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Evaluation of immunomodulatory activity of petroleum ether extract of seeds of *Celastrus paniculatus*

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ABSTRACT

Immunomodulation using medicinal plants provide an alternative to conventional chemotherapy of various diseases. Celastrus paniculatus is a member of Celastraceae family. The present study was undertaken to evaluate the immunomodulatory property of petroleum ether extract of seeds of Celastrus paniculatus (PECP) on immunological, hematological and oxidative stress parameters using pyrogallol induced immunosuppression model in rats. A dose of 500mg/kg was administered orally. The results of the study suggest that PECP stimulates humoral immunity as indicated by increase in antibody titre and cell mediated immunity as shown by mean percentage increase in paw volume. An increase in percent phagocytosis, hematological parameters like RBC count, WBC count, hemoglobin percentage and oxidative stress parameters such as superoxide dismutase, catalase, reduced glutathione and decrease in lipid peroxidation activities was observed. These findings lead to the conclusion that PECP has significant immunomodulatory and antioxidant property.

Key words: *Celastrus paniculatus*, Immunomodulatory activity, Humoral immunity, Pyrogallol.

INTRODUCTION

Immunity is the ability of the body to defend itself against specific invading agents such as bacteria, toxins, viruses and foreign tissues [1]. An immunomodulator is any substance that helps to regulate the immune system. This "regulation" is a normalisation process, so that an immunomodulator helps to optimize immune response [2]. Immune system dysfunction is responsible for various diseases like arthritis, ulcerative colitis, asthma, allergy, parasitic diseases, cancer and infectious diseases [3]. The degree to which the patient becomes abnormally susceptible to infections by this microbial environment depends on the extent of

immunosuppression.This immunosuppression allows opportunistic pathogens to overwhelm the host to cause secondary infection [4].

This problem can be overcome by boosting the immune system by the use of immunomodulatory drugs [5].Many medicinal plants are known to have immunomodulatory properties and maintain organic resistance against infection by re-establishing the body`s immune system such as Azadirachta indica [6], Terminalia chebula [7], Lawsonia alba [8]. The phytochemical constituents like diterpenoids, steroids, proteins, and tannins [9] are considered to exhibit immunomodulatory property.

In Indian context, there are some plants that have been traditionally used but have not been pharmacologically screened for their immunopotential property like Celastrus paniculatus. Celastrus paniculatus is a member of the Celastraceae family and is a large, woody climber with a yellow, corky bark. It grows throughout India and has been reported growing as high as almost 2,000 meters. The leaves are oblong-elliptic and the flowers are unisexual. The seeds which grow inside capsules, number from anywhere between 1-6 seeds per capsule and yield dark brown oil known as Celastrus oil or Malkanguni oil. The plant exhibits varying degrees of therapeutic values some of which are its use in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout, dyspepsia.

Pyrogallol is a strong generator of free radicals [10] and it is evidenced that it can suppress the immunity by means of oxidative stress [11]. The present study is aimed at investigating the immunomodulatory property of petroleum ether extract of seeds of Celastrus paniculatus using pyrogallol induced immunosuppression model in rats by evaluating the immunological, hematological and oxidative stress parameters.

MATERIALS AND METHODS

Plant material:
The seeds of Celastrus paniculatus were procured from the local commercial market in the month of October 2010 and authenticated by Dr. B. Ravi Prasad Rao, M.Sc., Ph.D, Department of Botany, Sri Krishna Devaraya University, Anantapur and voucher specimen (riper-08/11) is preserved in department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur.

Chemicals:
Pyrogallol, Normal saline, Gelatin, Sodium carbonate, DTNB, Tris-HCl buffer, Trichloro acetic acid, Thiobarbituric acid etc.

Experimental animals:
Wistar rats of average weight 180-250g were procured from bioneeds, Bangalore. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions i.e., 12:12 hour light/dark cycle at 25 ± 2^0C.

Ethical Approval:
The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/008/201**)** of Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, India, approved the animal experimental protocol.

All experiments were conducted according to the norms of committee for the purpose of control and supervision on experiments on animals (CPCSEA).

Preparation of petroleum ether extract
The seeds of Celastrus paniculatus were shade dried and reduced to coarse powder. The standardized coarse powder was evenly packed in the soxhlet apparatus and subjected to petroleum ether extraction. The extracts were filtered and the filtrate was concentrated by vaccum distillation. A dark brown colored oil was obtained. The percentage yield was found to be 38%.

Phytochemical investigation:
Preliminary phytochemical screening was performed on PECP.

