WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 10, 827-842 Research Article ISSN 2278 – 4357

EVALUATION OF ANTI-INFLAMMATORY ANTI PYRETIC ACTIVITY OF LEAVES EXTRACTS OF PLANT *PLACTYCLADUS ORIENTALIS* ON ALBINO MICE

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Article Received on
25 July 2017,

Revised on 15 August 2017,
Accepted on 05 Sept. 2017,

DOI: 10.20959/wjpps201710-10203

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ABSTRACT

In the present study, dried leaves of *Platycladus orientalis* plant were selected for preliminary phytochemical investigation and pharmacological evaluation for anti-pyretic & anti-inflammatory activity. Extraction of dried leaves of *Platycladus orientalis* was carried out by defatting powder with alcoholic/aqueous solvents. As a model of inflammation, carrageenan induced mice paw edema, dextran induced mice paw edema, histamine induced mice paw edema, formalin induced paw edema and cotton pellet induced granuloma in rats were utilized in the present study. In conclusion, the results of the present study demonstrated that ethanolic extract of *Platycladus orieantalis* produced dose related acute anti-inflammatory activity (Carrageenan, dextran, histamine and formalin), chronic anti-inflammatory activity (Cotton pellet) and antipyretic activity). These studies have shown that the ethanolic extract of Platycladus orrieantalis contains some active ingredients with the potential of being good anti-inflammatory and antipyretic agents. The results of the study were subjected to one way analysis of variance followed by student t-test for multiple comparisons. Values with $P < 0.05$ were considered significant.

KEYWORDS: Anti-inflammatory activity, anti-pyretic activity, pharmacological evaluation.

INTRODUCTION

For thousands of years plant and their derivatives are being used for treatment of pain and inflammation. During the last two decades, traditional systems of medicine and medicinal plant research have become topics of global interest and importance. Many studies have been carried out in search of suitable plant drugs for the management of pain & inflammation. There is great demand in developing and industrially developed countries to use alternative approaches to treat pain such as plant-based medicines, due to their cheaper rates and less or no side effects in comparison of synthetic drugs respectively. The plant drugs are frequently considered to be less toxic when compared to synthetic drugs.[1]

In the traditional system of Indian medicine various plant extracts are used for treating pain conditions**.** Synthetic drug can produce serious side effects and in addition, they are not suitable for use during pregnancy.[2]

The literature reveals that the anti-inflammatory and analgesic potential of *Platycladus* orientalis *(*Leaves) have been successfully used in Ayurvedic and other traditional formulations and found to be efficient and inexpensive as compared to synthetic drugs but not evaluated scientifically. In the present study, dried leaves of *Platycladus orientalis* plant were selected for preliminary phytochemical investigation and pharmacological evaluation for anti-pyretic & anti-inflammatory activity.

MATERIALS AND METHODS

Platycladus orientalis leaves were collected (from natural habitats) from local area Alwar, Rajasthan. Ethyl acetate, Benzene, Petroleum ether, Chloroform, Ethanol, Acetone, Diethyl ether and other chemical and solvents were of analytical grade/IP/equivalent gradeand procured from laboratory.

Extraction procedure

Defattation of powdered *P. orientalis* leaves

200g coarse leaves powdered leaves were defatted with 800 ml petroleum ether (60-80^{0}C) using soxhlet apparatus. Extraction was continued until a drop of solvent from siphon tube, when evaporated on filter paper, did not leave a greasy spot (approximately 10-12 cycles). After the defattation, mark was taken out from extractor and spreaded as a bed on a clean paper and dried till evaporation of petroleum ether. Mark was kept for ethanolic extraction. The light brown colored petroleum ether extract was collected and kept for analysis.

Ethanolic extraction of *P. orientalis* leaves

The dried mark obtained after defattation was packed in soxhlet apparatus and extracted with 800 ml ethyl alcohol in soxhlet apparatus. Extraction was continued until a drop of solvent from the siphon tube, when taken on TLC plate and sprayed with concentrated sulphuric acid, does not give a black spot. Dark brown extract thus obtained, was collected and solvent was evaporated under reduced pressure.

