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Pharmacological Evaluation of *Xanthoxylum oxyphyllum* Edgew Extract with Special Reference to Anti-inflammatory Activity

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A B S T R A C T

Inflammation is a localized physical condition, the immune reaction of the body tissues towards the harmful stimuli such as pathogens, damaged cells or irritants. Steroids and some other drugs have been used to alleviate inflammation but its regular use has been discouraged by the practitioners. The use of this plant in NE India is diverse but few pharmacological studies have been carried out to evaluate its anti-inflammatory activity. Therefore, the present study is undertaken to evaluate the anti-inflammatory properties of *Xanthoxylum oxyphyllum*. For evaluation of anti-inflammatory properties of the ethanolic extract of *X. oxyphyllum*, carrageenan induced rat paw edema model was used. Three doses of the extract showed significant reduction of paw edema in the later stages of carrageenan induced inflammation. Medium dose @30 mg/kg has shown maximum inhibition of paw volume edema. This might be due to inhibition of synthesis and/or release of inflammatory mediators in the late phase of inflammation. The ethanolic leaf extract of *Xanthoxylum oxyphyllum*, was evidenced to possesses anti-inflammatory properties but however, better activity was seen with medium dose @ 30 mg/kg body weight in both carrageenan induced paw edema model and Adjuvant Induced Arthritis model.

Introduction

Inflammation is a localized physical condition, the immune reaction of the body tissues towards the harmful stimuli such as pathogens, damaged cells or irritants. It is considered as a protective response which involves immune cells, blood vessels and molecular mediators and serves to destroy, dilute or otherwise neutralize harmful agents and repair damaged tissues. During inflammation, the specific body part becomes reddened, swollen, hot and often painful. Its main aim is to remove the early cause of cell injury, clearance of necrotic cells and tissue damaged from initial insult and the inflammatory process, and to trigger tissue repair. However, under certain conditions, inflammation may wander from its beneficial path and may become considerably more

harmful to the body, as in life threatening hypersensitivity reactions like rheumatoid arthritis, atherosclerosis, etc (Kleiman & Tuckerman, 2007).

Steroids and some other drugs have been used to alleviate inflammation but its regular use has been discouraged by the practitioners. Steroids are synthetic drugs that closely resemble cortisol, a hormone that our body produces naturally. Corticosteroids suppress the multiple inflammatory genes that are activated in chronic inflammatory diseases such as asthma, mainly by reversing histone acetylation of activated inflammatory genes through binding of liganded glucocorticoid receptors (GR) to coactivators and recruitment of histone deacetylase- 2 (HDAC2) to the activated transcription complex. At higher concentrations of corticosteroids GR homodimers also interact with DNA recognition sites to active transcription of anti-inflammatory genes and to inhibit transcription of several genes, linked to steroid side effects (Barnes P.J., 2006). Corticosteroids inhibit the formation of both PG's and leukotrienes by causing the release of lipocortin, which by inhibition of phospholipase A reduces arachidonic acid release. Hence by stopping the release of arachidonic acid we stop the whole mechanism of inflammation. (Kleiman & Tuckerman, 2007)

A very few pharmacological study has been carried out systematically to evaluate the anti-inflammatory activity of this plant. Hence keeping in mind the above shortcomings of the treatment, the present study is undertaken to evaluate the anti-inflammatory properties of *Xanthoxylum oxyphyllum.* The generic name is derived from ancient greek word *Xanthos* and *xylon* referring to a yellow dye made from the roots of some species (Buerton, 1994). Local names are mezenga, Timur and bhansi. The plant have been used by the tribals of north-east India since time immemorial for different purposes like keeping good health of the digestive, immune and joint health of body. It is used as local food and cultivated in India, southern China, Bhutan Nepal and Myanmar. Young shoots are used as vegetable and fruits are condiment in curries. The bark of the tree is tonic and aromatic and treats rheumatism and atonic dyspepsia. The bark is also administered in fevers. Fruits are prescribed for dyspepsia, asthma, bronchitis (Medhi *et al.*, 2013). Keeping in view the above mentioned facts and theories, it was felt necessary to select the plant for this research work with the objectives to find out the acute toxicity and anti-inflammatory activity of *Xanthoxylum oxyphyllum* extract.

