Original Research Paper

Volume-9 | Issue-1 | January-2019 | PRINT ISSN - 2249-555X

Pharmacology

ANTI-INFLAMMATORY ACTIVITY OF *PTEROSPERMUM ACERIFOLIUM* FLOWER IN WISTAR RATS.

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ABSTRACT In vitro as well as in vivo studies on anti-inflammatory activity of the methanolic extract of *Pterospermum acerifolium* flower was investigated. In-vitro study was screened by Hyaluronidase Inhibition Assay and In-vivo was studied using carrageenan-induced rat paw oedema model in which indomethacin at 10 mg·kg^{-1} (standard) and methanolic extract of *Pterospermum acerifolium* flower (Test) at 200 mg·kg^{-1}were administered orally to 18 Wistar rats 1 h before induction of oedema and compared with a negative control given 10 ml·kg^{-1} distill water. The results of in vitro study have shown that the flower is a highly potent inhibitor of hyaluronidase with 51.76% inhibition compared to 61.25% of Indomethacin at 1µg concentrations of the drugs respectively. The mean percentage inhibition of paw volume of rats treated with indomethacin was 91.1% followed by *Pterospermum acerifolium* treated group with 65.6%. These results suggest that *Pterospermum acerifolium* flower has anti-inflammatory property comparable with the standard drug and may be useful for the treatment of inflammatory conditions.

KEYWORDS : Plant extract, carrageenan, oedema

INTRODUCTION

Inflammation, although first characterized by Cornelius Celsus, a physician in first Century Rome, it was Rudolf Virchow, century who suggested a link between inflammation and cancer, cardiovascular diseases, diabetes, pulmonary diseases, neurological diseases and other chronic diseases. Extensive research within last three decades has confirmed these observations and identified the molecular basis for most chronic diseases and for the associated inflammation. The transcription factor, Nuclear Factor-kappaB (NF-κB) that controls over 500 different gene products, has emerged as major mediator of inflammation. In an attempt to identify novel anti-inflammatory agents which are safe and effective, in contrast to high throughput screen, the present day world has turned to "reverse pharmacology" or "bed to benchside" approach. Ayurveda, a science of long life, can serve as a "goldmine" for novel anti-inflammatory agents used for centuries to treat chronic diseases. Ethnic people of Upper Assam region are using *Pterospermum acerifolium* for the treatment of gum swelling, gingivitis, Rheumatic arthritis and tumourous growths. Its local name is "Moragach". Inflammation occurs worldwide in all races, sexes, age and climates. Inflammation is elicited as a local response of living mammalian tissues to injury due to any agent resulting in neurologic, vascular, humoral and cellular reactions within the site of injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues[1].

A good number of anti-inflammatory and analgesics drugs are available in the present era, among them some are expensive and some are having untoward effects such as dyspepsia and gastro intestinal bleeding and complications like tinnitus, vertigo, electrolyte imbalance, hepatic damage, renal damage etc. The establishment of certain herbal drugs, easily available in the vicinity capable of producing a marked anti-inflammatory effect will present an opportunity for the physicians to use these drugs in a safe and responsible way and thereby help patients to minimize their reliance upon more dangerous NSAIDs and other synthetic anti-inflammatory drugs. With this perspective, the work is carried out critically to assess and establish the anti-inflammatory effect of *Pterospermum acerifolium* flower.

MATERIALS AND METHODS

PLANT MATERIAL AND PREPARATION OF EXTRACT

Botanically identified fresh flowers of *Pterospermum acerifolium* were collected from the source plant in Koppal district, Karnataka. It was authenticated and a reference specimen was deposited in the herbarium vide Specimen no- Sl No-01, CRF/12/147 at Central Research Facility, Belgaum, Karnataka. The flowers were shade dried, powdered and was subjected to Soxhlet extraction using methanol as solvent. 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.

