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Research Article

IMMUNOMODULATORY EFFECT OF M. TRICUSPIDATUM

SANDEEP SINGH BHADORIYA*[1], ANKIT MANGAL[1], NARENDRA MANDORIYA[2]

Department of Pharmacy, Vikram University, Ujjain, India. Email: Sandeepbhadoriya10@gmail.com

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ABSTRACT

Background: Biochemical, haematological and Immunomodulatory effect of *M. tricuspidatum* in rat was studied.

Objective: To evaluate the immunomodulatory effect of *M. tricuspidatum* in rat.

Methods: Aqueous extract of *M. tricuspidatum* were administered oraly at doses of 100, 200 mg/kg/day for 45 days in wistar albino rats. Immunomodulatory effect and biochemical and haematological changes were tested by standard methods.

Results: Aqueous extract of the *M. tricuspidatum* showed increasing antibody production in dose dependent manner. It enhances the production of RBC, WBC and hemoglobin. It does not affect the biochemical parameters.

Conclusion: An oral administration of the aqueous extract of *M. tricuspidatum* showed immunomodulatory effect in rat.

Keywords: Immunomodulation, M.tricuspidatum, Antibody.

INTRODUCTION

The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 – 5000 B.C and Chinese used first the natural herbal preparations as medicines. In India, earliest references are available in Rigveda which is said to be written between 3500 – 1600 B.C[1]. Now a day large number of drugs in use are derived from plants, like morphine from Papaver somniferum, Aswagandha from Withania somnifera, Ephedrine from Ephedra vulgaris, Atrophine from Atropa belladonna etc. Plants play an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology[2]. Plant extracts are potentially curative. Some of these extracts can boost the humoral[3] and cell mediated immunity[4] against virsuses[5], bacteria[6], fungi , protozoa[7] and cancer[8].

Alkaloid, quinones, phenol carboxylic acid ester, simple phenol, tannins and terpenoids are claimed to have immunostimulatory activity[9].

Malvastrum tricuspidatum (L.) Family Malvaceae is one of the plant distributed world wide, also in the Indian subcontinent, commonly known as False mallow or Kharenti in hindi, is considered emollient, resolvent, and belchic. The leaves are applied to inflamed sores and wound; the flowers are given as a pectoral and diaphoretic[10]. *Malvastrum tricuspidatum* is used in traditional medicine as an antiinflamatory, analgesic, in jaundice, and ulcers[11]. Chemically reported to possess anti-inflammatory, analgesic[12], antibacterial [13], hypoglycemic[14] and antipyretic[15] by water extract of whole or arial part of plant. However, there is no scientific data on the *in vitro* and *in* vivo immunomodulatory activity of this plant. Therefore the present study has been undertaken to explore the immunomodulatory activity of various doses of aqueous extracts of Malvastrum tricuspidatum (L.) whole plant in experimental animal models.

MATERIALS AND METHODS

Plant extract

Fresh plants were cleaned, dried at 37 C for 3 days and powered well. From this dried powdered extracts were prepared.

Experimental designs

Animals were divided into three groups, each having five rats and treated accordingly, Group I: control

Group II: Animals treated with aqueous extract of *Malvastrum tricuspidatum* (100 mg/kg)

Group III: Animals treated with aqueous extract of *Malvastrum tricuspidatum* (200mg/kg)

Antigen Preparation

Crystalline Bovine Serum Albumin fraction V (BSA) was used as non cellular antigen for the present investigation. Soluble Bovine Serum Albumin (S - BSA) was prepared by overlaying the BSA powder in isotonic saline 1.0mg/ml of saline (0.15 N). It was allowed to dissolve without agitation and used as antigen.

Collection of Sheep red blood cells

SRBC were collected in Alserver's sol ution from animal husbandry without contamination. To avoid allogenic difference the Sheep red blood was used throughout the study. Immunization After 3 days of exposure to the toxicant, rats was immunized with optimum dose of 0.5 ml of antigen. The antigen was injected through the intraperitoneal route using 3ml tuberculin syringe. Secondary immunization was also done with the same dose of antigen through the same route on the 15th day after primary immunization. Antigen administration and serial bleeding of animals were always done between 2 – 4 pm to avoid circadian rhythmic variations on the immune response.

Blood Collection from Test Animal

Blood samples were collected from a tail vein by snipping the tip of the tail. The tip of the tail was cleaned with spirit and snipped with clean scissors. The blood was collected in EDTA rinsed vials for hematological studies and antigen-antibody titration.

