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EVALUATION OF IMMUNOMODULATORY ACTIVITY OF THE HYDROALCOHOLIC EXTRACTS OF PTEROSPERMUM ACERIFOLIUM FLOWERS IN WISTAR RATS

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ABSTRACT

Background: *Pterospermum acerifolium* Linn. is a widely used ethno-medicinal plant for various diseases in India. Various parts of this tree have been traditionally used as ethno medicine for a number of disorders including cancer. Most of immunostimulants and immunosuppressants in clinical use are the cytotoxic drugs which possess serious side effects. There is a growing interest to use herbal medicines as multi-component agents to modulate the complex immune system in the prevention of infections rather than treating the immune-related diseases. Thus the search for natural products of plant origin as new leads for development of potent and safe immunomodulator agents is gaining much major research interest. **Aims and Objectives:** The present study was conducted to evaluate the immunomodulatory activity of methanolic flower extract of *Pterospermum acerifolium in Wistar rats*. **Materials and Methods:** In the current study, various extracts of *Pterospermum acerifolium* flower were subjected to preliminary phyto- physicochemical studies. MEPA 200 and 500 mg/kg oral dose were selected for the study after conducting the acute dose toxicity study. We have used neutrophil adhesion test, haem agglutination antibody titre test, delayed-type hypersensitivity reaction test. Levamisole was used as standard immunomodulatory agent and Distilled water served as a control in all the tests. **Results**: In the in vivo studies, the successive methanol extract was found to exhibit a dose related increase in the hypersensitivity reaction, to the SRBC antigen, at concentrations of 200 and 500 mg/kg. It also resulted in a significant increase in the antibody titre value, to SRBC, at doses of 200 and 500 mg/kg in animal studies. There was an increment in WBC, lymphocyte, and neutrophil counts at a dose of 500 mg/kg body weight similar to the levamisole. The neutrophil adhesion was statistically significant ($p \leq 0.05$) for treatment groups that received 200 mg/kg bwt (25.73%) and 500 mg/kg bwt at 29.05%. The mean increment in footpad thickness was high (3.60±0.064 mm) after 4 h of injection of antigen in the footpad of rats dosed 500 mg/kg bwt and this later reduced to (1.52±0.28 mm) after 24 h. There was a dose-dependent increment in the mean haem agglutination antibody titre to sheep red blood cells (SRBC) from 17.50±1.48 HA units/µL for the 200 mg/kg bwt to 24.35±1.36 HA units/µL for the 500mh/kg body weight. **Conclusions:** The effect of this extract was comparable to the standard drug levamisole. *Pterospermum acerifolium* flower extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, allergic disorders, and may also serve as immunoadjuvant for vaccine therapy.

Keywords: hemagglutination, immunostimulant, sterculiaceae, wistar albino rats

INTRODUCTION

System of medicines like Ayurveda gives emphasis on promotion of health through strengthening host defences against different diseases. A number of medicinal herbs have long been used and reported to boost the immune system or to modulate it and they are used putatively to treat and prevent various disease conditions worldwide[1].

Currently, worldwide, there is an increase in diseases especially infectious diseases that requires efficient body defence mechanisms to control them through the process of immunomodulation[2].

Various allopathic drugs or medicines are used to modulate the immune system. However, these drugs are very expensive for poor people, they are not easily accessible, and in most cases they are associated with adverse drug reactions. As a result, the majority of people especially in the rural areas of the developing world turn to the use of alternative herbal medicines from medicinal plants that are widely accepted, accessible, cheaper, and assumed to have fewer side effects. The discovery and isolation of more specific immunomodulatory agents from plant origin possesses potential to counteract the side effects and high cost of synthetic compounds. *Pterospermum acerifolium* (Family Sterculiaceae) is a very large tree, 40-60 meters high, with wide-spreading branches. A preparation from its leaves has been used by rural practitioners, in Assam, to boost the immune system to fight a number of diseases. With above background, the study was taken up to determine the immunomodulatory activity of methanolic flower extracts of *Pterospermum acerifolium* in Wistar albino rats.