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

In Vitro Study Design[2]:

1) The HYALURONIDASE INHIBITION ASSAY was performed according to Ling et al (2003) and Sigma Protocol.
2) The assay medium consisting of 3 - 5U hyaluronidase (from Sigma –Aldrich, Bangalore) in 100µl 20mM sodium phosphate buffer pH 7.0 with 77mM sodium chloride, 0.01% BSA was pre incubated with different concentrations of the Methanolic Extract (in Dimethyl sulfoxide; DMSO) for 15 min at 37 °C.
3) The assay was commenced by adding 100µl hyaluronic acid (from Sigma –Aldrich, Bangalore; 0.03% in 300mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C.
4) The undigested hyaluronic acid was precipitated with 1ml acid albumin solution made up of 0.1% bovine serum albumin in 24mM sodium acetate and 79mM acetic acid, (pH 3.75).
5) After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm.
6) The absorbance in absence of enzyme, was used as the reference value for maximum inhibition.
7) The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound *vs.* absorbance in the absence of enzyme.
8) The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was preincubated with 5µl DMSO instead, and followed by the assay procedures described above.
9) Compound was tested in a range of 0.1µg -1µg in the reaction mixture.
10) Indomethacin (Indo) was used as reference standard.

IN VIVO:

Study design[3]:

1) Experimental study was carried out in accordance with the directions of the Institutional Animal Ethical Committee (IAEC) after obtaining permission at the Animal house, attached to KLEU'S, Shri B.M.K. Ayurved Mahavidyalaya, Shahpur, Belgaum-3, Karnataka.
2) 18 Healthy Adult Male Wistar rats of weighing from 200±2O grams wasobtained from Sri Venkateshwara Traders, Suppliers of Lab Animals, No-4304,13th Main,1st cross Subramanya Nagar, Bangalore, Registration No.: CPCSEA 237.
3) They were exposed to natural day and night cycles with ideal laboratory conditions in terms of ambient temperature (22±2ºc) and humidity (50-60 %) and fed on feed supplied by License Breeder.
4) 18 Healthy Adult Male Wistar rats were divided into 3 groups of 6 animals each.
5) Dose of the test drug was calculated using Paget and Barnes

(1969) table.

Table No. 1: Animal grouping

GROUP	TYPE	DRUG	DOSE
Group I	Control	Distill water	10 ml/kg
Group II	Standard	Indomethacin	10 mg/kg
Group III	Test	methanolic extract of flower	200mg/kg

1) After One hour 0.1ml freshly prepared 1% Carrageenan (Sigma type 1) in sterile Solution was injected to the sub-planter aponeurosis of the left hind limbs of each group to produce inflammation15.
2) Paw volumes were recorded with the help of Plethysmograph just after injection i.e. 0 hour and at regular intervals of 1 hour up to the 6th hour.

STATISTICALANALYSIS

Results were expressed as mean ± SEM. Analysis of the data was done using the one-way Analysis of Variance (ANOVA) followed by the Duncan multiple range test. The P value < 0.05 was considered significant in all cases.

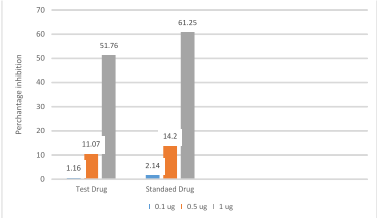
RESULTS

The results of in vitro study have shown that the flower is a highly potent inhibitor of hyaluronidase with 51.76% inhibition compared to 61.25% of Indomethacin at 1µg concentrations of the drugs respectively. This finding becomes important when we consider that expensive injections of hyaluronic acid into the synovial cavity are administered to patients with chronic arthritis (Tanaka et al 2002). Clearly, a safe and potent hyaluronidase inhibitor is urgently required in the clinic, and there are none! Such a level of potency of Pterospermum acerifolium (0.10 mg/ml inhibits both enzymes completely) has pharmaceutical significance for drug development programmes.

TABLE NO. 2: SHOWING PERCENTAGE INHIBITION OF HYALURONIDASE ENZYME

Sample	Test concentration (in µg)	O.D. at 600nm	% inhibition	
		Triplicates	Mean	
AMax	-	1.13		
		1.115	1.12	100
		1.114		
Pterospermum acerifolium	0.1	0.013		
		0.015	0.013	1.16
		0.012		
	0.5	0.129		
		0.123	0.124	11.07
		0.121		
	1	0.57		
		0.597	0.580	51.76
		0.572		
Indomethacin	0.1	0.023		
		0.021	0.024	2.14
		0.028		
	0.5	0.16		
		0.165	0.159	14.20
		0.153		
	1	0.69		
		0.68	0.686	61.25
		0.687		

