RESEARCH COMMUNICATION

Chemopreventive and Anticarcinogenic Effects of *Momordica Charantia* Extract

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Abstract

The aim of the study was to examine whether *Momordica* fruit extract (MFE) and *Momordica* leaves extract (MLE) might exert any chemopreventive effect in a two stage protocol in skin carcinogenesis with Swiss albino mice. The tumour incidence, tumour yield, tumour burden and cumulative no. of papillomas were found to be higher in the controls (without either extract) as compared to the MFE or MLE treated experimental groups. In a melanoma model, the mice which received fruit and leaf extracts of *Momordica* at the doses of 500 and 1000 mg/kg body weight for 30 days showed increase in life span of animals and tumour volume was significantly reduced as compared to control values. In cytogenetic studies, a single application of *Momordica* extracts at doses of 500, 1000 and 1500 mg/kg body weight, 24 hours prior the i.p. administration of cyclophosphamide, significantly prevented micronucleus formation and chromosomal aberrations in a dose dependent manner in bone marrow cells of mice. The present study demonstrate chemopreventive potential of *Momordica* fruit and leaf extracts on DMBA induced skin tumorigenesis, melanoma tumour and cytogenicity.

Keywords: *Momordica charantia* - skin anticarcinogenicity - micronucleus test - chromosomal aberrations

Asian Pacific J Cancer Prev, **11**, 371-375

Introduction

Laboratories studies and epidemiological evidence lead credence to chemopreventive strategy in attenuating the developing cancer in human beings (Bertram et al., 1987; Boone et al., 1990). Many nutrient and non nutrient dietary constituents of plant origin have evidence of chemoprevention by inhibiting and/or suppressing or reversing cancer incidence trend evoked by multitude of factors including environmental carcinogens. Karela/ bitter melon (*Momordica charantia*, MC) belongs to family *Cucurbitaceae*. *M. charantia* fruits contain charantin, pectin, Galactouronic and glycosides, saponin, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids (Miyahara, 1981). It is one of the important herbal drugs used in various systems of traditional medicine for several ailments i.e. antidiabetic, abortifacient, anthelmintic, contraceptive etc.

The chemical modulator(s) from bitter melon extracts obtained from leaves, fruits and tendrils were reported for their abilities to modulate the function of Pgp and the MDR phenotype in the multidrug-resistant human cervical carcinoma KB-V1 cells and wild type drug-sensitive KB-3-1 cells (lacking Pgp) (Seham et al., 2006). *Momordica charantia* was found effective on highly metastatic PC-3M prostate cancer cell line (Rao et al., 2004) There are few reports available on clinical use of MC in diabetes and cancer patients in traditional medicine (Grover and Yadav, 2004) Whole fruit extract of *Momordica* induced a significant increase in the hepatic levels of glutathione S-transferase (GST) and acid soluble sulfhydryl (-SH) after 14 or 21 days treatment in lactating dams (Singh et al., 1998) There are few reports which showed chemopreventive potential of *Momordica* whole fruit extract (Jilka et al., 1983; Singh et al.,1998). *Momordica* is an important drug used and there are lack of report about anticarcinogenicity and antimutagenicity of fruit and leaf extract, it is therefore we have undertaken to study the preventive effect of *Momordica* ext. using two stage protocols in skin papilloma model in Swiss albino mice and melanoma model in C57 Bl hybrid mice and also studied antimutagenicity of *Momordica* extract.

Materials and Methods

Animals:

Random bred male Swiss Albino mice of (6-8 weeks old) of 15-20 gms body weight were obtained from the animal colony of our research centre. They were kept on controlled temperature (22°C) and 12 : 12 hours light and dark cycle and were given synthetic pellet diet and water ad libitum. The experiment was approved by the institutional animal ethic committee before conduction of the experiments.

Chemicals:

Dimethyl benz(a)nthracene. Cyclophosphamide and croton oil was purchased from Sigma Chemical Co., USA and other chemical were reagents grade and were procured locally for the study.

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Preparation of Momordica charantia leaves and fruit extract.

Momordica fruit and leaves were collected from the local garden in September 2007 and was identified by the competent botanist of local laboratory at Bhopal. Fruit and leaves were air dried in shade without direct exposure to sun rays and it was powdered. The powder was mixed in 50 % methanol and kept in separating funnel for 6 hours. The supernatant was collected and this process was repeated until clear solution of supernatant was obtained. All supernatant was pooled together and dried to the powder at 40°C water bath. The powder was dissolved in double distilled water before the each treatment at required concentrations.