Plant Material

Sample collection and identification: The flowers of *Pterospermum acerifolium* belonging to the family Sterculiaceae were harvested during the flowering season (June to October) from the herbal garden of SJG Ayurvedic Medical College, Koppal, Karnataka, India. This plant was identified and authenticated from Central Research Facility, Belgaum, Karnataka, India and a voucher specimen number (CRF/12/147) was deposited.

Processing and extraction[3]:

The flowers were shade-dried in open air, pulverised using electric grinder to make powder and stored in airtight containers. The powder was subjected to successive Soxhlet extraction using solvents of varying polarity; petroleum ether (60oC-80oC), benzene, chloroform, acetone, methanol and water. The mixture was filtered on the 3rd day using a gauze cloth and the fine filtrate was obtained using Whatman No: 1 filter paper in a Buchner funnel. The filtrate was concentrated using a Büchi Rotavapor R-200 (Büchi Labortechnik, Flawil, Switzerland) into slurry which was further heated on water bath at 45 ± 5°C and stored in vacuum desiccator. The dry extract was stored at 4 °C until the immunomodulatory experimental bioassays were carried out. Concentrated stock solution of the *Pterospermum acerifolium* flower extract was prepared by dissolving 4 g of extract in 40 mL of distilled water to make a working concentration of 200mg/ 2mL and again 10 g of extract in 40 mL of distilled water to make a working concentration of 500mg/ 2mL respectively. Levamisole that was given at a dose of 50 mg/kg Body weight as the standard drug.

Preliminary Phytochemical Investigation: Preliminary phytochemical screening of extract was performed using standard procedures and tests with little modifications[4].

Animal Study design and setting: The study was a preclinical laboratory-based experimental study. It was conducted at Institute for Advanced Studies in Science and Technology, Boragaon, Assam.

Drugs and Chemicals: The levamisole (Cipla Limited - India) was purchased from Local Pharmacy, Guwahati, Assam, India and was used as standard. **A**lsever's solution and carageenan were purchased from Sigma Aldrich. Indian ink (Himedia). WBC diluting fluid (Nice Chemicals) and all other reagents used were of analytical grade and were checked to ensure that they were not expired before the experimentation.

Laboratory animals used in the study

Ethics Statement: The guidelines of the Institutional Ethics Committee and the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India, were followed. The study was approved by Central Animal Ethical Committee of KLEU, Belagum (Ref. No. 1017/C/ CPCSEA). Thirty-four disease-free Wistar male albino rats aged between 6 and 8 weeks (150-200 gm) were obtained from Sri Venkateshwara breeder, Regn. No.CPCSEA 237. They were maintained under standard laboratory conditions and temperature (25 °C±1 °C) and light/dark cycle (12 h light:12 h dark cycle). They received rat pellets and clean water ad libitum. The animals were acclimatized for 2 weeks before the experimental study was carried out.

Acute Toxicity Study (Limit Test)[5]: The methanolic extract of flower (MEPA) at a dose of 2000mg/kg was administered orally to one group and 5000mg/kg was administered orally to the other group of animals and were subjected to acute toxicity studies as per OECD 420 guidelines.

Immunomodulatory study: Twenty-four animals were divided into four groups. Each group comprised of a minimum of 6 animals. The plant extract was suspended

in water and was administered orally for 14 days. Group I (control) received water; group II received Levamisole @ 50 mg/kg body weight; group III received MEPA @ 200 mg/kg; group IV received MEPA@ 500 mg/kg body weight of the animal. For animal experiment methods of Bin-Hafeez *et al*. (2003) was followed. The dose volume was calculated to be not more than 2 ml of drug preparation per animal. Control animals received 2 ml of distil water.

Immunization[6]: All the rats were immunized by injecting 0.5 mL of sheep red blood cells (SRBC) pellets intraperitoneally (i.p.) on the 7th day of the experiment using an insulin syringe. The rats were pre-treated orally with vehicle or extracts for 14 days.

