

AN EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF MUCHAKUNDA FLOWER (*PTEROSPERMUM ACERIFOLIUM* WILLD) IN WISTAR RATS

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ABSTRACT

Medicinal plants represent potential sources for the discovery of new anti-inflammatory agents. Petroleum ether, Methanolic and Aquous extracts of *Muchakunda* (*Pterospermum acerifolium* Willd) were evaluated in vivo for their anti-inflammatory activities on carrageenan-induced paw oedema in Wistar albino rats. Some of the phytochemicals found in the extracts have previously been implicated as anti-inflammatory agents. The LD₅₀ of the extracts were investigated and found to be greater than 5000mg/kg. The Methanolic and Aquous extracts at doses 500 mg/kg showed modest anti-inflammatory activity. The aqueous extract demonstrated better inhibition of paw oedema compared with the methanolic extract at 500mg/kg after 4hrs. The activity of the standard drug, indomethacin at 10.0 mg/kg was significantly higher ($p < 0.05$) than those of the extracts. The results suggest that *Muchakunda* (*Pterospermum acerifolium* Willd) flower possess anti-inflammatory activity and will be useful in the search for novel anti-inflammatory agents.

Keywords: *Muchakunda*, *Pterospermum acerifolium* Willd., *in vivo*, anti-inflammatory, extracts, Indomethacin

INTRODUCTION: Inflammation, although first characterized by Cornelius Celsus, a physician in first Century Rome, it was Rudolf Virchow, a German physician in nineteenth 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. 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 bench side” approach. We found that Ayurveda, a science of long life, almost 6000 years old, can serve as a “goldmine” for novel anti-inflammatory agents used for centuries to treat chronic diseases.

I have seen the Adivasis of Upper Assam region using *Muchakunda* for the treatment of gum swelling, gingivitis, Rheumatic arthritis and tumourous

growths and the surprising thing is that they have obtained good results of it as analgesic and anti-inflammatory drug. There its local name is “Moragach”.

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 *Muchakunda* Flower (*Pterospermum acerifolium* Willd) through systematic experimentation and keen observations of results.

OBJECTIVES OF THE STUDY

1. To investigate Acute Oral Toxicity, LD₅₀ of Muchakunda Flower (*Pterospermum acerifolium* Willd.).
2. Fixation of doses for Anti-Inflammatory studies on the basis of LD₅₀ Studies.
3. To evaluate and compare the Anti Inflammatory activities of Petroleum Ether, Methanolic and Aquous Extracts of Muchakunda Flower (*Pterospermum acerifolium* Willd.).

MATERIALS AND METHODS

Source of the Trial drug Muchakunda (*Pterospermum acerifolium* Willd): Botanically identification of the Trial drug Muchakunda (*Pterospermum acerifolium* Willd) was done at Shree Shree Marishantaveer Herbal Garden of SJG Ayurvedic Medical College, PG Studies & Research Centre, Koppal-583231, Karnataka and marked as source plant. The source plant was taxonomically studied for the whole year.

Authentication of the Trial drug Muchakunda (*Pterospermum acerifolium* Willd): Specimens of flower, fruit, leaf and barks were submitted to the Central Research Facility, KLEU's Shri BMK Ayurved Mahavidyalaya, Belgaum-3 Karnataka. The source plant was authenticated to be *Pterospermum acerifolium* Willd by taxonomist and a reference specimen was deposited in the herbarium vide Specimen no- SI No-01, CRF/12/147.

Collection and preservation of the Trial drug Muchakunda (*Pterospermum acerifolium* Willd): Botanically identified fresh flowers of the Trial drug Muchakunda (*Pterospermum acerifolium* Willd) were collected from the source plant at Shree Shree Marishantaveer Herbal Garden of SJG Ayurvedic Medical College, PG Studies & Research Centre, Koppal-583231 Karnataka after authentication in the months of September and October 2011. The flowers were shade dried and preserved in air tight containers

at Rasashala attached to SJG Ayurvedic Medical College, PG Studies & Research Centre, Koppal-583231, Karnataka.

PREPARATION OF THE DRUGS

Extractions¹: The flowers were shade-dried in open air, pulverised using electric grinder to make powder and stored in airtight containers. The extraction procedures were carried out at Institute for advanced studies in science and technology, Boragaon, Assam following the already established extraction procedure of plant materials. The powder was subjected to successive Soxhlet extraction using solvents of varying polarity; petroleum ether, 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 anti-inflammatory experimental bioassays were carried out.

Preparation of 1 % Carrageenan

Solution: Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and by causing release of histamine, 5 HT, bradykinin and prostaglandins it produces inflammation and oedema. To 100 ml saline water, 1 gm of carrageenan powder is added and stirred well to get a uniform mixture of 1% carrageenan solution. This is used to induce inflammation.

