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Screening of analgesic activity *of Phoenix sylvestris* leaves in rodents

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ABSTRACT

Aim: The present study evaluated the central and peripheral analgesic activity of methanolic leaf extract of *Phoenix sylvestris* (PSLME) in swiss albino mice. **Method:** Peripheral and central analgesic activity was evaluated by tail immersion and acetic acid writhing in swiss albino mice. Dextropropoxyphene was used as a standard drug in the dose of 65mg/kg body weight in both models. PSLME was tested at 100 and 500mg/kg dose level. **Results:** The result revealed that methanolic extract exhibit 48% and 40.5% writhing inhibition at 500 and 100 mg/kg doses whereas ~30% tail withdrawal reflexes inhibition at 500mg/kg which was analogous to the standard drug dextropropoxyphene. **Conclusion:** Methanolic extract of leaves of *P. sylvestris* possesses both peripheral and central analgesic activity in experimental animal.

Keywords: Acetic acid, Analgesic activity, Phoenix sylvestris, Tail immersion.

INTRODUCTION

International Association for the Study of Pain (IASP) defined pain as a nasty sensory and emotional practice allied with actual or potential tissue damage, visceral distension, or other factors.[1,2] According to medical practitioner pain is nociceptive if pain is due to ongoing activation of the nociceptive system by tissue injury. In such situation, pain perception is a normal physiologic response (transduction, transmission, modulation and perception) mediated by healthy nervous system. Nociceptors are thin fibre like afferent neurons (C-fibers and A-delta) which are located in visceral tissues, skin and muscle, joints which are responsible noxious, chemical, mechanical or thermal stimuli. Recently transient receptor potential (TRP) receptors are under intensive investigation to get novel therapy for pain. Currently pain management is done by using Opioids or nonopioids (aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam) drugs.[3,4] These drugs carry side effects such as gastrointestinal bleeding, tolerance and dependence induced by opiates both acute and chronic therapy. The medicines which are produced from plant origin are being used ancient times without any side effects. Keeping this view in mind we have planned to undertake a complete ethno pharmacological research endeavor for the identification of herbal medicine for comparatively very less explored natural regimen in the management of pain. In our efforts we are exploring the potency of the desert plant *Phoenix sylvestris*is widely known as Wild Date Palm [5]. The plant *P. sylvestris* has been considered as traditional medicine to cure various ailments like abdominal complaints, fevers, loss of consciousness, constipation and in heart complaints [6, 7]. The Sap of the plant is nutritious, cooling and laxative where central tender part of the plant is used in gonorrhea. Root is useful in toothache, nervous debility and helminthiasis. The methanolic root extract of *P. sylvestris* is reported to have analgesic and diuretic activity [8-10]. Due to its great pharmacological properties central and peripheral analgesic activities of methanolic extract of leaves of *P. sylvestris* have been explored and reported in the present work.

MATERIAL AND METHODS

Collection and identification of plant material

The leaf parts of the plant were procured from Banasthali village, Tonk, Rajasthan. The plant was originally authenticated by Botany department of Rajasthan University (voucher specimen no. RUBL21103), Jaipur. A herbarium sheet of plant parts is prepared and deposited in Botany department, Rajasthan University, Rajasthan. The leaf part of the plant was air dried, powdered and subsequently stored in air tight container.

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Preparation of plant extract

50g of dried and coarse powder of leaves of *P. sylvestris* was extracted with methanol by cold maceration technique, for 24 hours, three times successively. Extracts were filtered and concentrate in rotavapor under reduced pressure. Extract was completely dried first on water bath then finally on vacuum. Percentage yield was calculated and crude extract was used for investigation [13].

Acute toxicity study

Acute toxicity studies of the PSLME were determined in albino mice, in accordance to OECD-420 guidelines Overnight fasted group of mice were administered with graded doses (50 -2000 mg/kg, *per os*) of extracts respectively. Mice were observed for alteration in behavior of animals, gait abnormality, signs of nervous manifestations, discomfort if any, up to 48 h.

Analgesic activity

Acetic acid induced writhing test

Analgesic behavior is observed by acetic acid induced writhing method which demonstrates a noxious stimulation in mice. In this method Control and test sample were given with the help of feeding needle orally. For the absorption of administered substances 30 minute time interval was given. Writhing was induced with the help of acetic acid solution (0.7%) which was given 0.2ml intraperitoneally to each animals of group. After an interval of 15 minutes no. of squirms (writhing) was counted for 5 minutes Dextropropoxyphene was used as reference standard drug [12, 13].

Tail immersion test

In this method, tail of an animal is immersed in hot water at the temperature of 55°C-55.5°C, flicking time was observed. Tail flicking time can be increased by administering any sample containing analgesic principle [14]. Dextropropoxyphene was used as reference standard drug.

