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Original Article

Anti-inflammatory and analgesic activity of methanolic bark extracts of Zanthoxylum Armatum

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ABSTRACT

A comprehensive study on the phytochemical contents and biological activities of different extracts from bark of *Zanthoxylum armatum* was studied. The extracts of the bark parts contained various levels of phenols, flavonoids and saponins. Present research work mainly focus on analgesic and antiinflammatory efficacy of methanolic bark extract of *Zanthoxylum armatum* belongs to the family Rutaceae. The analgesic activity was tested in albino mice by giving oral doses of 100, 200 and 300 mg/kg body weight using tail immersion method, acetic acid induced writhing and formalin induced analgesic effect. The outcome of the analgesic activity showed significant and dose dependent analgesic effect ($P<0.001$). The brink was found to be 60 min after treatment with 100, 200, and 300 mg/kg of the methanolic root extract. The activity produced by the extract was significantly lower than the standard drug used (Diclofenac sodium). The dose of the standard drug used is 100 mg/kg. The results clearly states that methanolic extracts showed delayed response towards thermally induced pain, decreased acetic-acid induced writhing and also showed significant inhibition in both the phases of formalin induced pain test. The analgesic activity of the extracts can be attributed to their central and peripherally mediated secondary messengers in the management of pain. The phytochemical constituents present in the bark extract may contribute for the analgesic activity. The results finally support the herbal remedies over the conventional dosages in the management of pain.

Key Words: *Zanthoxylum armatum*, Phytochemical screening, Analgesic activity, Antiinflammatory activity

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INTRODUCTION

Zanthoxylum armatum belongs to the family Rutaceae is an important traditional plant with lots of medicinal values growing in the tropical and temperate parts of the world [1, 2, 3]. It is extensively distributed in many parts of India. There are many species varying about 450 species [4], [5]. It is featured for many pharmacological values in the treatment of pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever in India [6, 7]. Scientific studies on extracts and formulations revealed anti-asthmatic, mast cell stabilization and anti-allergic effects of different parts of *Z. armatum* [8, 9]. This plant extract shows many traditional and ethno medicinal uses with eminent biological activities.

Analgesia is one of the most common problems around the world [10, 11]. The flexibility of conventional analgesic drugs in the market show many side effects like depletion of the mucosal layer in the stomach and thereby causing ulcers which lead to discomfort and change in the biological system[12, 13]. Most of the marketed analgesic drugs which are used in the tolerance of analgesia such as NSAIDS (Non-Steroidal anti-inflammatory drugs), Opiod analgesics, corticosteroids [14, 15]. Sustain release analgesics drugs etc are known for their common side effects like tolerance, dependence, increase in the lipid profile, obesity etc. So it is necessary to explore the folklore and find out an alternate remedy for the management of pain with fewer side effects.

According to World Health Organization (WHO), phytochemicals extracted from the plants would be the best source which could be formulated into variety of drugs [16, 17]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicinal compound; have continued to play a dominant role in the maintenance of human health, since ancient times. Most of the drugs derived from plants were developed because of their use in traditional medicine.

Preliminary chemical examination of the water soluble constituents of the stem bark of *Zanthoxylum armatum* (Linn) belongs to the family: Rutaceae, [18] commonly known as Toothache tree in English, Tejphal in Hindi and Tumboonalari in Malayalam was reported [19]. The aqueous extract was found to be containing free glucose and glycosidic principle in addition to a considerable amount of manitol. Examination of petroleum ether extract of the bark of *Z. armatum* the pet ether extract was found be contain gamma a-sitosterol which was established by mixing melting point determination with an authentic specimen [20, 21]. Different solvent extracts of *Z. armatum* showing antinociceptive, antiviral and antipyretic effects were reported of *Z. armatum* herbal formulations.

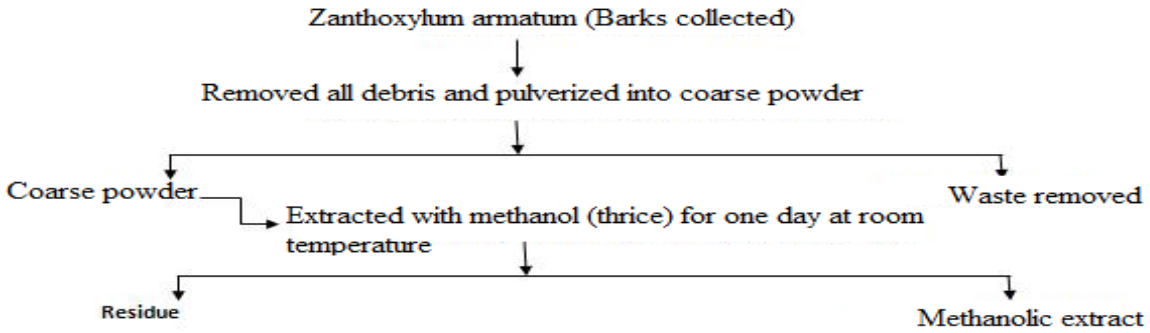
Based upon the existing chemical, clinical literature and folkaire claims, our objective were to gratifying the analgesic activity of bark extract namely methanol. Natural sources especially this genus it is necessary to find out alternate remedy for side effects caused by traditional system of medicine. The active principle which is responsible for the activity has to be found out by different chromatographic techniques.

