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Research Article

Immunomodulatory Effect of Fractions of Saponins from Tribulus Terrestris on Non-Specific Immunity Using *In Vitro* Phagocytosis

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Abstract

Immunomodulatory effect of many medicinal plants used in Indian system of medicines like *Withania Somnifera*, *Emblica Officinale*, *Aloe vera*, etc. , on the mitogen stimulated proliferation of human and animals peripheral blood mononuclear cells (PMBs) were investigated. No study was still done on phagocytic function of murine peritoneal macrophages by saponins isolated from *Tribulus terrestris*.

The objective of the present study is to investigate the immunomodulatory properties of saponins isolated from *Tribulus terrestris* on *in vitro* phagocytosis by murine peritoneal macrophages with regard to non-specific cellular immune responses.

PMNC's were isolated from the peritoneal fluid of the healthy rats respectively, and treated with the different fractions of saponins isolated from *Tribulus terrestris in vitro* with different fractions to see the engulfment of foreign particle by PMNC's. All the fractions of the saponin showed increased phagocytosis but the saponin fraction with Rf value *0.89* at different doses revealed that the *in vitro* phagocytic activity has been increased in dose dependent manner. The present study revealed the immunomodulating activity, which could be explained the traditional use of this plant in India.

Key Words: Medicinal plants, Immunomodulation, Polymorphonuclear cells Phagocytosis, Macrophages, Saponins.

Introduction

Immunology is probably one of the most rapidly developing areas of biomedical research and has great promises with regard to prevention and treatment of a wide range of disorders, inflammatory diseases of skin, gut, respiratory tract, joints and central organs (Ziauddin et al., 1996). Modulation of immune responses to alleviate disease has been of interest for many years and the concept of 'Rasayana' in Ayurveda is based on related principles (Charak Samhita, 1949). The healthy state is believed to be based on a sophisticated fine-tuning of the immunoregulatory mechanism (Mallurwar et al., 2006). Peoples are returning towards the use of natural therapies because the modern drugs are beyond the reach of common man and their indiscriminate use leads to health hazard (Kalia,2005). Immunomodulators of herbal origin appear to be a better alternative to overcome the above problem (Patwardhan et al., 1991). In recent years, immunostimulatory activity has been reported in a number of plants and plant derivatives such as polysaccharides, lectins, peptides, flavonoids, saponins and tannins in various *in vivo* models (Shivaprasad *et al*., 2006; Atal, 1985; Atal et al., 1986; Godhwani et al., 1988; Dua et al., 1989)

Tribulus terrestris, commonly known as Gokhru, is a shrub belonging to the Family Zygophyllaceae. Historically, *Tribulus terrestris* was used by the cultures of India and Greece as a rejuvenation tonic (Adaikan et al., 2000). It was also used as a therapy for a variety of health conditions affecting the liver, kidney, and cardiovascular ,immune systems, headaches, eye conditions such as itching, conjunctivitis and weak vision, and nervousness (Hu and Yao 2002, www.http://tribulusterrestris.com). The inhibitory effect of saponins from *Tribulus terrestris* on Bcap-37 breast cancer cell line in vitro were also studied (Zhong Yao Cai. 2003). Praveen Kumar et al., (1994) and Rao et al (1996) successfully demonstrated the immunomodulating activity of a combination of extracts of these plants. It has tonic and aphrodisiac properties (Stuart,1990).

Saponins are steroid or triterpenoid glycosides, common in a large number of plants and plant products that are important in human and animal nutrition. From the earlier reports, it had observed that saponins isolated from the Indian medicinal plants were described as possessing a potential usefulness as immunoadjuvant, increasing the immunogenicity of many vaccines. Saponins reportedly induced production of cytokines such as interleukins and interferons that might mediate their immunostimulant effects (Jie et al. 1984, Kensil 1996). It is likely that they interact with antigen-presenting cells to induce many of these responses (Barr et al. 1998).

In present study we have attempted to evaluate the immunomodulatory potency of the saponins isolated from the fruits Tribulus *terrestris* using macrophage phagocytosis in vitro which was subsequent events involved in the process of Immunomodulation.

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Material and Methods

Animals:

Inbred wistar rats of either sex, 3-4 weeks old were obtained from the National Institute of Nutrition, Hyderabad and were acclimatized for 3-4 weeks in the animal house of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, under standard conditions of temperature (23 ± 2°C), relative humidity (50 ± 5%) and light (10:14 h of light and dark respectively). The animals were housed in polypropylene cages containing sterile paddy husk (procured locally) as bedding and fed with standard animal feed and filtered, acidified water *ad libitum*. Seven to eight weeks old and weighing 150 to 200g were selected for the experiments. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care and was approved by the institutional ethical experimentation committee.

