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Original Article

Immunomodulatory effect of the saponins of Momordica Cymbalaria

Rosey Sarraf *, Suman Kumar Sah, Nagarathna P.K.M, Rahimuddin Syed, Shemin S. Dani, Priya Soman

Karnataka College of Pharmacy, Bangalore-64, Karnataka- India

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ABSTRACT

Momordica cymbalaria is a plant species that is almost ubiquitously found in different parts of the natives of tropical and subtropical Africa and Asia and Australia. Various preparations of it have been advocated in folk medicine as an abortifacient and for the treatment of diabetes mellitus. The present study investigates the Immunomodulatory potential of Methanolic extract of steroidal saponins of Momordica cymbalaria. An attempt has been made to assess the Immunomodulatory activity of purified saponin mixture (PSM) Methanolic extract of steroidal saponins of Momordica cymbalaria orally at dose of 25mg/kg was used as standard drug. The effect of steroidal saponin of Momordica cymbalaria on the immune system of rats and mice was evaluated by using different experimental models such as carbon clearance test, cyclophosphamides induced neutropenia, neutrophil adhesion test, effect of steroidal saponin of Momordica cymbalaria on serum immunoglobulins, mice lethality test, indirect haemagglutination test, hypersensitivity reaction, haemagglutination reaction and delayed hypersensitivity reaction test. Steroidal saponins of Momordica cymbalaria increased level of serum immunoglobulins. Hence it was concluded that saponins of Momordica cymbalaria increases both humoral immunity and cell mediated immunity. Steroidal saponins of Momordica cymbalaria increased both humoral immunity and cell mediated immunity.

Keywords: Momordica cymbalaria, humoral immunity, cell mediated immunity, Immunomodulatory.

INTRODUCTION

The immune system is a system of many biological structures and protects against disease. To function properly an immune system must detect a wide variety of agents known as pathogens (viruses to parasitic worms) and distinguish them from the organisms own healthy tissue. In many species, the immune system can be classified into subsystems such as humoral immunity and cell-mediated immunity. The immune system is the most complex biological systems in the body. At the time of infection, immune system go under the attack of a large number of viruses, bacteria and fungi [1]. There are two branches of immunity response: humoral immunity and cellular immunity [2].

Immunity disorders may affect both cellular and humoral components. An important role in the cellular immunity is played by reticuloendothelial system which mainly comprise of phagocytic cells whose function is to ensure elimination of senescent cells, pathogenic microorganisms and immune complex from blood and tissues and participate in lammation. This way they contribute to non-specific immunity [3]. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions. Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism against the invading antigens. Several plant products have been reported for Immunomodulatory activity and many formulations of these plant products are available to enhance the immune system. Plants are the essential and integral part in complementary and alternative medicine. Plants have the ability of the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are in turn used to restore health and heal many diseases [4, 5].

Momordica cymbalaria is traditionally used for the treatment of diabetes mellitus, rheumatism, ulcer, skin disease and diarrhea. The fruit of this plant have been reported to possess hypoglycemic, cardioprotective, Hepatoprotective, nephroprotective, immunodulatory and antioxidant properties. Owing to anthropogenic activities, such

Corresponding Author Address:** Rosey Sarraf, Karnataka College of Pharmacy, Bangalore-64, Karnataka- India; **E-mail id:** **roseysarraf99@gmail.com, sarrafrosey@gmial.com

as habitat destruction due to grazing and urbanization, and poor seed viability and germination, M. cymbalaria is under threat of extinction. This review focuses on the cultivation, nutritional and chemical composition, as well as medicinal and therapeutic properties of this plant. [6]

MATERIALS AND METHODS

Plant Material: The fresh roots of Momordica cymbalaria, fenzl were collected from Gadag district, Karnataka.

Drugs and Chemicals: Leishmann's stain, Indian ink and Gluteraldehyde purchased from Merck. WBC diluting fluid, zinc sulphate and barium chloride, cyclophosphamide, bovine serum albumin and Levamisole. All other chemicals and reagents used, were of analytical grade.