Normal Serum and Antiserum Collection

The blood was collected from the control and test animals by snipping the caudal vein rinsed with 1% EDTA and kept at room temperature for 20 min. The serum was separated by spinning down the clot at 3000 rpm for 15- 20 min and then collected in sterilized storage vials. It was kept at 57ºC in a water bath for 30mts to inactivate complement and stored at 20ºC until use.

Antibody Titration[16]

Passive Haemoagglutination Assay[16]

Chromic chloride method[16]

This assay was used to determine anti-BSA antibodies in the serum Two fold dilutions of the antiserum (50 µl per well) were made with saline in 'U' bottom microtitre plate 50µl of 2% BSA coupled SRBC in saline was added to each well. For effective mixing the microtitre plate was hand shaken and incubated for an overnight at 37 ºC. The highest dilution of the serum samples showed detectable

macroscopic agglutination was recorded and expressed as Log 2 antibody titre of the serum.

Coupling of BSA to SRBC

The chromic chloride method for immunological purposes was followed[17]. In this present study, CrCl3 used as a coupling agent for the coupling of BSA to SRBC. Fresh sheep erythrocytes were washed thrice by using phosphate buffered saline and stored at 4ºC. One volume of the chromic chloride solution was added to an equal volume of the protein antigen in 0.15M saline and then added to one volume of packed red cells immediately. Then it was mixed well and kept at room temperature for 4 min.The coupled red cells are then washed three times in 10-20 volumes of 0.11 M NaCl and resuspended in 0.15 M NaCl with 2 % BSA .

Haematological analysis

The fresh whole blood samples were used for the estimation of leucocyte, erythrocyte counts and haemoglobin, RBC, WBC[16].

Biochemical tests

Total plasma protein, albumin, globulin, alkaline phosphatase, SGOT, SGPT were analysed by Semi auto analyzer (Chem 400).

RESULTS

Administration of aqueous extract of (100 mg/kg) and 200 (mg/kg) *Malvastrum tricuspidatum* produced dose dependent significant increased in antibody titre compared to control. The results were given in the table (1).

Haematological changes WBC, RBC count in *Malvastrum tricuspidatum* treated groups was significantly higher compared with the control group during the experimental period Fig.1. and 2. Haemoglobin content also increased.

Biochemical analysis

The results showed that the increasing level of total protein in low and high dose of Malvastrum *tricuspidatum* treated animals. When compared to control, albumin level was not significantly changed for both low and high dose. SGOT was slightly increased for low doe. But when compared to control, significant changes were not observed in high dose. SGPT was decreased during the study period for both low and high dose.

ALP was increased for both low and high dose during the experimental period. The results were given in the table. 1.

Table 1: Shows effect of *Malvastrum Tricuspidatum* in biochemical parameters of Wistar Albino rat

Biochemical Parameter	Exposure (Days)	Control	100 mg/kg	200 mg/kg
Protein (g/dl)	0	6.34 ± 0.45	6.3 ± 0.52	6.58 ± 0.50
	15	6.06 ± 0.19	6.36 ± 0.39	6.68 ± 0.51
	30	6.2 ± 0.61	6.4 ± 0.56	6.84 ± 0.38
Albumin (g/dl)	0	4.16 ± 0.35	4.12 ± 0.43	4.06 ± 0.13
	15	4.16 ± 0.21	4.1 ± 0.32	4.12 ± 0.13
	30	4.26 ± 0.15	4.12 ± 0.19	4.2 ± 0.16
Globulin (g/dl	0	2.18 ± 0.53	2.18 ± 0.28	2.52 ± 0.4
	15	1.9 ± 0.1	2.26 ± 0.42	2.56 ± 0.50
	30	1.94 ± 0.56	2.28 ± 0.52	2.64 ± 0.27
SGOT (U/L)	0	55.5 ± 7.00	58.78 ± 11.51	56.9 ± 8.40
	15	57.34 ±5.02	64.14 ± 10.13	58.32 ± 8.45
	30	60.06 ±5.81	70.78 ± 9.60	59.6 ± 8.55
SGPT (U/L)	0	22.76 ±4.05	20 ± 1.17	20.86 ± 3.07
	15	23.42 ±3.91	20.2 ± 0.85	21.32 ± 3.44
	30	24.2 ± 4.05	20.76 ± 0.96	21.58 ± 3.22