Plant material and extract preparation:

Fruits of *Tribulus terrestris* was collected from the suburbs of Bhopal in the month of August-October and authenticated at the State Forest Research Institute, Jabalpur, India. The freshly collected roots from the plant were shade dried at room temperature and grinded to powder and extracted with Soxhlet apparatus using 50% alcohol for 48 hrs or till 12 cycles are completed. The extract was then concentrated on a rotatory evaporator below 40^0 C.

Isolation of saponins from plant material:

The mode of extraction of plant material depends on the texture and water contents of the plant material being extracted and the substance to be extracted. Since saponins are polar compound they are extracted in alcohol or with water. In the present study keeping this in view and the result obtained from the previous studies, the plant material was extracted in 50% alcohol. Isolation of compound was done as described in the fig-1 and TLC analysis was performed as described by Stahl et al (1963).Briefly, solvent system used for isolation of saponins is toluene: ethyl acetate (7:3) and chromatograms were sprayed with the anisaldehyde-sulfuric acid reagent; three spots were detected as fraction I, Fraction II and Fraction III with Rf value 0.40, 0.54, 0.89 respectively. After elution of the spots the quantification of each drug was done by spectrophotometer and immunomodulatory activities of these fractions were analyzed using in vitro phagocytic activities.

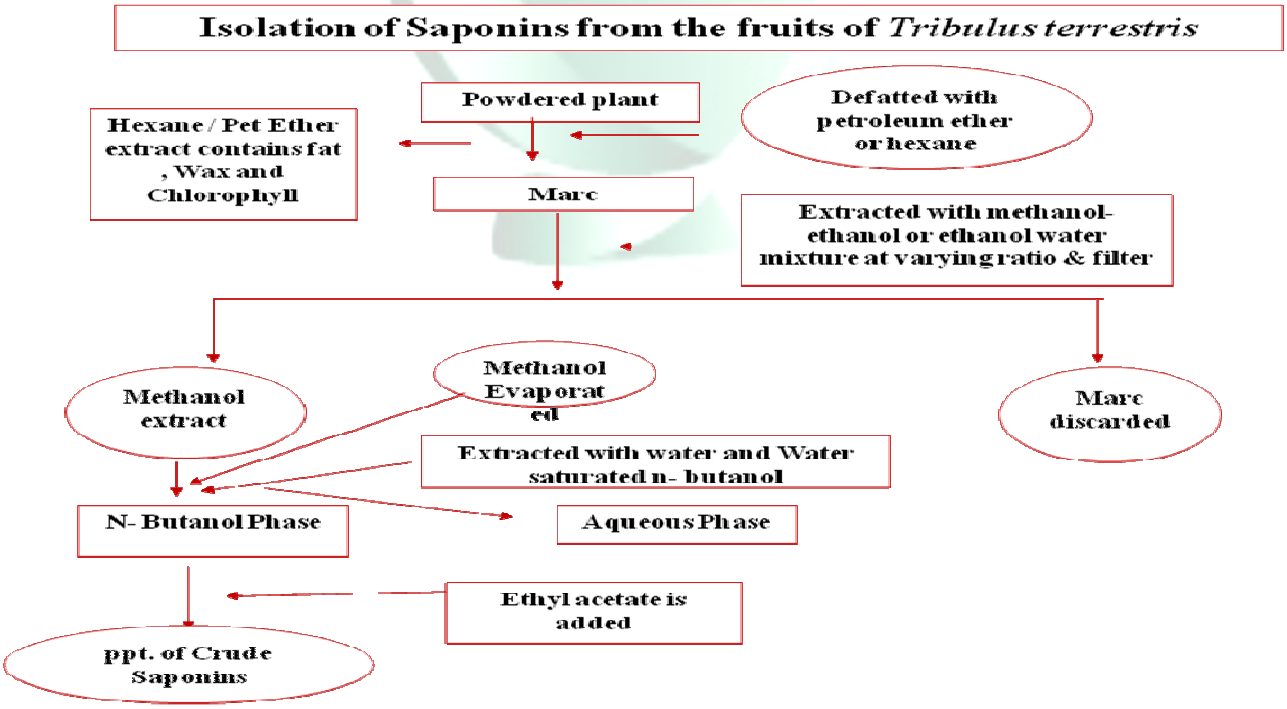
The following parameters were studied:

In vitro phagocytosis test:

Collection of peritoneal exudates cells (PECS):