Equipment used:

- Ultra universal bioamplifer
- Weighting balance
- Shimadzu model U.V 1800
- Auto analyzer NOVA 2021
- Digital Cam
- Cooling Whirlpool
- Centrifuge meter
- Nephelo meter
- Vernier Calipers
- Micro titer plates
- pH Tutor

Acute Oral Toxicity Study for Steroidal Saponins of Momordica Cymbalaria [7]**:** Colony breed female mice (20-25gm) were maintained under controlled standard animal house condition with access to food and water ad libitum. The mice acclimatized for 5 days and then kept for fasting overnight. Animals were weighed then limit and main test were performed in accordance with OPPTS guidelines. The limit test was carried out first at 5000 mg/kg body weight for one animal and if animal died, main test was performed. If the animal survived, two more animals were dosed, if both survived then the test is terminated.

The main test was performed with an initial dose of 175 mg/kg body weight and half log units (corresponding to progressing of 3.2) and following sequence is followed: - 175, 550, 1750 and 5000 mg/kg body weights. The first animal was to be dosed with 175 mg/kg body weight. If animal is died, a much low dose is tested. If animal survived, then two more animals are dosed, the decision is made after 48 hr. survival pattern of all animals. If animal survives, then the main dose was terminated. If animal dies, two more animals are dosed and observed. Moribund animals or animal in pain are humanely killed and considered as animal died. The maximum volume of liquid administered at one time depends on size of the test animal. In mice, the volume should not normally exceed 1ml per 100 g body weight.

The dosing was stopped when one of the following stopping criteria is met:

1. 3 consecutive animals survive at the upper bond.
2. 5 reversals occur in any 6 consecutive animal tested.
3. At least 4 animals have followed the first reversal and the specified like hood ratios exceed the critical values.

The control rats received vehicles (tween 80 1%p.o) only.

Neutrophil adhesion test [8, 9]**:** On the 14th day of drug treatment, blood samples were collected (before challenge) by puncturing the retro-orbital plexus into heparinized vials and analyzed for total leucocyte counts (TLC) and differential leucocyte counts (DLC) by fixing blood smears and staining with Field stain I & II-Leishmann's stain. After initial counts, blood samples were incubated with 80 mg/ml of nylon fibers for 15 min at 37oC. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and % neutrophil gives neutrophil index (NI) of blood sample.

Cyclophosphamide induced Neutropenia[10]**:** Swiss albino mice received Saponins of MC or vehicle orally for 10 days. On 10^{th} day, neutropenia dose of Cyclophosphamide (200 mg/kg, SC) was injected and this day was labelled as day zero. Blood was collected, the total leukocyte count (TLC) and DLC were performed prior to and on day 3 after injection of Cyclophosphamide. The TLC and neutrophil counts (%) in treated groups were compared with the values of the control group.

Carbon clearance test [11, 12] **:** Swiss albino mice were administered with Saponins of MC and or vehicle orally for 10 days. 48 hours after the last dose of the drug, animals of all the groups received intravenous injection of (0.3 ml per 30 g) Indian ink (colloidal carbon) Via the tail vein. Blood samples were withdrawn from each animal by retro-orbital plexus at an interval of 0 and 15 min after the ink injection. A 50-µl blood sample was mixed with 4 ml of 0.1% sodium carbonate solution and the absorbance of this solution was determined at 660 nm. The phagocytic index K was calculated using the following formula:

$K = (Log_eOD1 - Log_eOD2)/15$

Where, OD1 and OD2 are the optical densities at 0 and 15 min, respectively.

Serum Immunoglobulin [13]: The drugs were administered to Wister rats orally for 21 days. Six hours after the last dose of drug, blood was collected and the serum was used for immunoglobulin level estimation following a method described by Mullen (1975). Briefly, for every sample of serum to be analysed, a control tube containing 6 ml of distilled water and a test tube containing 6 ml of zinc sulphate solution were prepared. To each, 0.1 ml of serum was added from a pipette. They were inverted to enable complete mixing of the reagents and left to stand for 1 hr. at room temperature. The first tube served as blank and the second tube was taken as sample. The turbidity developed was measured using a digital nepheloturbidity meter. The turbidity obtained (sample-blank) was compared with that obtained with standard barium sulphate (BaSO4) solution. The standard BaSO4 solution was prepared by adding 3 ml of barium chloride solution (1.15% w/v) to 97 ml of 0.2 N sulphuric acids. The turbidity obtained with this solution was expressed as 20 zinc sulphate turbidity (ZST) units.