Chromatographic Conditions² The Methanolic extract of Flower powder was subjected for High Performance Thin Layer Chromatography.

Acute Toxicity Study (Limit Test)

Study design³:

- a) 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.

a) Fifteen Healthy Female nulliparous and non- pregnant Wister albino rats weighing 150-200g will be selected and grouped into two groups of five animals each.

b) After acclimatization for seven days' animals will be housed at room temperature of 22°C ($\pm 3^{\circ}\text{C}$), relative humidity 50-60%, exposed to 12 hours day, 12 hours night cycles and fed with standard pellet and ad-libitum.

c) They were fasted prior to dosing by withholding food but not water overnight.

d) Animals were weighed.

e) Petroleum ether (PE), Methanolic and Aquous extracts of *Pterospermum acerifolium* at a dose of 2000mg/kg were administered orally to first three groups was administered orally.

f) After administration food were withheld for further 3 hours.

g) Animals were observed upto 14 days for any signs of toxicity and death.

h) As there was no apparent signs of toxicity or death was found even at a dose of 5000mg/kg in all the extracts, the dose of 500mg/kg was fixed as per the provision of 1/10th of the dose administered in Toxicity Studies for further studies.

ANTI-INFLAMMATORY STUDY

Study design⁴:

a) 30 Healthy Male Wister albino rats weighing 150-200g. Male Wister albino rats aged between 60-90 days.

b) Animals grouped into Five groups of six animals each.

c) After acclimatization for seven days animals were housed at room temperature of 22°C ($\pm 3^{\circ}\text{C}$), relative humidity 50-60%, exposed to 12 hours day, 12 hours night cycles and fed with standard pellet and ad-libitum.

d) They were fasted prior to dosing by withholding food and water overnight.

e) Animals were weighed before administration of drugs.

f) Normal saline at a dose of 25ml/kg, Indomethacin at a dose of 10 mg/kg and Petroleum ether (PE), Methanolic and Aquous extracts of *Pterospermum acerifolium* at a dose of 500mg/kg body weight were administered orally with the help of a gastric catheter of suitable size sleeved onto a syringe nozzle at a constant volume to all the groups once as a stat dose. Dose of the standard drug was calculated using Paget and Barnes (1969) table.

g) After One hour of dosing, 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 inflammation.

h) Paw volumes were recorded with the help of Plethysmograph 30 minutes before administration of carrageenan and thereafter, readings were taken hourly until the 4th hour past plant extracts administration. The anti-inflammatory activities were calculated as the degree of paw oedema (*e*) and the degree of paw oedema inhibition (*i*) using the formulae:

$$e = (E_0 - E_t / E_0) \times 100\% \quad i = (e_0 - e_t / e_0) \times 100\%, \text{ where}$$

E_0 = The paw volume at the baseline

E_t = The paw volume at a particular reading time of the right hind paw.

e_0 = the degree of paw oedema of untreated

e_t = The degree of paw oedema of test group.

i) Results were expressed as percentage increase in paw volume in comparison with the initial paw volumes and also in comparison with the control group.

j) Results were reported as mean \pm SD, the test of significance ($p < 0.005$) after statistical analysis using One way ANOVA followed by Post hoc Scheffe's test.

Table. 1 Grouping of Animals

Group	No. of Rats	Drug	Form	Dose (orally)	Purpose
Group I	6	Normal saline	Liquid	25ml/kg b.w.	Normal for diuretic activity
Group II	6	Indomethacin	Powderd tablet in Saline.	10mg/kg + 25ml/kg NS b.w.	Standard for diuretic activity
Group III	6	<i>Pterospermum acerifolium</i>	Petroleum ether extract (PE)	500mg/kg+25ml/kg NS b.w.	<i>Pterospermum acerifolium</i> PE for antiinflammatory activity
Group IV	6	<i>Pterospermum acerifolium</i>	Methanolic extract (ME)	500mg/kg+25ml/kg NS b.w.	<i>Pterospermum acerifolium</i> ME for antiinflammatory activity
Group V	6	<i>Pterospermum acerifolium</i>	Aquous extract (AE)	500mg/kg+25ml/kg NS b.w.	<i>Pterospermum acerifolium</i> AE for antiinflammatory activity

RESULTS AND DISCUSSION:

Preliminary phytochemical screening of the extracts showed that saponin, phenolics, reducing sugars, triterpenes and phytosterols were present in both Methanolic and Aquous extracts. Flavonoids and Steroids were found to be present in Aquous extract. It can be said that, the steroids which get extracted in aqueous extract show the anti-inflammatory activity.