Immunomodulatory Studies:

Group treatments and dosing of animals: 24 rats were divided into four groups of six animals each.

Group 1: Control - 0.5% CMC 10 ml/kg by oral route for 14 days

Group 2: PA methanolic extract - 200 mg/kg/day by oral route for 14 days,

Group 3: PA methanolic extract - 200 mg/kg/day by oral route for 14 days,

Group 4: Standard-Levamisole - 50 mg/kg/day by oral route for 14 days.

The rats were pre-treated orally with vehicle or extracts for 14 days. On 14th day of drug treatment, blood samples were collected by puncturing retro-orbital plexus into heparinised vials and analyzed for performing different tests such as total haemocyte count, differential count, blood glucose, haemoglobin, Determination of neutrophil adhesion and Haemagglutination antibody titre. **Blood glucose test** was carried out with **Trinder's method[14], Hemoglobin test** by **Cyanmethemoglobin method-CMG method, Total serum protein and albumin: globulin ratio** was estimated by Biuret method and absorbance was read at 540 nm in a UV spectrophotometer. **Total leukocyte count** was determined by WBC diluting fluid using Haemocytometer. **Haemagglutination antibody titre** was done in accordance with Bin-Hafeez *et al.,* 2001.

Neutrophil Adhesion Test[15]: Blood samples were incubated with 80 mg/ml of nylon fibres for 15 min at 37°C. The incubated blood samples were again analysed for TLC and DLC. The product of TLC and percent neutrophil gives neutrophil index of blood sample. The percent neutrophil adhesion was calculated as shown below:

Neutrophil adhesion (%) = [(NIu − NIt)/NIu] ×100, where NIu is the Neutrophil index of untreated blood samples and NIt is the Neutrophil index of fibre treated blood samples.

Determination of Delayed type hypersensitivity: On the 14th day, the animals were challenged by subcutaneously injecting 0.1 mL of 1×108 SRBCs into the left hind footpad of the rats. The extent of delayed-type hypersensitivity (DTH) response in the rats was determined by measuring the footpad thickness after 6h and 24h of challenge using Plethysmograph. The difference in the thickness of the right hind paw and the left hind paw was then used as a measure of DTH reaction and was expressed as a mean percent increment in thickness/oedema.

Statistical Analysis

Values were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA (Graph pad prism version 6) followed by Dunnets *post-hoc* test and values of $P < 0.05$ were considered to be statistically significant.

RESULTS:

Preliminary Phytochemical Analysis: All the extracts obtained after successive soxhlet extraction had acidic pH other than aquous extract. The highest extractive value of aquous extract indicates the presence of high amount of water soluble polar compounds. Different solvent extractive values of Muchakunda flower in Pet. Ether, Benzene, Chloroform, Acetone, n Butanol, Methanol, Ethanol, and Water are 1.29, 1.35, 2.06, 4.94, 7.82, 9.12, 9.44, and 12.9 % respectively. Preliminary phytochemical investigation of methanolic extract of *Pterospermum acerifolium* flower (MEPA) showed the presence of alkaloids, proteins, carbohydrates, titerpenoids, flavonoids, glycosides, tannins and sterols.

HPTLC Report: 2 spots of orangish-yellow with Rf values 0.27 and 0.85 were seen under long UV before derivatization. They were not seen under visible light. Violet blue 5 spots were seen under visible light (after derivatization) with Rf values of 0.04, 0.14, 0.27, 0.70 and 0.85. Under long UV only 2 spots of blue and red respectively at Rf 0.04 and 0.27 were seen.

Acute Toxicity Study (Limit Test): The MEPA did not show any toxic reactions and mortality at dose of 2000 mg/kg and 5000 mg/kg. No changes in food consumption,

water intake or behaviour (tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma) were observed in the female rats after dose administration. Hence, MPEA 200 mg/kg and 500 mg/kg ($1/10^{th}$ of LD_{50} study) were taken as treatment doses for the current study.

