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Evaluation of *Momordica charantia* ghrita for Immunomodulatory Activity*

¹V. Prasad, ²V. Jain and ¹A.K. Dorle
¹Department of Pharmaceutical Sciences,
Nagpur University Campus, Nagpur, India
²NDVMPs College of Pharmacy, Nashik, India

Abstract: *Momordica charantia* ghrita contains cow's ghee and *Momordica charantia*, as its main constituents and rock salt. In the present study an attempt has been made to evaluate *Momordica charantia* ghrita for immunomodulatory activity. MG was administered orally at doses of 175 and 350 mg/kg/day to healthy rats. Assessment of immunomodulatory activity was carried out by testing the humoral (antibody titre) and cellular (foot pad swelling) immune responses to the antigenic challenges with sheep RBCs and by neutrophil adhesion test. Orally administered MG showed a significant increase of test parameters viz., neutrophil test, Haemagglutinating Antibody Titre (HAT) and Delayed Type Hypersensitivity (DTH) response. In rats immunized with sheep RBC, MG enhanced the humoral antibody response to the antigen and significantly potentiated the cellular immunity by facilitating the foot pad thickness response to sheep RBC in sensitized rats. There was dose dependent increase in immunomodulation, there was significant improvement compared to control group and the differences were statistically significant in 350 mg/kg/day, therefore *Momordica charantia* ghrita promises strong utility in clinical practice as an effective immunostimulant.

Key words: MG (*Momordica charantia* ghrita), HAT (Haemagglutinating Antibody Titre), DTH (Delayed Type Hypersensitivity), NI (Neutrophil Index), CMI (Cell Mediated Immunity)

Introduction

In recent years there has been an upsurge in the clinical use of indigenous drugs. Herbal preparations, originally used in the traditional systems of medicine, are now being investigated and effectively tried in a variety of pathophysiological states (Shah *et al.*, 1997). Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs which are without the side effects, especially those belonging to the traditional systems of medicine like Ayurveda, homeopathy, etc. emphasis is also laid on the integration of indigenous health care systems with modern health facilities (Ziauddin *et al.*, 1996).

Ayurvedic medicines are largely based upon herbal and herbo-mineral preparations, either single active constituent or in combination (Polyherbal) having specific diagnostic and therapeutic principles (Kulkarni and Karande, 1998). Greater emphasis has been made directed towards research for herbal formulations which can be helpful in the management of stress related disorders. One of the main approaches in Ayurvedic medicine is to increase the body's natural resistance/stress known as rasayana (rejuvenation) (Pallabi *et al.*, 1998).

Corresponding Author: V. Prasad, Department of Pharmaceutical Sciences, Nagpur University Campus, Nagpur, India
Tel: +91-522-2212411-18 Fax: +91-522-2223938, 2223405

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Panchgavya is a term used in Ayurveda to describe the five important bovine products viz., milk, curd, ghee, urine and dung. Several formulations based on each one of these components are reported in Ayurvedic texts with medical claims. These compositions individually as well in combination have been ascribed several therapeutic values (Oyebola, 1993; Shah, 1997).

Ghrita formulation is a ghee containing formulation claimed in traditional practices. The ingredients of the *Momordica charantia* Ghrita are momordica charantia, cow's ghee and rock salt. The present study was carried out to evaluate the immunomodulatory potential of Ghrita formulation. However, no systemic study has been attempted to confirm the traditional practice of Ghrita formulation for immunomodulatory activity, literature regarding this herbal formulations documented only few pharmacological actions and clinical uses of herbal formulation.

Momordica charantia L., a climber belonging to family Cucurbitaceae, is commonly known as bitter gourd or bitter melon in English and karela in Hindi. Momordica means, "to bite" is referring to the jagged edges of the leaf, which appear as if bitten. All parts of the plant, including the fruit, taste bitter. The fruit is oblong and resembles a small cucumber (Basch *et al.*, 2003). The young fruit is emerald green that turns to orange-yellow when ripe. This plant is cultivated throughout the tropics, particularly in India, China, East Africa and South America and used in many countries as a folk remedy for various ailments (Duke, 2002).

In India, various medicinal properties are claimed for *Momordica charantia* L. namely antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative. It is used for treatment of dysmenorrhea, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhoea, piles, pneumonia, psoriasis, rheumatism and scabies. Several constituents of plant include charantin (mixture of sterol glycosides); vicine (pyrimidine nucleoside) and p-insulin (polypeptide) are reported as the active ingredients. The vivid orange-colored and oblong-shaped fruits of the plant are especially used as drug and vegetable (Grover and Yadav, 2004). Unripe fruits of the plant are mainly used for diabetes. *Momordica charantia* has shown immunomodulatory activity (Marabe *et al.*, 2003).

