



RESEARCH ARTICLE

**IMMUNOMODULATORY ACTIVITY OF ETHANOLIC EXTRACT OF BITTER GOURD
[*MOMORDICA CHARANTIA*] IN EXPERIMENTAL MODELS**

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Received 15 May 2014; Accepted 28 May 2014

ABSTRACT

Ethanollic Extract of *Momordica Charantia*, commonly known in India as bitter gourd is used to verify its immunomodulatory activities. Immunomodulatory activity of ethanolic extract at doses of 25, 50 and 100mg/kg body weight for 5 days was evaluated on body weight, relative organ weight, delayed type hypersensitivity (DTH) response and haemagglutination titre (HT) in Male Charles Foster rats. No significant weight gain difference was recorded in various groups of animals. Significant increase in DTH response at the dose of 50mg/kg. In the HT test, plant extract showed stimulatory effect in all doses. This change was significant at 50mg/kg body weight. No mortality occurred in tested doses. Thus bitter gourd or *Momordica Charantia* showed a stimulatory effect on both humoral and cellular functions in mice.

Key words: *Momordica Charantia*, DTH, Haemagglutination titre, Immunity response, Toxicity

INTRODUCTION:

Bitter gourd is a functional vegetable with beneficial effects on health. Bitter gourd is a popular vegetable in South –East Asian countries including subcontinent of India. In view of the fact of an outburst and volcanic biological eruption of Diabetes in India due to various biological and non-biological factors, bitter gourd is used as an antidiabetic plant in a mass scale.

But there has been a growing pool of evidences that the underlying cause of various antibacterial, antiviral and biochemical metabolism intervening mechanisms is because of various immunomodulatory activities.

The various medicinal effects of bitter gourd, as it is popularly called or *Momordica Charantia* are as follows. Its treatments of cell cultures or feeding trials with laboratory animals show it does have glucose lowering properties. It influences overall glucose metabolism in the body. It can act as protective agent from non-communicable diseases. It has been shown to reduce hypertension, plasma cholesterol and plasma lipids. It also helps in weight loss. There is also evidence that it can act as an anti-oncological agent to fight against cancer. *Momordica Charantia* or bitter gourd has also been established to be useful in treatment of piles. It is of medicinal value in blood disorder like blood boils, scabies,

itching, psoriasis, ring worm and other fungal infections. It is effective in diarrhea in summer.

The immunomodulatory effect of this indigenous medicinal plant is taken into account and investigated because of the fact that immunomodulatory effect has been found to be the underlying mode of action behind different spans of diseases from liver disease to heart diseases, from autoimmune diseases to disturbances in CNS.

The immune system is involved in the etiology as well as pathophysiologic mechanism of many diseases. Modulation of the immune responses to alleviate the diseases has been of huge interest for many years. Medicinal plants are a rich source of substances which are claimed to induce para-immunity, the non-specific immunomodulation of essentially granulocytes, macrophages, natural killer cells and complement functions. Because of the concerns about the side effects of conventional medicine, the use of natural products as an alternative to the conventional treatment in healing in different diseases is of growing interest. Medicinal plants are being used not only from a very ancient Indian history of medicines, but also from the point of less side effects, decreased drug abuse and resistance related to different groups of drugs.

A large number of plants and their isolated active ingredients have been shown to possess antioxidant, anti-inflammatory, anticancer antimicrobial and immunomodulatory effects. Some of the plants with established immunomodulatory effects are *Viscum album*, *Panax ginseng*, *Asparagus racemosus*, *Azadirachta indica*, *Tinospora cordifolia*, *Polygala senega* and *Osmium sanctum*. There is no scientific data about the immunomodulatory effect of bitter melon which is being investigated in this report.

MATERIALS AND METHODS:

Plant Extract: The plant was collected from the local market in Kolkata and the plant identified by Botanical survey of India. A voucher specimen of the plant material is being kept in the Biochemistry Department of the concerned author.

Preparation of Ethanolic Extract: The ethanolic extract was prepared using 95% ethanol and 0.90Kg/l of solvent. The ethanol extraction was made at room temperature of 37°C in rotary evaporator under sealed condition. The extracts with suitable adsorbents were stored at 4°C until further experiments.

