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Full Length Research Paper

Evaluation of *Zanthoxylum armatum DC* for *in-vitro* and *in-vivo* pharmacological screening

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The crude ethanolic extract of leaves(ZLE) and fruits (ZFE) of *Zanthoxylum armatum* were scrutinized for acute toxicity and antipyretic activities while crude ethanolic and *n*-hexane extract of the leaves (ZLE, ZLH) and fruits (ZLE, ZLH) of this plant were screened out for their phytotoxic and cytotoxic potential. The extract were found to be safe at a dose of 500, 1000 and 2000 mg/kg body weight. The antipyretic activity of leaves and fruits were tested on mice using Brewer's yeast induced pyrexia model, both the extract, that is, ZLE and ZFE were found to active in lowering pyrexia in the tested animals. The antipyretic effect of fruits was more significant than leaves. ZFE showed a percent inhibition of 83.84, 80.70 and 44.18 at the dose of 300, 200 and 100 mg/kg, respectively at the 3rd hour while ZLE showed a percent inhibition of 85.42, 77.19 and 46.18 respectively at the dose of 300, 200 and 100 mg/kg, respectively. The antipyretic action was observed to be maximum at the 3rd hour of the dose, which remain significant up to 5th hour. The ZLE, ZLH, ZFE and ZFH also showed significant potency as phytotoxic and cytotoxic agent. Phytotoxicity and cytotoxicity were found to be dose dependant and showed maximum efficiency at 1000 µg/ml. It is concluded that *Z. armatum* a potential antipyretic, phytotoxic and cytotoxic medicinal plant. Further investigations are required to identify and isolate its active principals responsible for its pharmacological activities.

Key words: *Zanthoxylum armatum,* antipyretic, phytotoxic, cytotoxic.

INTRODUCTION

Different bioassays are in practice to suggest immense advantages for screening out various extracts from medicinal plants for different ailments (Srirama et al., 2007). Fever is not a disease but a symptom of disease; it is mostly associated with infections caused by bacteria, virus or fungi. A large number of antipyretic drugs are available, but due to their side effects natural antipyretic agents are necessary as they are safe with fewer side effects. The brine shrimp lethality assay was considered as a expedient investigation for preliminary detection of toxicity, cell-line toxicity and anti tumor activity (Meyer et al., 1982; Anderson et al., 1991). The brine shrimp assay is also very useful for the isolation of biogenic compounds from plant extract against cancer (Sam, 1993; Al-Fatimi et al., 2007). The world is still in search of and in the process of developing farming techniques, which are sustainable for environment, crop production and protection as well as socio-economic points of view (Barkatullah et al., 2009). Phytotoxic assay helps in screening out natural sources having improved characteristics for weedicides search (Atta-Ur-Rahman et al., 2001). *Zanthoxylum armatum* is a small tree or large spiny shrub with compound leaves, blooming in March April, prefer semi shady or no shade for their growth. Locally it is called Dambara (Pashtu), Dambrary, Tamur (Urdu) and wing leaf prickly ash (English) and traditionally it is used as a tonic, carminative, condiment, stomachic, anthelmintic (Verma and Khosa, 2010), Insecticidal (Tiwary et al., 2007), for tootheache, abortifacient, antifertility agent (Shah and Khan, 2006), antiseptic, disinfectants, deodorant (Shinwari et al., 2006) antipyretic and anti diarrheal. It improves speaking power and increase

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Abbreviations: ZLE, crude ethanolic extract of leaves; **ZLH,** n-hexane extract of the leaves; **ZFE,** crude ethanolic extract of fruits.

saliva secretion (Ahmad et al., 2006). Fruits and seeds of this plant are used in fever, dyspepsia and skin diseases (Khare, 2007). Because of large number of its traditional uses, it is necessary to screen out *Z. armatum* for its pharmacological potential. This study is carried out to investigate acute toxicity, antipyretic, phytotoxic and cytotoxic potential of the leaves and fruits of *Z. armatum*.

