RESULTS

Micronucleus assay

The inhibitory effect of *M. charantia* against clastogenicity induced by cyclophosphamide is shown in Tables 1, 2, 3 and Figure 1. In the present study, *M. charantia* showed time dependent inhibitory effect on the frequency of MN in PCE as well as NCE. Decrease in P/N ratio due to Cyclophosphamide was also inhibited by *M. charantia*. Inhibition was found to be more between 24 and 48 h after clastogenic challenge and less there after.

Chromosomal aberration test

As shown from Table 4 and Figure 2b, there was a statistically significant increase in chromosomal aberrations in reponse to cyclophosphamide (100 mg/kg). *M. charantia* significantly inhibited the frequency of various chromosomal aberrations (Figure 2c) and the decrease in mitotic index induced by clastogen.

DISCUSSION AND CONCLUSION

The bone marrow micronucleus test is one of the most suitable genotoxicity tests. Other tests include chromosomal aberration, peripheral blood micronucleus, and

Table 1. Effect of *M. charantia* after 24 h of clastogenic challenge.

Group	% MNPCE	%MNNCE	P/N
Control	0.46 ± 0.036	0.25 ± 0.030	0.909 ± 0.008
Cyclophosphamide (100 mg/kg)	2.57 ± 0.092^{+}	0.88 ± 0.041^{+}	0.625 ± 0.009^{+}
M. charantia	0.57 ± 0.030	0.34 ± 0.029	0.928 ± 0.010
M. charantia + Cyclophosphamide (100 mg/kg)	1.23 ± 0.566*	0.51 ± 0.013*	0.818 ± 0.011*

n=6, Values are expressed in Mean ± SEM, One way ANOVA followed by Turkey's multiple comparison test. $^{+}$P<0.001 Vs Control, *P<0.001 Vs Cyclophosphamide.

Table 2. Effect of *M. charantia* after 48 h of clastogenic challenge.

Group	% MNPCE	%MNNCE	P/N
Control	0.46 ± 0.036	0.25 ± 0.030	0.909 ± 0.008
Cyclophosphamide (100 mg/kg)	3.71 ± 0.133^{+}	1.32 ± 0.048^{+}	0.481 ± 0.007^{+}
M. charantia	0.57 ± 0.030	0.34 ± 0.029	0.928 ± 0.010
M. charantia + Cyclophosphamide (100 mg/kg)	1.53 ± 0.020*	0.64 ± 0.016*	0.767 ± 0.014*

n=6, Values are expressed in Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison test. $^{+}$P<0.001 Vs Control, *P < 0.001 Vs Cyclophosphamide.

Table 3. Effect of *M. charantia* after 72 h of clastogenic challenge.

Group	% MNPCE	%MNNCE	P/N
Control	0.46 ± 0.036	0.25 ± 0.030	0.909 ± 0.008
Cyclophosphamide (100 mg/kg)	4.36 ± 0.049^{+}	1.88 ± 0.026^{+}	0.399 ± 0.008^{+}
M. charantia	0.57 ± 0.030	0.34 ± 0.029	0.928 ± 0.010
M. charantia + Cyclophosphamide (100 mg/kg)	1.82 ± 0.038*	0.83 ± 0.019*	0.711 ± 0.010*

n=6, Values are expressed in Mean ± SEM, One way ANOVA followed by Tukey's multiple comparison test. $^{+}$P<0.001 Vs Control, *P<0.001 Vs Cyclophosphamide.

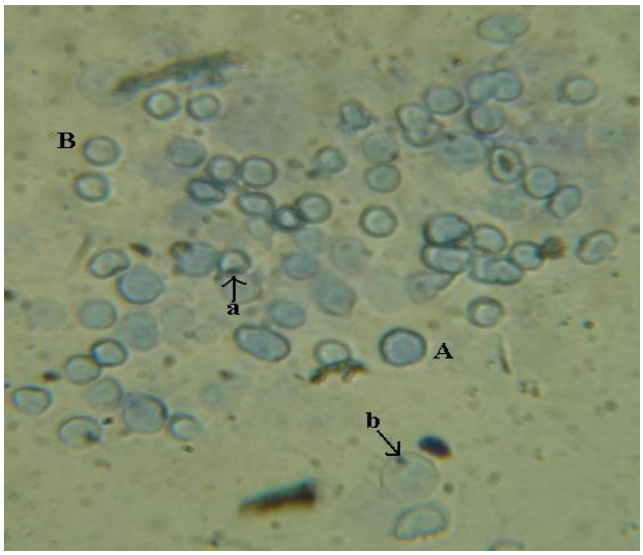

Figure 1. Effect of MC on micronucleus assay.
A-Polychromatic Erythrocyte (PCE), **B**-Normochromatic Erythrocyte (NCE).
a-Micronuclei in PCE, **b**-Micronuclei in NCE (M.G.& G, x100).

Table 4. Effect of *M. charantia* on chromosomal aberrations and mitotic index.