Materials and Methods

The materials and techniques used for performing the experiments are internationally acceptable. Adult healthy Wister rats and albino mice were procured from an animal dealer in Kolkata. All the animals were caged in small groups of 3 animals per cage. Animals had free access to standard balanced ration and clean drinking water *ad libitum*, and were kept in standard laboratory conditions.

The use of experimental animals and the study protocol was duly approved by Institutional Animal Ethics Committee (IAEC) of the college. The plant *Xanthoxylum oxyphyllum* was authenticated as *Zanthoxylum oxyphyllum* Edgew. by the Taxonomist via collection number 5176. The powdered leaves of *X. oxyphyllum* were processed for optimum ethanolic extract. Phytochemical tests were conducted on ethanolic extract of *X. oxyphyllum* as per standard procedures described by Sofowara (1993), Trease and Evans (1989) and Harborne (1998).

Experimental protocol for evaluation of anti-inflammatory property of ethanolic extract of *X. oxyphyllum*

30 rats were taken for this experiment and they were distributed into 5 groups with 6 animals per group (Table 1). They were kept together for a week for acclimatization and were fed and watered adequately. Group I served as normal control and were given the vehicle i.e. 20% tween 80 solution @1ml/rat. Group II served as standard and were given Meloxicam suspension @1mg/kg to all the 6 rats. Group III, IV & V served as low, medium and high dose groups and were given 10, 30 & 100 mg/kg of the extract respectively dissolved in 20% tween 80 solution orally. After 30 minutes of administration of the drugs and extract along with the vehicle acute inflammation was induced by sub plantar injection of 0.1 ml of freshly prepared 1% carrageenan suspension in normal saline in the left hind paw of rats in each group. The paw volumes of left hind paw of each of the rats were measured using plethysmometer just before injection i.e. at "0" hrs. and then at "1st", 2nd, 3rd, 4th, 5th, 6th, and 24th hour after carrageenan injection and recorded. The percent inhibition of rat paw edema was calculated after each hour of carrageenan injection for up to 6 hrs. and again after 24 hours.

Statistical analysis

The values were expressed as mean ± SEM and were subjected to statistical analysis by employing ANOVA using the software SAS (Table 4).

Results and Discussion

Xanthoxylum oxyphyllum extract

The dried and powdered leaves of *X. oxyphyllum* were subjected to extraction following standard techniques. The ethanolic extract yield was found to be 6.38 g per 100 grams of dry powder, respectively.

Phytochemical analysis

The extracts of *X. oxyphyllum* were qualitatively analyzed for the presence of different phytochemical properties. The extract was found to contain Alkaloids, Terpenoids, Flavonoids, Steroids, Tannins, Glycosides, phenols. It was found not to contain any Saponins.

Evaluation of ethanolic extract of *X. oxyphyllum* for its anti – inflammatory activity using carrageenan induced rat paw edema model

The anti-inflammatory property of the ethanolic extract of *X. oxyphyllum* was evaluated using carrageenan induced rat paw edema model. The paw volumes and paw volume difference compared with "0" hour are displayed in Table 2 & 3 along with percent inhibition. In the control group, the animals showed a biphasic reaction. The swelling increased instantly within the first hour and then it subsided in the second only to be back in the third hour and continued increasing till the fifth hour and then it subsided. The paw volume was again measured after 24 hours of carrageenan injection. The resulting paw volumes at time t0, t1, t2, t3, t4, t5, t6 and t24 are 0.92 ± 0.11, 1.23 ± 0.09, 1.18 ± 0.11, 1.26 ± 0.15, 1.27 ± 0.18, 1.51 ± 0.04, 1.26 ± 0.08 and 1.17 ± 0.08 respectively.

Paw volumes observed in Standard group where Meloxicam was used as a standard are 0.97 ± 0.09, 1.00 ± 0.09, 1.07 ± 0.06, 1.19 ± 0.02, 1.24 ± 0.02, 1.38 ± 0.09, 1.29 ± 0.00, 1.15 ± 0.02 at time t0, t1, t2, t3, t4, t5, t6 and t24 respectively. Inhibition of paw volume swelling by Meloxicam was maximum in the

first hour as 90%. Meloxicam inhibited paw volume considerably in the first 1-2 hours. Later on from the third hour, paw volume increased significantly as shown in the Table 3.

