



Research Article

EVALUATION OF THE ANALGESIC ACTIVITY OF THE LEAF METHANOLIC EXTRACT OF *ZANTHOXYLUM OVALIFOLIUM* WIGHT

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Article Received on: 19/03/19 Approved for publication: 26/04/19

DOI: 10.7897/2230-8407.1006199

ABSTRACT

Background: *Zanthoxylum ovalifolium* is a medicinal shrub used by some communities of Karnataka, India, in treating fever, headache and other ailments. Objectives: The present study is to evaluate the analgesic activity of the *Z. ovalifolium* methanolic leaf extract. Method: Standard in-vivo methods such as acetic acid-induced abdominal writhing, tail immersion and hot plate analgesic methods using albino Wistar mice. Dose levels such as, 100, 200 and 400 mg/kg body weight of the extract were given orally by gastric gavage and the obtained results was compared with a standard acetylsalicylic acid (aspirin) (400 mg/kg) and negative control. Statistical analysis was done using Prism using one-way ANOVA. Result: In the acetic acid-induced writhing model, the *Z. ovalifolium* methanolic extract (400 mg/kg) and the reference drug significantly ($P < 0.0001$) decreased the number of abdominal constrictions in the tested mice. The percentage inhibition of the abdominal constriction was improved from 0% (100 mg/kg) to 40% at the highest dose of the extract (400 mg/kg). In the tail immersion method, the *Z. ovalifolium* methanolic extract (400 mg/kg) appreciably ($P < 0.0001$) increased the pain reaction time. In hot plate method the extract and Aspirin drug showed significant antinociceptive effect in the tested animals ($P < 0.0001$) which increases the mean pain reaction time at the doses of 200 and 400 mg/kg. From One-way ANOVA followed by Dunnett's multiple comparison test showed significant results in all tested analgesic experiments with P value is less than 0.0001 against control. Conclusion: *Z. ovalifolium* methanolic extract demonstrated significant analgesic activity which influencing both central and peripheral pain suppression pathway.

KEYWORDS: *Zanthoxylum ovalifolium*, writhing, hot plate, acetylsalicylic acid.

INTRODUCTION

Pain is an uncomfortable situation due to damage of the cell or organ associated with actual or potential tissue damage. It is also a warning signal and primarily protective in nature but in serious conditions it may leads to lot of discomfort. Pain suppression is the most important therapeutic priorities to satisfy the suffering patient.¹⁻² Analgesic drugs are used to suppress the pain which are notably opiates and non-steroidal anti-inflammatory drugs. Synthetic compounds even though cure pain effectively causes serious adverse effects such as ulceration, gastrointestinal bleeding, sleepiness, nausea etc.³⁻⁴ Due to these adverse effects of the synthetic drugs there is the need of bioactive compounds from the natural origin especially from medicinal plants as an alternative with no side effects.

Zanthoxylum ovalifolium belongs to the family of *Rutaceae*. It is an erect shrub up to 8-10 m high. The bark is thorny smooth, brownish or yellowish, Stems pubescent and brown. The leaves are simple glabrate, elliptic-obovate, petiole axillary, puberulus. The flowers are panicle white. The fruits are lenticular or flattened globose with shining surface, containing 1 seed embedded. It grows in dense dry deciduous to moist deciduous forests throughout Western Ghats, India, the tree flowers from May to June and fruits from October to November. Other common names include: Thnry yellow wood (English), Aramadalu (Kannada), Rijayi (Telugu).⁵

Different parts of the tree have been used in folkloric medicine for treatment of different diseases such as headache and pains (bark), fever, fever, headache and also carcinogenic. An essential oil is extracted from the stem and fruit.⁶

The present study was therefore undertaken to examine the painkilling activity of the leaf of *Z. ovalifolium* with the aim of demonstrating its folkloric use to treat pains and headaches.

MATERIALS AND METHODS

Plant collection and Identification

The leaf of *Z. ovalifolium* was collected from Sringeri forest and the voucher specimen number KU/AB/RN/PP/002/2017 was deposited in the department of Applied Botany, Kuvempu University herbarium repository.

Preparation of Plant Extract

The leaf of *Z. ovalifolium* was shade dried for 20-30 days and pulverized using sterile pestle and mortar into a coarse powder of about 1mm in diameter. The extraction was done by Soxhlet extraction method by methanol for 48 hours and the filtrate concentrated to dryness and the extract stored in a refrigerator at 15°C until time of use.