MATERIALS AND METHODS

Plant materials

Leaves and fruits of *Z. armatum* were collected from Charkotli hills Batkhela, then rinsed, dried and pulverized into powder. The materials were then separately macerated with ethanol and *n*-hexane solvent in a tank for fourteen days with constant shaking. This procedure was repeated thrice. The extract was filtered and the filtrate was concentrated using rotary evaporator at low temperature and high pressure. This crude extracts of both parts were then used for acute toxicity, antipyretic activity, phytotoxicity and cytotoxicity.

Animals

Swiss albino mice (20 to 25 g) of either sex were used in the experiments. The animals were kept in cages and housed under standard condition of 12: 12 h light/dark cycle and were fed with NIPRD formulated food and had water *ad libitum*.

Acute toxicity studies

The acute toxicity study was carried out for the crude ethanolic extract of leaves (ZLE) and fruit (ZFE) of the selected plant. The study was carried out using albino mice weighing 20 – 25 g of either sex. The animals were randomly distributed into six groups each of six animals. The animals were acclimatized to the laboratory conditions before the commencement of experiment. All the animals were deprived from food overnight, the control group received normal saline and the remaining II – IV groups were treated with 500, 1000 and 2000 mg/kg body weight respectively with crude ethanolic extract of leaves, while group V-VII groups were treated with 500, 1000 and 2000 mg/kg respectively with ethanolic extract of fruit. The animals were observed continuously for the first 4 h and then for the next 24 (Khan et al., 2010).

Yeast-induced pyrexia

The antipyretic activity of ethanolic extract of *Z. armatum* leaves and fruits were evaluated using albino mice (25 - 30 g) of either sex. The animals were acclimatized to the laboratory condition before the start of experiment; all the animals were divided in eight groups each of six. All groups were fasted overnight while allowed free accesses to drinking water. Groups I and II received saline and paracetamol as control and standard drug while the remaining groups received 100, 200 and 300, mg/kg respectively of crude ethanolic extract of *Z. armatum* leaves and fruits. Normal temperature was recorded using digital thermometer before injecting 20% aqueous suspension of Brewer's yeast (10 ml/kg s.). After 18 h, rectal temperature was recorded and corresponding groups were injected with above doses. Rectal temperature was recorded periodically at 1, 2, 3, 4 and 5 h of drugs administration. The percent reduction in pyrexia was calculated by using the following formula.

$$\text{Percent reduction} = (B - C_n)/(B - A) \times 100$$

Where, A (normal rectal temperature), B (temperature after yeast injection) and C_n is body temperature after 1, 2, 3, 4 and 5 h (Taesotikul et al., 2003).

Phytotoxicity

The phytotoxic activity of ZLE, ZLH, ZFE and ZFH of *Zanthoxylum armatum* were evaluated using *Lamna minor* as test species following recommended procedure (Attaurahman et al., 2001). 15 mg of respective extract was dissolved in 15 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to the flask (3 flasks for each concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml respectively. The solvent was allowed to evaporate overnight under sterilized condition in laminar flow. 20 ml of E. medium was added to each flask. Other flasks (3 for each) were supplemented with E. medium and standard drug (Atrazine) served as negative and positive control. To each flask ten plants with 2-3 fronds were transferred and kept all the flasks under about 12 h day light conditions. Plants were observed daily and on the seventh day the numbers of fronds were counted. The % growth inhibition was recorded with reference to the negative control using the following formula;

$$\text{Inhibition \%} = \frac{(100 - \text{Number of fronds in test sample})}{\text{Number of fronds in negative control}} \times 100$$

Cytotoxicity

The phytotoxic activity of ZLE, ZLH, ZFE and ZFH of *Z. armatum* were tested using brine shrimp assay following the method of Attaurahman et al. (2001). About 20 mg of respective extract was dissolved in 2 ml of respective solvent and from this solution transfer 5, 50 and 500 µl to vials (3 vials /concentration). This concentration was equivalent to 10, 100 and 1000 µg/ml, respectively. The solvent were allowed to evaporate overnight. 5 ml with seawater water solution (38 g/L) were added to each vial. After 36 h of hatching and maturation of larvae as nauplii, 10 larvae were transferred to each vial using a Pasteur pipette. The vials were then placed at room temperature (25-27°C) under illumination. Other vials were supplemented with brine solution served as positive controls.