Graph 1: % Inhibition of Hyaluronidase Inhibition Assay



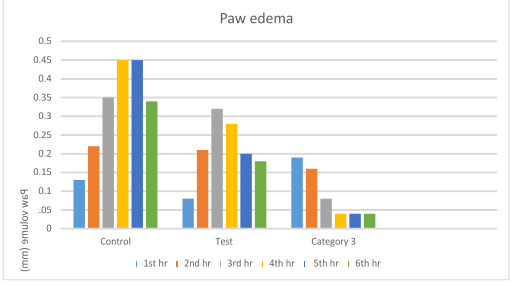
In Vivo study have shown that the Control group animal showed significant and continuous increase in edema till the end of 5th hour. The biphasic response was evident from the pattern of increase. The Standard drug Indomethacin (10 mg/kg) started showing significant decrease in edema at the end of 3rd hour. The test drug *Pterospermum acerifolium* (200mg/kg) started showing significant decrease in the edema, at the end of 4th hour.

TABLE NO- 3: MEAN OF THE PAW VOLUMES

Groups	Paw volume (ml)					
	1st hr	2nd hr	3rd hr	4th hr	5th hr	6th hr
Control	0.13 ± 0.016	0.22 ± 0.36	0.35 ± 0.024	0.45 ± 0.034	0.45 ± 0.024	0.34 ± 0.0371
Test	0.08 ± 0.02	0.21 ± .055	0.32 ± 0.039	0.28 ± 0.032*	0.20 ± 0.033*	0.18 ± 0.0354*
Standard	0.19 ± 0.035	0.16 ± 0.023	0.08 ± 0.0213*	0.04 ± 0.0119*	0.04 ± 0.0296*	0.04 ± 0.0115*

Values are mean + SEM; n=6 in each group. One-way ANOVA revealed F=23.563, P=0.05 indicating significant difference between groups. Further multiple comparisons using Dennett's t-test showed significant difference among treated and control groups. *Mean difference (+SEM) is significant at the 0.05 level.

Graph 2: Paw edema



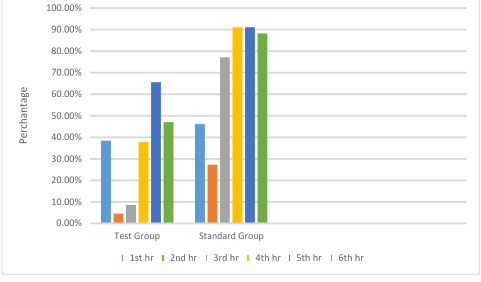
The oedema inhibitory activity was calculated according to the following formula-

Percentage inhibition:% Inhibition=100(1–Vt/Vc), where Vt =Volume in Test,
Vc = Volume in Control

Table No-4: Percentage inhibition of Paw edema

Group	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
Group II (Test)	38.46 %	4.55 %	8.57 %	37.78 %	65.6 %	47.06 %
Group III (Standard)	46.15 %	27.27 %	77.14 %	91.11 %	91.1 %	88.24 %

GRAPH 3: PERCHANTAGE INHIBITION OF EDEMA



DISCUSSION AND CONCLUSION

It is well known that carrageenan-induced paw oedema is characterized as a biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 hr after carrageenan injection), chemical mediators such as histamine and serotonin play a role, while in the second phase (4 h after carrageenan injection), kinnins and prostaglandins are involved4. In this test,

development of edema (inflammatory response) is a biphasic event with a maintenance phase in between. The initial phase is primarily mediated by histamine and serotonin, but platelet activating factor and arachidonic acid metabolites also play an important role. The Control group animal showed significant and continuous increase in edema till the end of 5th hour. The biphasic response was evident from the pattern of increase. The Standard drug (10 mg/kg) started showing significant decrease in edema at the end of 3rd hour. The test drug (200 mg/kg) started showing significant decrease in the edema, at the end of 4th hour and maximum at 5th hour. Pterospermum acerifolium seems to inhibit prostaglandins and arachidonic metabolites mediated phase. It has shown considerable anti-inflammatory activity against kinnins, prostaglandin and arachidonic acid metabolites.

In conclusion, methanolic extract of Pterospermum acerifolium flowers could serve as an alternative anti-inflammatory therapy in managing inflammatory conditions or as complementary therapy allowing patients to take smaller doses of conventional anti-inflammatory drugs, thereby minimizing the side effects of these standard drugs is promising and warrants further study.

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