Academic Sciences

Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Issue 4, 2011 ISSN - 0974-2441

Research Article

IMMUNOMODULATORY ACTIVITY OF METHANOLIC EXTRACT OF *THESPESIA POPULNEA* LEAVES IN WISTAR ALBINO RATS

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Received: 28 Aug 2011, Revised and Accepted: 21 Sep 2011

ABSTRACT

The work has been carried out to find out immunomodulatory potential of methanolic extract of leaves of *Thespesia populnea* (Family: Malvaceae). The methanolic extract of *T. populnea* (METP) was given at doses of 100, 200, and 400 mg/kg b.w, p.o. Levamisole (50 mg/kg b.w, p.o) was used as a standard immunomodulatory drug whereas Cyclophosphamide (30 mg/kg b.w, p.o) was used as a standard immunosuppressant drug. The measurement of immunomodulatory property was carried out by Delayed Type Hypersensitivity (DTH), Humoral antibody (HA) titre response to SRBC, and Cyclophosphamide induced myelosuppression. Results of present study clearly indicate that Methanolic extract has shown potentiation of DTH response at 400 mg/kg b.w, p.o ($P<0.01$). The increasing doses of methanolic extract have shown the augmentation of antibody titre. Cyclophosphamide induced immunosuppression was counteracted by METP, i.e. WBC counts have reached to normal values at a dose of 200 mg/kg b.w, p.o ($P<0.001$). Phytochemical screening suggest the presence of flavonoids, triterpenoids, Proteins, Amino acids, phenolic and steroidal compounds. The immunomodulatory activity of plant may be attributed to these phytoconstituents. From the above study we concluded that METP stimulate both cellular and humoral immunity. So the plant can be explored for the search of new complimentary therapeutic agent.

Keywords: Immunomodulatory, *Thespesia populnea* , Malvaceae, DTH, HA titre, Myelosuppression.

INTRODUCTION

Immunology is one of the most developing areas of biomedical research. It also opens the doors of immense hopes and major advances in the prevention and treatment in wide range of disorders. It also defined as the study of mechanism of defense of the body against harmful invading agents. Arthritis, ulcerative colitis, asthma, allergy, parasitic and infectious diseases are primarily considered as immunologic disorders[1, 2]. Severe side effects and cost of the allopathic medicines have aggravated the necessity of research of drugs which are without side effects especially belonging to the traditional systems of medicines. Herbal medicines have been the foundation of treatment and cure for various ailments. Natural products provide an excellent material for the discovery and development of novel immunomodulatory compounds.

Thespesia populnea (Malvaceae) is a large tree found in costal and tropical regions of the India. The medicinal importance of this plant is mentioned in famous primordial texts of Ayurveda such as Dravyaguna[3]. Flavonoids, glycosides, and phytosterols are major constituents of *T. populnea*[4]. Leaves contain lupeol, β-sitosterol, lupenone[5], quercetin, ferulic, syringic, melilotic acid[6]. Traditional leaves are used to treat swollen joints confirm its anti-inflammatory property[7, 8]. Gossypol is the main color pigment present in this plant[9]. Bark and flowers possess hepatoprotective and antioxidant activity [10, 11].

MATERIALS AND METHODS

Plant Material Collection and Extract Preparation

Fresh leaves of *T. populnea* were collected from Ahmednagar district of Maharashtra. Authentication of plant was done at Botanical Survey of India, Pune, (Voucher number SWITTHP20). Shade dried leaves were crushed and cold macerated with methanol for 7 days. Solvent was filtered after 7 days and evaporated in open air.

Qualitative Phytochemical Screening [12]

Methanolic extract of plant was screened for the presence of various phytoconstituents *viz*, alkaloids, carbohydrates, flavonoids and phenolic compounds, proteins, amino acids, terpenoids, steroids, and tannins as per the standard procedure. Results are presented in Table 1.

Animals used

Wistar albino rats (Approx 150 to 180 gm) were procured from Gentox Bioservices, Hyderabad. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad (Reg. No.1175/ac/08/CPCSEA).

Antigens

Sheep Red blood cells (SRBC) were collected in Alsever's solution from animal house of National Institute of Nutrition, Hyderabad. It was washed 3-4 times with large quantity of sterile and pyrogen free saline[13].

Acute Toxicity Studies

Methanolic extract of leaves was tested for acute toxicity studies as per procedure given in OECD guidelines. Rats (n=6) were starved for overnight and fed orally with the increasing doses of extract (10, 40, 100, 400, 1000 and 2000 mg/kg .b.w). Animals were observed for next 14 days for behavioral changes and mortality.

Delayed Type Hypersensitivity[14, 15, 16]

Antigen Challenge

On 0^{th} day, all groups were sensitized with 0.1 ml of SRBC containing 1×10^8 cells, intraperitonially.

Experimental Design

Animals were divided into six groups each containing 6 animals.

Group I - Control, 1% Gum Acacia suspension in saline

Group II - Negative control i.e. Cyclophosphamide, 30 mg/kg b.w p.o (4^{th}, 5^{th}, 6^{th} day).