Statistical analysis

Data was analysed using one way ANOVA followed by Tukey-Kramer posthoc test. Values were considered statistically significant at $p < 0.05$. After 24 h the data were collected and analyzed with Finney computer program to determine LD50 values with 95% confidence intervals (Taesotikul et al., 2003; Saeed et al., 2010).

RESULTS AND DISCUSSION

ZLE and ZFE were injected at the dose of 500, 1000 and 2000 mg/kg body weight. No death was observed at all the doses showing that the plant is safe for human use (Table 1).

Pyrexia may be the outcome of infection or due to tissue damage, inflammation and other illness. Antipyretics

Table 1. Acute toxicity test of *Zanthoxylum armatum* in mice, assisted for 24 h.

Group	Dose	Dead	Survived	Gross effect
Saline	10 mg/Kg	-	All	-
Leaves	500	-	All	-
	1000	-	All	-
	2000	-	All	-
Fruit	500	-	All	-
	1000	-	All	-
	2000	-	All	-

Table 2. Effect of ethanolic crude extract of *Zanthoxylum armatum* fruits and leaves at 100, 200 and 300 mg/kg i.p. in.

Treatment	Dose (mg/kg)	Rectal temperature (°C)						
		After administration of drug						
		Normal (A)	After 24 h (B)	1 h (C1)	2 h (C2)	3h (C3)	4 h (C4)	5 h (C5)
Saline	10 ml	36.66± 0.21	38.93± 0.23	38.81 ± 0.12	38.88 ± 0.13	38.88 ± 0.22	38. 78 ± 0.18	38.75 ± 0.35
Paracetamol	150	37.10± 0.08	38.8± 0.04	37.51**±0.01	37.46**±0.03	37.32**±0.02	37.35**±0.28	37.45**±0.04
ZFE	100	37.17± 0.28	38.83± 0.29	38.57*±0.12	38.30*± 0.17	38.10*±0.10	38.17*±0.06	38.20*±0.20
	200	36.73± 0.21	38.63±0.55	38.30*±0.35	37.60*± 0.35	37.10**±0.10	37.17**±0.15	37.30**±0.06
	300	37.17± 0.31	38.47 ±0.21	38.17*±0.15	37.23**±0.32	37.10**±0.10	37.17**±0.55	37.17**±0.44
ZLE	100	37.11±0.17	38.77± 0.25	38.43*±0.12	38.13* ± 0.12	37.00* ± 0.10	37.00*±0.10	38.07*± 0.21
	200	36.63± 0.15	38.53 ±0.47	38.27*± 0.32	37.47**±0.31	37.07**±0.06	37.10**±0.10	37.20**±0.21
	300	36.93± 0.06	38.53 ±0.15	37.97**±0.06	37.40**±0.10	37.17**±0.23	37.27**±0.32	37.37**±0.40

Values are reported as mean ±S.E.M. for group of six animals. The data was analyzed by one way ANOVA followed by Dunnett's test. Asteriks indicated statistically significant values from control. *P<0.05, **P<0.01.