Pharmacological investigation of ethanolic extracts for anti-inflammatory and antipyretic activity

Adult wister rats (150-180g) of either sex were procured and housed in the animal house of Alwar College of Pharmacy, with 12 hrs light and 12 hrs dark cycles. Standard pellets obtained from Hafed, Rohatak, India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad *libitum*. After randomization into various groups, the rats were acclimatized for a period of seven days under standard environmental conditions of temperature, relative humidity and dark/light cycle. All the animal experiments were conducted according to the ethical norms approved by CPCSEA, at Alwar College of Pharmacy.

Evaluation of anti-inflammatory activity

Drug dose

The doses considered for the experiment on rat for the anti-inflammatory and antipyretic experiments were carried out in two different doses of 100 and 200 mg/kg body weight.

Preparation of test drug and standard drug

Test drug (Ethanolic extract of *Platycladus orientalis*) and standard drug (Acetyl salicylic acid) were prepared as a suspension in distilled water using mortal & pestle.

Animal grouping

The animals were divided into four groups for anti-inflammatory studies. Each group consisted of six animals of either sex. The groups were:

Group I: Negative control - Distilled water

Group II: Test drug- Ethanolic extract of *Platycladus orientalis* -100 mg/kg bodyweight (POE-100)

Group III: Test drug- Ethanolic extract of *Platycladus orientalis* -200 mg/kg bodyweight (POE-200)

Group IV: Positive control- Standard drug acetyl salicylic acid - 10 mg/kg bodyweight (ASA-10)

Carrageenan induced rat paw edema

Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% 0.1ml of 1% carrageenan in normal saline in the hind paw of rats 1 h after the administration of the test drug as well as positive and negative controls. The paw volume was measured at 1 h, 2 h and 3 h after carrageenan injection, using plethysmograph.

Dextran induced rat paw edema

The animals were treated in a manner similar to that of carrageenan induced paw edema model. 0.1 ml 1% dextran was used for the study. Paw volume was measured as mentioned incarrageenan induced paw edema model at 1 h, 2 h and 3 h.

Histamine induced rat paw edema

In this model paw edema of mice was induced by sub plantar injection of 0.1ml of 1% freshly prepared histamine in normal saline and the paw oedema was measured as mentioned in carrageenan induced paw edema model. The paw volume was measured at 0.5 h, 1 h, 2 h and 3 h.

Formalin induced paw edema

The test drug was administered once daily for seven consecutive days to allthe groups. On seventh day, initial paw volume was measured before drug administration. After 1 h of drug administration, paw edema of the mice was induced by subplantar injection of 0.1 ml of 3% formalin solution in normal saline. Paw volumes were measured at 3 h, 24 h and 48 h after formalin injection as described earlier in carrageenan model.

Cotton pellet induced granuloma in rats

Cotton pellet induced granuloma formation in rats was performed for chronicanti-inflammatory study. This model represents the exudative and proliferative phases of inflammation. The cotton pellets weighing 100 mg were made by rolling of cotton piece and sterilizing by autoclaving. The rats were anaesthetized with ether; dorsum was shaved clear and swabbed with 70%(v/v) alcohol. Midline incision of 1 cm was made in the intrascapular region. A small tunnel was made on either side of the incision with the help of a small blunt forceps. Sterile cotton pellet (100 mg) was implanted in each tunnel. Air was removed from

the tunnel and then incision was closed with sutures. The test drugs were administered for 7 consecutive days starting from the day of implantation. The rats were sacrificed on the 8th day, cotton pellets were removed and cleaned of extraneous tissue and dried by placing them in a hot air oven overnight at 80^0C and then weighed. The difference between the initial weight and the final weight of the pellet after drying was taken as the granuloma tissue weight. The results were expressed as mg granulation tissue formed per 100 g body weight.

Evaluation of antipyretic activity

At first, pyrexia was induced injecting aqueous suspension of brewer's yeast into Swiss albino mice with almost uniform body temperature and then antipyretic activity of plant extracts was evaluated on them. The test animals were divided into four groups i.e. group I receiving normal saline 1 mg/ml p.o as control, group IV receiving paracetamol at a dose of 100 mg/kg b.wp.o as standard, group II and group III receiving ethanolic extract of leaves *Platycladus orientalis* respectively; both at a dose of 100 and 200 mg/kg b.wtp.o. All the drugs were given as freshly prepared aqueous suspension. The initial rectal temperature of the Swiss albino mice was recorded using digital thermometer. Mice were made hyper thermic by subcutaneous injection of 20% brewer's yeast suspension in double distilled water at a dose of 1 mL/100g b.w. When the temperature was believed to be at its peak (18 hours after yeast injection) the rectal temperature of the mice was recorded again. The experimental animals that showed a rise in rectal temperature of at least 2°C were selected for the test. The plant extracts, paracetamol and control vehicle were given orally and rectal temperature of animals was recorded at 1 hour interval for 3 hours following the administration of the test substances.