Momordica charantia was selected as it is widely distributed easily available through out subcontinent and also cost effective. In the developing countries, its fruit in form of vegetable are taken in diet from centuries. It has also shown to clinically effective in various clinical studies regarding its antidiabetic potential.

Ghee was selected as it is widely used and easily available through out subcontinent and also cost effective. In the developing countries, ghee is used for cooking food items and it was used as the material for roasting the vegetables. It has also shown to be clinically effective in various clinical studies regarding its memory enhancing potential and shown to possess immunomodulatory activity (The Wealth of India, 1995). Literature survey revealed that no scientific investigation has been made in regard to the immunomodulatory activity of ghee.

Therefore the aim of the present study was to evaluate the formulation made of ghee and *Momordica charantia* for immunomodulatory activity in said experimental models.

Materials and Methods

Materials

Momordica charantia fresh fruits were collected in the month of June from the local areas of Nalgonda, Andhra Pradesh, India. The plant was authenticated by Botanist, Department of Botany, Railway Degree College and Secundrabad, India. The fresh fruits were cut into small pieces. The fresh

fruit pieces were then dried in shade pulverized by a mechanical grinder and powdered using mesh size 40-50 μm . Ghee was obtained as the gift sample from Go-vighyan anusandhan kendra and used as such in formulation.

Animals

Male Wistar rats (150-200 g) were used, animals were housed under standard conditions of temperature ($23\pm 1^\circ\text{C}$), 12 h light/dark cycle and fed with standard pellet diet (Gold Muhor brand, Lipton India Ltd.) and water *ad libitum*. Fresh Sheep Red Blood Cells (SRBCs) in alsevers solution were obtained from Nagpur veterinary college, Nagpur.

Antigen

SRBC collected in alsevers solution, washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5×10^9 cells mL^{-1} for immunization and challenge.

Treatment

MG was prepared in laboratory in a simple mortar using pestle; the animals were divided into three groups consisting rats served as control (Group I). The herbal formulation was fed orally at a dose of 175 mg/kg/day (Group II) and 350 mg/kg/day (Group III) for assessment of immunomodulatory effect.

Neutrophil Adhesion Test

On the 14th day of the drug treatment, blood samples were collected (before challenge) by puncturing the retro orbital plexus into heparinised vilas and analyzed for Total Leukocyte Counts (TLC) and Differential Leukocyte Counts (DLC) by fixing blood smears and staining with field stain I and leishmans stain. After initial counts, blood samples were incubated with 80 mg mL^{-1} of nylon fibers for 15 min at 38°C . The incubated blood samples were analyzed for TLC and DLC. The product of TLC and % neutrophil gives Neutrophil Index (NI) of blood sample (Wilkinson, 1978). Percent neutrophil adhesion was calculated as shown below:

$$\text{Neutrophil adhesion (\%)} = (\text{NI}_T - \text{NI}_U / \text{NI}_U) \times 100$$

Where, NI_U = Neutrophil index of untreated blood sample

NI_T = Neutrophil index of treated blood sample

Haemagglutinating Antibody (HA) Titre

Rats of group II and III were pretreated with MG for 14 days and each rat was immunized with 0.5×10^9 SRBC/rat by i.p. route, including control rats. The day of immunization was referred as day 0. The animals were treated with MG for 14 more days and blood samples were collected from each rat on day 15 for HA titre. The titre was determined by titrating serum dilutions with SRBC (0.025×10^9). The micro titre plates were incubated at room temperature for two hours and examined visually for agglutination. The reciprocal of the highest dilution of serum showing 50% agglutination has been expressed as HA titre (Mitra *et al.*, 1999).

Delayed Type Hypersensitivity (DTH) Response

Six animals per group (control treated) were immunized on day 0 by i.p. administration of 0.5×10^9 SRBC/rat and challenged by a subcutaneous administration of 0.025×10^9 SRBC/mL into right hind foot pad on day +14. MG was administered orally from day -14 until day +13. The DTH response was measured at 24 h after SRBC challenge on +14 day and expressed as mean percent increase in paw volume (plethysmometrically) (Puri *et al.*, 1993).

Statistical Analysis

The data were analysed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. $p < 0.05$ were considered significant.