Animals: Swiss albino female mice (20-25g) were bred and were maintained under standard laboratory conditions [25± 2°C, 12:12 hr photo-period]. Commercial pellet diet and water were given *ad libitum*. All experiments were performed under the compliance of the concerned University guidelines and under the Central Government Experimentation Ethical Committee Guidelines.

Dosage: The plant extracts were dissolved in normal saline and was administered *i.p* for 5 days at doses of 25, 50 and 100 mg/kg body weight. The dose volume was 0.2ml. Control animals received the same volume of normal saline.

Body weight, lymphoid organ weight: Animals were divided into four groups (I –IV). Each group comprised of six animals. Group I (Control) received normal saline. Group II –Group IV received plant extract at 25, 50 and 100 mg/kg body weight. The dose volume was 0.2ml. Control animals received the same volume of normal saline.

Body weight, lymphoid organ weight _Animals were divided into four groups as above. The animals were sacrificed 24 hr after the last dose. Body weight gain (percentage) and relative organ weight (Percentage) and relative organ weight (organ weight/100 g of body

weight) of kidney, liver and spleen were determined for each animal.

Assessment of humoral immune functions-Animals of all the groups treated in the above manner were challenged with 0.2 ml of 10% sheep red blood cells (SRBC) *i.p.* on the 10th day of initiation of the experiment and the haemagglutinin titre was studied as per Bin-Hafeez *et al.*

Haemagglutinin titre (HT) assay – Haemagglutinin titre assay was performed using the procedure of Bin Hafeez *et al.* On the fifth day after immunization, animal was anaesthetised and the cardiac blood was collected from each animal for serum preparation. Serum was diluted in 96 –well microtitre plates and mixed with 50 µl of 1% SRBC suspension in PBS. Plates were kept at room temperature for 2 hrs. The value of antibody titre was considered the highest serum dilution showing visible agglutination.

Delayed type hypersensitivity response –The DTH response was determined using the method of Raisuddin *et al.* with some modifications. On the day of termination of the treatment, animals were immunized with 1x 10⁹ SRBC, subcutaneously. On the next day, the animals were again given 1x10⁸ cells in the left hind footpad by injection. The same volume of normal saline was injected to the right footpad as trauma control for nonspecific swelling. Increase in footpad thickness was measured 24 hrs after the challenge using dial caliper. Dexamethasone (0.2 mg/kg/day for 5 days) was used as positive group.

Liver function and blood parameters: Activities of serum glutamate oxaloacetate (SGOT), SGPT or serum glutamate pyruvate transaminase and blood parameters (RBC, WBC and Hb) were estimated.

Statistical Analysis: Statistical analysis was performed using one – way ANOVA. The significance was expressed as p≤0.05. The values are expressed as mean ± SE.

RESULTS:

Effect of extract of *Momordica Charantia* on body weight and lymphoid organ weight.

None of the studied doses of the plant extract of *Momordica Charantia* showed toxicity or mortality in the extract treated animals. No significant difference in the body weight gain were recorded in various groups of animals. The extract did not alter the relative weight of kidney and liver in tested dose, however a significant increase was observed in the relative weight of spleen in Group IV (p <0.05) (Table 1)

Table 1: Effect of different doses of Ethanolic Extract of *Momordica Charantia* on the relative organ weight (g) of mice

[Values are Mean ± SEM from 6 mice in each group]

Group	Relative organ weight (g)		
	Spleen	Liver	Kidney
<i>Momordica Charantia</i> L (25 mg/kg)	0.53 ± 0.07	5.1 ± 0.02	1.09 ± 0.03
<i>Momordica Charantia</i> L (50mg/kg)	0.54 ± 0.09	5.3 ± 0.07	1.11 ± 0.06
<i>Momordica Charantia</i> L (100 mg/kg)	0.63 ± 0.06 *	5.1 ± 0.10	1.10 ± 0.02
Control	0.41 ± 0.02	4.7 ± 0.06	1.09 ± 0.03
P < 0.05 when compared to control			

Effect of plant extract [*Momordica Charantia*] on humoral immunity parameters:

In haemagglutination test , doses of 25 , 50 and 100 mg/kg showed titre values of 1:4.1,1:5.3 and 1:4.2 respectively , while the titre values of control was 1:2.1. The increase in the titre values was significant (p<0.05) in 50 mg/kg .