Paw volumes observed in the Test 1 group i.e. low dose (10mg/kg) group are 0.52 ± 0.03, 0.74 ± 0.05, 0.74 ± 0.05, 0.78 ± 0.05, 0.77 ± 0.06, 0.76 ± 0.07, 0.64 ± 0.04, 0.61 ± 0.03 at time t0, t1, t2, t3, t4, t5, t6 and t24 respectively. Inhibition of acute inflammation by low dose of the drug was maximum in the later phase of inflammation i.e. in the fifth, sixth and after 24 hours as 58.64%, 63.90% and 61.22% respectively.

In group Test 2 i.e. medium dose (30mg/kg), paw volumes recorded are 0.52 ± 0.03, 0.74 ± 0.05, 0.74 ± 0.05, 0.78 ± 0.05, 0.77 ± 0.06, 0.76 ± 0.07, 0.64 ± 0.04 and 0.61 ± 0.03 at time t0, t1, t2, t3, t4, t5, t6 and t24 respectively. Inhibition of paw volume swelling by medium dose (30mg/kg) was the best among all doses and even better than the standard Meloxicam after 1-2 hours of carrageenan injection. A significant amount of paw volume inhibition was seen from the start of the experiment and can be expressed as 46.01%, 67.37%, 71.94%, 74.28%, 88.37%, 75.11% and 95.10% after 1, 2, 3, 4, 5, 6 and 24 hours of carrageenan injection, respectively, described in the graph in Table 3. Maximum inhibition was seen from 4th to 5th hour (88.37%) and also after 24 hours (95.10%) (Fig. 1 and 2).

Table.1 Grouping of animals to evaluate the anti-inflammatory property of ethanolic ext

Group	No. of animals	Treatment
I	6	Normal control(0.1 ml of 1% carrageenan in left hind paw+vehicle @20% tween 80 solution)
II	6	Standard (0.1 ml of 1% carrageenan in left hind paw + Meloxicam @ 1mg/kg)
III	6	Test-1 (0.1 ml of 1% carrageenan in left hind paw + low dose of extract @ 10 mg/kg)
IV	6	Test-2 (0.1 ml of 1% carrageenan in left hind paw + medium dose of extract @ 30 mg/kg)
V	6	Test-3 (0.1 ml of 1% carrageenan in left hind paw + high dose of extract @ 100 mg/kg)

Table.2 Effect of ethanolic extract of *X. oxyphyllum* and meloxicam on carrageenan induced rat paw edema

GROUP	PAW VOLUME (ml)							
	0 Hr	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	24 Hr
Control	0.92±0.11c-m	1.23±0.09a-e	1.18±0.11a-g	1.26±0.15a-e	1.27±0.18a-d	1.51±0.04a	1.26±0.08a-e	1.17±0.08a-h
Standard	0.97±0.09b-l	1.00±0.09b-l	1.07±0.06a-j	1.19±0.02a-f	1.24±0.02a-e	1.38±0.09a-b	1.29±0.00a-c	1.15±0.02a-i
Test 1	0.52±0.03m	0.74±0.05i-m	0.74±0.05i-m	0.78±0.05 g-m	0.77±0.06g-m	0.76±0.07h-m	0.64±0.04k-m	0.61±0.03l-m
Test 2	0.66±0.01j-m	0.83±0.04e-m	0.75±0.02g-m	0.76±0.01f-m	0.75±0.03g-m	0.73±0.01h-m	0.75±0.01g-m	0.68±0.01j-m
Test 3	0.78±0.06g-m	1.08±0.09b-i	0.89±0.07d-l	0.96±0.08c-l	0.97±0.1c-l	1.00±0.14b-k	1.07±0.10b-i	0.93±0.1c-l

Table.3 Effect of ethanolic extract of *X. oxyphyllum* and meloxicam on carrageenan induced rat paw edema indicating paw volume difference and edema inhibition