Experimental design

(A) Skin bioassay protocol: The experiment was conducted as per the method reported by Berenbrum (1975) and standardized by us (Agrawal et al,2009). The animals were randomly divided in to 8 groups. Each group comprises of 6 animals. Mice were shaved in 2cm 2 area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatment was provided topically on shaved area using the following protocol.

Group 1 (Untreated control) No treatment was given.

Group 2 (Vehicle control) 100 µl acetone 2 times/week was given up to 8 weeks

Group 3 (DMBA Alone) 104 µg DMBA was dissolved in 100 µl acetone and single application was given

Group 4 (DMBA + Croton Oil) 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards 1 % Croton oil was applied on skin 2 times a week up to 8 week.

Group 5 (DMBA + *Momordica* fruit ext. + Croton Oil) 104 µg DMBA was dissolved in 100 µl acetone and single application was given after one week, the 100 µl of Momordica fruit extract (MFE) at the dose of 500 mg/kg body weight was given one hour before the each application of 1 % croton oil 2 times a week up to 8 weeks.

Group 6 (DMBA + *Momordica* leaves ext. + Croton Oil) 104 µg DMBA was dissolved in 100 µl acetone and single application was given after one week, the 100 µl Momordica leaves extract (MLE) at the dose of 500 mg/kg b. wt. was given one hour before the each application of 1 % croton oil 2 times a week up to 8 weeks

Group 7 Croton oil alone 1 % Croton oil was applied on skin 2 times in a week up to 8 weeks.

Group 8 (*Momordica* leaves ext. Alone) The 100 µl Karela leaves extract at the dose of 500 mg/kg b. wt. was given 2 times a week up to 8 weeks

The animals of all groups were kept under observation and tissues were fixed in neutral formalin for gross and microscopic changes.

(B) Melanoma model: Melanoma cell line were obtained from National Cell science centre, Pune and maintained in our laboratory. The C57 Bl hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were obtained from the animal colony of our institute. They were kept on controlled temperature (22˚C) and 12: 12 hours light and dark cycle and given standard mouse pellet diet and water ad Libitum. Cell suspension having total $5x10^5$ cells/ animal were injected. After implantation of the melanoma cell line, animal were kept under observation and experiment was started after 10 days when the tumours were seen. The treatment was given orally for 30 days and tumour volume and survival time of each animal was recorded. The following groups were maintained.

Control Group: This group consisted of four mice. The melanoma cell line (B6F10) were injected subcutaneously (S.C.) in all four mice.

Test Group: This group was divided into two sub groups. Each group consisted of four animals. The melanoma cell line was injected by S.C. route. The tumour bearing mice were orally given dose of 500 mg/kg body weight in 50 % methonolic extract of fruit and leaves extract of momordica as standardized by us in earlier experiments (Agrawal et al, 2009).

Cytogenetic study

Cytogenetic damage in bone marrow cells was studied with reference to chromosomal aberrations and micronuclei induction.

(A) Chromosomal aberrations analysis: For the chromosomal aberrations assay, the Momordica fruit and extract at different dose levels i.e. 500,1000 and 1500 mg/kg body weight in the volume of 0.2 ml was injected 24 hours before the treatment of cyclophosphamide. The positive control group received single i.p. injection of 50 mg/kg cyclophosphamide in 0.9% saline. Colchicines (4 mg/kg b.wt) was administered intraperitoneally 2 hours before the harvest of the cells. Animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as per modified method of Preston et al (1987) for chromosomal aberrations and method of Schmid (1975) and standardized by us (Agrawal et al., 1998; 1999) for micronucleus evaluations. The femur was excised and the bone marrow was extracted in 0.56 % KCl. The harvested cells were incubated at 37°C for 20 minutes and then centrifuged for 10 minutes at 1000 rpm. Cells were fixed in Carney's fixative (Methanol: Acetic acid, 3:1) and burst opened on a clean slide to release the chromosomes. The slides were stained with 5 % Giemsa solution for 15 minutes and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 x 10 X) for each group. Different types of chromosomal aberrations such as chromatid breaks, gaps, pulverization, polyploidy, centromeric association etc. were scored and expressed as % chromosomal aberrations.