Statistical analysis

The results obtained from antipyretic activity test were expressed as the mean ± SEM. The results were analyzed using one-way ANOVA followed by using Dunnett's t-test. Values with $P < 0.05$ were considered significant.

RESULT AND DISCUSSION

The results of screening of anti-inflammatory activity of *Platycladus orientalis* in carrageenan induced rat paw edema. *Platycladus orientalis* was selected for further pharmacological studies. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageen an model is mainly mediated by histamine, serotonin and

increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (3 h) is associated with neutrophil originated free radicals, such as hydrogen peroxide, superoxide and hydroxyl radicals, as well as prostaglandin release.

The results of anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in carrageenan induced paw edema is shown in Table 1 .POE-100 group showed anti-inflammatory activity at 1 h (4.17%,), 2 h(9.3%,) and 3 h (36.06%,). POE-200 group showed significant decrease in paw volume at 1 h (37.50%,), 2 h (47.55%,) and 3 h (47.68%,). Acetyl salycylic acid group showed significant decrease in paw volume at 1 h (30.45%,), 2 h (50.48%,) and 3 h (51.57%). POE-100 showed less anti-inflammatory activity than reference drug acetyl salycylic acid at 1 h, while POE-200 group showed more anti-inflammatory activity same as reference drug acetyl salycylic acid at 1 h 2 hand 3h.

Thus, it can be concluded that ethanolic extract of *Platycladus orientalis* has potentanti-inflammatory activity in carrageenan induced rat paw edema in early phase (2 h) and also in later phase (3 h).

It is well established that carrageenan and dextran induce rat paw oedema by different mechanisms. Dextran is a polysaccharide of high molecular weightthat induces anaphylactic reaction after injection in rat's extremities, which is characterized by extra vasation and oedema formation, as a consequence of liberation of histamine and serotonin from mast cells.

The results of anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in dextran induced paw edema edema is shown in Table 2. POE-100group andPOE-200 group showed significant anti-inflammatory activity in dextran induced paw edema model when compared with the control group. POE-200 group showed significant decrease in paw volume at 1 h (31.20%), 2 h (51.64%) and 3 h (47.36%).Acetyl salycylic acid group showed significant decrease in paw volume at 1 h (23.61%), 2 h (25.81%) and 3 h (53.68%). Ethanolic extract of *Platycladus orientalis* showed anti-inflammatory activity in dose dependent manner. The results tend to suggest that the anti-inflammatory activity of the ethanolic extract of *Platycladus orientalis* possibly backed by its antihistamine or anti-serotonin activity.

The histamine is a basic amine related with inflammatory and allergic process causing, among several effects, both vasodilatation and increase of vascular permeability. The results

of anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in histamine induced paw edema is shown in Table 3. Anti-inflammatory activity of POE-100and POE-200 groups was statistically significant at1 h, 2 h and 3 has compared with the control group. POE-200 group showed significant decrease in paw volume at 1 h (13.88%) and significant at 1 h (26.74%) and 3 h (42.10%). Reference drug acetyl salycylic acid group showed highly significant decrease in paw volume at3 h (567.84%), 2 h (43.02%) and 1 h (30.61%) as compared with the control group. Thus POE-100 groups did not show higher anti-inflammatory activity than reference drug acetyl salycylic acid group.

The results of anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* formalin induced paw edema. It is well known that inhibition of formalin induced paw edema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis. Thus formalin-induced paw edema is a model used for the evaluation of an agent with antiproliferative activity. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation. The administration of POE-100, POE-200 and acetyl salycylic acid daily for 7 days successfully inhibited edema induced by formalin. POE-100 group showed decrease in paw volume at 1 h (25.02%), 2 h (29.06%) and 3 h (45.35%). POE-200 group showed decrease in paw volume at 1 h (41.06%), 2 h (49.50%) and at 3 h (54.67%). Acetyl salycylic acid group showed decrease in paw volume at 1 h (43.05%), 2 h (49.64%) and 3 h (54.67%). POE-100and POE-200 groups showed almost similar anti-inflammatory activity at1 h, 24 h and 3h. Thus, from the results, it can be concluded, that ethanolic extract of *Platycladus orientalis* has higher anti-inflammatory activity in formalin induced paw edema test. POE showed significant decrease in paw volume till 3h with both doses, which suggests its long duration of action.