Indirect haemagglutination test [10]: Rats were pre-treated with the drugs for 14 days and each rat was immunized with 0.5×109 sheep red blood cells (SRBCs) intraperitoneally, including control rats. The day of immunization was referred to as day 0. The drug treatment was continued for 14 more days and blood samples were collected from each rat at the end of the drug treatment and the titre value was determined by titrating serum dilutions with SRBC (0.025×109 cells) in micro titre plates. The plates were incubated at room temperature for 2 hr and examined visually for agglutination. The minimum volume of serum showing haemagglutination was expressed as haemagglutination (HA) titre.

RESULTS

Ten kgs of dried powered roots of Momordica cymbalaria has yielded 450gms of Methanolic extract. 100gms of Methanolic extract yielded 1.9mg of steroidal saponin. Hence the yield is 0.0019% of crude powder.

Phytochemical analysis of saponins of Momordica cymbalaria: Test for saponin: The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15minutes. The formation of 1cm layer of foam shows the presence of saponins.

Acute oral toxicity studies of saponin of roots of Momordica cymbalaria:

TREATMENT	DOSE mg/kg BODY WEIGHT					INFERENCE
	175	500	1000	1750	5000	
Control (Tween 80%)	0	0	0	0	0	Stop dosing
Steroidal Saponin	0	0	0	X	-	Stop dosing

0= Survived; X=Died

Effect of saponins of Momordica cymbalaria (50mg/kg) on neutrophils adhesion properties in Wister rats: Addmistration of saponins of MC (50mg/kg per day / 14days) significantly ($p<0.001$) increased neutrophils adhesion index when compared to control group and therefore neutrophils adhesion index 20.82. Addmistration of Levamisole (2.5mg/kg per day/14days) significantly ($p<0.001$) increased neutrophils adhesion index when compared to control group. Addmistration of saponins of MC (100mg/kg per day / 14days) significantly ($p<0.001$) increased neutrophils adhesion index when compared to control group.

GROUPS	TREATMENT	NEUTROPHILS ADHESION INDEX
1	Control-distilled water	16.57 ± 0.45
2	Levamisole	31.78±0.5372
3	Saponins of MC (50mg/kg per day / 14days)	20.82 ± 0.3250
4	Saponins of MC (100mg/kg per day / 14days)	25.17 ± 0.2120