After 24 hrs of last injection, peritoneal macrophage were obtained as following, 15 ml of normal saline was injected intraperitoneally to the rats, the abdomen was massaged for 5 minutes and the peritoneal exudate cells (PECS), consisting of 60% lymphocytes & 40% macrophages, were collected in a 5ml syringe,

Schematic Diagram For Isolation of Saponins From Crude Extract By Fractionation Method



allowing recovery of 90-95 of the injected volume Resting macrophages identified by morphology and neutral red staining, were counted and adjusted in RPMI-1640 with 10% FCS to 80-100 x 10^6 macrophage \ ml. Cellular disability was routinely measured before and after each experiment by the trypan blue exclusion test. In all cases disability was higher that 95%. All incubations were done at 37^0C in a humidified atmosphere of 5% CO_2.

Preparation of Candida albicans (Yeast) suspension:

Yeast (heat inactivated yeast, 50 ^{0}C. for 1 hr) was used as the test microorganism.

Slide Preparation method for Phagocytosis:

The Macrophages (Cell density adjusted to 1.0 x 10^6 cells/ml using MEM.) were mixed with 1.0 x10^6 cells/ml of yeast cells and incubated at 37^0C for one hour in CO_2 atmosphere, in the presence of the saponin fractions (50, 100, 200 µg/ml) and control without saponin fractions. Cytosmears were prepared after incubation, fixed with methanol, stained with Giemsa stain and studied under 100X 'oil immersion objective' to determine the phagocytic activity of PMN cells. Macrophages (100 nos.) were scanned and the cells with ingested microorganisms were counted. The parameters evaluated were percentage phagocytosis (percentage of PMN cells involved in phagocytosis) and phagocytic index (ratio of number of yeast cells engulfed to the total number of macrophages).

Statistical evaluation :

All the data's are expressed as the Mean ± SEM and analyzed by Student's "t" test.

Result

Effect of fractions on In vitro phagocytic test:

The in vitro phagocytosis using yeast as antigen was measured, by treating the PMNC's with the different fractions of saponins isolated from *Tribulus terrestris* and without saponins. All the fractions of saponins have showed phagocytosis at different doses but the fraction with Rf value 0, 89 has exhibited a significant increase in percent phagocytosis in a dose dependent manner. It has showed highest phagocytic activity at dose 200 µg/ml, which is found to be two fold increase, as compared to the control as in fig - 4 and 5

Discussion and Conclusion:

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years. Modulation of immune responses to alleviate disease has been one mechanism of interest. A number of medicinal plants have been shown to stimulate or inhibit immune responses (Craig, 1999; Block and Mead, 2003; Agarwal and Singh, 1999).

The present study demonstrates, for the first time, the immunostimulant potential of saponins, isolated from fruits of *Tribulus terrestris* by above method described. The result of PMN function test showed a significant increase in the percentage phagocytosis. This indicates that the saponins obtained from TT enhance the phagocytic efficacy of the PMN cells by causing more engulfment of the yeast cells, thereby stimulating a non-specific immune response. As neutrophils form the first line of host defense by virtue of their ability to phagocytosed invading microorganism and foreign body, they have a major role in modulating the immune function. The stimulation of neutrophils results in an increase in the immediate non specific cellular immune response.

The augmentation of the immune system may be brought about by increasing the activities of macrophages, T-lymphocytes and B-lymphocytes (Reizenstein and Mathe 1984). At low to moderate doses, commonly used therapeutic drugs such as doxorubicin, taxol, vincristine and vinblastine were shown to enhance the immune status of the host by activation of macrophages and lymphocytes (Mihich and Ehrke 1991, Mullins et al 1997, Mihich 2000a, 2000b)

The activation of macrophages may have a significant role in increasing the immunity since they are principle effector cells of the immune system (Gershon et al 1967). Suppression of the tumor growth, inflammation due to activation of macrophages by biological response modifiers has been demonstrated by earlier studies (Aoyogi et al 1977, Umezawa 1977, Jastrand and Blomgren 1982). The interleukin secreted from the activated macrophages have shown to modulate the secretion of immunoglobulins from B-lymphocytes (Golab et al 2000). Saponins appear to act similarly on T or B cells, as indicated by humoral and cell mediated response studied earlier (Tilwari et al., 2011a, 2011b).

The result of the present investigation, conclude that saponins had shown the augmentation of the host immune system, which may appear to be mediated through direct activation of macrophages and a possible stimulation of non specific immunity at low and non toxic dose. It was already reported that the naturally occurring saponins have immunomodulatory activity.

