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PHARMACOLOGICAL EVALUATION OF METHANOLIC EXTRACT OF *ZANTHOXYLUM OXYPHYLLUM* EDGW. WITH SPECIAL REFERENCE TO ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

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ABSTRACT: The objective of this study was to evaluate the anti-inflammatory and analgesic activities of the methanolic extract of the aerial parts of *Zanthoxylum oxyphyllum*, a plant used in Assamese traditional medicine. Successive solvent extraction were carried out with the plant and the methanolic extract of the plant was evaluated for its anti-inflammatory activity by carrageenan induced paw edema and analgesic activity by hot plate method at the doses of 250mg/kg and 500mg/kg *p.o* (per oral) in wistar rats. The result shows that the extract at dose of 250 mg/kg and 500 mg/kg has significant reduction in the the paw edema ($P<0.05$ and $P<0.01$) in a dose dependent manner when compared to the control. The extract have shown less analgesic effect at the dose of 500 mg/kg when compared to the control group. These inhibitions were statistically significant ($p < 0.05$). The results of this study demonstrated the anti-inflammatory and analgesic effects of the methanolic extract in animal models and justify traditional use of this plant in the treatment of pain and inflammatory conditions.

Key words: *Zanthoxylum oxyphyllum,* Anti-inflammatory activity, Indomethacin, Analgesic activity, Hot plate method, Diclofenac sodium.

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INTRODUCTION

Inflammation is a defensive attempt of living tissue to remove the injurious stimuli (Parmar N et al, 2012) and to initiate the healing process that leads to local accumulation of plasmatic defense mechanism that helps body to protect itself against allergens, infection, toxic chemicals, burns, or other noxious stimuli. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Sosa S et al, 2002). The endogenous mediators like serotonin, histamine, prostaglandins, bradykinin etc. are released during inflammation. These mediators even in low quantities can induce pain. Most of the analgesics are associated with the antiinflammatory drugs which helps in relieving pain by reducing inflammation as opposed to opioids which affect the central nervous system (Parmar N et al, 2012).

Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Amberkar M.B et al, 2011). Drugs, which help in reducing or removing pain are called as painkiller or analgesics. Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli that shows classical signs of pain, heat, redness, swelling and loss of function. So we can say that inflammation is related to pain.

Nature has provided a complete store-house of remedies to cure all aliments of mankind (Ravi V et al, 2009). Plants as source of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines (Stiffness M, Douros J, 1982).. Over 50% of all modern clinical drugs are natural products that play a vital role in modern drug development in the pharmaceutical industry (Baker D.D et al, 2007) including treatment of inflammation and pain.

Synthetic substitutes have no doubt taken over but they may result in many adverse effects such as respiratory distress, drowsiness, gastrointestinal bleeding, ulceration, nausea etc. (Laurence D.R et al, 1997, Mate G.S et al, 2008). Due to these conditions there is a need to search the bioactive components from medicinal plants which may be used as alternative for anti-inflammatory and analgesic drugs with little or no side effects.

Zanthoxylum oxyphyllum, under the family Rutaceae, commonly known as Mezenga in Assam grow in tropical and temperate regions (Pioani J.R, 1993). They are represented by thorny, dioecious shrubs or small trees with dense foliage and prickly trunk and leaves with a strong and pungent taste (Chayee C.C et al, 1996). Leaves are imparipinnate. Flowers are globose, glabrous and dull red in colour. Seeds are solitary and shining black. The tender shoots are cooked and eaten as a vegetable in Assam which acts as blood purifier, help in reducing incidence of leucoderma and are useful against stomach trouble (Buragohain J et al, 2011). Fruits are sweetish bitter, hot and digestible, appetizer, anthelmintic and remove pain, tumors, useful in gastric problem, headache, body ache, fever, cold and cough and also used for the purification of blood. The bark is considered as stomachic and digestive stimulant. It is also administered in fevers as a sudorific (Kanjilal, U.N 1992, Kirtikar, K.R, Basu, B.D, 1993). The stem bark yielded indoquinazoline alkaloid and is commonly applied in rheumatism, varicose ulcers, varicose veins, skin diseases and leg pains. Moreover it is also used in relieving inflammation, fevers and hypotension (Arun K.K.V, Paridhavi M, 2012). The bark and root extract of *Z. oxyphyllum* has been shown to have antiproliferative activity against the growth of human keratinocytes (Kumar S, Muller K, 1999). Besides it has stimulant, stringent and digestive properties and is also used in dyspepsia and diarrhoea (Medhi K, et al 2009).