(B) Micronuceus assays: The femur of mice were dissected out and the bone marrow was flushed out in HBBS solution as described by us earlier (Agrawal et al., 1998) . The smear was made in precleaned slides, air dried and fixed in absolute methanol. The slides were stained with Maygrunwald and Giemsa stain. About 2000 cells were counted and number of micronucleated polychromatid and Normochromatid erythrocytes cells were scored. PCE/NCE ratio was also calculated. The data are presented in MNPCE+SE. The statistical significance

Table 1. Effect of M. Charantia leaves and fruit extract on tumour incidence in skin papilloma model

Groups	No. of Weeks			
	4th	8th	12th	16th
DMBA* + Croton Oil**	1/6 (16 %)	3/6 (50%)	5/6 (83%)	6/6 (100%)
DMBA* alone	0/6	0/6	0/6	0/6
Croton oil**	0/6	0/6	0/6	0/6
DMBA* + Croton Oil** + M. leaves extract	0/6 (0 %)	1/6 (16 %)	3/6 (50%)	4/6 (60%)
M.Leaves extract (500mg/kg b wt)	0/6	0/6	0/6	0/6
DMBA* + Croton Oil** + M. fruit extract	0/6	0/6	1/6 (16%)	2/6 (33%)
M.fruit extract (500mg/kg b wt)	0/6	0/6	0/6	0/6
Untreated control	0/6	0/6	0/6	0/6

*Single application of DMBA was given at the dose of 4 mg/ kg body weight; **1 % croton oil was given one hour after application of Momordica extract

Table 2. Cumulative No. of Papilloma on M.Charantia Leaves and Fruit Extract Treatment

Groups	Dose	Day of 1st Appear	Cumulative No. of Papilloma
DMBA* + Croton Oil**	104 µg/ animal + 1 %	On 29th day	40
DMBA alone*	104 µg/ animal	-	0
Croton oil**	1 %	-	0
DMBA* + Croton Oil **+M. leaves extract	104 µg/ animal + 1 % + 500 mg/kg b wt	On 71th day	6
Leaves alone	500 mg/kg b wt	-	0
DMBA* + Croton Oil** +M. fruit extract	104 µg/ animal + 1 % + 500 mg/kg b wt	70 days	5
M.Fruit alone	500 mg/kg b wt	-	0

*Single application of DMBA was given at the dose of 4 mg/ kg body weight; **1 % croton oil was given one hour after application of Momordica extract

was evaluated using Student's t test.

Results

The tumour incidence, tumour yield, tumour burden and cumulative no. of papillomas were found to be higher in the control (without M E treatment) as compared to experimental animals (ME treated). The first appearance of papillomas was also delayed in DMBA + ME + Croton oil group (53 and 45 days in group 5 and 6 respectively) as compared to DMBA + Croton Oil group (27 days). Significant prevention in no. of papillioma was observed in DMBA + ME + Croton oil group (50 and 67 % tumour in group 5 and 6 respectively) as compared to DMBA + Croton Oil group (100 % tumour) The cumulative no. of papilliomas was also reduced in DMBA + MFE/MLE + Croton oil group (6 and 3 in group 5 and 6 respectively) as compared to 12 papillomas in DMBA + Croton Oil group (Table 1 and 2) .

In another experiment the chemopreventive effect of Momordica extract was also studied using melanoma tumour model in the C57 Bl mice. The mice which received fruit and leaves extract of Momordica at the dose of 500 and 1000 mg/kg body weight for 30 days showed increase in life span of animals and tumour size was significantly reduced in Momordica extract treated mice as compared to control. The tumour volume was significantly reduced to 35 % and 48 % in Momordica extract treated mice as compared to 135 % in untreated control animals (Table 3).

In cytogenetic studies, single application of Momordica fruit and leaves extract (Karela ext.) at the dose of 500,

Table 3. Effect of Fruit and Leaves Extract of Momordica in Melanoma Tumor bearing Mice

Groups	Dose (mg/kg b wt)	Tumor Volume	Mean Survival (in mm)	ILS (%)	IR (%)
Untreated Group	-	1638 ± 345.5	17.5	-	-
M.Fruit	500	441± 10*	28	60	74
M.Leaves	500	445± 26.1*	27	54	73

*Denotes stastical significance at p<0.05 when compared with untreated control group; Each group contains 4 mice; ILS: Increase in Life span, IR: Tumour Growth Inhibition Rate

