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Immunomodulatory effect of *Tricosanthes Dioica* Roxb

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ABSTRACT

Objective: Aqueous extract of *Tricosanthes dioica* Roxb.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 *Tricosanthes dioica* Roxb. 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 *Tricosanthes dioica* Roxb. showed immunomodulatory effect in rat.

1. 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].

Trichosanthes dioica Roxb. (Family: Curcurbitaceae) isdioecious, climber with perennial root stock. It was found wild in the plains of North India from Punjab to Assam; it is also extensively cultivated all over the warmer region of India, particularly in Uttar Pradesh, Bihar, West Bengal and Assam for its fruit [10-12]. Brief description of *T. dioica* is found in the charaka samhita , sushruta samhita [13].Leaves of the plant are considered to be rich source of bioactive compounds with many medicinal properties such as blood sugar lowering effect in experimental rat models [14], mild diabetic human subject [15] antifungal activity and antibacterial activity [16-18] . 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 *Trichosanthes dioica* whole plant in experimental animal models.

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2.Materials and methods

2.1 Plant Material

Trichosanthes dioica Roxb was procured from agriculture college, Indore, India. The identity of the plant was confirmed through Dr. S.C Mahajan, Department of Pharmacognosy, Mahakal Institute of pharmaceutical studies, Ujjain. A voucher specimen of the plant has been deposited in the Herbarium-cum-museum of Mahakal Institute of pharmaceutical studies.

2.2 Plant extract

2.2.1 Preparation of aqueous extract

Aqueous extract of *Trichosanthes dioica* Roxb.was prepared by maceration method. Powdered plant material was macerated for 72 h with occasional shaking in distilled water. It was then filtered. The solvent was evaporated under vacuum. Yield of aqueous extract of *Trichosanthes dioica* Roxb. was 4.2 % w/w. Preparation of drug solutions: TD (Trichosanthes dioic) extracts suspended in1% CMC

solution , and Control group given 0.5 ml of 1% CMC solution

2.2.1 Experimental designs

Animals were divided into three groups, each having five rats and treated accordingly,

Group I: received 0.5 ml of 2 % w/v sodium carboxy methyl. cellulose suspension p.o. for 14 days as a control group.

Group II: Animals treated with aqueous extract of *Trichosanthes dioica* (100 mg/kg) p.o

Group III: Animals treated with aqueous extract of *Trichosanthes dioica* (200mg/kg) p.o

2.3. Pharmacological screening

2.3.1 Phagocytic assay (PM′ function test)

Ficoll Hypaque density gradient method was used for isolation of neutrophils from blood collected in heparinised tube from peripheral vein of healthy rats. Afterward dextral sedimentation was performed and supernatant with more than 90 % PMN cells were collected and used cell density was 1×10^6cells/ml in MEM.Candida albicans in concentration of 1×10^6cells/ml was used for present study. PMN cells and C.albicans were mixed and incubated in 5 % CO2 at 37 ℃ with different extract at different concentration. After incubation cytosmears were prepared and fixed in methanol and stained with Giemsa stain. Prepared slides were observed under 100X magnification for determination of phagocytic activity. Hundred neutrophil were analyzed and it was how many cells ingested microorganism. Percentage of PMN cells involved in phagocytosis (percentage phagocytosis) and ratio of number of microorganism engulfed to the total number of neutrophils (phagocytic index) was calculated for ascertaining in-vitro phagocytic activity.[19]

2.3.2 Acute toxicity study

Acute oral toxicity study was performed as per OECD 423 guidleines. Extract (CEE) was given upto the maximum dose of 2000 mg/kg and animals were observed for mortality. .[19]

2.3.3 Delayed type hypersensitivity test

To measure cellular immunity delayed type hypersensitivity test was performed. SRBC was used as antigen in present study. In group I (six rats) vehicle was administered orally for five days. In group II and III was administered orally at the dose of 100 mg/kg, and 200 mg/kg respectively for five days. TDE and Vehicle was administered on each two day before immunization, on the day of immunization and on each two day after immunization (i.e. −2,−1,0,+1+2). Rats were immunized by injecting 0.1 ml of SRBS subcutaneously into the right hind paw on day zero. Animals were challenged seven day later with same amount of SRBC into the left hind paw. Change in paw thickness was measured using digital caliper at 4th and 24th hour after challenge. .[19]

2.3.4 Hemagglutination reaction

To measure humoral immunity hemagglutination test was performed. In group I (six rats) vehicle was administered orally for five days. In group II and III was administered orally at the dose of 100 mg/kg, and 200 mg/kg respectively for five days. T.DE and vehicle was administered on each two day before immunization, on the day of immunization and on each two day after immunization (i.e. −2,−1,0,+1+2). Rats were immunized by intraperitonealinjection of 0.5 ml SRBC. On the tenth day after immunization blood sample was collected by retro orbital puncture. Hemaggluination titer assay was performed for antibody level determination. Serial dilution of serum and 0.1% bovine serum albumin (100 μl: 100 μl) was prepared in sterile saline. One volume (100 μl) of 0.1 % SRBC in saline was added and mixed. They were allowed to settle at room temperature for 90 min till control tube showed a negative pattern (a small button formation). The value of highest serum dilution showing visible hemagglutination was considered as antibody titer. .[19]

2.3.5 Biostatical analysis

All data were analysed by One Way ANOVA followed by Dunnet′s test. $P<0.05$ was considered as level of significance.