Estimation of Total leukocyte count (TLC) and differential count (DLC): When compared to control group total leukocyte count was found to be increased in the low dose (200 mg/kg) of drug administration. However, increase in the drug dose has further decreased the total leukocyte count. The percentage of neutrophils were found high in low dose (200 mg/kg) of drug tested with lowest percentage in high dose of a drug as shown in Table 1.

Estimation of Glucose, Haemoglobin, Protein and Albumin-Globulin ratio: Similar to other parameters glucose level is also found to be at higher concentration of 110 mg/dl in low dose group (200 mg/kg). Whereas increased concentration of the drug had reduced the blood glucose level. Similar trend is noticed for haemoglobin, serum protein and albumin globulin ratio. Haemoglobin is found to be high in group where drug administration was 200mg/kg as shown in Table 1.

Estimation of Hem agglutination antibody titre: Among the animals which received drug at concentration 200 mg/kg and 400 mg/kg respectively showed high antibody titre against SRBC. However, the increased concentration of drug administered did not show proportionate increase in the haemagglutination antibody titre as shown in Table 1.

Table 1:

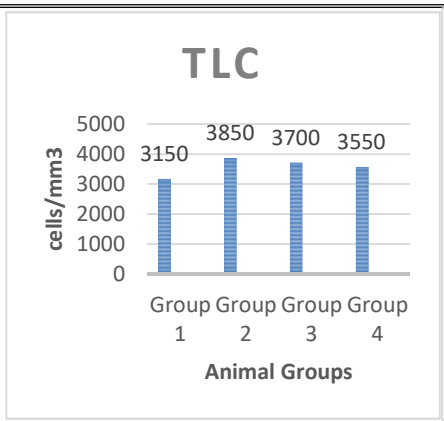
Group	Drug	TLC (cells/mm^3)	DLC (%)					Glucose % mg/dl)	Haemoglobin % (g/dl)	Serum protein (g/dl)	Albumin globulin ratio	HA titre units/µl
			L	M	N	E	B					
1	Distilled ater	3150	41	4	51	3	1	96	5.1	7.1	6.2	6.64±0.36
2	Levamisole	3850	31	5	61	2	1	126	8.8	8.6	8.2	25.11±0.70
3	MEPA 200mg/kg	3700	42	3	52	2	1	110	6.7	7.6	7.3	17.50±1.48
4	MEPA 500mg/kg	3550	41	5	51	2	1	105	4.5	7.3	7.2	24.35±1.36

Neutrophil Adhesion Test: Incubation of blood with nylon fibres produced a decrease in the neutrophil counts due to adhesion of neutrophils to the fibres. Pre-treatment with MEPA 200 mg/kg and 400 mg/kg evoked a significant increase in the *in vitro* neutrophil adhesion to nylon fibres as compared to the control group as shown in Table 2.

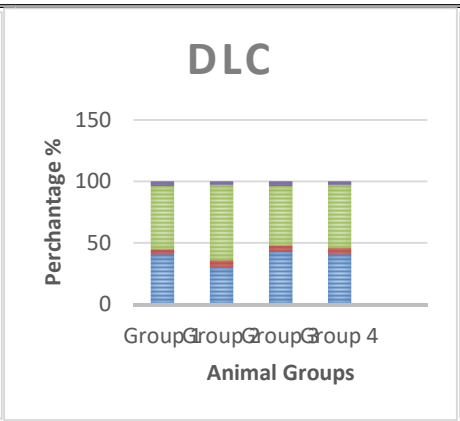
Determination of Delayed Type Hypersensitivity: The effect of methanolic extract and standard drug on the DTH response in Wistar rats using SRBCs as antigen, administration of methanolic extract of PA at the dose of 200 mg/kg and 400 mg/kg and levamisole 50 mg/kg treatments which were given orally for 14 days showed significant increase in paw oedema compared to control group. The standard drug levamisole showed the maximum increase in paw volume compared to all groups as shown in Table 2.