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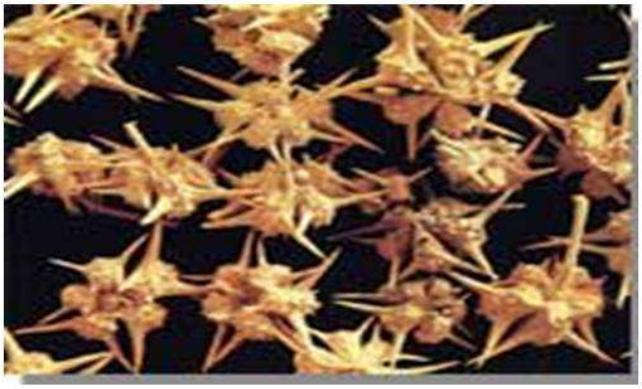
References

1) Ziauddin M., Phansalkar N., Patki P., Diwanay S., Patwardhan B. (1996). Studies on the Immunomodulatory effects of Ashwagandha. J Ethanopharmacol,50:69-76

2) Charak Samhita, (1949) .Charak Samhita, Translated by Shree Gulabkunverba Ayurvedic Society. In:,Jamnagar, India, p. 58



Scientific classification

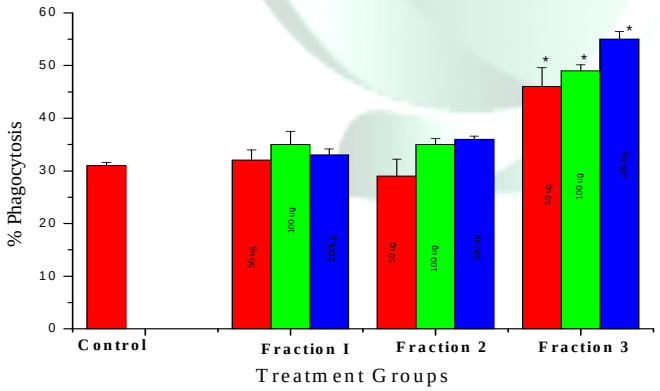
Kingdom : *Plantae*
Division : *Magnoliophyta*
Class :*Magnoliopsida*
Order : *Sapindales*
Family : *Zygophyllaceae*
Genus : *Tribulus L.*
Species :*Tribulus terrestris L.*

Fig 1 Tribulus terrestris fruits used in the study with its scientific classification

Fig: 2 Control wistar rats used in the study

Fig: 3 collection of peritoneal fluid from rats.

Fig 4: Effect of different fraction of saponins isolated from *Tribulus terrestris* on *in vitro* phagocytic function of murine peritoneal macrophages.



Values are Mean ± SEM, n = 6 in each group,
***p<0.05 when compared with control**

3) Mallurwar V.R., Joharapurkar A.J., Durgakar K.J. (2006). Studies on Immunomodulatory Activity of *Mucuna prurient.* Indian J Pharma Educ Res,40:205

4) Kalia A.N. (2005). Text Book of Industrial Pharmacognosy. Oscar publication.

5) Patwardhan B., Kalbag D., Patki P.S. (1991). Search of immunomodulatory agents: A review. Indian Drugs,28:249

6) Shivaprasad H.N., Kharya M.D., Rana A.C., Mohan S., (2006). Preliminary immunomodulatory activities of aqueous extract of Terminalia chebula. Pharmaceutical Biology,44: 32–34.

7) Atal C.K., Dubey R.K. and Singh J.(1985). Biochemical basis of enhanced drug bioavailability by piperine:Evidence that piperine is a potent inhibitor of drug metabolism. Journal of Pharmacology and Experimental Therapeutics,232:258-262.

8) Atal C.K., Sharma M.L., Kaul A., Khajuria A. (1986). Immunomodulating agents of plant origin. I: Preliminary screening. J Ethnopharmacol, 18, (2):133-41

9) Godhwani S., Godhwani J.L., and Vyas D.S. (1998). Ocimum sanctum: A preliminary study evaluating its immunoregulatory profile in albino rats. Journal of Ethnopharmacology, 24: 193-198.

10) Dua S.K.(1989). "Electret method for continuous measurement of radon in water" Health Physics, 68:110-114

11) Adaikan P.G., Gauthaman K., Prasad R.N., Ng S.C. (2000). Proerectile pharmacological effects of Tribulus terrestris extract on the rabbit corpus cavernosum. Ann Acad Med Singapore, 29, (1): 22-26

12) Hu K. and Yao X. (2002). Protodioscin (NSC-698 796): its spectrum of cytotoxicity against sixty human cancer cell lines in an anticancer drug screen panel. Planta Med, 68,(4):297-301.