Effect of plant extract on cell mediated immunity parameters (DTH):

-Plant extract at dose of 100mg/kg showed a significant increase in comparison to control animals (p<0.05) (Table A). In this experiment, dexamethasone has decreased

DTH response significantly in comparison to control (p<0.05).

Effect of plant extract on liver enzymes and blood parameters:

There was no significant elevation in the levels of SGOT and SGPT as a result of treatment with *Momordica Charantia* at any of the doses used in this study (p<0.05) (Table 2). No significant differences in blood parameters and WBC were recorded in this study (p<0.05) (Table 2). No significant differences in blood parameters , RBC, Hb and WBC were recorded between test groups .

Table (A): The effects of various doses of extract of *Momordica Charantia* extract on delayed hypersensitivity (DTH) response in mice immunized with sheep red blood cells in comparison to dexamethasone and control [Values are Mean ± SE of footpad thickness from 6 mice in groups. p<0.05 when compared with the control animals]

Dose (mg/kg body weight)	25	50	100	Dexamethasone	Control
Footpaw Oedema (mm)x100	3.10	5.10	14.20	1.10	2.20

Table 2: Effect of extract of *Momordica Charantia* on liver enzymes , WBC , Hb, RBC

[Values are mean ± SE from 6 mice in each group]

Group	Treatment	WBC(10 ³ /mm ³)	Hb(g/dL)	RBC(10 ⁶ /mm ³)
I	<i>Momordica Charantia</i> Extract (25mg/kg)	4.7± 1.1	11.6±1.1	9.1±0.4
II	<i>Momordica Charantia</i> Extract (50mg/kg)	4.6± 1.1	12.1±1.1	9.3±0.3
III	<i>Momordica Charantia</i> Extract (100mg/kg)	4.9± 1.1	11.9±1.1	8.9±0.2
IV	Control	4.9± 1.4	11.6±1.4	9.1±0.4

Table 2A: Effect of extract of *Momordica Charantia* on enzymes viz. SGOT and SGPT

[Values are mean ± SE from 6 mice in each group]

Group	Treatment	SGPT(IU/L)	SGOT(IU/L)
I	<i>Momordica Charantia</i> Extract (25mg/kg)	52.2 ±11.6	147.7± 25.0
II	<i>Momordica Charantia</i> Extract (50mg/kg)	47.2 ±18.2	131.2±19.2
III	<i>Momordica Charantia</i> Extract (100mg/kg)	44.8 ±08.2	128.8 ±20.0
IV	Control	48.8±11.0	132.2 ±22.2

DISCUSSION:

Momordica Charantia has primarily been described as an antidiabetic plant in traditional system of medicine. But as, the underlying cause of many diseases has been found to be regulation of immunomodulatory activities, therefore the present investigation was recorded to find the immunomodulatory effect of the plant, which is found to have positive effects. In the present study stimulatory effects have been found to be on both humoral and cellular immunity.

In HT test, *Momordica Charantia* showed an increased response in all doses, but the increase was significant only in the dose of 50mg/kg. This activity could be due to the presence of various phytochemicals, which increase the humoral response, by stimulating the macrophages and B-lymphocytes subsets involved in antibody synthesis, the details of the phytochemicals-their individual mode of action, mechanism and interaction are subject to further and detailed investigation. But considering the practical aspect of reverse pharmacology, often the unified and synergistic results of the compounded composition of the phytochemicals are much potent and active in their actions than the individual phytochemicals.

It appears that 50mg/kg is the optimum dose in mice in humoral immunity, at the experimental level, in this particular experimental model, which is subject to change at different quantitative and qualitative level and application in commercial models, which are subject to further regulation, modulation and investigation.

Estimation of LFT or liver function test does not reflect any toxic effect which was concomitant with any significant increase in relative weight of liver. No significant changes regarding SGOT or SGPT level was observed at the experimental level. Results also revealed no significant difference in blood parameters.

In DTH test, the DTH RESPONSE, which directly correlates with the cell-mediated immunity (CMI), was found to be the highest at the maximum dose (100mg/kg) tested in the extract. The mechanism behind this elevated DTH during the CMI responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lymphokines, attracting more scavenger cells to the site of reaction.

An increase in DTH response indicates a stimulatory effect of the plant which has occurred on the lymphocytes and accessory cell types required for the expression of this reaction.

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