Cotton pellet granuloma test is a chronic inflammation model commonly used to evaluate the anti-proliferative activities of drugs. Tissue granulation, one of the distinctive features of chronic inflammation, which is composed of marked infiltration macrophages and neovascularization, was induced by subcutaneous implantation of biomaterials. The implanted material induces a host's inflammatory response and modulates the release of inflammatory mediators which finally leads to tissue proliferation and granular formation.

The results of anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in cotton pellet induced granuloma is shown in Table 5. In the present study, POE-100 and POE-200 groups showed dose dependent activity and markedly inhibited granuloma

formation surrounding the pellets compared with the control group. POE-100group showed significant decrease in granuloma formation with 19.73% while POE-200 group showed significant decrease in granuloma formation 26.92% with which was almost near to the reference drug acetyl salycylic acid (32.71%) group. Thus, the results showed that ethanolic extract of *Platycladus orientalis* potent anti-inflammatory activity in chronic inflammatory model.

The effect of ethanolic extracts of leaves of *Platycladus orientalis* mice is presented in Table 6. In this test, the extracts at a dose of 200 mg/kg b.wt significantly attenuated hyperthermia up to 3 hours in mice. Throughout the experiment, the leave extract reduced temperature from 39.06°C to 38.8°C, 38.5°C and 38.1°C in 1st, 2nd and 3rd hour respectively and caused maximum reduction of temperature in 2nd hour. It was found that the anti-pyretic properties of the extracts were comparable to that of the standard drug paracetamol. It was clearly understood from the study that the observed anti-pyretic effects of the extracts were similar in both magnitude and time course.

In this study, the extracts were observed to inhibit yeast induced pyrexia. The extracts may have reduced temperature of the test animals by decreasing brain concentration of prostaglandin E2 especially in the hypothalamus through its action on COX-3 or by causing the enhancement of the production of the body's own antipyretic substances like vasopressin and arginine. This effect must be due to the presence of the different phytochemical constituents in the extracts.

Table 1: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in carrageenan induced paw edema.

Treatments	Dose (mg/Kg, P.O)	After 1h		After 2h		After 3h	
		Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition
Control	Saline	0.72±0.02	-	0.86±0.01	-	0.95±0.02	-
ASA	300	0.50±0.03**	30.55	0.42±0.03 **	50.48	0.46±0.03**	51.57
POE	150	0.69±0.04^{ns}	4.17	0.78±0.02^{ns}	9.3	0.60±0.03 **	36.84
POE	300	0.45±0.03**	37.50	0.45±0.01**	47.67	0.50±0.04**	47.36

b.wt. n=6, ns- non significant , *****p<0.05- significant, ******p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals.

POE 200- *Platycladus orientalis* ethanolic extract 200mg/kg b.wt, POE 200- *Platycladus orientalis* ethanolic extract 400mg/kg b.wt 400mg/kg b.wt, ASA-300- Acetyl salycylic acid 300 mg/kg.