13) Zhong Yao Cai. (2003). Effect of Tribulus terrestris on Cancer cells, 26 ,(2):104-6

14) Praveen kumar V., Kuttan R. and Kuttan G., Tumouri E. (1994) . J. Exp Biol, 80: 306

15) Rao C.S., Gopumadhavan S., Chauhan B.L., Kulkarni R.D., Mitra S.K. (1996). Immunotherapeutic modification by an Ayurvedic formulation Septilin. Indian J Exp Biol, 32: 553-558

16) Stuart H., Rev G.A. (1990). Chinese Materia Medica. Taipei. Southern Materials Centre A translation of an ancient Chinese herbal. Shanghai: The American Presbyterian Mission Press, 1911

17) Jie Y.H., Cammisuli S.& Baggiolini M. (1984). Immunomodulatory effects of Panax ginseung C. A. MEYER in the mouse. Agents and Actions, 15: 386–391.

18) Kensil C.R. (1996) . Saponins as vaccine adjuvants. Critical Reviews in Therapeutic Drug Carrier Systems, 13: 1–55.

19) Barr I.G., Sjolander A. & Cox J.C. (1998). ISCOMs and other saponin based adjuvants (Review). Advanced Drug Delivery Reviews, 32: 247–271.

20) Stahl S. M. and Meltzer H. Y. (1963). A kinetic and pharmacologic analysis of 5-hydroxytryptamine transport by human platelets and platelet storage granules: comparison with serotonergic neurons. J. Pharmac. exp. Ther., 205:118-132.

21) Boyum A., (1968). Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest ,21:77-89

22) Craig W.J. (1999). Health-promoting properties of common herbs. *Am J Clin Nutr*, 70: 491S-9S.

23) Block K.I. and Mead M.N. (2003). Immune system effects of Echinacea, ginseng, and astragalus: a review. *Int Cancer Ther*, 2: 247-67.

24) Agarwal S.S., Singh V.K. (1999).Immunomodulators: a review of studies on Idian medicinal plants and synthetic peptides: Part I. Medicinal plants. *Proc Indian Natl Sci Acad*, 65:179-204.

25) Reizenstein P. and Mathe G. (1984) . Immunomodulating agents. In : Immune modulation agents and their Mechanisms, Fenichel R.L. and Chirigos M.A. (eds) . Vol. 25, Marcel Decker, Inc., New York and Basel : 347-361

26) Mihic, E. and Ehrke M.J. (1991). Immunomodulation by anticancer drugs. In:Devita V.T., Helman S., Rosenberg S.A., eds Biologic therapy of cancer Philadelphia, PA:JB Lippincott, 776-786.

27) Mullins D.W., Walker T.M., Berger C.J. and Elgert K.D. (1997). Taxol mediated changes in fibrosarcoma induced immune cell function: Modulation of antitumor activities. Cancer Immunol Immunother., 45: 20-28

28) Mihich E. (2000a). On the immunomodulating effects of anticancer drugs and their therapeutic exploitation. Jpn. J. Clin. Oncol., 3:469-471

29) Mihich E. (2000b). Historic overview of biologic response modifiers . Cancer Invest, 18:456-466

30) Gershon R.K., Carter R.I. and Lane N.J. (1967) . Studies on homotransplantable lymphomas in hamsters. IV . Observation on macrophages in the expression of tumor immunity. Am .J. Pathol., 51:1111

31) Aoyagi T., Ishizuka M., Takeichi T. and Umezawa H. (1977). Enzyme inhibitors in relation to cancer therapy. J. Antibiotic, 30:121-132

32) Umezawa H. (1977). Recent advances in bioactive microbial secondary metabolites. J. Antibiot., 30 :138-163.

33) Jarstrand C. and Blomgren H.(1982). Increased granulocyte phagocytosis after oral administration of bestatin, a new immunomodulator. J. Clin. Lab. Immunol., 7:115-118

34) Golab J., Zagozdozon R., Stoklosal T., Kaminski R., Kzar K. and Jakobisiak M. (2000). Direct stimulatin of macrophages by IL-12, IL-18-a bridge too far? Immun. Lett., 72:153-157

35) Tilwari A., Shukla N.P., Uma devi P. (2011a): Effect of five medicinal plants used in Indian system of medicines on immune function in Wistar rats. African Journal of Biotechnology Vol. 10 (73),: 16637-16645.

36) Tilwari A., Shukla N.P., Uma devi P. (2011a): Immunomodulatory activity of the aqueous extract of seeds of Abrus precatorius Linn (Jequirity) in mice. Iran.J.Immunol. 8(2):96-103

Fig 5: Plate : Photomicrograph showing peritoneal macrophages isolated from the peritoneal cavity of rats stained with giemsa stain.