are most commonly uses as fever reliving agents. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point elevates and a drug like paracetamol regulating the body temperature. The use of brewer yeast for fever production is a well know and convenient method used for assessment of antipyretic activity of medicinal plants or other chemicals. Both parts showed a dose dependent antipyretic action. The antipyretic action of ZFE was 83.84, 80.70 and 44.18% at the dose of 300, 200 and 100 mg/kg respectively at the 3rd hour of the dose while ZLE showed a percent inhibition of 85.42, 77.19 and 46.18 at the dose of 300, 200 and 100 mg/kg, respectively (Table 2, Figures 1 and 2). The maximum antipyretic action observed at the 3rd hour of the dose and remained significant up to 5 hours. A compound Hesperidin has been isolated from one *Zanthoxylum* species, which has antipyretic, analgesic and anti-inflammatory activities (Santos and Moreno, 2004). Literature reports also suggested that coumarinolignans present in *Z. avicennae*, have anti-inflammatory activity (Liu et al., 2007; Chen et al., 2008). These compounds might be present be present in this plant and the antipyretic potential of ZLE and ZFE might be due to these compounds. These result also seemed to influence prostaglandin- biosynthesis, which has a thermo-regulatory effect (Milton, 1982). The inhibition of prostaglandin synthesis could be the

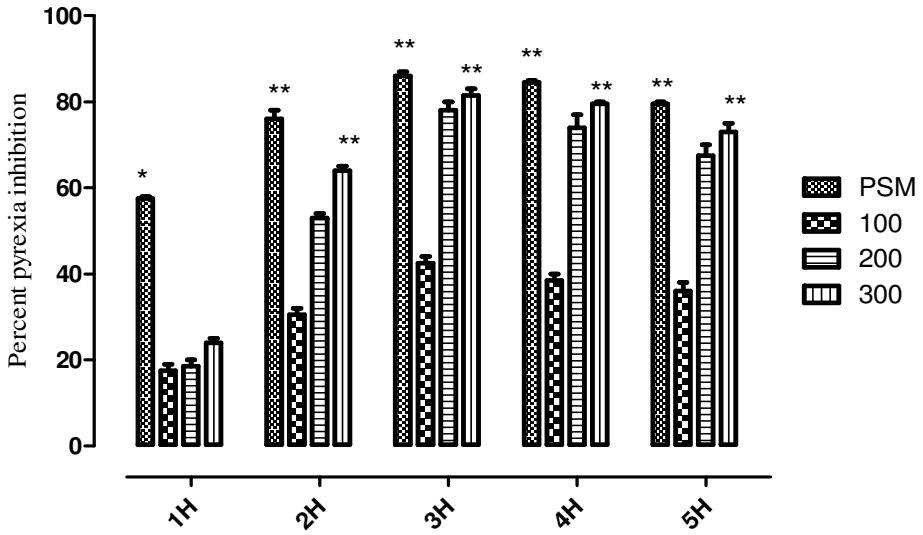

Figure 1. Effect of ZFE on brewer yeast pyrexia in mice treated with 100, 200 and 300 mg/kg extract.