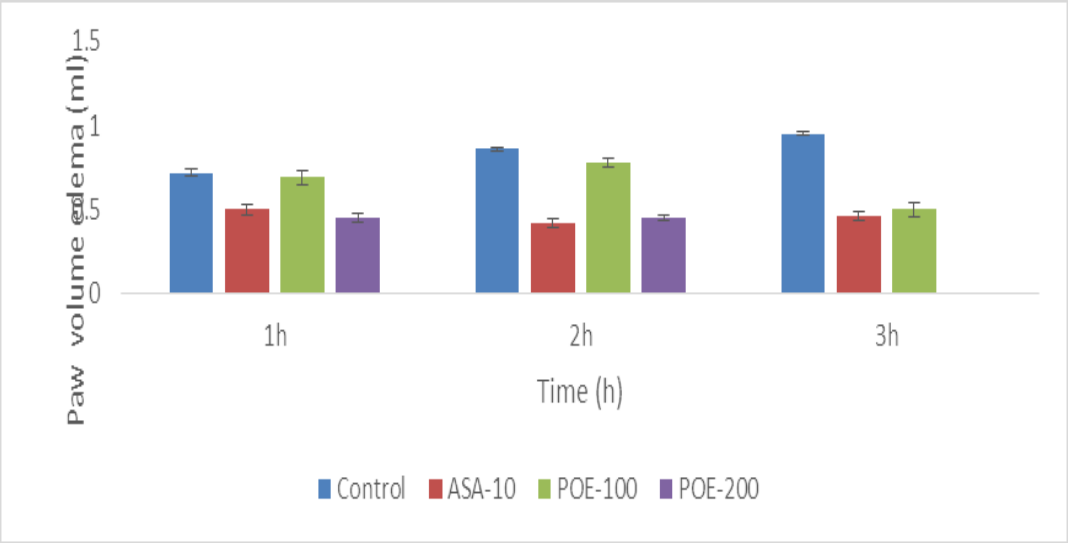

Fig. 1: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in carrageenan induced paw edema.

Table 2: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis*in dextran induced paw edema.

Treatments	Dose (mg/Kg, P.O)	After 1h		After 2h		After 3h	
		Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition
Control	Saline	0.72±0.04	-	0.86±0.01	-	0.95±0.02	-
ASA	300	0.55±0.02**	23.61	0.64±0.02**	25.81	0.44±0.03**	53.68
POE	150	0.60 ± 0.01^{ns}	16.66	0.72 ± 0.01^{ns}	16.27	0.65±0.04**	31.57
POE	300	0.49±0.03**	31.90	0.42±0.03**	51.64	0.50±0.02**	47.36

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals.

POE 100- *Platycladus orientalis* ethanolic extract 100mg/kg b.wt, POE 200- *Platycladus orientalis* ethanolic extract 200mg/kg b.wt 400mg/kg b.wt, ASA-300 Acetyl salicylic acid 300mg/kg b.wt.

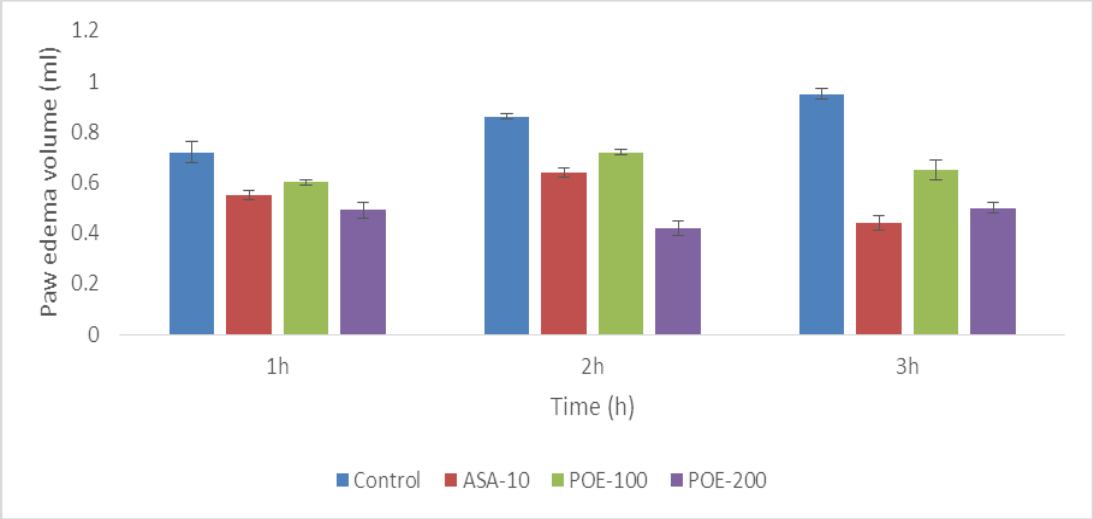

Fig. 2: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis*in dextran induced paw edema.

Table 3: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in histamine induced paw edema.

Treatments	Dose (mg/Kg, P.O)	After 1h		After 2h		After 3h	
		Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition
Control	Saline	0.72±0.02	-	0.86±0.01	-	0.95±0.04	-
ASA	300	0.40±0.03**	30.61	0.49±0.04**	43.02	0.41±0.03**	56.84
POE	150	0.62±0.04^{ns}	13.88	0.63±0.01^{ns}	26.74	0.55±0.02**	42.10
POE	300	0.52±0.03**	27.17	0.52±0.03**	39.53	0.49±0.01**	48.42

n=6, ns- non significant, *****p<0.05- significant, ******p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals.