Animals

In this study we used mice having weight between 180-250 gm. Animals were kept in well ventilated animal house maintained at standard environmental conditions (temperature 25±2^{0}C relative humidity: 55-65% and 12 h light/dark cycle) at department of pharmacy, Banasthali University, Rajasthan. Mice were kept with standard diet with complete access to water during entire experiment.

Grouping and dosing

Four groups of animals were selected having six animals of either sex in each group. Vehicle (1 % Tween 80 in water) administered orally at a volume of 10 ml/kg in a control group. Test groups were pre-treated orally with methanolic leaf extract (100 and 500 mg/kg), while reference drug was administered to positive control group.

RESULTS AND DISCUSSION

PSLME did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose. This study is the first report related to central analgesic activity of *Phoenix sylvestris* leave extracts. The analgesic activity was assessed through acetic acid writhing and tail immersion assays in mice. The antinociception activity was evaluated by acetic acid induced writhing responses. Central nociception and peripheral action was produced by administration of acetic acid intraperitoneally leads to release of endogenous mediators and non steroidal anti-inflammatory drugs blocked it [11]. The methanolic extracts showed significant (P<0.001) reduction (22.27 to 11.56) in writhing and stretching induced by acetic acid at the doses of 500 mg/kg dose which is comparable to standard drug (22.27 to 8.96) dextropropoxyphene (Table 1). The dose-dependent protective effects of methanolic extract exhibit 48% and 40.5% writhing inhibition at 500 and 100 mg/kg doses (table 1). The protective effect for dextropropoxyphene was ~60% (standard drug) and this action was comparable with methanol extractat100 and 500mg/kg doses.This action might be due to blockade of the release of endogenous substances. Central pain mechanism is controlled by brain and spinal cord. pain and inflammation targets the dorsal part of the spinal cord which contain substances such as somatostatine, P, endogenous opioids and other inhibitory harmones. tailimmersion models are the well documented methods for measuring the central analgesic effects of drugs through opoid receptor [11]. It is also established that tail immersion models are the well-established methods for measuring the central analgesic effects of drugs through opoid receptor [16]. Our present study demonstrated that PSLME were protective effective against tail immersion method at100 and 500mg/kg doses which were comparable with standard drug dextropropoxyphene (Tables 2). The methanolic extracts showed non significant inhibition at the doses of 100 and 500mg/kg doses with respect to control. The positive control, dextropropoxyphene demonstrated~40% inhibition at therapeutic dose, whereas methanolic extracts showed ~30% inhibition upto 500mg/kg dose. According to Table 2, it was evident that methanolic extracts had non significant analgesic activity which was slightly lower than dextropropoxyphene. Narcotic analgesics are active against both peripheral and central pain, while non steroidal anti-inflammatory drugs inhibit peripheral pain [12]. Our findings suggested that methanolic extracts of *P. sylvestris* may act like central as well as narcotic analgesic drugs.

Table 1: Effects of PSLME on acetic acid induced writhing behavior in mice

Drug (dose)	Writhing	% inhibition
Control	22.27± 0.57	-
Dextropropoxyphene (65mg/kg)	8.96± 0.32***	59.76
PSLME 100	13.24± 0.40***	40.54
PSLME 500	11.56± 0.48***	48.09

Values are expressed as Mean ± SEM, n=6, Data analyzed by One-way ANOVA followed by Dunnette's test *** P < 0.001.

Table 2: Protective effect of PSLME on tail withdrawal reflexes induced by tail immersion method in mice

Drug (dose)	Before treatment	After treatment	% inhibition
Control	6.73±0.23	6.73±0.23	00
Dextropropoxyphene (65mg/kg)	6.62±0.15^{ns}	11.56±0.20^{ns}	42.73
PSLME 100	6.67±0.35^{ns}	7.89±0.52^{ns}	15.46
PSLME 500	6.59+ 0.20^{ns}	9.40±0.62^{ns}	29.89

Values are expressed as Mean ± SEM, n=6, Data analyzed by One-way ANOVA followed by Dunnette's test ^{ns}

CONCLUSION

From the present study we come to conclusion that the methanolic extract of leaves of *P. sylvestris* possesses both peripheral and central analgesic activity in experimental animal. However, further study need to carry out isolation and characterisation of the bioactive compound(s) and determination of the exact mechanism of action.

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Author Contribution

Sarvesh Paliwal and Swapnil Sharma conceptualized the research. Pankaj Jain executed the research work and wrote the manuscript. Sonika Jain and all the other authors read, improved and approved the manuscript.

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