Group III - Standard, Levamisole, 50 mg/kg b.w, p.o (1^{st} to 7^{th} day)

Group IV - Methanolic extract, 100 mg/kg b.w, p.o. (1^{st} to 7^{th} day)

Group V- Methanolic extract, 200 mg/kg b.w, p.o. (1^{st} to 7^{th} day)

Group VI - Methanolic extract, 400 mg/kg b.w, p.o (1^{st} to 7^{th} day)

On 7^{th} day, prior to injection right hind footpad thickness was measured with Micrometer screw gauge (Mitutoyo Digimatic). Then animals were challenged with 1% SRBC (20 µl) into the right hind footpad. On 8^{th} and 9^{th} day footpad thickness was again measured. Difference between prior and post injection footpad thickness was reported as DTH response.

Humoral Antibody (HA) Titre Response to SRBC[14, 16]

Experimental design was done same as mentioned in Delayed type hypersensitivity model. On 7th day before challenge, blood was withdrawn from retro-orbital plexus of each animal. Blood was centrifuged, and serum was separated. Serial two fold dilution were made i.e 50 µl of serum was added to 1st well of 96-well micro titre plate containing 50µl normal saline. To this 1% SRBC (50µl) dissolved in normal saline was mixed. From 1st well 50µl of diluted serum was added to 2nd well containing 50µl normal saline and 50µl 1% SRBC. Such a dilutions were done till 24th well. Plates were incubated at 37ºC for 1 hr. Highest dilution that has shown visible agglutination was considered as haemagglutination antibody titre.

Cyclophosphamide Induced Myelosuppression: [13, 17]

Group I - Control, 1% Acacia suspension in saline

Group II - Negative control, Cyclophosphamide 30 mg/kg b.w p.o (11th, 12th, 13th day).

Group III - Standard, Levamisole 50 mg/kg b.w, p.o (1st to 13th day)

Group IV - Methanolic extract, 100 mg/kg b.w, p.o. (1st to 13th day)

Group V - Methanolic extract, 200 mg/kg b.w, p.o. (1st to 13th day)

Group VI - Methanolic extract, 400 mg/kg b.w, p.o (1st to 13th day)

On 0th day, blood was withdrawn from retro-orbital plexus of animals of each group and subjected to haematological parameter determination. Drugs were feed as per the schedule given above from 1st to 13th day. Cyclophosphamide (30mg/kg, p.o) was given to all animals on 11th, 12th and 13th day, 1 hr after extract administration except control and standard group. On day 14th, again blood was withdrawn from retro- orbital plexus of animals of each group and subjected to haematological parameter determination and restoration of are observed.

Statistical Analysis

Values are Mean ± SEM, n= 6 rats in each group. Results obtained were statistical analyzed by using one-way ANOVA followed by Dunnett's multiple comparison test. P<0.05 was considered a significant value.

RESULTS AND DISCUSSION

The main objective of this study was to explore the immunomodulatory potential of methanolic extract of leaves of *T. populnea* with reference to its immunostimulatory properties in animal models. Phytochemical screening of extract has shown the presence of flavonoids, tannins, and phenolic compounds. It also revealed the presence of traces of carbohydrates, steroids and terpenoids (Table1).

Acute Toxicity Studies:

No behavioral changes or mortality were observed after 14 days of extract administration. So 1/10th of the dose i.e 200 mg/kg, b.w has been selected for the present study.

Delayed Type Hypersensitivity:

Dose dependant increase in paw edema after 24 hrs of challenge was observed in all extract treated groups when compared to control. Significant increase in paw thickness was observed at the dose levels of 100 mg/kg (P<0.05), 200 mg/kg (P<0.01) and 400 mg/kg (P<0.01) after 24 hrs. DTH response was lowered after 48 hrs but significant (P<0.01) in all groups when compared to control animals. After 72 hrs footpad thickness was normal. Potentiation of DTH response (P<0.01) was observed in Cyclophosphamide treated animals because it damaged short lived suppressor T cells in immune system. Levamisole, a standard immunomodulatory drug, has shown maximum potentiation of DTH response (P<0.001). Cell mediated immunity was calculated using Delayed Type Hypersensitivity test in animals. DTH requires the unambiguous recognition of a given antigen by activated 'T' lymphocytes, which consequently proliferate and liberate cytokines. These interns induce vasodilatation, increases vascular permeability, macrophage activation, accumulation and produce inflammation. It augments phagocytic activity and increases concentration of lytic enzymes for more effective killing so results increase in footpad thickness.

Increase in DTH response of animals revealed the stimulatory effect of ME on T lymphocytes. (Table 2)

Humoral Antibody (HA) Titre Response to SRBC

In Humoral antibody titre, (Table 3), doses of 100, 200, and 400 mg/kg showed titre value of 204.8±31.35, 281.6±62.70, and 332.2±77.2 respectively. Immunosuppressant group i. e. animals treated with cyclophosphamide have shown significant reduction of haemagglutination titre (2.4±0.4) as compared to control group (8.8±1.96). Immunostimulation of humoral response by standard immunomodulatory drug levamisole has resulted in higher antibody titre (P<0.01).