Table 2

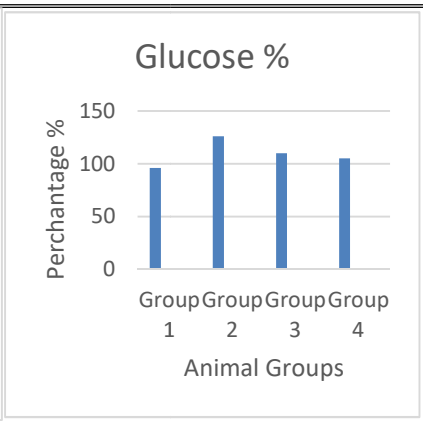
Group	Drug	Neutrophil index		Neutrophil adhesion %	Paw edema (mm)	
		Before treatment	After treatment		4 h (mean±SD)	24 h (mean±SD)
1	Distilled water	218.22±1.55	180.36±2.23	17.34±2.01	1.13±0.024	0.12±0.01
2	Levamisole	365.66±2.01	235.55±1.23	35.55±1.54	5.11±0.032	4.56±0.89
3	MEPA 200mg/kg	355.86±2.55	264.51±1.56	25.73±1.12	2.82±0.94	1.95±0.56
4	MEPA 500mg/kg	399.23±1.33	283.22±1.55	29.05±1.55	3.60±0.064	1.52±0.28



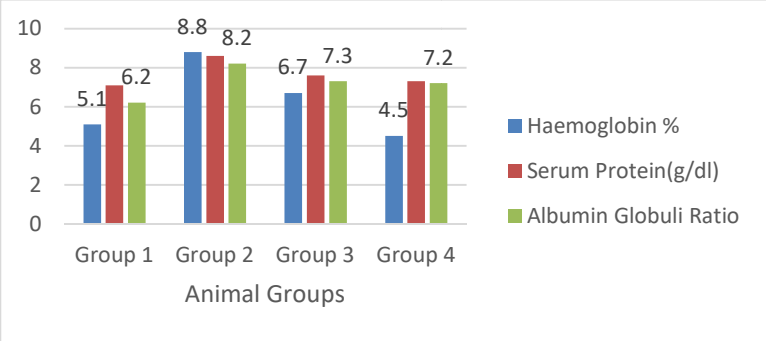
Graph 1: Showing TLC



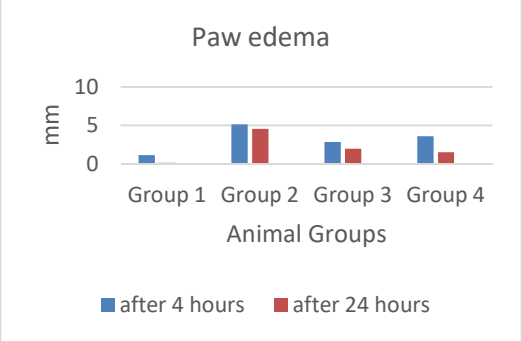
Graph 2: Showing DLC



Graph 3: Glucose Perchantage



Graph 4: Hb %, Serum Protein, Albumin-Globulin Ratio



Graph 5: Showing Paw Edema

DISCUSSION

Acute dose toxicity study, in which the animals treated with the MEPA at a higher dose of 2000 mg/kg and 5000mg/kg, did not produce any significant toxicity signs, behavioural changes, body weight changes or macroscopic findings during observational period. So the LD50 of MEPA should be more than 5000 mg/kg.

