Research Article

Investigations on hydroalcoholic extract of *Zizyphus oenoplis* for analgesic and anti-nociceptive activity

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Abstract

Objectives: The aim of the present study was to investigate analgesic and anti-nociceptive activity of hydroalcoholic extract of *Zizyphus oenopli* leaves. **Materials and Methods**: Hydralcoholic extract of *Zizyphus oenoplis* leaves was tested for phytochemical analysis. Swiss mice were selected for study of analgesic and anti-nociceptive activity of *Zizyphus oenoplis* extract at doses 50, 100 and 200 mg/kg p.o. Various models viz. acetic acid induced writhing model, Eddy's hot plate and tail flick model were selected for analgesic study. Another model tail immersion model was used for anti-nociceptive study. **Results**: Effect of *Z. oenoplis* extract in acetic acid induced writhing model, found significant at 200 mg/kg dose and comparable to the standard group Diclofenac sodium (25mg/kg). In case of hot plate model, the maximium activity was observed at 90 min at dose of 200 mg/kg, which was less than the reference drug morphine sulfate (1.5 mg/kg i.p.). The analgesic effect on the tail flick model was found comparable with morphine sulfate in dose dependent manner. Anti nociceptive effect of *Z. oenoplis* extract in formalin-induced paw licking model, was found significantly in the early phase at 100 and 200 mg/kg p.o. and comparable to the Diclofenac sodium (25mg/kg) group. **Conclusion**: *Z. oenoplis* found effective in both type non-narcotic and narcotic nociception models which suggesting its possible activity via peripheral and central mechanism. The activity can be concluded due to presence of alkaloids and terpenoids in hydroalcoholic extract of *Z. oenoplis*.

Keywords: *Zizyphus oenoplis*, analgesic, acetic acid, Eddy′s hot plate, tail flick

Introduction

Herbal medicine is growing for treatment of many disorders used by the population. In general survey an estimate 80-90% of the world's population uses herbal medicines for cure diseases. A large number of synthetic drugs are used at present for analgesic and antinociceptive effect with many side effects (Ahmad et al., 1992).

Pain is one of the common situations that affect the overall quality of life. Presently there is a large number of analgesic opioid and synthetic drugs are available for analgesic and anti nociceptive effect but due to lack of safety and more side effects, such as ulcers, bleeding, and dependence, limit their clinical applications. The basic concept behind nociception involved a complex interaction of peripheral system and CNS which extend from the skin, the viscera and the musculoskeletal tissues to the cerebral cortex (Jage, 2005).

Ziziphus oenoplia (L) Mill (Family-Rhamnaceae) is also known as *Rhamnus oenoplia* or Jackal Jujube distributed throughout the tropical region of India, Malaysia and Australia. A straggling shrub often semi scan dent by its prickles, and young branches are rusty. Leaves numerous, 2.5-6.5 by 2-2.5 cm, ovate, acute to mentose tips, galabarous, densely silky with hair, petioles 6-8 mm long flowers 12-20 sub sessile calyx hairy outside, petals obviate. Fruits are edible. The bark, fruits, leaves and stems of plant are extensively used in the rural area for stomache, hypotensive, diuretic, wound healing, antibacterial, anti-inflammatory and analgesic (Kirtikar and Basu, 2005). It is scientifically proof for antiulcer activity of *Zizyphus oenoplis* roots in rats by Jadhav et al(2011). Chemical examinations of *Z. oenoplis* were reported presence of cyclopeptide alkaloids. A decoction of the root bark is reported to promote the wound healing (Munda tribe). The fruit is used as an ingredient in the preparation of stomach ache pills. Based on traditional uses of this plant the present

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study has been designed to analgesic and antinociceptive activity of *Z. oenoplis* barks.

Materials and Methods

Collection of Plant material

The leaves of *Zizyphus oenoplis* were collected from the college campus of GRKIST (Pharmacy), Jabalpur, District of Madhya Pradesh, India and authenticated in Tropical Forest Research Institute, Jabalpur. The plants leaves were cleaned well and dried under shed at room temperature for extraction (Gupta et al., 2015).