3. Results

Administration of aqueous extract of (100 mg/kg) and 200 (mg/kg) *Trichosanthes Dioica* produced dose dependent significant increased in Phagocytic index and antibody titre compared to control. The results were given in the table 1 and table 2.

Table1

pmn function test for different extracts of *trichosanthes dioica* in wistar albino rat.

Treatment	Concentration	% Phagocytosis	Phagocytic index
Control	−−−	28.66±3.66	1.6±0.09
T.D 100 mg	1.0	31.67±2.58	1.69±0.11
	2.0	32.5± 3.15	1.76±0.12
	3.0	33.33±4.23	1.78±0.13
T.D 200 mg	1.0	32.67±3.39	1.93±0.10 a
	2.0	36.17±1.94a	2.05±0.24 a
	3.0	38±4.56a	2.12±0.32 a

*All data presented in mean ± SD

a$P<0.05$ as compared to control group.

3.1 Effect of TD on Phagocytic index

Phagocytosis represents an important innate defence mechanism against ingested foreign materials. The blood monocytes, neutrophils and macrophages are specialized phagocytic cells. In PMN test an increase in phagocytic index in dose dependant manner was observed, which may be due to increase in production of phagocytic cells stimulated by TD extract. The TD extract shows significant increase in phagocytic index ($P< 0.05$) at the dose 100 mg and 200 mg. The result indicates the role of TD in activation of non specific immunity.

3.2 Effect of TD on Haemagglutination Antibody Titre

3.2.1 Haemagglutination Antibody Titre

The haemmagglutinating antibody (HA) titer value was significantly ($P<0.05$) increased in animals that received vaccination along with TD extract at two different doses (100mg and 200 mg) compared to animals that received vaccination alone (Control). The results of Hypersensitivity reaction test suggest that TD extract affects humoral immunity as shown by its effect in the indirect

haemagglutination test. The results showed that levels of circulating antibodies are increased if the test animals are pretreated with TD extract.

Table 2

Effect of extracts of *trichosanthes dioica* of in hypersensitivity reaction test

Treatment	Haemagglutination Antibody Titre
	Titre value
Control	193.33 ± 44.96
T.D (100 mg)	248.5 ± 59.82
T.D (200 mg)	595.83 ± 252.87 a

Each group consist of six animal

Data presented in Mean ± SD, a $P<0.05$ as compared to vehicle treated group.

4. Discussion

In fighting against various diseases immune system plays an important role. Modulation of this immune response may help in treating and preventing many diseases. Agents that can modulate immunity in the presence of an impaired immune responsiveness can provide supportive therapy inmany treatment approaches.[19]. Phagocytosis is an important part of immunity. It was observed that TDE elevated percentage phagocytosis at all selected dose thus for investigation on humoral immuneresponsehaemagglutination antibody titre (HAT) methodology was used. Augmentation of thehumoral immune response to SRBCs by extract TDE evidenced by increase in the antibody titres in the blood of rats. B lymphocytes,plasma cells, IgG and IgM are the components involvedin the complement activation, opsonization, and neutralization of toxins.[19]Thus they become anintegral part of humoral immune system. Thus from HAT test it can be postulated that TDE showed its effect by modulating any of these factors involvedin humoral immune system.

SRBC-induced delayed type hypersensitivity was used to assess the effect of thefraction on cell-mediated immunity. Cell mediated immunity involves effector mechanisms carried outby T lymphocytes and lymphokines. Cell mediated immunity responses are critical to defenceagainst infectiousorganisms, infection of foreign grafts, tumor immunity and delayed-type hypersensitivity reactions [19]. Thus stimulatory effect of CEE on delayed type hypersensitivity test revelaed that extract was having stimulatory effect on T lymphocytes. *Trichosanthes dioica* 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 *Trichosanthes dioica* was studied in wistar albino rat.

In this present study, *Trichosanthes dioica* 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 *Trichosanthes dioica*

The present study suggests that the aqueous extract of *Trichosanthes dioica* stimulate the antibody production in rat.

Conflict of interest statement

We declare that we have no conflict of interest.

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