Fig.1. Photograph of *Zanthoxylum oxyphyllum* plant

As no pharmacological study has been carried out systematically to evaluate the anti-inflammatory and analgesic activity of *Z. Oxyphyllum* till date therefore an attempt has been made to study the Analgesic activity of the plant in correlation with the Anti-inflammatory activity on experimental animal models.

MATERIAL AND METHODS

Plant material

The plant samples were collected from Tinsukia district of Assam during the month of November, 2015. Healthy aerial parts of the plant were collected for the study since microbial and other infections may hamper the metabolites produced by the plant. The plant was identified by Prof. L.R. Saikia, Department of Life Sciences, Dibrugarh University. A voucher specimen is stored in our laboratory for future reference as specimen L.Sc. 432.

Preparation of extract

The plant sample collected was properly cleaned, shade dried and grounded to coarse powder. The powdered sample was then continuously extracted by successive solvent extraction using different solvents viz., Petroleum Benzene, Ethyl acetate, Methanol using Soxhlet apparatus. The methanolic extract of *Zanthoxylum oxyphyllum* (MEZO) was evaporated on water bath at 60^0C to a sticky mass of crude extract.

Acute toxicity study

Acute oral toxicity test was performed as per Organization for Economic Co-operation and Development (OECD) guidelines (OECD, 2000) Approval No. IAEC/DU/89 dtd. 27/3/15. Experiments were performed using healthy wistar rats weighing 100-120 gm. Test substance at a volume of 0.5ml/kg body weight of the animal was administered orally at a dose of 500, 1000 and 2000 mg/kg body weight. All the animals showed no mortality after 48hr of administration of the plant extract. The parameters (like tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma etc.)were normal even at the highest dosage of 2000mg/kg body weight of the test animal throughout the study period of 3 days. This clearly indicated that the Methanolic extracts of *Zanthoxylum oxyphyllum* do not produce toxicity. The medium lethal dose (LD50) of the extracts is higher than 2000mg/kg body weight and hence, in a single dose administration, the plant extract has no adverse effect.

Experimental Animals

Wistar rats weighing between 100-150 gm were used in the experimental study. The animals were kept properly in hygienic cages and provided with food and water. Healthy animals were used in the experiment. They were kept on fasting overnight before the experimentation. The experimental protocol was approved by the Institutional Animal Ethical Committee of Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam-786004(Regd.No. 1576/GO/a/11/CPCSEA) through Approval No. IAEC/DU/89 dtd 27/3/15.

Carrageenan-induced paw edema (Acute Model)

This method is the most widely used method for the evaluation of anti-inflammatory drugs. Wister rats were divided into five groups having five animals in each group[18]. Animals of group-1 received normal saline solution, animals of group-2 (carrageenan control group) received normal saline solution, animals of group-3 (standard drug treated group) received indomethacin (10 mg/kg, i.p.), animals of group-4 received *Z. oxyphyllum* extract (250 mg/kg, p.o.) and animals of group-5 received *Z. oxyphyllum* extract (500 mg/kg p.o.). Vehicle, standard drug and test compound were administered 30 min prior to carrageenan injection. After 30 min, 0.1 ml of 1% (*w/v*) solution of carrageenan in 0.9% normal saline solution was injected into the plantar region of right hind paw. The paw volume of each rat from all groups was measured at 0, 30, 60, 120, 180, 240min and 24hours after carrageenan injection. The inflammation was measured in terms of ml i.e. displacement of water by paw edema using a digital plethysmometer.