POE 100- *Platycladus orientalis* ethanolic extract 100mg/kg b.wt, POE 200- *Platycladus orientalis* ethanolic extract 200mg/kg b.wt 400mg/kg b.wt, ASA-300 Acetyl salicylic acid 300mg/kg b.wt.

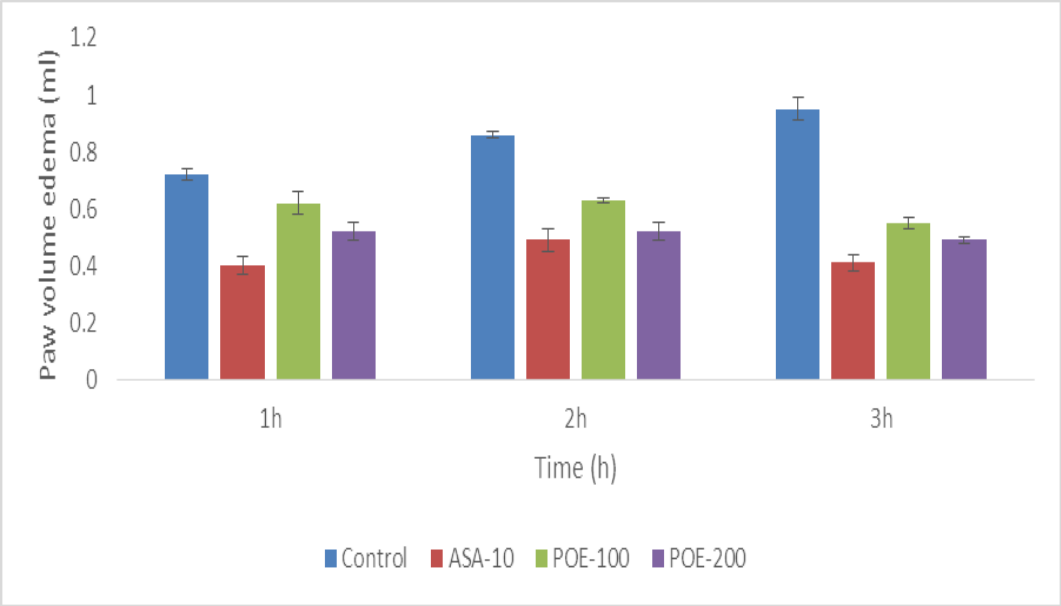

Fig. 3: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis* in histamine induced paw edema.

Table 4: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis*in formalin induced paw edema.

Treatments	Dose (mg/Kg, P.O)	After 1h		After 2h		After 3h	
		Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition	Paw edema volume (ml)	% Inhibition
Control	Saline	0.72±0.02	-	0.86±0.01	-	0.95±0.01	-
ASA	300	0.39±0.03**	48.83	0.41±0.04**	52.29	0.37±0.04**	61.05
POE	150	0.54±0.04 ns	25.02	0.61±0.03 ns	29.06	0.52±0.03 **	45.35
POE	300	0.41±0.03**	43.05	0.47±0.02**	45.34	0.43±0.02**	54.73

n=6, ns- non significant, *p<0.05- significant, **p<0.01-more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animals.

POE 100- *Platycladus orientalis* ethanolic extract 100mg/kg b.wt, POE 200- *Platycladus orientalis* ethanolic extract 200mg/kg b.wt 400mg/kg b.wt, ASA-300 Acetyl salicylic acid 300mg/kg b.wt.