*Treatment**:***
Preliminary studies revealed that the maximum tolerable dose of pyrogallol was 100mg/kg, i.p. for 7 days at which no mortality could be seen up to 30 days. Hence, the experimental group was divided into four subgroups (n=6). The normal group received only the vehicle, the negative control group received a dose of pyrogallol i.e. 100mg/kg, i.p., once daily up to 7 days. The test group received the extract at a dose of 500mg/kg, p.o. and the standard group received levamisole at a dose of 50mg/kg, p.o. from day 1 to day 22. Besides the above treatments, the rats from all the groups received sheep red blood cells (SRBC) (0.5x109 cells/100g, i.p.) on day 7 and 13, as the antigenic material to sensitize them for immunological studies.

Study of immunomodulatory activity:
1.) Immunological parameters:
i.) *Humoral immune response* [12]:
On day 13 and 20, blood was withdrawn from the retro-orbital plexus of all antigenically challenged rats. Twenty-five µl of serum was serially diluted with 25 µl of phosphate-buffered saline. SRBC (0.025x109 cells) were added to each of these dilutions and incubated at 37^{o}c for one hour. The rank of maximum dilution that exhibited haemagglutination was considered as antibody titer. The level of antibody titer on day 13of the experiment was considered as the primary humoral immune response and the one on day 20 of the experiment was considered as the secondary humoral immune response.

ii.) *Cellular immune response* [12]:
This was assayed by the footpad reaction method. The oedema was induced in the right paw of the rats by injecting SRBC (0.025x109 cells) in the sub-plantar region on day 20.The increase in paw volume in 48hrs i.e. on day 22 was assessed on digital plethysmometer (UGO Basile 7150). The mean percentage increase in paw volume was considered as delayed type of hypersensitivity

and as an index of cell- mediated immunity. The volume of left hind paw, injected similarly with phosphate-buffered saline, served as a control.

iii.) *Carbon clearance* [13, 14, 15]:
Rats were injected with 0.1ml of carbon ink suspension (1.6v/v in 1%gelatin dissolved in saline) through i.v. route via the tail vein. The blood samples (50µl) were taken at intervals of 0mins and 15mins after injection and dissolved in 0.15%w/v disodium EDTA (50µl).25µl of sample was mixed with 2ml of 0.1% sodium carbonate solution. Absorbance was read at 660nm taking 0.1% sodium carbonate solution as blank.

2.) Hematological Parameters:
The blood was collected from the retro-orbital plexus using heparinised capillary tubes and hematological tests were carried out. The WBC count was done by Turke's method [16], RBC by Hayem's method [17] and haemoglobin by Sahli's method [18].

3.) Oxidaive stress parameters:
i.) Lipid peroxidation (LPO) [19]:
To 2.0 ml of the 5% suspension of RBC in 0.1 M phosphate-buffered saline, 2.0 ml of 28% trichloroacetic acid was added and centrifuged. 1.0 ml of 1% thiobarbituric acid was added to the supernatant, heated in boiling water for 60 min and then cooled. The absorbance was measured at 532 nm. Lipid peroxidation was calculated based on the molar extinction coefficient of malondialdehyde (MDA) (1.56 x 10**5**), and expressed in terms of nanomoles of MDA/g Hb.

ii.) Superoxide dismutase (SOD) [20]:
It was estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 µl of the lysate, 75 mM of Tris-HCl buffer (pH 8.2), 30 mM EDTA and 2mM of pyrogallol were added. An increase in absorbance was recorded at 420 nm for 3 min in spectrophotometer (Schimadzu 1601). One unit of enzyme activity is 50% inhibition of the rate of auto oxidation of pyrogallol as determined by change in absorbance/min at 420nm .The protein content of lysate was estimated by Lowry's method [21] and the activity of SOD is expressed as units/mg protein.

iii.) Catalase (CAT) [22]:
Catalase activity was determined in erythrocyte lysate using Aebi's method with some modifications. The erythrocyte lysate (50 µl) was added to a cuvette containing 2.0 ml of phosphate buffer (pH 7.0) and 1.0 ml of 30 Mm H_20_2. Catalase activity was measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H_20_2 - 43.6 M/cm was used to determine the catalase activity. One unit of activity is equal to one millimole of H_20_2 degraded per minute and is expressed as units per milligram of protein.

iv.) Reduced glutathione (GSH) [23]:
Blood glutathione was measured by addition of 0.2 ml of whole blood to 1.8 ml distilled water followed by 3.0 ml of precipitating mixture (1.67 g metaphosphoric acid, 0.2 g EDTA and 30 g NaCl to make 100 ml of solution). It was centrifuged at 5000 rpm for 5 min and 1.0 ml of the filtrate was added to 1.5 ml of the phosphate solution, followed by the addition of 0.5 ml of DTNB reagent. The optical density was measured at 412 nm using a spectrophotometer.

RESULTS

Preliminary phytochemical investigation revealed the presence of steroids and diterpenoids (Table1). Pyrogallol induced immune-suppressive rat model was used because the dynamic and complex nature of the immune system in which a drug elicits its effect can be detected more reliably after immune challenge. The results of the present study suggest that PECP stimulates humoral immunity as indicated by an increase in antibody titre value (Table2) and cell mediated immunity as shown by mean percentage increase in paw volume (Table 2) and an increase in macrophage induced phagocytosis in carbon clearance test (Table2).