Plan and Objective: The aim and objective of the work was to identify the analgesic activity of the methanolic bark extract of *Zanthoxylum armatum* in the management of pain response against the experimental models. The analgesic activity is determined by tail immersion method, acetic acid induced writhing model and formalin test

MATERIALS AND METHODS

Collection and authentication of plant material: The bark parts of *Zanthoxylum armatum* were collected from the local areas of Araku, Visakhapatnam district which belongs to state of Andhra pradesh in the month of September and October. It was authenticated by Dr. S.B.Padal, Dept. of Botany, Andhra University and Sample specimen was kept in our laboratory for future reference. The collected barks are garbled and freed from the unwanted dust material. The barks are first washed with tap water followed by distill water to remove all the debris attached to the barks. Then these are allowed to dry in shade and after drying the barks are pulverized into a course powder and required amount of the extract is taken for preparation of the extract and the remaining powder is stored in a tightly closed container for future reference.

Preparation of Plant extracts: To 1 Kg of *Zanthoxylum armatum* bark powder, 2 liters of solvent, viz. methanol was added consequently for preparing the extract (flow chart-1). Extraction with the solvent was done for 24 hours at 27^0C, after maceration the supernatant of each solvent was recovered by filtering through whatmann filter paper. This process was repeated thrice and the respective solvent from the supernatant was evaporated in a rotavapor (BUCHI) to obtain crude extract which are to be stored at 4^0c until used for evaluation.



Flow chart-1: Schematic representation of showing methanolic extraction procedure for *Zanthoxylum armatum* bark powder.

Experimental animals: Analgesic activity was carried out on 2 weeks aged swiss wister albino mice weighing 25-30 grams were procured from Mahaveer enterprises, Hyderabad, Telangana state. The animals are taken utmost care and housed in standard cages, with 5 mice in each cage, under a 24 hr a day (12 hr day and 12 hr night) catalog at an optimum room temperature (25-30^{0}c) so as to make the rats acclimatize to the laboratory conditions. The experimental animals were fed with water and food *ad libitum* before a week prior to the commencement of the experiment. The experimental animals were treated according to the rules of institutional and international guidelines mentioned for the use of experimental animals.

Screening of suitable animals for the experiment: Total number of albino mice used for this experiment was 20 weighing between 150-250grams. These total rats were grouped in three groups containing 5 albino rats in each group. The tail of the rat was immersed in the water which is maintained at 55 ± 5^{0}C withdraws its tail normally in 3-6 seconds when exposed to hot water., if any animal fails to withdraw its tail in that stipulated reaction time those rats were rejected for the study. The rats which are normal and suitable for the experiment were labeled accordingly.

Preparation of Standard drug: Diclofenac sodium is used as a standard drug in this method. 400mg diclofenac sodium tablets were procured and powdered. Then 40ml of distilled water was added to get 10mg of diclofenac in 1ml of solution. Group I is maintained as standard group which received 0.1ml/ 100 gram body weight of albino rats.

Tail immersion assay [22]: The experimental animals are allowed to acclimatize to the lab conditions, in the cage for about half an hour prior to experimentation. Analgesic activity is performed by the method described by Chandrashekar et al, with slight modification. The mice selected for the experiment are divided into five groups of four animals each. Group I mice received standard drug Diclofenac sodium 10mg/kg. Group II, III, IV mice were pretreated with 100-300 mg/kg (i.p) of root bark methanolic extract of *Zanthoxylum armatum*. Group V received physiological salt solution (PSS) as control. After half an hr of administration of the drug, each mouse was held in a position such that the tail is extended out of the cage. The tail was marked about 3 cm and was immersed into the water bath which was maintained at temperature 55 ± 5^{0}C. The mouse has given response within few seconds which was recorded as the withdrawal time. The time period was evaluated at 0, 30, 60, 90 and 180 minutes with 0 minute being the initial reading. The mean increase in the reaction time after administration of the drug indicates the potency of the extract and the standard. To prevent the damage to the tail of the mice, the immersion time was limited to 10 seconds.

Analgesic activity of *Zanthoxylum armatum* by Acetic acid induced writhing method [23]: Acetic acid induced writhing assay method was performed by using 25 rats which were divided as 5 rats per group into five groups. The writhing were induces as described in koster et al with minor modification. The standard group received the standard drug 100mg/kg body weight of diclofenac sodium. Control group received 0.3ml physiological saline solution and the test groups received 100,200 and 300 mg/kg of methanolic root bark extract of *Zanthoxylum armatum* intra peritonea (i.p). Firstly the experimental rats are allowed to fast for 15hrs and after that the treatment was initiated. After 60 min of the treatment, the rats were administered with acetic acid (0.6%, v/v in normal saline, 10 mL/kg), which

acts as a source for the contraction of the abdominal muscle (Writhing). The stretching contractions and jerking of the hind limb was noted between 5 to 30 minutes after the administration of the acetic acid. The standard group rats are compared with that of the test and control group. Inhibition of the writhing activity was taken as the mark of analgesia and the percentage of writhing inhibition is calculated.