Results

Momordica charantia ghrita was evaluated for immunomodulatory effect. MG showed a significant increase in neutrophil adhesion ($p < 0.05$) at dose of 350 mg/kg/day in rats. Similarly dose dependent effect was observed on humoral and cellular immunity showing no significant effect with a dose of 175 mg/kg/day (Table 1). With the dose of 350 mg/kg/day the values of HA titer and DTH response were 435.09 ± 0.45 and 35.11 ± 0.34 , respectively, (Table 2) in comparison to the corresponding figures of 110.08 ± 1.11 and 11.15 ± 0.67 , respectively for the untreated control. These differences were statistically significant ($p < 0.05$). The treatment induced marked enhancement of humoral and DTH response in the animals. From the study it may be inferred that MG promotes immunomodulation and thus rationalizing its traditional claim.

Discussion

Ghee based preparations are becoming increasingly popular for a variety of diseases and infective conditions, primarily influencing the host defense mechanism. Immunomodulatory agents of plants and animal origin enhance against a pathogen by activating the immune system. In the present study *Momordica charantia* ghrita when orally administered, significantly increased the adhesion of neutrophil to nylon fibers which correlates to the process of margination of cells in blood vessels. The neutrophil adhesion was significantly increased with the dose of 350 mg/kg/day when compared to untreated control.

MG titre did not show any significant change with 175 mg/kg/day of MG administration. However, a significant increase was observed at a dose of 350 mg/kg/day with almost 5 fold increase compared to control untreated animals ($p < 0.05$). The augmentation of the humoral response as evidenced by an enhancement of antibody responsiveness to SRBC in rats as consequence of both pre and post immunization drug treatment indicates the enhanced responsiveness of macrophages and b-lymphocytes subsets involved in antibody synthesis (Mungantiwar *et al.*, 1999). The DTH response, which is a direct correlate of Cell Mediated Immunity (CMI), was found to be increased by 30% at a dose of 350 mg/kg/day of the herbal formulation. During CMI responses, sensitized

Table 1: Effect of herbal formulation on neutrophil adhesion in rats

Treatment mg/kg/day	TLC (10 ³ /mmm ²) (A) UB	TLC (10 ³ /mmm ²) (A) FTB	Neutrophil (%) (B) UB	Neutrophil (%) (B) FTB	Neutrophil Index (A×B) UB	Neutrophil Index (A×B) FTB	Neutrophil adhesion (%)
Control	6.24±7.21	5.80±6.11	42.20±11.12	37.00±6.91	275.65±66.33	242.16±32.67	20.75±7.49
HF(175)	7.42±7.52	6.67±6.23	51.64±5.23	42.56±3.25	391.20±59.23	289.35±36.22	24.42±5.62
HF(350)	7.82±6.45	5.92±2.33	52.23±9.73	43.30±6.11	398.31±35.23	341.80±83.96	28.12±5.26

*All the values are mean±SD of rats in each group. One-way ANOVA followed by Tukey-Kramer comparisons test; * $p < 0.05$ VS Group 1, $F(2, 15) = 9.4$, TLC = Total Leukocyte Count; UB = Untreated blood; FTB = Fiber Treated Blood

Table 2: Effect of herbal formulation on HA titre and DTH response to antigenic challenge by sheep RBCs in rats

Treatment (mg/kg/day)	HA Titre	DTH response (% increase in paw volume)
Control	80.44±2.21	10.82±3.56
HF (175)	229.32±1.11*	22.38±5.54*
HF (350)	465.10±0.68*	30.11±0.35*

All the values are mean ± SD of rats in each group. One-way ANOVA followed by Tukey-Kramer comparisons test; * $p < 0.05$ VS Group 1, $F(2, 15) = 4.1$ for H.A titre and $F(2, 15) = 10.2$ for DTH response

T-lymphocytes, when challenged by the antigen, are converted to lymphoblasts and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory reaction). In our studies, foot volume was enhanced after MG treatment suggests cell mediated enhancement (Sen *et al.*, 1992).

Increase in both, HA titre and DTH response indicated the *Momordica ghrita* potentiates humoral as well as the cellular immunity. One of the explanations forwarded to justify the beneficial effects of indigenous drugs in diseases states is the non specific enhancement of immune states of the organism (Patil *et al.*, 1998). The immunostimulant activity of *Momordica charantia* was known there was no documentary evidence. In conclusion, the results obtained in the present study have shown the immunomodulatory activity of MG *in vivo*, further studies are warranted for the understanding the exact mechanisms responsible for immunomodulation.

Conclusions

Momordica charantia has shown significant immunomodulatory effect in animals. Therefore clinical studies as a potential immunostimulant is further warranted. Its efficacy as antidiabetic agent has been demonstrated in many clinical studies. *Momordica charantia* safety is assured as it has been consumed in diet from centuries. Therefore, we assume that clinical studies with *Momordica charantia ghrita* in immunomodulation will result in positive outcome.

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