Treatment	t1 Mean	t2 Mean	t3 Mean	t4 Mean	t5 Mean	t6 Mean	t7 Mean
Control	0.31±0.14ab	0.26±0.08ab	0.34±0.07ab	0.35±0.21ab	0.59±0.13a	0.34±0.05ab	0.25±0.13ab
Standard (%inhibition)	0.03±0.01b (90.24)	0.11±0.04b (59.22)	0.23±0.1ab (32.83)	0.27±0.12ab (22.14)	0.42±0.11ab (29.09)	0.35±0.1ab (2.96)	0.18±0.11ab (25.51)
10mg/kg (%inhibition)	0.22±0.05b (27.37)	0.22±0.07 ab (13.91)	0.26±0.04 ab (22.38)	0.25±0.04 ab (28.09)	0.24±0.06 ab (58.64)	0.12±0.03b (63.90)	0.1±0.03b (61.22)
30mg/kg (%inhibition)	0.17±0.05 ab (46.01)	0.08±0.02b (67.37)	0.09±0.01b (71.94)	0.09±0.04b (74.28)	0.07±0.01b (88.37)	0.08±0.01b (75.11)	0.01±0.01b (95.10)
100mg/kg (%inhibition)	0.3±0.05ab (1.89)	0.11±0.03b (55.98)	0.18±0.05b (46.26)	0.19±0.05b (46.19)	0.23±0.09 ab (61.25)	0.3±0.06ab (12.09)	0.15±0.07b (38.77)

Table.4 Anova table representing treatment with ethanolic extract of *X. oxyphyllum* and meloxicam on carragenan induced rat paw edema

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	44	11.481646	0.260946	10.1743
Error	155	3.975376	0.025648	**Prob > F**
C. Total	199	15.457022		<.0001**

**Significant at P (<.001)

Fig.1 Graph representing mean change in paw volume of rats when treated with ethanolic extract of *X. oxyphyllum* and meloxicam in carrageenan induced rat paw edema test

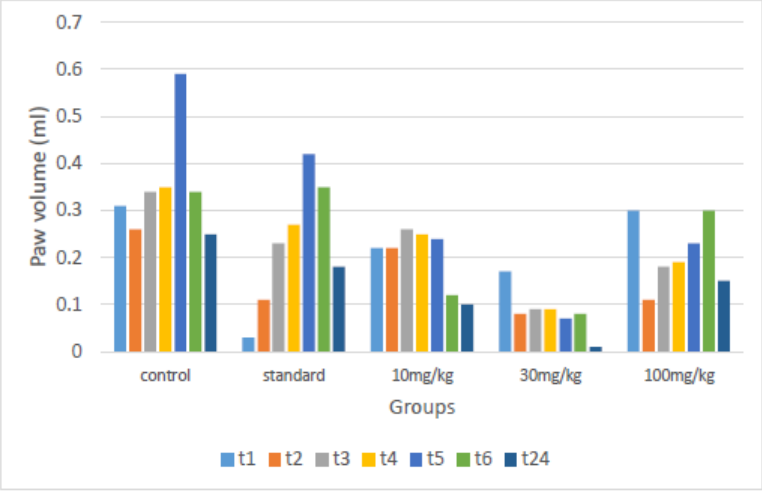
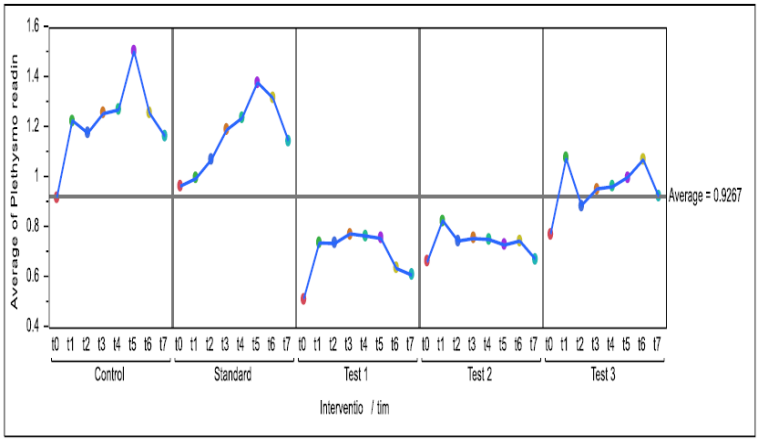


Fig.2 Graph representing mean paw volume of rats when treated with ethanolic extract of *X. oxyphyllum* and meloxicam in carrageenan induced rat paw edema test