Animals

Albino Wistar mice weighing 21-30 g obtained from laboratory animal units of the NITTE Pharmacy College, Mangalore were used for the experiment. The animals were kept in stainless steel cages and housed at 25- 27°C with free access to feed and water. Ethical guidelines governing the use of animals for conducting experiments was strictly followed and was approved by Institutional Animal Ethical Committee (IAES) (Number: NGSM/IAES/2017-18/40).

Acute Toxicity Test

Twenty five Wistar mice of both sexes were selected and grouped into five of five mice each and named from A-E. Each group of mice were dosed with 100, 500, 1000, 2000 and 3000 mg/kg respectively orally through gastric gavage. After the treatment the dosed mice was provided free access to feed and water. A period of 24 hours for selected after the extract dose to observe signs of toxicity and mortality.

Acetic Acid induced abdominal Writhing Method

This study was carried out using standard protocol.⁷⁻⁸ Group of five Wistar mice of both sexes were divided into five groups and fasted for 12 hours. Grouping was made as follows; the A group mice were treated with tween 20 solution 10 ml/kg (negative control group), group B mice were treated with 400mg/kg acetylsalicylic acid (Aspirin) (positive control group) whereas groups such as, C, D and E received 100, 200, 400 mg/kg of methanolic extract of *Z. ovalifolium* respectively by gastric gavage. After one hour of extract and drug administration approximately 0.7% glacial acetic acid (10 ml/kg) was given to the intraperitoneal region of tested mice to induce abdominal constrictions or writhes. After glacial acetic acid administration the recording time of 30 minutes was taken to observe the number of writhes in each mouse. The reduced number of abdominal writhing in tested animal was considered as degree of analgesia and was calculated using the formula.¹¹

$$\text{Degree of analgesia} = \frac{\text{Mean control} - \text{Mean treated group}}{\text{Mean of control group}} \times 100$$

Table 1: Effect of leaf methanolic extract of *Z. ovalifolium* on Acetic Acid-Induced Writhing in Mice

Group	Treatment mg/kg	Mean number of writhing	% of protection
A	Tween 20 solution	45.6±6.22	0
B	Aspirin (400 mg/kg)	15.42±7.85***	50
C	ZO 100 mg/kg	44.3±8.32	0
D	ZO 200 mg/kg	28.34±4.29**	9
E	ZO 400 mg/kg	20.75±7.93***	40
	One-way ANOVA	F value	407.4
		P value	<0.0001

Each value is the mean ± S.E.M. of five rats.; *P < 0.05, **p<0.01, ***P <0.0001 vs. control, One-way ANOVA followed by Dunnett’s multiple comparison test.

Table 2: Effect of leaf Methanolic Extract of *Z. ovalifolium* on Tail Flick Response in Mice

Group	Treatment mg/kg	Mean pain ± S.E.M (seconds)
A	Tween 20 solution	1.89±1.20
B	Aspirin (400 mg/kg)	6.23±1.60***
C	ZO 100 mg/kg	2.32±1.48
D	ZO 200 mg/kg	3.09±2.35**
E	ZO 400 mg/kg	4.02±0.17***
	One-way ANOVA	F value
		P value

Each value is the mean ± S.E.M. of five rats.; *P < 0.05, **p<0.01, ***P <0.0001 vs. control, One-way ANOVA followed by Dunnett’s multiple comparison test.

Tail Immersion Test

The Tail Immersion Test was followed by standard protocol.⁹ Group of five Wistar mice were divided into five groups. All the selected Wistar mice were fasted for 12-13 hours and provided sterile distilled drinking water with *ad libitum*. The A group of animals were pre-treated with 10ml/kg tween 20 solution 60 minutes before tail immersion considered as negative control, for group B animals 400 mg/kg acetylsalicylate acid (aspirin) considered as positive control and for remaining groups the concentration of 100, 200, 400 mg/kg of *Z. ovalifolium* extract were dosed. After dosing the drug and the extracts the tail of each group of the mice was dipped into 50 -55 °C maintained water bath and pain reaction time (PRT) was noted by recording time taken for the mice to flick its tail or withdraw it from the warm water. The cut off time was put at 15 seconds.