Percentage inhibition of paw oedema = (1–Vt/Vc) × 100

Where, Vc represent average increase in paw volume (average inflammation) of the control group of rats at a given time; and Vt was the average inflammation of the drug treated (i.e. plant extracts or test drug) rats at the same time (Kulkarni S.K, 1999).

Hot plate method

The paws of mice and rats are very sensitive to heat at high temperatures which can damage the skin which results in jumping, licking of the paws and withdrawal of the paws. The analgesic activity of the extract, is usually measured by hot-plate method (Hemamalini K et al., 2010). The hot plate is made up of electrically heated surface. The animals are placed on the hot plate and the time is recorded by a stop-watch until its responses starts. The hot plate was maintained at 55±0.5^{o}C. All test drugs including the standard i.e., Diclofenac sodium were provided to the respective group rats prior to the experiment. The reaction time was measured as the interval from the animal placed on the hot plate until the moment animal licked its feet or jumped. To avoid any thermal injury to the paws, a cut off time of +10s was maintained. The reaction time was recorded before and after +10, +30, +60min following administration of test or standard drug.

Table 1: Anti-inflammatory activity of *Zanthoxylum oxyphyllum* by Carrageenan-induced paw edema

Groups	Dose	Paw Volume(ml)(Mean±S.E.M)						
		0min	30min	1hr	2hr	3hr	4hr	24hr
Normal	Normal Saline	0.44 ± 0.01	0.42 ± 0.14	0.42 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.45 ± 0.01	0.44 ± 0.01
Control	Carrageenan (0.1ml)	0.42± 0.06	0. 59± 0.14	0.63 ± 0.01	0. 65 ± 0.01	0.70 ± 0.03	0.73 ± 0.16	0.80 ± 0.14
Standard	Indomethacin (10mg/kg)	0.41 ± 0.04	0.54 ± 0.08	0.56 ± 0.03	0.53 ± 0.04*	0.52 ± 0.03**	0.44 ± 0.02**	0.42 ± 0.04 **
I	250mg/kg	0.44 ± 0.01	0.54 ± 0.03	0.52 ± 0.02 *	0.49 ± 0.02 **	0.49 ± 0.01**	0.47 ± 0.02**	0.46 ± 0.01**
II	500mg/kg	0.43 ± 0.02	0.52 ± 0.02	0.49 ± 0.02**	0.47 ± 0.02**	0.45 ± 0.01**	0.45 ± 0.02**	0.44 ± 0.02**

All values are given in mean±SEM, (n=5) ANOVA*p<0.05, **p<0.01, when compared to carragenan control group.

Table 2. Percentage (%) inhibition of paw oedema volume

Groups	Dose	%Inhibition					
		30min	1hr	2hr	3hr	4hr	24hr
Standard	Indomethacin (10mg/kg)	23.53	28.57	47.83	60.71	90.32	97.36
I	250mg/kg	41.17	61.9	78.26	82.14	90.32	94.73
II	500mg/kg	47.06	71.43	82.61	92.86	93.55	97.37