An immunomodulatory effect of any immune substance would first be seen as a change in leukocyte count and differential count. In the present study group 3 which received lower concentration (200 mg/kg) of drug showed highest leukocyte count showing the initial triggering of blood cell to mount a potent immune response. The results are further strengthened with highest percentage of neutrophil being circulated in the group receiving lower concentration (200 mg/kg) of drug. Serum protein and serum albumin globulin ratio is one of the earliest indicators of normal serum chemistry of an individual. A change in serum protein concentration and albumin ratio would hint us about the altered immune response status of the individual. Accordingly, in the present study serum protein level and albumin globulin ratio is found to be similar in case of control and higher concentration (500 mg/kg) of the drug but in the lower concentration (200 mg/kg) of drug test the group showed increase in serum protein and albumin ratio showing that higher immune response might have contributed to the serum protein in terms of different molecules such as immunoglobulins and other humoral factors. Similar results were observed for glucose and haemoglobin. Haemoglobin is also one of the important parameter that would reveal the health status of the individual. So in the present case group 3 receiving the low dose (200 mg/kg) of the drug show a better health index based on haemoglobin. Neutrophil adhesion test is widely used to check the effect of various test drugs in cell-mediated immune reactions. The adhesion of neutrophil to nylon fibres indicates the migration of cells in the blood vessels and the number of neutrophils reaching the site of inflammation[17]. Both doses of MEPA were found to enhance the adhesion of neutrophil in to the fibre. This might be due to the upregulation of the integrins, present on the surface of the neutrophils through which they adhere firmly to the nylon fibres[18].The HA titre test was performed to study the effect of MEPA on the humoral immune system. Antibody molecules, a product of B-lymphocytes and plasma cells, are central to humoral immune responses; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonisation, neutralisation of

toxins, etc[19].The results of HA titre test showed that pre-treatment with both doses of MEPA significantly increased the circulating antibodies. CMI responses are critical to defence against infectious organisms, infection of foreign grafts, tumour immunity and DTH reactions[19]. In the current study DTH model was used to evaluate the effect of MEPA in CMI reaction. Therefore, increase in paw oedema in rats in response to T-cell-dependent antigen revealed the stimulatory effect of MEPA in CMI. It is possible that the presence of phytoconstituents such as taraxerol, friedelin, friedelin-3-one, β-sitosterol and β-sitosterol glucoside, β-amyrin, 24β-ethylcholest-5-en-β-O-cellobioside, Kaempferol, kaempferide-7-O-β-D-glucopyranoside, kaempferol-3-O-β-D-galactopyranoside, luteolin and luteolin-7-O-β-D-glucoside as reported by previous studies might be responsible for the observed immune stimulatory ability. Further, Studies are required to gain more insights into the possible mechanism of action. The effect of this extract was comparable to the standards drug levamisole.

CONCLUSION

The results of the present investigation showed that MEPA is a potent immunostimulant, stimulating both the specific and non-specific immune mechanisms. The effect of this extract was comparable to the standards drug levamisole. MEPA flower extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, various allergic disorders, and may also serve as immunoadjuvant for vaccine therapy. Further studies are required to elucidate the exact mechanism of immunomodulatory activity of *Pterospermum acerifolium* Linn.

REFERENCES

1. Gulati K, Ray A, Debnath PK, Bhattacharya SK. Immunomodulatory Indian medicinal plants 2002; 2:121–31
2. UNICEF; Available at: http://www.unicef.org/publications/files/ UNICEF_ Annual Report_2008_EN_072709.pdf].
3. Marjorie, CC. Separation of mixtures by extraction: experimental organic chemistry. Vol. Section I–VI. New York: W. A. Benjamin Inc; 1967. p. 1-204.
4. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med 2010; 10:21.
5. OECD GUIDELINE FOR TESTING OF CHEMICALS, Adopted: 17th December 2001.
6. Thomas L, Asad M, Hrishikeshavan HG, Chandrakala GK. Effect of centchroman on cellular and humoral immunity. Indian J Physiol Pharmacol 2007; 51:387-94.
7. Sumalatha *et al*. / Journal of Applied Pharmaceutical Science 2 (09); 2012: 098-107
8. Wilkinson PC. Neutrophil adhesion test. In: Vane JK, Ferreria SH, editors. Handbook of Experimental Pharmacology. 1st ed. Berlin: Springer Verlag; 1978. p. 10 9.

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