Hot Plate Method

The Hot Plate Method was followed by standard protocol.¹⁰⁻¹¹ Group of five albino mice of both sexes were randomly grouped into five groups, fasted for 12-13 hours with adequate clean water provided *ad libitum*. The A group of animals were pre-treated with 10ml/kg tween 20 solution 60 minutes before tail immersion considered as negative control, for group B animals 400 mg/kg acetylsalicylate acid (aspirin) considered as positive control and for remaining groups the concentration of 100, 200, 400 mg/kg of *Z. ovalifolium* extract were dosed. Each group of the tested mice was placed on 55°C maintained hot plate. A stop watch was used to note the pain reaction time (PRT). The pain stimulus such as, jumping, raising and licking of hind foot was considered as the stimulus for the heat of hot plate. The observation time is restricted to 15-20 seconds.

Data Analysis

The result was presented as mean ± SEM and analyzed using one-way Analysis of Variance (ANOVA). The difference between the means was tested with Dunnett’s multiple comparison test and values of p <0.0001 were considered statistically significant.

Table 3: Effect of leaf Methanolic Extract of *Z. ovalifolium* on hot plate Response in Mice

Group	Treatment mg/kg	Observation time (min)			
		Mean drug reaction time± S.E.M (sec)			
		30 min	60 min	120 min	180 min
A	Tween 20 solution	3.6±1.46	2.28±0.93	2.757±1.12	1.45±0.59
B	Aspirin (400 mg/kg)	3.233±1.32	7.95±1.20***	8.902±1.18***	8.188±0.48***
C	ZO 100 mg/kg	2.983±1.21	2.933±1.19	3.038±1.24	1.175±0.47
D	ZO 200 mg/kg	3.13±0.08	4.635±0.06**	5.86±0.24**	3.24±0.26**
E	ZO 400 mg/kg	3.17±0.04	6.24±0.03***	7.96±0.14***	7.98±0.25***
One-way ANOVA	F value	2.185	2557	10580	2419
	P value	0.1441	< 0.0001	< 0.0001	< 0.0001

Each value is the mean ± S.E.M. of five rats.; *P < 0.05, **p < 0.01, ***P < 0.0001 vs. control, One way ANOVA followed by Dunnett's multiple comparison test.

RESULTS

Plant Extraction

The yield of the methanolic extract was 25 grams with 700 grams of dry leaf material and was dark in color.

Acute Toxicity Test

Even after 24 hours at the dose of 3000 mg/kg concentration of methanolic extract of *Z. ovalifolium* produced no death or signs of toxicity in tested animals in turn proved the toleration in tested animals.

Acetic Acid Induced Abdominal writhing method

The effect of *Z. ovalifolium* extract on the acetic acid- induced abdominal writhing in mice is showed in Table 1. The result shows that the leaf methanolic extract in different concentration showed dose dependent analgesic activity. Methanolic extract at 100 mg/kg concentration showed negligible reduction of abdominal writhing, but in 200 mg/kg concentration moderately reduced abdominal writhing in mice, whereas, in 400 mg/kg concentration showed appreciable reduction of abdominal writhing which is comparable to the reference drug aspirin (400 mg/kg). The extract showed dose dependent analgesic activity in inhibition of abdominal writhing. Furthermore, one-way ANOVA with Dunnett's multiple comparison test detect significant for methanolic extract at the doses of 400 mg/kg versus reference drug (aspirin) and control group (Table 1).

Tail Immersion Method

The result of tail immersion test in mice is showed in Table 2. The result shows that the leaf methanolic extract at the dose of 100 mg/kg concentration showed negligible analgesic activity whereas, in 200 mg/kg concentration showed moderate analgesic activity in tested animals. 400mg/kg concentration showed appreciable analgesic activity but not good as reference drug aspirin. The methanolic leaf extract at 400 mg/kg concentration and the standard drug significantly increased the pain reaction time (PRT) when compared to the negative control group. Moreover, one-way ANOVA with Dunnett's multiple comparison test showed significant results for methanolic extract at the doses of 400 mg/kg versus reference drug (aspirin) and control group (Table 2).

Hot Plate Method

The result of the effect of *Z. ovalifolium* methanolic leaf extract on the hot plate method is showed in Table 3. The result shows that there was no significant pain reaction time (PRT) for the methanolic extracts at 100 mg/kg concentration whereas, at

200 mg/kg concentration showed moderate hot plate response in tested mice. The extract at the dose 400 mg/kg significantly increased the pain reaction time producing a better effect than the rest of the methanolic extracts concentrations along with the reference drug aspirin (400 mg/kg). After treatment up to 30 min there is no significant pain withstanding effect observed in tested mice, but after 60-180 min there is appreciable analgesic activity was noticed in the animals treated with 400 mg/kg dose methanolic extract and in standard drug. Furthermore, one-way ANOVA with Dunnett's multiple comparison test showed significant results for methanolic extract at the doses of 400 mg/kg versus reference drug (aspirin) and control group (Table 3).