Table 4. Effect of M.charantia Fruit and Leaves Extract on Micronucleus Formation in Mouse Bone Marrow Cells

Groups	Dose (mg/kg b wt)	MNPCE ± SE	MNPCE ± SE	PCE / NCE Ratio ± SE	PCE / NCE Ratio± SE
		fruit	leaves	fruit	leaves
Cyclophosphamide [Positive Control]	50	3.30 ± 0.56	3.30 ± 0.56	0.455 ± 0.219	0.455 ± 0.219
M. Charantia Extract + CP	500 + 50	1.6 ± 0.547*	1.16 ± 0.4*	1.03 ± 0.136	1.06 ± 0.02
M. Charantia Extract + CP	1000 + 50	1.001 ± 0.707*	1.25 ± 0.48*	0.347 ± 0.11	1.09 ± 0.06
M. Charantia Extract + CP	1500 + 50	0.748 ± 0.20*	1.1 ± 0.07*	0.457 ± 0.101	1.5 ± 0.7
M. Charantia Extract alone	500	0.285 ± 0.11	0.75 ± 0.4	1.00 ± 0.70	1.06 ± 0.03

*Denotes stastical significance at p < 0.05 when compared with positive control group. Each group contains 4 mice

Table 5. The Effect of M.charantia Fruit and Leaves Extract on Chromosomal Aberration in Mouse Bone Marrow Cells

S.N.	Treatment	Chromosomal Aberration (%)	Chromatid Break (%)	Chromatid Fragmentation (%)	Chomatid Gap (%)	Chromatid Ring (%)	Chromtid Association	% Prevention
1.	Cyclophosphmide (50 mg / kg)	66.5 ± 2.5	18	17	10	13	9	-
2.	Momordica Fruit Ext. + CP (500 mg/kg +50)	21.8 ± 2.4*	6	8	3	5	Nil	67.2
3.	Momordica Fruit Ext. + CP (1000 mg/kg +50)	16.1 ± 2.0*	5	6	3	2	Nil	75.9
4.	Momordica Fruit Ext. + CP (1500 mg/kg +50)	8.2 ± 1.7*	3	2	2	Nil	1	87.6
5.	Momordica Fruit Alone (500 mg/kg)	11.9 ± 2.2*	3	5	2	1	1	81.9

*Denotes stastical significance at p< 0.05 when compared with positive control group; Each group contains 4 mice

1000 and 1500 mg/kg dry weight, 24 hours prior the i.p. administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in dose dependent manner in bone marrow cells of mice as compared to Cyclophosphamide group (Table 4). The dose dependent protection was also observed in chromosomal aberrations assay in bone marrow cells of mice in Momordica extract treated mice as compared to known mutagen, Cyclophosphamide treated groups (Table 5).

Discussion

Our studies indicate chemo preventive activity of 50 % methonolic extract of Momordia charantia fruit, and leaves on DMBA and croton oil induced skin papilliomagenesis in male Swiss albino mice. Thus a significant reduction in tumour burden, tumour incidence, and cumulative number of papillomas was noted in momordica extract treated animals as compared to the animals treated with single topical application of DMBA alone and croton oil. The antimutagenic and chemopreventive activities of Momordica extract was reported on prostate cancer cell line (Rao et al., 2004) and skin carcinogenesis protocol (Singh et al., 1998). Recent studies indicated that compounds with antioxidant and anti-inflammatory properties as well as certain phytochemicals can inhibit tumour initiation, promotion and progression in experimental animals models (Perchellet and Perchellet, 1989; Chesson and Collins, 1997) . The effect of Momordica charantia on certain key hepatic enzymes was also reported using male Sprague-Dawley rats. The hepatic enzymes such as Serum gamma-glutamyl transferase and alkaline phosphatase which were found to be significantly elevated following oral administration of both the fruit juice and the seed extract .The report on elevation of hepatic enzymes suggest that Momordica extract help in detoxification of Xenobiotics which is an important for antimutagenicity and anticarcinogenicity studies. The Momordica charantia is an important vegetables and medicinal plant which is being used as antidiabetic drug in traditional medicine. So its finding on antimutagenicity and anticarcinogenicity may be an important alternative medicine for chemotherapy of cancer treatment.

Acknowledgement

The authors are thankful to Shri Madan Mohan Joshi, Chairman and Dr. K.V. Pandya, Director of Jawharlal Nehru Cancer Hospital and Research Centre for providing necessary facilities to carry out the above work.

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