Neutrophil adhesion

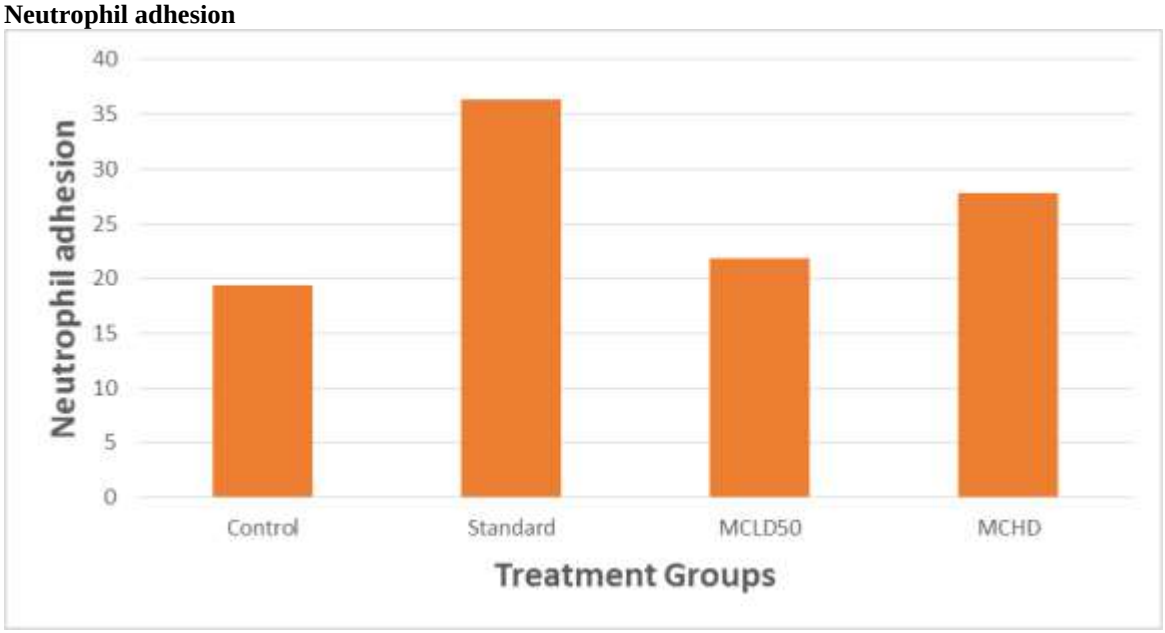


Table showing effect of saponins of Momordica cymbalaria of (50, 100mg/kg, per day/5days) on phagocytic index in Swiss albino mice.

GROUPS	TREATMENT	PHAGOCYTIC INDEX
1	Control-distilled water (1ml/kg, per day/ 5days)	0.00778±0.0001068
2	Levamisole (2.5mg/kg, po/day/5days	0.01488±0.0003455
3	Saponins of MC (50mg/kg per day / 5days)	0.01156±0.0002561
4	Saponins of MC (100mg/kg per day / 5days)	0.0121±0.0001732

Phagocytic Index

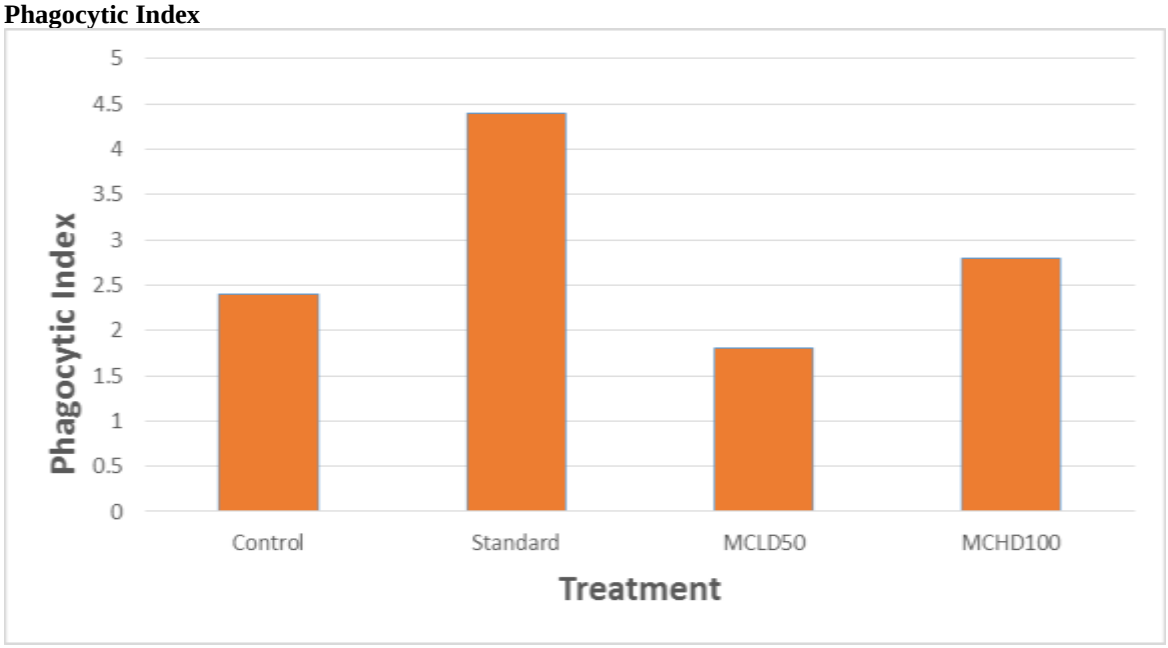


Table showing effect of saponins of Momordica cymbalaria of (50, 100mg/kg, per day/10days) on cyclophosphamide induced neutropenia in Swiss albino mice:

GROUP	TREATMENT	NO. OF LEUCOCYTES AFTER CYPM TREATMENT	% REDUCTION OF LEUCOCYTES	NO. OF NEUTROPHILS AFTER CYPM TREATMENT	% REDUCTION OF NEUTROPHILS
1	Control-distilled water (1ml/kg, per day/ 10days)	2555±106.3	52.33±0.8993	16.17±0.4773	67.29±1.624
2	Levamisole (2.5mg/kg, po/day/10days	1093±42.12	24.05±0.4645	6.33±0.4216	25.39±1.067
3	Saponins of MC (50mg/kg per day / 10days)	2073±53.30	46.01±0.3850	9.667±0.3333	39.30±0.9886
4	Saponins of MC (100mg/kg per day / 10days)	1758±125.9	42.84±0.6141	8.333±0.494	32.76±1.168

Cyclophosphamide Neutropenia

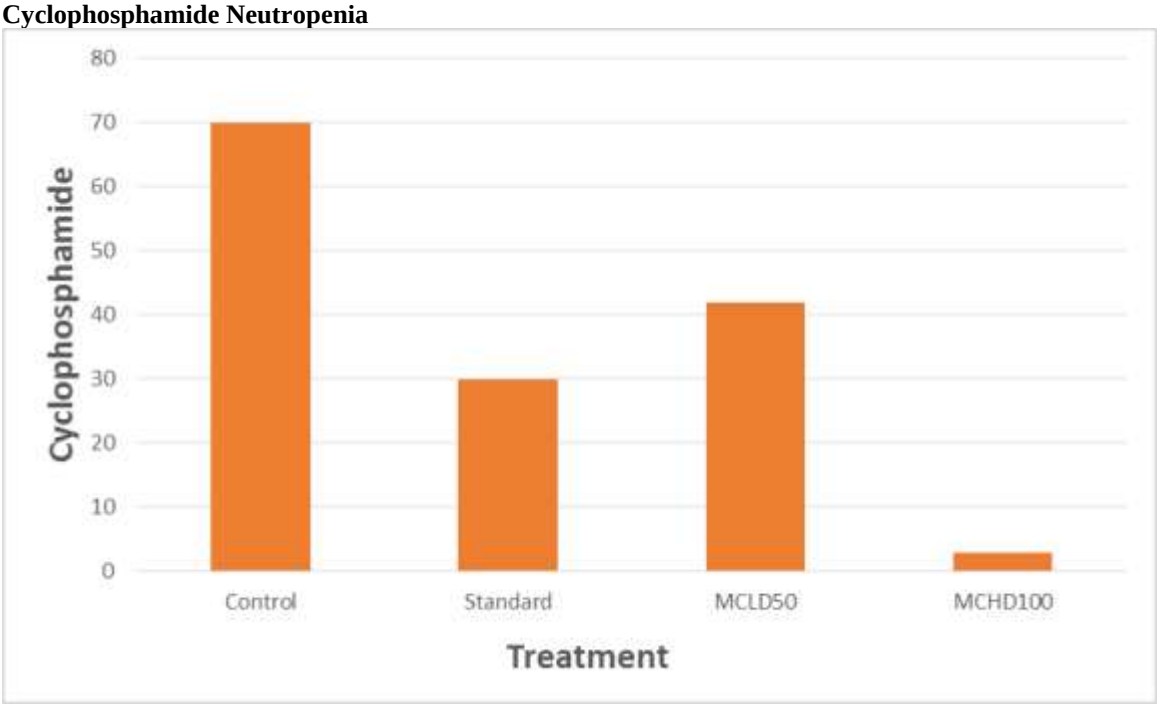


Table showing effect of saponins of Momordica cymbalaria of (50, 100mg/kg, per day/21days) on Serum Immunoglobulin levels in Wister rats:

GROUPS	**TREATMENT**	**SERUM IMMUNOGLOBULIN LEVEL (ZST-UNITS)**
1	Control-distilled water (1ml/kg, per day/ 21days)	19±0.8563
2	Levamisole (2.5mg/kg, po/day/21days	34.17±1.276
3	Saponins of MC (50mg/kg per day / 21days)	22±0.8165
4	Saponins of MC (100mg/kg per day / 21days)	34.33±1.256