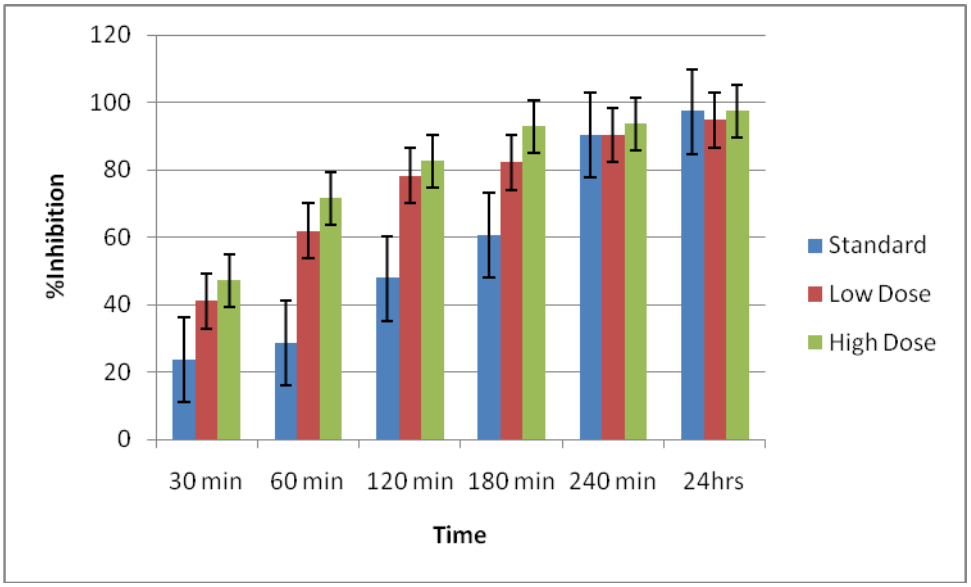

Fig.2: Percentage (%) inhibition of paw oedema volume.

Table 3. Hot plate method for analgesic activity

Groups	Dose	Reaction time(secs) (Mean ± S.E.M)			
		0min	10mins	30mins	60mins
Control	Normal saline	4.13± 0.66	5.10 ± 1.05	5.11 ± 1.08	4.73 ± 0.22
Standard	Diclofenac Sodium (20mg/kg)	4.17± 0.70	7.33± 0.27	7.83 ± 0.49	9.18 ± 0.47 *
I	250mg/kg	4.15 ± 0.68	4.79 ± 0.77	6.68 ± 1.21	7.98 ± 1.82
II	500mg/kg	4.19 ± 0.42	8.12 ± 1.07	10.99 ± 1.05	11.77 ± 0.81

Results are presented as mean ± SEM, (n=5), *p<0.05, **p<0.01 Dunnet test as compared to control

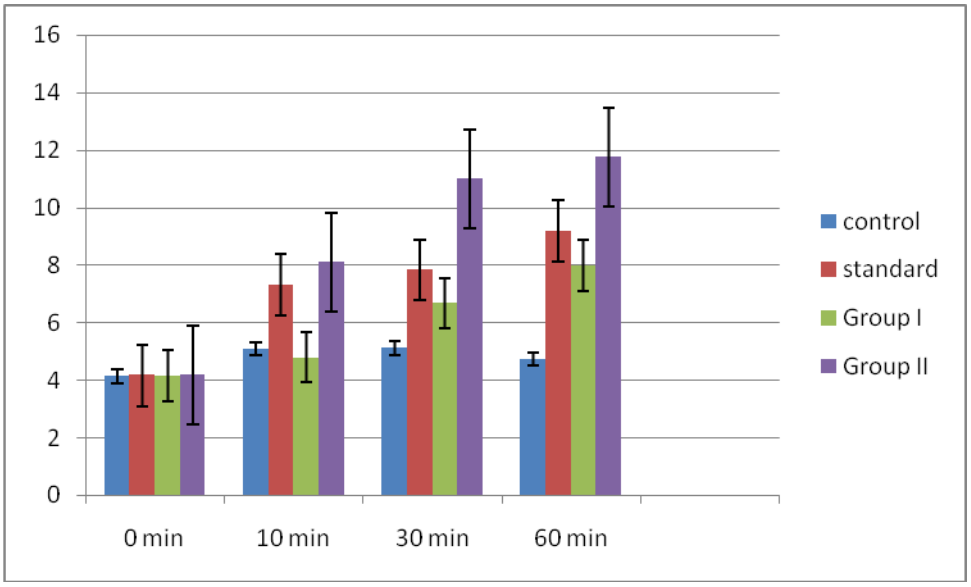

Fig.3: Graphical representation of Analgesic effect of methanolic extract of *Z. Oxyphyllum* in pain using Eddy's Hot plate method

Statistical Analysis

The statistical analysis were carried out based on the expression of numerical data as mean ±S.E.M. The statistical significance between control and treated groups were analyzed using analysis of variance (ANOVA) followed by Dunnet's test, where p values less than **0.05** were considered to be significant.