Formalin test [24, 25]: Formalin test is used to determine acute pain. This method was done as described by shibata et al with minor deviations. This method involves the injection of 20ml of 5% formalin subcutaneously (sc) to the right hind paw of mice to produce pain response. The time (in seconds) taken by the mice to lick and bite the hind paw was taken as the indicator of pain induction. The responses are measured from 0-5 minutes in the early phase and 25-40 minutes after the administration of formalin injection. The experimental mice selected suitable for the experiment were divided into four groups of five animals each. Group I, II, III received the methanolic extract 100-300 mg/kg intra peritoneal route (i.p) administered 60 minutes prior to formalin injection. Group IV received the standard drug (diclofenac sodium) 10mg/kg (i.p) was administered 30 minutes prior to formalin injection. Group V received Physiological Salt Solution (PSS) as control.

RESULTS AND DISCUSSIONS

The analgesic effect produced by bark methanolic extract by performing tail immersion method is well elucidated in Table I. Methanolic extract showed % inhibition of the pain at 47.8, 17.2 and 35.9 tail withdrawals by inducing 100, 200, 300 mg/kg doses respectively. While the standard drug showed % inhibition of 62.6 at 100mg/kg dose which shows that the extract produced less effect when compared with that of the sample. Methanolic extract showed significant (P<0.001) dose dependent inhibition of analgesia. The recordings were done keeping in mind about the precaution to be taken to prevent damage to the tissue of the tail.

Acetic acid induced writing assay results is well illustrated in table II. The methanolic extract showed percentage inhibition of writhing (30 minutes) of 5.41, 16.5, 39.2 writhing responses at 100mg, 200mg, 300mg dose. The results obtained are found to be significant (P<0.001) when compared with that of the control and standard doses. The extract showed significant inhibition at high dose level of 300mg/kg, but the effect produced by the extract is found to be inferior to the effect produced by Diclofenac sodium (100mg/kg) which is standard drug used in the study of the effect.

Formalin test results were given in table III. There was a significant dose-dependent inhibition in both phases of the formalin-induced pain response in mice, with a more potent effect in the second phase. Diclofenac sodium also inhibited pain in both phases, but its effect on the first phase was not significantly (P > 0.001) different from that produced by 300 mg/kg of the extract.

Table I: Effect of methanolic extract of *Z. armatum* on tail immersion test

Group	Dose(mg/kg)	Before drug Administration	After drug administration	% Inhibition
Control	--	2.2± 0.65	2.3± 0.32	--
Z. armatum bark extract	100	1.9± 0.30	1.8± 0.50	47.8
	200	2.0 ± 1.65	2.5± 0.73*	17.2
	300	1.9± 1.78	2.6± 0.63*	35.9
Diclofenac sodium	100	2.2± 0.65	3.5± 0.26*	62.6

Values are means± S.E.M. * P < 0.001, significantly different from control; Student's t-test (n = 5).

Table II Effect of acetic acid induced writhing in albino rats when administered with Standard dose, Methanolic extract at different doses of *Z. armatum*

Experimental group	No. Of writhing (30 minutes)	% inhibition of writhing
Diclofenac sodium (100mg/kg b.w)	67.5±1.00*	66.2
Control (0.3 ml normal saline)	72.2±0.49	---
Z.armatum (100mg)	62.7 ±1.48	5.41
Z.armatum (200mg)	50.8 ±0.25*	16.5
Z.armatum (300mg)	23.9 ±1.12*	39.2

Values are means ±S.E.M. * P < 0.001, significantly different from control; Student's t-test (n = 5).

Table III Effect of methanolic extract of *Z. armatum* on the early and late phase of Formalin-induced pain in rat.

Group	Dose (mg/kg)	0-5 min	% inhibition	15-30 min	% inhibition
Control	--	92±1.3		99±1.3	
Z. armatum	100	90±2.1	2.2	63±1.5*	36.5
	200	82±2.3*	10.1	45±1.5*	54.1
	300	70±1.7*	22.4	34±2.0*	65.2
Diclofenac sodium	100	65±2.5*	27.5	20±2.2*	79.2

Values are means ± S.E.M. * P < 0.001, significantly different from control; Student's t-test (n = 5).

CONCLUSIONS

Anti-inflammatory and analgesic activity performed in experimental objects was found to be significant by the administration of methanolic bark extract of *Zanthoxylum armatum*. Standard drug used was diclofenac sodium, test dose (300mg/kg) has produced significant effect but during course of time, the reaction time for tail withdrawal has gradually decreased. Abdominal contractions induced by acetic acid were used to evaluate the peripheral analgesic activity; the pain may be due to action of various interactive secondary messengers like PG (Prostaglandins), TNF which are responsible for nociceptive activity. The phytochemical constituents like alkaloids and terpenoids may contribute to the following effect, Literature review states that terpenoids inhibits both peripheral and centrally mediated analgesia. There is no evidence of toxicity found in the course of study. Thus it is found to be safe when compared with that of conventional dosage forms.

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