Preparation of extract and phytochemical screening

Fresh leaves of *Zizyphus oenoplis* were cleaned and dried under shade, grinded and stored for further studies. Powdered leaves were extracted with 70% alcohol for 72 hrs by soxhlet apparatus (Lodhi et al., 2010). The solvent was filtered and filtrate was concentrated in rotary evaporator at 50-60° C under reduced pressure to obtain dark brown residue. *Z. oenoplis* extract was transferred to store for further use. The yield of extract was calculated. Freshly prepared extract was subjected to standard phytochemical screening tests for various constituents by standard methods (Kokate et al., 2010; Lodhi et al., 2013).

Acute toxicity study

Swiss mice (80-100 g) were selected for acute toxicity study. The animals were left for free access to food and water. The animals were acclimatized to the laboratory conditions for at least five days prior to the experiments. They were fasted overnight before the experiment and were housed with alternating light dark cycle of 12 h each in animal room. According to organization of Economic Corporation Development (OECD) guidelines (Jhariya et al., 2015), *Z. oenoplis* extract was administered orally in doses of 200, 400, 600, 800 and 1000 mg/kg b.w.to the group of mice (n=6). The observations of percentage mortality were recorded up to a period of 24h. The animals were observed for any behavioral changes like hyperactivity, convulsion, sedation, respiration, loss of lighting reflex, salivation and urination. Animal studies were conducted according to protocol approved by Institutional ethical Committee.

Pharmacological screening

Animal Protocol

Swiss mice (80-100 gm).were selected for the analgesic and antinociceptive activity studies. These animals were kept in the animal room in a controlled room temperature at 24 $\pm 1^0$C with free access to food and water ad libitum. All animals were divided into four groups containing each of 6 animals. The animals were divided into following groups:

- Ø Group I was control and given vehicle (2% tween 80) p.o. .
- Ø Group II was Standard and given Diclofenac sodium (25mg/kg) by intra peritoneal injection.
- Ø Group III & IV were test group given hydralcoholic extract (200 & 300 mg/kg b.w.).

Preparation of dosage form

The suspension of hydroalcoholic extract was prepared by mixing the extract with 2% Tween 80 in a beaker. Diclofenac sodium was given as 25mg/kg body weight of animal being used for the experiment. In control group given only vehicle 2% tween 80 solution.

Acetic acid induced writhing in mice

Briefly the total number of writhes recorded following intraperitoneal administration of 0.1 ml of 1% (v/v) Acetic acid over a period of 20 min, Observation was started from 5 min after acetic acid injection. The animals were pretreated with oral dose of alcoholic extract (100 & 200 mg/kg) 60 min before administration of acetic acid. Standard group pretreated with Diclofenac sodium (25mg/kg) (Barua et al, 2011).

Effect on reaction time of mice using hot plate

The hot plate method, consists of a electrically heated surface having controlled temperature of 55- 56 ^{0}C. The animals were placed on the hot plate and the time until animals jump down from the hot plate was recorded using a stop-watch. The latency is recorded before and after 30 min following intraperitoneal administration of the standard or the test compound (Patel et al, 2014).

Effect of *Z. oenoplis* on tail flick time in mice

Exposing tip of tail of mice to radiant heat through heated electric Nichrome wire resulted in flicking of the tail from heated surface. Pretreatment of animal with reference drug Diclofenac sodium (25mg/kg) and *Z. oenoplis* (100 and 200mg/kg) were observed the effect on reaction time (tail flick time), after 30 minutes of treatment (Patel et al, 2014).

Effect of *Z. oenoplis* on mice using tail immersion method

In this method the mice were restrained and the tail was immersed in a hot water bath maintained at 52±0.5^{0}C .The reaction time to flick the tail from the hot water was recorded for each mice. The reaction time was recorded initially without drug treatment and time interval 30 min after the test drug treatment. A change in mean reaction time between these two readings is an indication of possible antinociceptive response. A cut-off time of 15s was maintained to prevent any injury to the tail (Umamaheswari et al, 2006).

Results and discussion

Phytochemical studies

The phytochemical analysis of hydroalcoholic extract was revealed the presence of alkaloids, glycosides, phenolic compounds and terpenoids. The yield of hydroalcoholic extract was 6.25% w/w.

Acute toxicity study

Animals were observed initially after dosing at least once during the first 30min, periodically during the first 24h. Additional observations like changes in skin and fur, eyes, mucous membranes and also respiratory, circulatory, autonomic, central nervous systems and behavioural pattern were also observed. Attention was also given to observations of tremors and convulsions. There was no toxic effect was observed up to 1000 mg/kg dose of extract.