RESULTS AND DISCUSSION

The result shows that the plant extract at dose of 250 mg/kg and 500 mg/kg b.w.has significant reduction in the carrageenan induced paw edema (**P<0.01) in a dose dependent manner when compared to control. The inhibition of the extract was found to be almost similar to that of the standard drug, Indomethacin (10 mg/kg, i.p.). The higher dose of the extract (500 mg/kg) exhibited better anti-inflammatory activity comparable to Indomethacin while lower dose of the extract (250 mg/kg) exhibited slightly low anti-inflammatory activity.

Carrageenan induced rat paw edema is frequently used to assess the anti-edematous effect of natural products for evaluating anti-inflammatory drugs (Panthong A et al, 2003). This edema depends on the participation of kinins and polymorphonuclear leucocytes with their pro-inflammatory factors including prostaglandins (Damas J et al, 1986). Present study finding supports the traditional claims and provides a scientific basis for anti-inflammatory effect of *Zanthoxylum oxyphyllum* in inflammatory diseases. So it is hoped that these studies will further aid to the efforts of developing new medications for the treatment of inflammatory diseases.

The results for the analgesic activity were expressed as "mean increase in latency after drug administration ±S.E.M" in terms of seconds. In the analgesic assay of MEZO, it was observed that the extract at 500mg/kg and 250mg/kg showed analgesic activity but not comparable to the standard drug Diclofenac sodium. The extracts of the plants showed less latency time than the Diclofenac sodium (20 mg/kg) in the hot plate test in a dose related manner. Effects of methanolic extract of *Zanthoxylum oxyphyllum* were determined in interval of 0, 10, 30, 60min. The specified plant extract at 500mg/kg have shown more analgesic effect than the dose of 250 mg/kg i.e., if we increase the concentration of the doses we can achieve a good analgesic.

Carageenan induced paw edema in rats is most frequently used experimental model for assessing the anti-inflammatory effect of compounds or natural products (Panthong A et al, 2003).

Inflammatory diseases have been reported to occur because of the various mediators like prostaglandins, kinins, leukotrienes, platelet activating factor etc. The development of carageenan induced edema is biphasic. The first phase is partly attributed to the trauma of injection and due to the release of histamine, serotonin compounds and the second phase is attributed to the production of bradykinin, protease, prostaglandins and lysosome. Anti-inflammatory agents inhibit the cyclooxygenase involved in prostaglandin synthesis (Seibert K et al, 1994). The doses 250mg/kg and 500mg/kg of methanolic extract of *Z. Oxyphyllum* produced significant reduction of carrageen induced paw edema at 2hr and more. Therefore it can be said that the inhibitory effect of the crude extract is due to the inhibition of cyclooxygenase enzyme thereby inhibiting the production of prostaglandins and inflammation related compounds. Subsequent inhibition may be due to the inhibition of histamine and serotonin compounds. Earlier researchers reported the presence of flavanoids, alkaloids, sterols and terpenes in the *Zanthoxylum* species which is responsible for diverse biological properties such as antioxidants, anti-inflammatory, antibacterial etc. (Andersson C.M et al, 1996, Harbone J.B, Williams C.A 2000, Adesina S.K 2005, Patino O.J 2004, Patino O.J (2004, Waterman P.G, Grundon M.F (1983). Lupeol and sitosterol, a dietary triterpene which has gained wide attention in pharmaceuticals is found in most species of the genus *Zanthoxylum* which is extensively studied for its inhibitory effects on inflammation under in vitro and in animal models of inflammation (Saleem M, 2009). These metabolites may be responsible for narcotic analgesic, anti-inflammatory and antidiabetic activity of the plant. The steroids β-sitosterol, stigmasterol and campesterol associated with sitosterol may also contribute to the analgesic activity observed Malairajan P et al, 2006).

CONCLUSION

The present study have demonstrated that *Z. Oxyphyllum* has significant anti-inflammatory activity at the specified doses but to be comparable with the standard analgesic drug Diclofenac, we have to increase the concentration of the doses used to justify its traditional use to treat inflammation and pain. At present, there are no reports on investigation to identify the active components present in methanolic extract of *Zanthoxylum oxyphyllum.* Further study is required in order to understand the precise mechanism and to identify the active components, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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