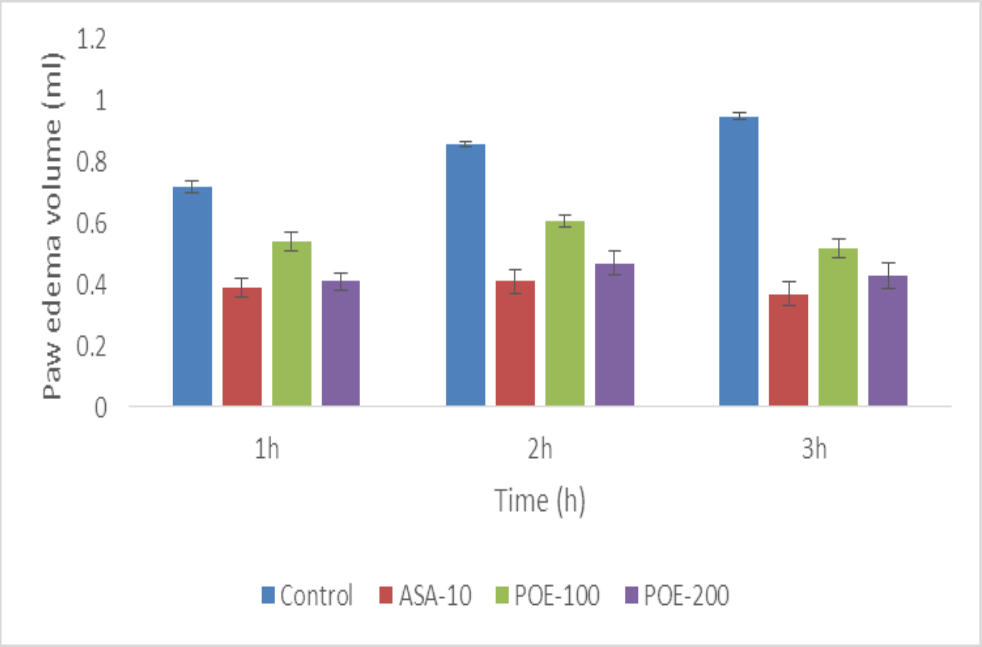

Fig. 4: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis*in formalin induced paw edema.

Table 5: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis*in cotton pellet induced granuloma formation.

Treatments	Dose (mg/Kg, P.O)	Pellet weight g/100g Body weight	% Change
Control	Saline	0.162 ± 0.012	
ASA	300	0.109 ± 0.016*	32.71
POE	150	0.131± 0.010*	19.13
POE	300	0.114± 0.006**	29.62

SD = n=6, ns- non significant, *****p<0.05- significant, ******p<0.01-more significant v/s control, SEM= standard error mean, standard deviation, n= number of animals.

POE 100- *Platycladus orientalis* ethanolic extract 100mg/kg b.wt, POE 200- *Platycladus orientalis* ethanolic extract 200mg/kg b.wt 400mg/kg b.wt, ASA-300 Acetyl salicylic acid 300mg/kg b.wt.

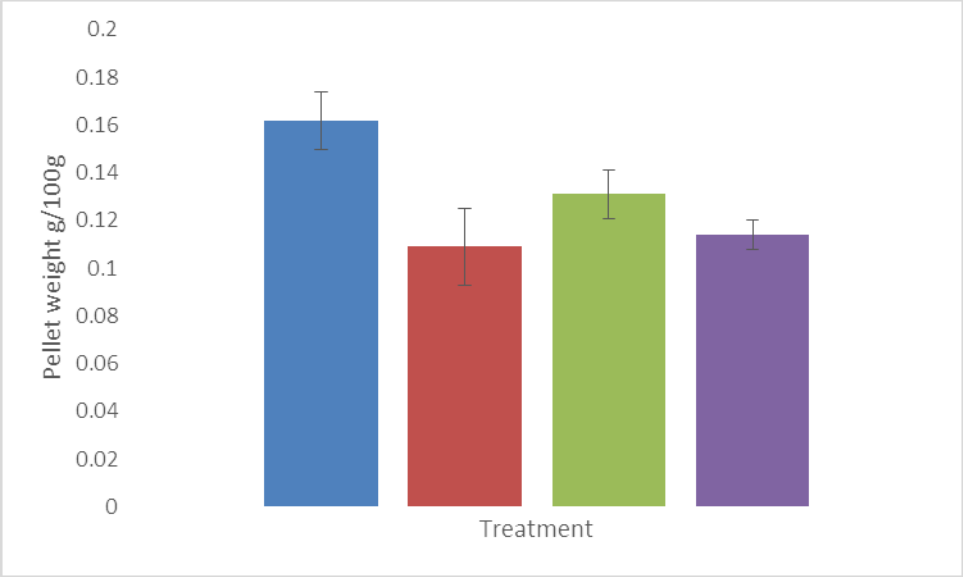

Fig. 5: Anti-inflammatory activity of ethanolic extract of *Platycladus orientalis*in cotton pellet induced granuloma formation.