Serum Immunoglobulin

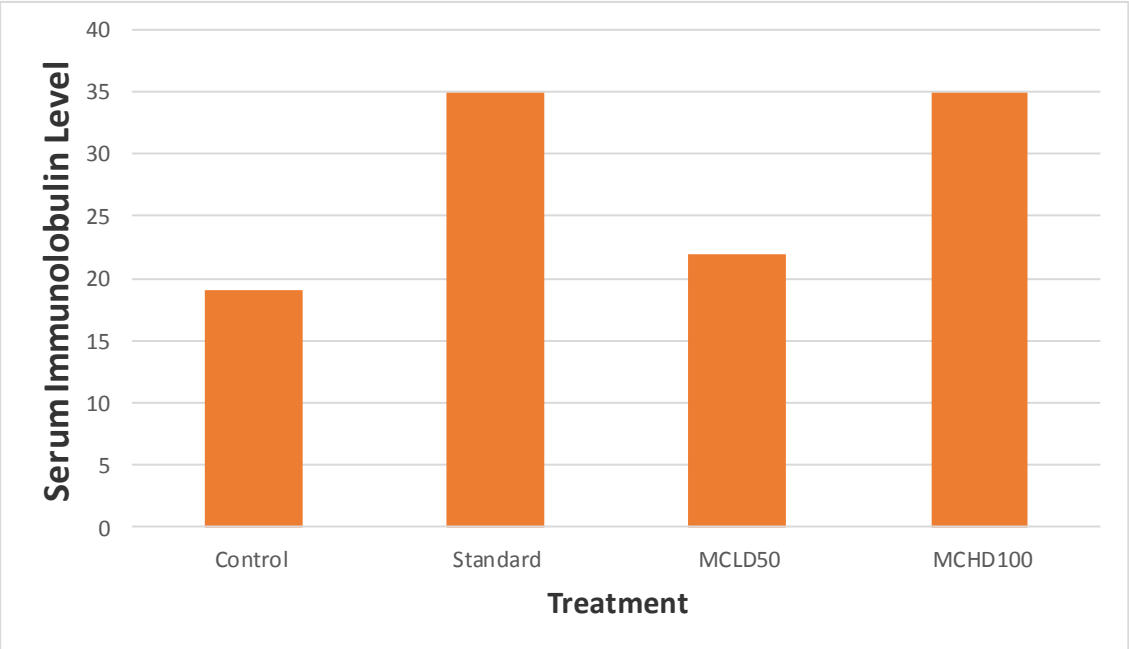
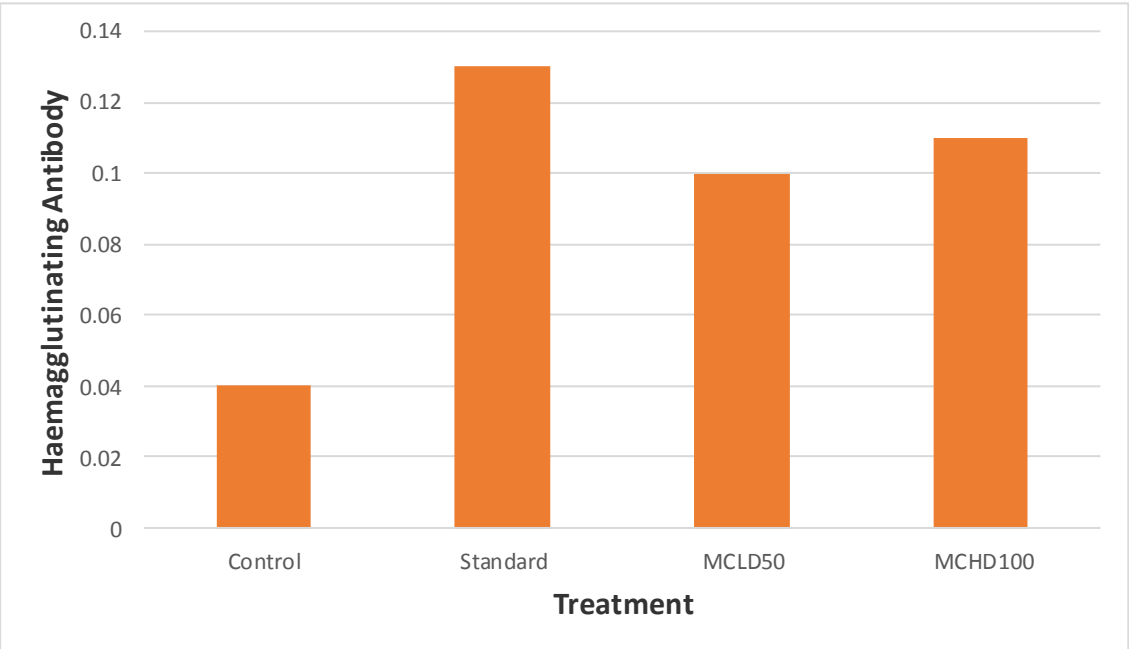