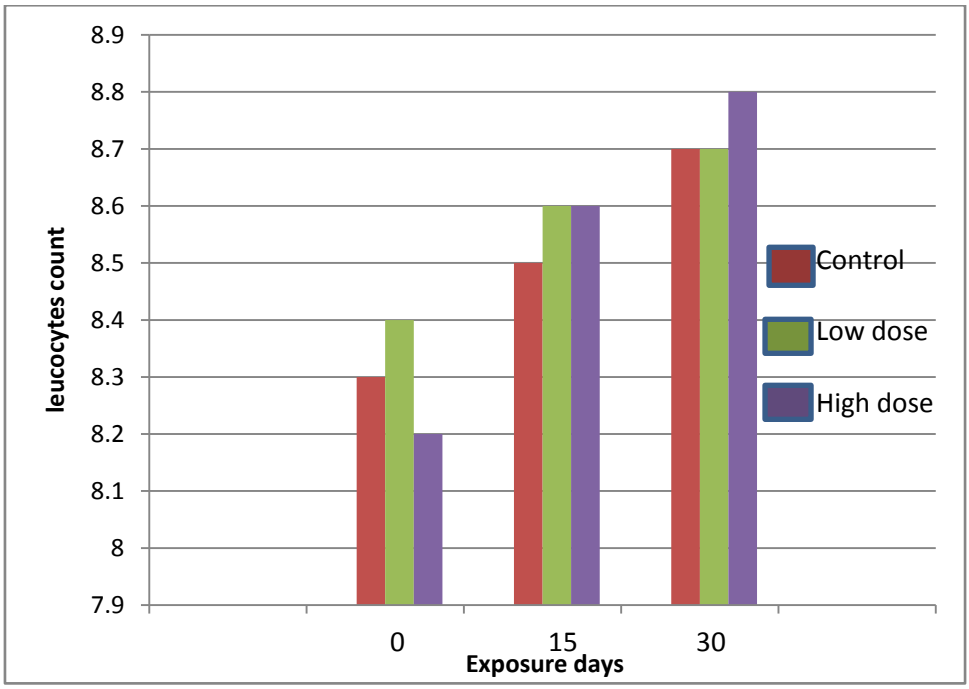

Fig. 1: Shows effect of *Malvastrum tricuspidatum* on leukocyte count of Wistar albino rat

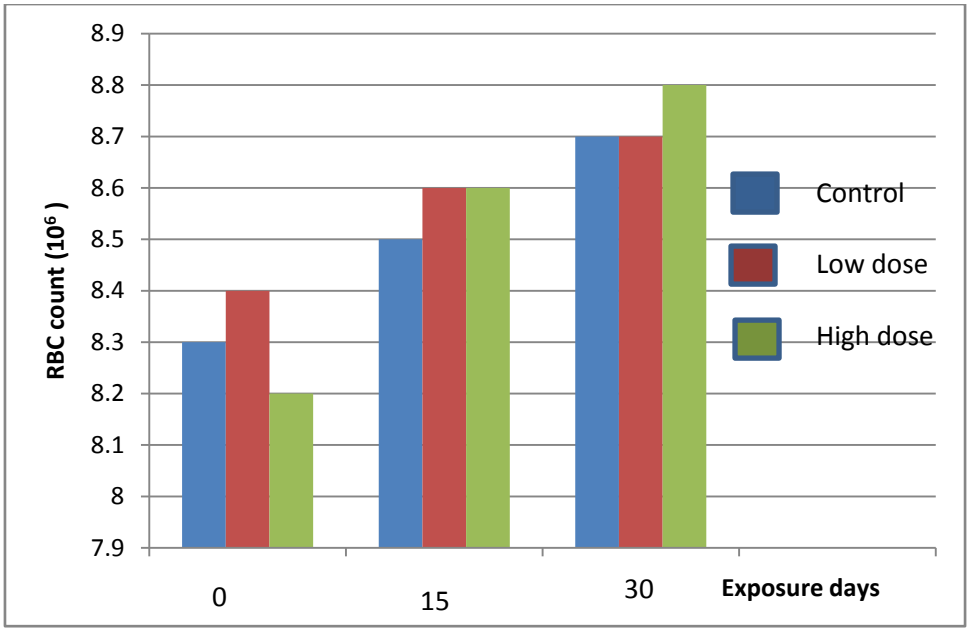

Fig. 2: Shows effect of *Malvastrum tricuspidatum* on leukocyte count of Wistar albino rat.

DISCUSSION

Earlier workers reported that the drug having immunomodulatory effects show cutaneous reaction which is attributed to liberation of lymphokines, skin reactive factor and monocytes, chemotactic factor from sensitized T- cells.14 Thickening and reddening of skin in the immunized animals are attributed to vasodilation that causes increase capillary permeability of local influx of mononuclear cells at the site of inoculation18. Alkaloid, quinones, phenol carboxylic acid ester, simple phenol, tannins and terpenoids are claimed to have immunostimulatory activity.