Analgesic and Antinociceptive studies

A tissue injury or injury is related with pain and inflammation. Analgesic drugs can work on peripheral or central nervous system. Peripherally acting analgesics work by blocking the impulse generation at chemoreceptors of pain. The centrally acting analgesics increse the threshold for pain, and also alter the physiological response to pain so that patient's anxiety and apprehension will be suppress (Bhutia et al., 2010). In acetic acid induced writhings, hydroalcoholic extract inhibited upto 68.51% writhings of test group in dose depended manner (Table 1).

Table 1. Effects of *Z. oenoplis* on acetic acid induced writhings in mice

Group	No. of writhings	% Inhibition of writhings
Control (Vehicle)	52.63 ±4.26	–
Standered (Diclofenac sodium 25mg/kg)	11.24± 1.23	78.64
Z. oenoplis (100mg/kg)	20.18± 1.72	61.65
Z. oenoplis (200mg/kg)	16.57±1.28	68.51

Table 2. Effect of *Z. oenoplis* on reaction time of mice using hot plate

Group	Jump time from hot plate	%increase in jump time
Control	9.76± 1.30	-
Standered (Diclofenac sodium 25mg/kg	25.34 ± 2.21	61.48%
Z. oenoplis (100mg/kg)	17.62 ± 2.39	44.60%
Z. oenoplis (200mg/kg)	24.63 ± 2.41	60.37%

Acetic acid induced writhing response is a simple and reliable model and also affords rapid investigation of peripheral type of analgesic action. Acetic acid causes inflammatory pain by increasing capillary permeability and liberating endogenous substances that excite pain nerve ending. Acetic acid is also known to increase prostaglandins peripherally. The mechanism of analgesic activity of *Z. oenoplis* could be probably due to the blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to the NSAIDs. Thus, the reduction in the number of writhing indicates that *Z. oenoplis* might exert anti-nociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandin. The hot plate model for nociceptive reaction toward thermal stimuli in mice is a well-validated model for detection of opiate analgesics drugs from spinal origin (Vogel, 2002).

Table 3. Effect of *Z. oenoplis* on tail flick time in mice using

Groups	Tail flick time (sec)	% Increase in tail flick time
Control	4.3 ± 0.7	-
Standard (Diclofenac sodium 25mg/kg)	15.7± 2.9	72.6%
Z. oenoplis (100mg/kg)	8.4 ± 1.2	48.80%
Z. oenoplis (200mg/kg)	11.5 ± 1.9	62.60%

Table 4. Effects of *Z. oenoplis* on mice using tail immersion method

Groups	Mean increase in reaction time
Control	2.75 ±0.14
Standard (Diclofenac sodium 25mg/kg)	14.26 ± 1.7
Z. oenoplis (100mg/kg)	7.59 ± 0.83
Z. oenoplis (200mg/kg)	9.76±1.16

Hydroalcoholic extract significantly increases reaction time with 200 mg/kg dose and comparable to the standard group (Table 2). The tail flick model is also used to evaluate analgesic agents acting through central nervous system. Tail flick time was increased 62.60% by 200 mg/kg dose of extract which was comparable to the standard group of animals (Table 3). Same as in tail immersion method, the reaction time was significantly differs from extract treatment group in dose dependent manner (Table 4). In this study, *Z. oenoplis* completely abolishes the early phase at both doses (100 and 200 mg/kg). *Z. oenoplis* also decreased the reaction time in both doses in the late phase also which might suggest that *Z. oenoplis* causes partial inactivation of NMDA and non-NMDA receptors. Analgesic activity of

hydroalcoholic extract of *Z. oenoplis* in the present study showed significant effect in both narcotic and non narcotic models. The activity of hydroalcoholic extract was more prominent in non narcotic models. The extract contains flavonoids, terpenoids, alkaloids and glycosides. Hence, significant analgesic activity in hydroalcoholic extract might be attributed to the presence of these bioactive principles.

Conclusion

In conclusion, study can be interpreted that hydroalcoholic extract of *Z. oenoplis* possesses promising analgesic and anti-nociceptive properties, which are probably peripherally mediated via prostaglandin inhibition as well as central inhibitory mechanism. This may be of potential benefit for management of pain. There are additional studies required to isolate the active components from the leaves of *Zizyphus oenoplis*. Study on its mechanism of action to ascertain their analgesic and anti-nociceptive properties will throw light on mode of action.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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