Our Ayurvedic dosage form of *kwatha* can be equated with Aquous extract of modern pharmacology. Thus, it can be said that, the Phytosterols or steroids as well as Flavonoids which get extracted into Methanolic and Aquous extracts show better anti-inflammatory activity. The result slightly varies from those reported by Olonisakin *et al.* (2004), Okokon *et al.* (2005) and Mathur *et al.* (2009). The variation may be attributed to differences in plant location, mode of

extraction as well as season of plant sample collection. Generally, the crude nature of the extracts could be responsible for their low anti-inflammatory activities compared with the standard control. Further separation of the various phytochemicals found in the crude could yield better results.

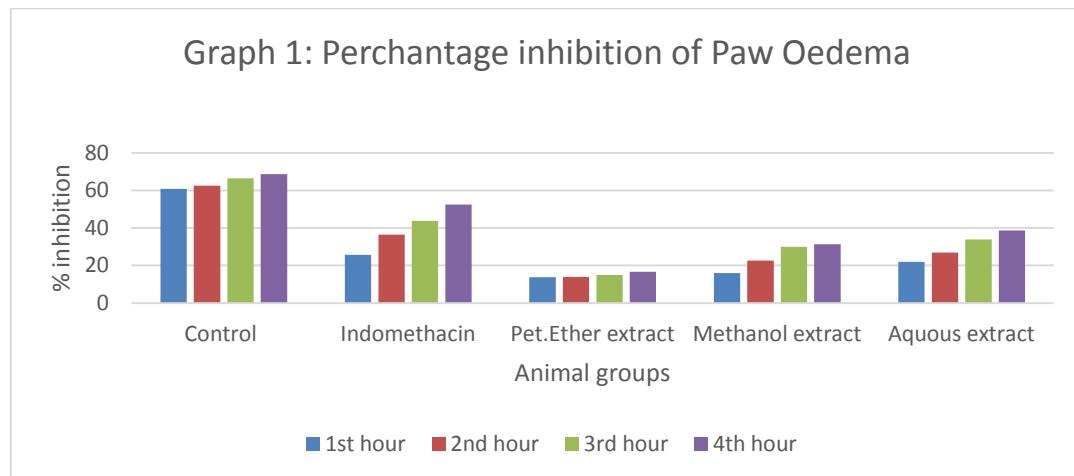
Two spots of orangish-yellow with Rf values 0.27 and 0.85 were seen under long UV before derivatization. They were not seen under visible light. It is observed that, Rf values of the phyto-constituents corresponds to Flavonoids which may be responsible for Anti Inflammatory activity of *Pterospermum acerifolium*.

The LD50 is greater than 5000 mg/kg and may be classified as practically nontoxic and within the acceptable margin of safety (Hodge and Sterner scale) at the recommended dose. Thus 1/10th (i.e. 500mg/kg) was selected for the study.

Table. 2: Mean degree of paw oedema inhibition (%)

Group	1 st hour	2 nd hour	3 rd hour	4 th hour
Control	60.88	62.46	66.43	68.65
Indomethacin	25.72	36.44	43.72	52.40
Pet.Ether extract	13.70	13.89	14.96	16.70
Methanol extract	15.97	22.69	29.99	31.33
Aquous extract	21.99	26.81	33.90	38.56

Degree of paw oedema inhibition after treatment with standard/extracts. * Values significantly different from standard drug indomethacin ($p<0.05$)



From the graph, the degree of paw inhibition by the standard drug (indomethacin, 10.0 mg/kg) increased significantly with time as compared to the test groups. Among the test groups (plant extracts), however, the aqueous treatment inhibited paw oedema with time better than the methanolic and petroleum ether extracts. 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 h 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 involved. Aqueous and Methanolic flower extracts seems to inhibit prostaglandins and arachidonic metabolites mediated phase. Thus it can be said that *Pterospermum acerifolium* has shown considerable anti-inflammatory activity against kinnins, prostaglandin and arachidonic acid metabolites.

CONCLUSION: Aqueous and Methanolic flower extracts of *Pterospermum acerifolium* have shown modest anti-inflammatory activity on carrageenan-induced paw oedema. This investigation suggests that *Pterospermum acerifolium* is

a potential candidate for the discovery of new anti-inflammatory agents.

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Source of support: Nil Conflict of interest:

None Declared

Cite this Article as :Biswa Jyoti Bora et al :An Evaluation of Anti inflammatory Activity of Muchakunda Flower(*Pterospermum Acerifolium* Willd.) in Wistar Rats]www.ijaar.in: IJAAR, VOLUME III ISSUE XI NOV –DEC 2018 Page No:1639-1643