Malvastrum tricuspidatum is found throughout the semitropical and tropical parts of India. This is used as medicinal plant in Ayurveda and Siddha systems of medicine. It has anti-inflammatory, analgesic and immunostimulatory properties. In this present study immuno modulatory effect of *Malvastrum tricuspidatum* was studied in wistar albino rat.

In this present study, *Malvastrum tricuspidatum* showed increasing antibody production. It may be the release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs by *Malvastrum tricuspidatum*.

The present study suggests that the aqueous extract of *Malvastrum tricuspidatum* stimulate the antibody production in rat. It enhances the production of WBC, RBC and Haemoglobin.

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REFERENCES

1. Sirkar NN. 1989.Pharmacological basis of Ayurvedic therapeutics. In: Cultivation and utilization of medicinal plants. Editors: Atal CK and Kapoor BM (Published by PID CSIR).
2. Borchers, A.T., Sakai, S., Henderson, G.L., Harkey, M.R., Keen, C.L., Stern, J.S., Terasawa, K., Gershwin, M.E 2000. Shosaiko-to and other Kampo (Japanese herbal) medicines: a review of their immunomodulatory activities. J. Ethnopharmacol. 73: 1-13.
3. Rehman, J., Dillow, J.M., Carter, S.M., Chou, J., Le, B.B., Maisel, A.S. 1999. Increased production of antigen-specific immunoglobulins G and M following in vivo treatment with the medicinal plants Echinacea angustifolia and Hydrastis canadensis. Immunol. Lett. 68: 391-396.
4. Upadhyay, S., Dhawan, S., Garg, S., Talwar, G.P. 1992. Immunomodulatory effects of neem (Azadirachta indica) oil. Int. J. Immunopharmacol. 14: 1187-1193.
5. Calixto, J.B., Santos, A.R., Cechinel Filho, V., Yunes, R.A. 1998. A review of the plants of the genus Phyllanthus: their chemistry, pharmacology, and therapeutic potential Med. Res. Rev. 18: 225-258.
6. Boyanova L, Neshev G. (1999). Inhibitory effect of rose oil products on Helicobacter pylori growth in vitro: preliminary report. J. Med. Microbiol. 48: 705-706.
7. Sharma, P, Sharma, J.D. 1998. Plants showing antiplasmodial activity from crude extracts to isolated compounds. Indian J. Malariol. 35: 57-110.
8. Wong, C.K., Leung, K.N., Fung, K.P., Choy, Y.M., 1994. Immunomodulatory and anti-tumour polysaccharides from medicinal plants. J. Int. Med. Res. 22: 299-312.
9. Wagner H and Jurcic K. Assays for immunomodulation and effects in mediators of inflammation. In: Hostettman K, Editor. Methods in plant biochemistry, London: Academic press; 1991, Vol.6, p. 195-217.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. I, International book distributors, Dehradun (India); 1987.p. 652-658.
11. Sebestian MK, Bhandari MM. Journal of Ethnopharmacology 1984; 122: 223.
12. Khonsung P, Nantsupawat S, Jesadanont SN, Chantharateptawan V, Panthong A. Anti-inflammatory and Analgesic activities of water extract of *Malvastrum coromandelianum* (L.) Garcke. Thai J. Pharmacol 2006, Vol 28 (3) 8-15.
13. Sittiwet C, Jesadanont S, Pongpech P, Naenna P, Pongsamart S. Antibacterial activity of *Malvastrum coromandelianum Garcke* against Methicillin-sensitive and Methicillin-resistant strains of Staphylococcus aureus. Current Research in Bacteriology 2008; 1 (1): 42-45.
14. Andrade-Cetto A, and Heinrich M. Mexican plants with hypoglycemic effect used in the treatment of diabetes. Journal of Ethanopharmacol 2005; 99 (3):325-328.

15. Dahanukar SA, Kulkarni RA, and Rege NN. Pharmacology of medicine plants and natural products. Indian J. Pharmacol 2000 ;32:81-118.
16. R.Caroline Jeba, Rama Vaidyanathan, G.Rameshkumar. Immunomodulatory activity of aqueous extract of Ocimum sanctum in rat. IJPBR 2011; 2(1):33-38.
17. Goding, J.W. 1976. The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. J Immunol Methods 10:61-66.
18. Vaibhav d. wahi, A. Pharmacological Study Of Tinospora Cordifolia As An Immunomodulator. Int J Curr Pharm Res2010; 2(4): 52-5