Studies suggests that extract at a higher dose level 400mg/kg (P<0.01) is capable to influence the role of immunoglobulins resulting in activation of dendritic cells or pre B- cells resulting in activation of antibodies which provide the higher agglutination titre against SRBC.

Table 1: Preliminary Phytochemical Screening of Methanolic Extract of *T. Populnea* Leaves

Phytoconstituents	METP
Alkaloids	-
Carbohydrates	+
Glycosides	+
Flavonoids	+++
Tannins and Phenolic compounds	++
Proteins & Amino acids	+
Triterpenoids	+
Sterols	+

[+] Positive, [-] Negative

Cyclophosphamide Induced Myelosuppression

Cyclophosphamide treatment for the period of 3 days showed significant reduction in WBC count (3.22± 0.48, P<0.001) and thereby exerted immunosuppressant effect. Suppressive effect of Cyclophosphamide was protected by administration of METP. Combined treatment of extract and myelosuppressive drug at all doses showed restoration of WBC count when compared to myelosuppressive drug. But total WBC count was regained normal values only at a dose of 200 mg/kg on 14th day of study (P<0.001). Levamisole (P<0.001) has shown marked potentiation of WBC counts when compared with 0th day count. Increasing doses of extract have not showed much restoration of WBC counts. Results shown in table 4 have revealed that administration of methanolic extract of plant could stimulate the haemopoetic system.

Table 2: Table shows effect of Methanolic extract *T. Populnea* on Dth reactivity

Group	Drug	Dose (mg/kg)	Mean footpad thickness (mm)	
			24 hrs	48 hrs
Group I	Control	1% gum acacia	0.0231±0.0020	0.02±0.123
Group II	Cyclophosphamide	30 mg/kg, bw, p.o	0.8902±0.066**	0.4242±0.04**
Group III	Levamisole	50 mg/kg, bw, p.o	1.5764±0.084***	0.893±0.230**
Group IV	ME treated	100mg/kg, bw, p.o	0.4821 ± 0.087*	0.4631 ±0.0821**
Group V	ME treated	200 mg/kg, bw, p.o	0.6878 ± 0.149**	0.5736 ±0.123**
Group VI	ME treated	400 mg/kg, bw, p.o	1.257 ± 0.114**	0.7442 ± 0.136**

Comparison of Group I (Control) with Group II, III, IV, V, VI; Where, ***P<0.001 Extremely Significant, **P<0.01 Very Significant, *P<0.05 Significant

Table 3: Effect of methanolic extract of *T. Populnea* on Antibody Titre in rats

Group	Drug	Dose (mg/kg)	Titre Level
Group I	Control	1% gum acacia	8.8±1.96
Group II	Cyclophosphamide	30 mg/kg, b.w, p.o	2.4±0.4^{ns}
Group III	Levamisole	50 mg/kg, b.w, p.o	819.2±125.41**
Group IV	ME treated	100 mg/kg, bw, p.o	204.8±31.35^{ns}
Group V	ME treated	200 mg/kg, bw, p.o	281.6±62.70*
Group VI	ME treated	400 mg/kg, bw, p.o	332.2±77.2**

Group II to VI were compared with Group I; Where, ^{ns}P>0.05 not significant,*P<0.05 Significant, **P<0.01 Very significant

Table 4: Effect of Cyclophosphamide Induced Myelosuppression on WBC Counts

Group	Drug	Dose (mg/kg)	WBC count (×10^3/mm^3) (Mean ± SEM)	
			(0 day)	(14day)
Group I	Control	1% gum accacia	9.55 ± 0.737	9.3± 0.456
Group II	Cyclophosphamide	30 mg/kg, bw, p.o	10.1 ±0.287	3.22± 0.48**a
Group III	Levamisole	50 mg/kg, bw, p.o	9.738 ± 0.428	10.68± 0.22***b
Group IV	ME treated	100 mg/kg, bw, p.o	9.50 ± 0.445	8.15 ± 0.119**b
Group V	ME treated	200 mg/kg, bw, p.o	10.75 ± 0.839	9.45±0.573***b
Group VI	ME treated	400 mg/kg, bw, p.o	9.95 ± 1.109	8.875 ±0.77**b

a: Group II was compared with Group I; b: Group III to VI were compared with Group II

Where, **P<0.01 Very Significant, ***P<0.001 Extremely Significant

CONCLUSION

Extensive literature survey revealed that flavonoids, polyphenolics, and terpenoids possesses anti inflammatory, antioxidant, neuroprotective property and also useful for prophylactic and therapeutic treatment of allergy. METP stimulates both cellular and humoral immune system and also showed potentiating effect on haemopoetic system in myelosuppressant model. So this effect of plant is may be due to the presence of flavonoids, polyphenolics, and terpenoids which may modulate one of these mechanisms. Immunomodulation using medicinal plants can provide an alternative to conventional chemotherapy for a variety of diseases, so along with leaves other parts of plant can be screened for the immunomodulatory activity.

ACKNOWLEDGEMENT

The authors are very grateful to The Principal, Dr. C.V.S Subramanyam and M. Gangaraju, Associate Professor, Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India for providing animal house facility. Author expresses her gratitude to JNTUH, Hyderabad for financial support.

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