Table showing effect of saponins of Momordica cymbalaria of (50, 100mg/kg, per day/28days) on Indirect Haemagglutination reaction in wistar rats:

GROUPS	TREATMENT	HA TITER VALUE
1	Control-distilled water (1ml/kg, per day/ 28days)	6.436±0.2719
2	Levamisole (2.5ml/kg, per day/ 28days)	0.9177±0.1159
3	Saponins of MC (50mg/kg per day / 28days)	2.451±0.2509
4	Saponins of MC (100mg/kg per day / 28days)	1.147±0.1431

Indirect Haemagglutination



DISCUSSION

Immunomodulatory agents of plant and animals origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. The results of the present study suggest that steroidal saponins of Momordica cymbalaria potentiates humoral immunity as shown by its effect in the indirect haemagglutination reaction and it also potentiates cell mediated immunity as shown by its effect in the neutrophil adhesion, carbon clearance, cyclophosphamide induced neutropenia.

The indirect haemagglutination test was performed to confirm the effect of steroidal saponins of Momordica cymbalaria on the humoral arm of the immune system. Saponins of Momordica cymbalaria at the doses and Levamisole showed that levels of circulating antibodies are significantly increased. The estimation of serum immunoglobulin levels was used to evaluate the increase in serum immunoglobulin production after the administration of the drugs. Immunoglobulin are antibodies the react specifically with the antigen. Saponins of Momordica cymbalaria at both the doses showed a significant increase in the serum immunoglobulin levels.

Antibody molecules which are secreted by plant cells mediated the humoral immune response. This augmentation of the humoral response to SRBC indicated an enhanced responsiveness of the macrophages and T and B lymphocyte subsets involved in antibody synthesis. Saponins of Momordica cymbalaria at both the doses and Levamisole showed a significantly increase in the haemagglutination titter value signifying increase in antibody titre.

Steroidal saponins of Momordica cymbalaria at both doses showed significant increase in the neutrophil adhesion to nylon fibers. This might be due to the up regulation of the β2 integrin, present on the surface of the neutrophils through which they adhere firmly to the nylon fibers. Hence it was inferred that saponins of Momordica cymbalaria causes stimulation of neutrophils towards the site of inflammation. Steroidal saponins of Momordica cymbalaria at 50mg/kg, po/10days and 100mg/kg, po/day/10days caused 39.3% and 32.50% decrease in cyclophosphamide induced neutropenia suggesting that it attenuates the effect of cyclophosphamide on the haemopoetic system.

Cells of the reticuloendothelial system (RES) play important role in the clearance of particles from the bloodstream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation. Steroidal saponins of Momordica cymbalaria at both doses and Levamisole showed increase in the phagocytic index. Hence these agents may increase the activity of the reticuloendothelial system.

Steroidal saponins of Momordica cymbalaria had stimulated both humoral as well as cellular arms of immune system. Finding of the present study showed an overall stimulatory effect of Steroidal saponins of Momordica cymbalaria on both humoral and cellular immunity.

CONCLUSION

The results of the present study show that the Steroidal saponins of Momordica cymbalaria affects on both humoral and cellular immunity. Steroidal saponins of Momordica cymbalaria increased the levels of serum immunoglobulins and increased the circulating antibody titter in indirect haemagglunation test. On the other hand, it showed significant increase in the phagocytic index in carbon clearance assay, a significant protection against Cyclophosamide induced neutropenia and increased the adhesion of neutrophils in the neutrophil adhesion test. Exhinbits a dose related increase in the early hypersensitivity reaction to the SRBC (sheep red blood cells) antigen. It also resulted in a significant increase in the antibody titter value of SRBC in experimental animals. Hence it was concluded that Steroidal saponins of Momordica cymbalaria on both humoral and cellular cell mediated immunity.

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