Table 16: Anti-pyretic activity of ethanolic extract of *Platycladus orientalis* in brewer's yeast induced pyrexia.

S.N	Treatment	Dose mg/Kg	Normal rectal temperature ^{O}C mean ±S.E	Temperature 18hrs after Yeast induced pyrexia	Temperature after treatments (hours)				% activity
					1st Hour ^{O}C	2nd Hour ^{O}C	3rd Hour ^{O}C	4th Hour ^{O}C	
1	Control		37.2 ± 0.05	38.09 ± 0.24	39.6 ± 0.05	39.7 ±0.07	39.9 ±0.04	39.7* ±0.06	
2	The ethanolic extract	100	37.3 ± 0.09	39.08 ± 0.34	38.6 ± 0.24*	38.8 ±0.12**	38.5 ±0.09	38.01 *±0.06	3.82
3	The ethanolic extract	200	37.6 ± 0.13	39.06 ± 0. 72	38.8 ± 0.41**	38.5 ±0.20**	38.01 ±0.15**	37.8 ±0.21*	4.55
4	Paracetamol	10	37.3 ± 0.28	38.10 ± 0.16	38.5 ± 0.28*	37.9 ± 0.34**	37.9 ±0.26**	37.6*± 0.17**	4.80

n=6, ns- non significant, *****p<0.05- significant, ******p<0.01-more significant v/s control, SEM= standard error mean,

SD = standard deviation, n= number of animals

POE 100- *Platycladus orientalis* ethanolic extract 100mg/kg b.wt, POE 200- *Platycladus orientalis* ethanolic extract 200mg/kg b.wt 400mg/kg b.wt, Paracetamol -300 Paracetamol 300mg/kg b.wt.

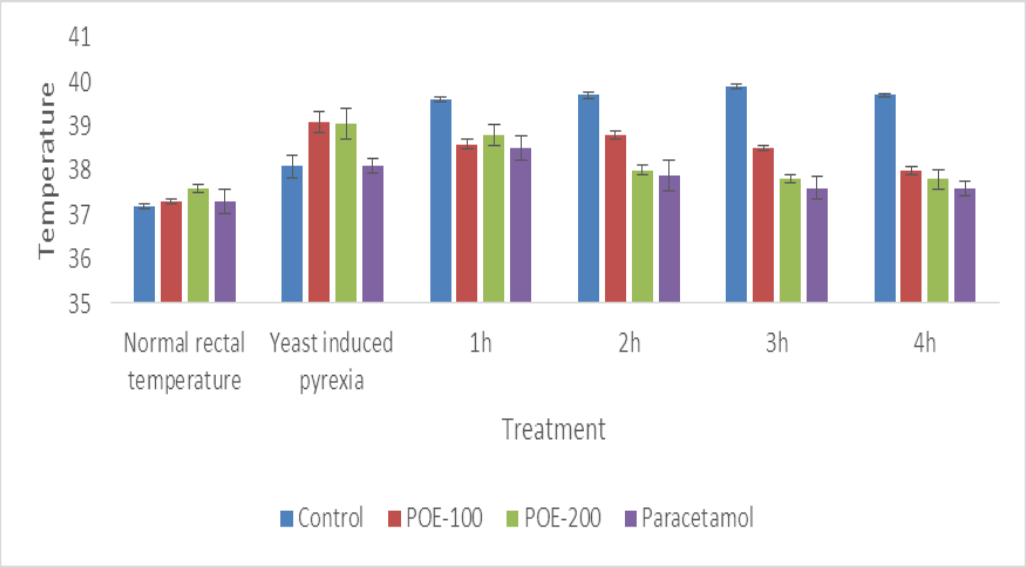

Fig. 6: Anti-pyretic activity of ethanolic extract of *Platycladus orientalis* in brewer's yeast induced pyrexia.

CONCLUSION

In conclusion, the results of the present study demonstrated that ethanolic extract of *Platycladus orrieantalis* produced dose related acute anti-inflammatory activity (Carrageenan, dextran, histamine and formalin), chronic anti-inflammatory activity (Cotton pellet) and antipyretic activity). These studies have shown that the ethanolic extract of *Platycladus orrieantalis* contains some active ingredients with the potential of being good anti-inflammatory and antipyretic agents.

ACKNOWLEDGEMENTS

Authors are profusely thankful to Alwar Pharmacy College, Alwar (Raj.), India staff for their constant and perennial support.

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