TABLE-1: Phytochemical investigation of PECP

Phytochemicals	PECP
Carbohydrates	-
Proteins	-
Flavanoids	-
Tannins	-
Diterpenoids	+
Steroids	+
Saponins	-
Polyphenols	-
Aminoacids	-
Glycosides	-
Alkaloids	-

+ = Present - = Absent

Table-2 : Effect of PECP on immunological parameters

Group	Drug Treatment	Immunological response			
		Humoral immune response (antibody titer)		Cellular immune response	Carbon clearance (% phagocytosis)
		Primary	Secondary		
NORMAL	Vehicle	224.3±6.173	235.7±2.028	0.8333±0.03333	87.83±0.7172
NEGATIVE CONTROL	Pyrogallol 100mg/kg i.p. daily for 7 days	83±4.583####	77.33±0.8819####	0.2967±0.02333####	45.53±1.615####
TEST	Pyrogallol 100mg/kg i.p. daily for 7 days + PECP 500mg/kg p.o. daily for 22 days	228.7±1.764****	239.3±0.8819****	0.9033±0.03180****	89.30±0.3000****
STANDARD	Pyrogallol 100mg/kg i.p. daily for 7 days + levamisole 50mg/kg p.o. daily for 22 days	233±1.528****	242.7±1.202****	0.8733±0.01202****	91.10±0.6807****

*#### - P < 0.001 when compared to normal **** - P < 0.001 when compared to negative control*

PECP exhibited good protection by increasing all the hematological parameters. WBC count, RBC count, and % hemoglobin values observed were increased compared to the untreated control groups (Table 3).

Pyrogallol which is a strong generator of superoxide radicals might impair the immune response through oxidative stress. It is observed that daily oral administration of PECP (500 mg/kg) significantly prevented the influence of pyrogallol on immune responses which is evidenced by

decrease in lipid peroxidation and increase in superoxide dismutase, catalase and reduced glutathione activity (Table 4).

Table-3 Effect of PECP on haematological parameters

Group	Drug Treatment	Parameters		
		Haemoglobin gm%	Red blood cells count Cells/10^3/cumm	White blood cells count ells/million/cumm
NORMAL	Vehicle	16.4±0.057774	5.267±0.08819	6.3±0.05774
NEGATIVECONTROL	Pyrogallol 100mg/kg i.p. daily for 7 days	11.47±0.4055####	2.5±0.05773####	2.603±0.08950####
TEST	Pyrogallol 100mg/kg i.p. daily for 7 days + PECP 500mg/kg p.o. daily for 22 days	18.30±0.05773****	6.267±0.08819****	6.837±0.04631****
STANDARD	Pyrogallol 100mg/kg i.p. daily for 7 days + levamisole 50mg/kg p.o. daily for 22 days	17.80±0.05773****	5.367±0.1202****	6.370±0.1453****

- P < 0.001 when compared to normal ***** - P < 0.001 when compared to negative control*

TABLE-4 Effect of PECP onoxidative stress parameters

Group	Drug Treatment	Parameters			
		Lipid peroxidation MMDA/gHb	Reduced glutathione µmol/ghb	Superoxide Dismutase Units/mg protein	Catalase Units/mg Protein
NORMAL	Vehicle	97.13±0.5457	7.000±0.4163	28.77±0.3283	114.9±0.9207
NEGATIVE CONTROL	Pyrogallol 100mg/kg i.p. daily for 7 days	117.9±0.9262####	2.400±0.05774####	14.10±0.2646####	86.40±0.6429####
TEST	Pyrogallol 100mg/kg i.p. daily for 7 days + PECP 500mg/kg p.o. daily for 22 days	88.53±0.5812****	7.467±0.1764****	30.17±0.3480****	116.8±0.8090****
STANDARD	Pyrogallol 100mg/kg i.p. daily for 7 days + levamisole 50mg/kg p.o. daily for 22 days	85.47±0.6936****	6.967±0.1202****	28.10±0.2646****	118.4±0.9735****

- P < 0.001 when compared to normal ***** - P < 0.001 when compared to negative control*

DISCUSSION

Celastrus paniculatus is known for several medicinal uses and has been investigated for different pharmacological properties. However, there is no systematic study of its immunomodulatory activity. Hence in the present study, the immunomodulatory activity of petroleum ether extract of seeds of this plant was investigated. The study affirms that the PECP is effective immunomodulatory agent. Further investigations are needed to explore the exact active constituents and mechanisms of action of immunomodulatory activity

CONCLUSION

The petroleum ether extract of seeds of Celastrus paniculatus has protected the animals against pyrogallol induced immunosuppression indicating its profound immunomodulatory activity.

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