In group Test 3 i.e. high dose (100mg/kg) group, paw volumes recorded are 0.78 ± 0.06, 1.08 ± 0.09, 0.89 ± 0.07, 0.96 ± 0.08, 0.97 ± 0.1, 1 ± 0.14, 1.07 ± 0.1 and 0.93 ± 0.1 at time t0, t1, t2, t3, t4, t5, t6 and t24, respectively. Paw volume inhibition is not as remarkable as medium dose and anti-inflammatory property of ethanolic extract of *X. oxyphyllum* is not dose dependent as in high doses it doesn't give better results as compared to low and medium doses but significantly differs from the standard as can be seen from Table 3. Paw volume inhibition was maximum in the period from 4th – 5th hour i.e. 61.25 % and also from Ist – 2nd hour i.e. 55.98%.

The ethanolic extract of the indigenous plant *Xanthoxylum oxyphyllum* was evaluated for anti-inflammatory properties. Phytochemical analysis of the ethanolic extract of leaves of *Xanthoxylum oxyphyllum* revealed the presence of phytochemicals like alkaloids, terpenoids, flavonoids, steroids, tannins, glycosides and phenols. It was not found to contain Saponins. It has been reported that the ethanolic extract of leaves and seeds of *Xanthoxylum oxyphyllum* possesses an abundance of Glycosides, Coumarins, Flavonoids, Phenols and Tannins. They reported a presence of phenols and flavonoids in leaves when compared to seeds (Ayangla *et al.*, 2016).

The ethanolic extract of the leaves of *Xanthoxylum oxyphyllum* was tested for anti-inflammatory activity. Carrageenan induced paw edema is also one of the most effective models for evaluation of anti-inflammatory effect of new agents. The edema produced by carrageenan is caused by release of mediators' like vasoactive amines, prostaglandins, bradykinin and leukotrienes. So, the reduction of pain and inflammation is caused by effectively inhibiting the synthesis and/or release of these mediators. The first phase of inflammation is mediated by the release of mediators like histamine, bradykinin etc. and usually lasts for 1-2 hours after the carrageenan injection. It can also be due to the trauma associated with the injection of carrageenan in sub-plantar region of the paw. The second phase of inflammation is mediated by substances like leukotrienes and prostaglandins etc. The control group showed a biphasic reaction i.e. a sharp rise in inflammation at start and then a fall in swelling after the first hour and then again, a rise in second hour and kept on increasing till the 5th hour. After the 5th hour, inflammation subsided.

The ethanolic extract of *Xanthoxylum oxyphyllum* was administered to the respective test group rats @ 10, 30 & 100 mg/kg and acute inflammation was measured through paw volume change using water displacement digital

plethysmometer. all the three doses have shown significant reduction in paw volume, with better paw volume edema inhibition than the standard group in the later phase of inflammation i.e. after 1-2 hours. Maximum inhibition was shown by the dose @ 30 mg/kg with 88.37% inhibition at 5th hour and 95.10% inhibition after 24 hours as compared to 29.09% (5th hour) and 25.51% (after 24 hrs.) of standard, respectively. Ethanolic extract of *Xanthoxylum oxyphyllum* showed better effect on delayed phase of inflammation, which may be probably be due to inhibition of prostaglandins and leukotrienes. The decrease in the anti-inflammatory effect at high dose i.e. 100 mg/kg may be due to an inverted "u" phenomenon where, as the dose increases, the effect decreases creating an inverted u-shaped dose-response curve. Similar type of dose-response was found by Virdi *et al.*, (2013) where they noticed that aqueous extract of *Momordica charantia* plant can reverse alloxan induced diabetes with doses as low as 20 mg/kg and suggesting that higher doses may not only be ineffective but may also cause toxicity.

In conclusion from all the findings of the present study, it may be concluded that the acute oral toxicity of ethanolic extract of leaves of *Xanthoxylum oxyphyllum* was found to be less than 2000 mg/kg. 1000 mg/kg of the extract was finalized as the safe dose. *X. oxyphyllum* possesses significant anti-inflammatory activity in lower doses. Further studies need to be directed towards fractionation and isolation of the active principles present in the extracts for chemical finger printing for identification of the pharmacophores/ lead biomolecule(s) and safety evaluation for exploring the therapeutic potential of the plant

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