Group	Treatment	Types of aberrations				Total no. of aberrations	Mitotic index
		Rings	Exchanges	Breaks	Minute		
1.	Normal control	1.16 ± 0.307	0.16 ± 0.166	0 ± 0	1.5 ± 0.223	2.83±0.307	3.51 ± 0.108
2.	Cyclophosphamide (100 mg/kg) p.o., (24 h)	5.16 ± 0.307^{+}	1.83 ± 0.307^{+}	5 ± 0.365^{+}	4.16 ± 0.307^{+}	15.83±0.477^{+}	1.03 ± 0.022^{+}
3.	Cyclophosphamide (100 mg/kg) p.o., (48 h)	5.83 ± 0.307^{+}	2.16 ± 0.307^{+}	5.66 ± 0.333^{+}	5.16 ± 0.307^{+}	18.83±1.014^{+}	0.93 ± 0.026^{+}
4.	Cyclophosphamide (100 mg/kg) p.o., (72 h)	6.16 ± 0.166^{+}	2.5 ± 0.223^{+}	5.83 ± 0.307^{+}	5.66 ± 0.210^{+}	20.16±0.654^{+}	0.83 ± 0.018^{+}
5.	*M. charantia* (900 mg/kg) p.o.,	1.16 ± 0.307	0.16 ± 1.66	0 ± 0	1.5 ± 0.223	2.83±0.307	3.67 ± 0.144
6.	*M. charantia* + Cyclophosphamide (100 mg/kg) p.o., (24 h)	2.16 ± 0.307*	1.0 ± 0.258	1 ± 0.258*	1.66 ± 0.210*	5.83±0.477*	3.23 ± 0.091*
7.	*M. charantia* + Cyclophosphamide (100 mg/kg) p.o., (48 h)	2.5 ± 0.223*	1.5 ± 0.223	1.33 ± 0.210*	1.83 ± 0.307*	7.16±0.307*	3.00 ± 0.081*
8.	*M. charantia* + Cyclophosphamide(100 mg/kg) p.o., (72 h)	2.66 ± 0.210*	1.66 ± 0.210	1.66 ± 0.210*	2.33 ± 0.333*	8.33±0.333*	2.74 ± 0.104*

sperm morphology tests. The antimutagenic activity of *M. charantia* was evaluated by measuring their inhibitory effect on cyclophosphamide induced mutagenesis.

Upon administration of cyclophosphamide there was significant rise in % MNPCE, % MNNCE (Figure 1) and it was time dependent, indicating cyclophosphamide induced chromosomal damage in mouse bone marrow cells. These fragmented chromosomes were condensed to form micronuclei which are not included in the main nucleus (Hayashi et al., 1994). Administration of *M. charantia* alone do not produce any significant variation in % MNPCE and % MNNCE indicating that, it is devoid of any genotoxicity. *M. charantia* decreased the cyclophosphamide induced formation of micronuclei in PCE and NCE, which may be due to the inhibition of cyclophosphamide induced chromosomal damage. The inhibition was more between 24 and 48 h and less there after. This may be due to the metabolism of the drug after 48 h.

Cyclophosphamide produced a significant, time dependent decrease in the P/N ratio, which may be due to increase in NCEs which signals a cytotoxic effect. *M. charantia* significantly inhibited the same, by decreasing the formation of NCE. The inhibition increased linearly at 24 and 48 h after cyclophosphamide. But *M. charantia* could not bring P/N ratio to normal level. A regimen of M.C treatment/administration for more than seven days may bring P/N ratio to normal level. *M. charantia* is devoid of any genotoxicity as it does not produce any significant variation in P/N ratio after administration of MC alone.

In chromosomal aberration test, there was a significant, time dependent rise in the total no. of chromosomal aberrations-rings, breaks, exchanges and minute, of cyclophosphamide treated animals (Figure 2b), when compared with normal control animals (Figure 2a). Cyclophosphamide gets metabolized to phosphoramide mustard and acrolein before it can act as a mutagenic agent to promote chromosomal aberrations (Hales, 1982). Chromosomal aberrations are due to lesions in DNA caused by phosphoramide mustard which lead to discontinuities of the DNA helix. *M.charantia* significantly inhibits the cyclophosphamide induced chromosomal aberrations (Figure 2c), which may be due to inhibition of cyclophosphamide induced chromosomal damage. The inhibition was more at 24 h and less thereafter. *M. charantia* is devoid of any genotoxicity as it does not produce any significant variation in total no. of chromosomal aberrations after administration of MC alone.

In our study, we found a significant decrease in mitotic index of cyclophosphamide treated animals, which can be due to the affected cell division in the bone marrow (Goncalves et al., 2008). *M. charantia* significantly inhibits the disturbances in the cell division of mouse bone marrow.

M. charantia possess antimutagenic activity.

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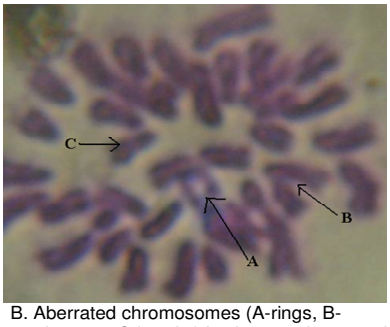
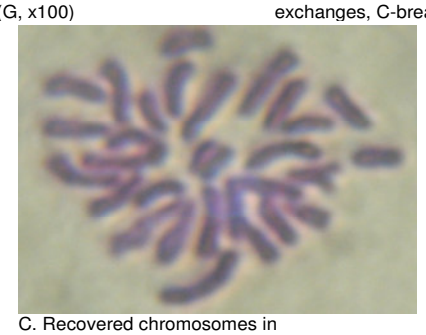
A. Normal chromosomes in control mice (G, x100)



B. Aberrated chromosomes (A-rings, B-exchanges, C-breaks) in clastogenic control



C. Recovered chromosomes in *Momordica charantia* treated mice (G, x100)

Figure 2. Cytogenetic evaluation of MC.

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