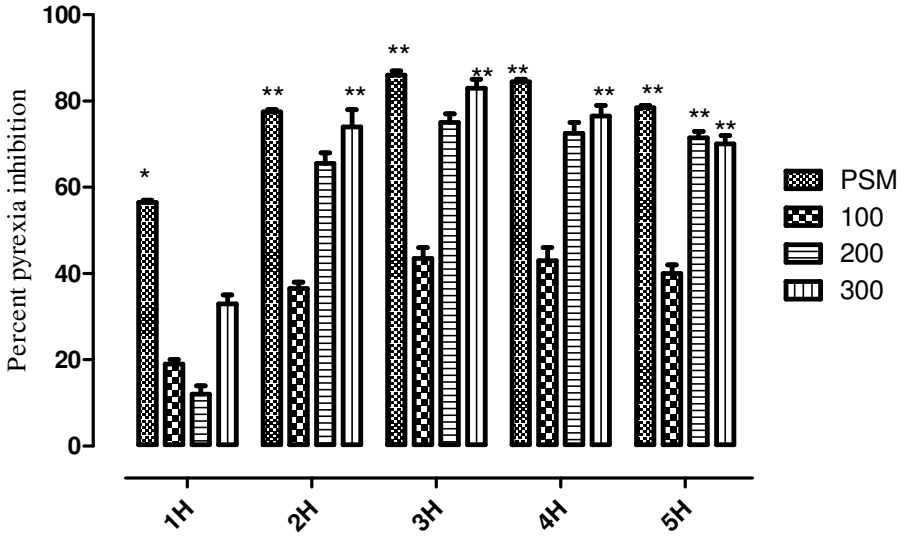

Figure 2. Effect of ZLE on brewer yeast pyrexia in mice treated with 100, 200 and 300 mg/kg extract.

possible mechanism of antipyretic action of these extracts.

Pakistan is an agriculture country yielding high quality of cereals. Due to poor weed control strategies, large quantity of these crops may be damaged. The extent of damage caused by the uncontrolled weeds is usually more than that of insects and diseases but its effects are unseen. They also reduce the crops yield due to competition for sunlight, water and fertilizer. In addition, weeds provide habitat for insects which help in spreading the disease. So weeds controlling is very essential for the increasing the production of various crops. In this regard phytotoxic screening of The crude ethanolic and n-hexane extract of both parts of *Z. armatum* were carried out using *Lamna minor* as test species. Both parts showed significant phytoinhibition. A significant inhibition was observed at all doses and was found to be dose dependant. The ZFH showed 100% inhibition at 1000 µg/ml which is outstanding action. The ZLH also showed excellent activity (90.99±1.36). These results suggested that *n*- hexane extract of both parts have some active principle with phytotoxic potential. Our result of phytotoxic

Table 3. Phytotoxic activities of *Zanthoxylum armatum* fruit and leaves.

Code	10 µg/ml	100 µg/ml	1000 µg/ml
N/ control	0.00±0.00	0.00±0.00	0.00±0.00
P. control	87.82±1.11	87.82±1.11	87.82±1.11
ZFE	56.23±3.64	79.46±0.79	94.15±2.28
ZFH	62.72±2.03	89.76±2.80	100.00±0
ZLE	68.61±0.69	66.59±3.09	78.72±2.96
ZLH	48.66±1.15	52.43±4.45	90.99±1.36

All values were expressed as mean ±S.E.M.

Table 4. Cytotoxic activities of *Zanthoxylum armatum* fruit and leaves.

Code	10 µg/ml	100 µg/ml	1000 µg/ml	LD_{50} values
N/ control	0.0.±0.00	0.0.±0.00	0.0.±0.00	-
P. control	100.00±0.00	100.00±0.00	100.00±0.00	-
ZFE	30.66±5.77	70.33±5.77	90.00±0.00	65.05
ZFH	20.67±5.77	55.33±5.77	77.00±0.00	63.30
ZLE	42.67±5.77	76.67±5.77	94.00±0.00	20.00
ZLH	46.67±5.77	60.00±10	93.33±5.77	19.34

All values were expressed as mean ±S.E.M.

activity indicates that the ZFH and ZLH have potential as herbicides or weedicides. Further study is needed to explain phytotoxic mechanism and to identify and quantify the active principle. It may also be investigated in detail to test its efficacy as a weeds, pests and disease control agent.

Cytotoxic screening is the preliminary step in investigation for formulation of anti-cancer drug. The crude ethanolic and n-hexane extract of leaves and fruits of *Z. armatum* were evaluated using brine shrimps. Both parts showed significant cytoxicity (Table 4). Significant inhibition was observed at all doses and was found to be dose dependent (Table 3). The ZFE and ZLE showed outstanding mortality rate. These results suggested that both parts have some active principal with cytotoxic potential. These results recommend the existence of some bioactive compounds, which might be helpful for further investigations to identify and quantify the active cytotoxic principal. All these results for *Z. armatum* suggested that further detailed investigations are required for particularized detection of more specific pharmacologically bioactive constituents.

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