DISCUSSION

To evaluate the analgesic activity of *Zanthoxylum ovalifolium* methanolic extract three *in vivo* anti-nociceptive models such as, acetic acid-induced abdominal writhing, tail immersion method and hot plate models were used because to test the analgesic activity of methanolic extracts to different stimulus such as, thermal (hot plate or tail immersion method), chemical (acetic acid-induced writhing method). The methanolic leaf extract of *Z. ovalifolium* produced no death or signs of toxicity even at the dose of 3000 mg/kg which suggests that the extract was well tolerated by the mice we can safely use the extract to that concentration extent.

In acetic acid induced abdominal writhing method the methanolic extract of *Z. ovalifolium* showed dose dependent abdominal writhing suppression, means that increase in the extract concentration also increases the analgesic activity. The injection of 0.7% glacial acetic acid in the Intraperitoneal region of the tested animals produced abdominal writhing in this experiment. It has been noticed that the level of dose is directly influencing the percent reduction of abdominal constrictions. It has been documented that, the sample which suppress the abdominal constriction is directly influencing the suppression of prostaglandin synthesis.¹² (Table 1)

In tail flick method the methanolic extract of *Z. ovalifolium* showed dose dependent analgesic activity. In 100 mg/kg concentration (2.32±1.48) the methanolic leaf extract showed negligible pain reaction time which is almost nearer to the negative control (1.89±1.20) whereas, in 200 mg/kg concentration (3.09±2.35) the methanolic leaf extract showed moderate pain withstanding effect. Similarly, in 400 mg/kg concentration (4.02±0.17) the methanolic leaf extract showed significant pain reaction time which is comparable with the Aspirin (6.23±1.60) (Table 2).

In hot plate method the methanolic extract of *Z. ovalifolium* showed dose dependent analgesic activity. In initial

observation time (30 min) there is no significant pain withstanding effect was observed in all the tested samples in all the tested animals but, in the successive observation time intervals (60, 120, 180 min) *Z. ovalifolium* extract showed dose dependent pain withstanding effect with considerable pain reaction time, in that only 200 and 400 mg/kg concentration of crude extracts were showed statically significant (Table 3).

Both tail immersion and hot plate models were tested to evaluate central analgesic effect in suppressing prostaglandin synthesis.¹³ From the results, it is proved that the methanolic extract of *Z. ovalifolium* showed appreciable antinociceptive in all tested models. The analgesic activity of *Z. ovalifolium* methanolic extract is due the influence of unknown compound(s) present in it. The antinociceptive action of the *Z. ovalifolium* methanolic extract is mediated through central and peripheral mechanism which is proved through our results.

The values obtained from the three analgesic experiment was subjected to One-way ANOVA followed by Dunnett's multiple comparison test showed significant results with P value is less than 0.0001 for *Z. ovalifolium* methanolic extract (400mg/kg) against control in turn proved the positive analgesic effect of methanolic crude extract.

CONCLUSION

From the results it is concluded that, the methanolic leaf extract of *Zanthoxylum ovalifolium* proved appreciable analgesic activity in 200 and 400 mg/kg concentrations which is comparable with the standard reference drug. The mechanism of analgesia is through inhibiting prostaglandin pathway or by through peripheral pain mechanism. To conclude the exact pathway more work is needed to isolate the bioactive compound(s) which has antinociceptive properties.

ACKNOWLEDGEMENT

Authors will acknowledge Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shankaraghatta, Bhadravati, Shimoga, Karnataka and Dr Vijay Kumar, Assistant Professor, NITTE, Pharmacy College, Mangalore for providing facilities to do the experiment.

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Cite this article as:

Pavani P et al. Evaluation of the analgesic activity of the leaf methanolic extract of *Zanthoxylum ovalifolium* Wight. Int. Res. J. Pharm. 2019;10(6):33-36 <http://dx.doi.org/10.7897/2230-8407.1006199>

Source of support: Nil, Conflict of interest: None Declared

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