ANALGESIC ACTIVITY OF CRUDE HYDRO-ALCOHOLIC EXTRACT OF SALVADORA PERSICA ROOT

Mangal Sain Hooda* and Rishi Pal
Janta College of Pharmacy, Butana, (Sonepat), 131302. Haryana, India.

ABSTRACT
Salvadora persica has been widely used for its reported biological activity in indigenous system of medicines. The main objective of the present investigation is to evaluate the analgesic activity of hydro-alcoholic extract of Salvadora persica root on mice. Analgesic activity of hydro-alcoholic extract of Salvadora persica root at a dose of 100 mg/Kg, 200 mg /Kg and 400 mg /Kg were evaluated against drug pentazocine at a dose of 17.5 mg/Kg. Adult albino mice and rats of either sex of six numbers in each group were under taken for study and evaluated by eddy’s hot plate and tail immersion method. The all doses of Salvadora persica root crude hydro-alcoholic extract were found to produce significant (P<0.05) analgesic activity. In eddy’s hot plate and tail immersion, both method showed significant activity (P<0.05) after 30 minutes. The result showed significant analgesic activity against stimuli.

KEYWORDS: Salvadora persica root, Crude hydro-alcoholic extract, Toothbrush tree, Miswak, Tail immersion method, Pentazocine.

INTRODUCTION
The use of plant products is increasing in many segments of the population. According to an estimate, 80% of the world’s population relies upon plants for their medication. Most of the synthetic drugs used to present for analgesic and anti-nociceptive effect cause many side and toxic effects. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for development of novel drugs.¹

Pain is a multidimensional experience that is essential for the maintenance and preservation of an individual. It warns of the danger of body harm and alerts to trauma and injury. Pain is
a specific enterocceptive sensation; it can be perceived as arising from a particular portion of the body, its temporal properties can be detailed, it can be differentiated qualitatively (for example, as stinging, pricking, burning, throbbing, dull or aching) and it involves dedicated subsets of peripheral and central neurons. The experience of pain has a distinctly unpleasant character, that is, an affective or motivational aspect that can be distinguished from its discriminative sensory aspects and from the long-term emotional experience of ‘suffering’. The unpleasantness ranges in intensity from the discomfort of a cold room, fatigued muscles or colonic tension to the excruciating agony of a severe burn, toothache, gallstone or migraine. Under normal circumstances, primary afferent pain fibres activate particular central pathways that engage protective mechanisms at several functional levels: autonomic, homeostatic, moto-ric, behavioural and mnemonic. However, injury or disease can alter the balance of this system and result in persistent, pathological pain. Analgesic substances, such as aspirin and morphine, that interact with the transmitters and modulators of the pain system are helpful for many people with pain, but there is a great need for the development of better methods for the alleviation and control of both acute (immediate) and chronic (long-term, pathological) pain. Types of pain: Acute, Chronic, Survived, Physiological, Pathological, Vascular, Bone & Joints pain and Myalgia.\[2\]

*Salvadora persica* Linn (Salvadoraceae) is used in a prospective way. *Salvadora persica* (SP) also known as miswak, toothbrush tree and mustard tree, distributed mainly in tropical and subtropical Asia. The plant is a large, evergreen profusely branched shrub, or a small tree up to 4–6m tall. SP has been used commonly as toothbrush to strengthen the gums.\[3\] The fresh root bark and leaves have been used in folk medicine for the treatment of a wide range of medical problems such as cough, asthma, scurvy, piles, leprosy, gonorrhea, headache and hepatic disorders.\[4\]

Various phytochemical studies on SP reported the presence of alkaloids salvadoreine, trimethylaine, and salvadoricine\[5\], flavonoids (quercitin), triterpenes, phytosterols, and traces of vitamin C.\[6\] Essential oil from the roots of SP contains benzyl isothiocyanate (70%) with other components such as a-pinene, camphene, benzialdehyde, b-pinene, myrcene, d-3-carene, limonene, terpinolene, benzyl nitrile, umbellulone, b-elemene, g-murolene, myristicin, b-caryophyllene and longifolene.\[7\] Various pharmacological activities on SP including, in vivo antimicrobial activity especially on lactobacilli and streptococcus mutans\[8\], with moderate secretary activity significantly high acetyl cholinesterase inhibiting ability, antifertility
activity in male rats.\textsuperscript{[9]} Aqueous and alcoholic extracts from leaves of SP reduce elevated urinary oxalate levels and deposition of stone-forming constituents in the kidneys of calculogenic rats.\textsuperscript{[10]} In our previous study, the activities of hydro-alcoholic root extract of SP being a powerful antioxidant, free radical scavenger, and lipid per-oxidation inhibitor, and as an excellent total antioxidant capacity, on the bases of in vitro studies.\textsuperscript{[11]} In the present study, we aimed to explore the analgesic activity of hydro-alcoholic extract of \textit{Salvadora persica} root in rats and mice.

**MATERIALS AND METHODS**

**Chemical details and identification of plant materials**

All chemicals were of analytical grade. Pentazocine (Fortwin, Ranbaxy) was used for this study. The root and stem of \textit{Salvadora persica} were collected in March- April month from Kharainti, Meham Teh. Rohtak. District, Haryana (India), was authenticated by Dr. Ashok Sharma (M.D.) (Dravya Guna Vigyan), Prof. & Head of Department. Shri Baba Mastnath Ayurvedic Degree College, Asthal Bohar, Rohtak.

**Extraction process**

\textit{Salvadora persica} shed dried plant material (root) was grinded and powdered material (100 g) was used for extraction. The hydro-alcoholic and aqueous extracts were prepared by hot continuous percolation method in a Soxhlet apparatus. Both extracts were collected separately and dried in vacuum system. Hydro-alcoholic extract with 70\% ethanol (Root-HA, yield: 11.65\%) and Water extract with water (Root-W, yield: 12.25\%). Both extracts were condensed by re-distillation and dried in vacuum desiccators to obtain a final extract residue.

**Experimental animals**

Healthy adult Wister albino rats and albino mice were selected for the study. Animals were housed in polypropylene cages, maintained under standard conditions (12 hours light/dark cycle; 25± 30 °C; 45-55\% humidity). They were fed with standard pellet diet and water \textit{ad libitum}. The Institutional Animal Ethical Committee of Janta College of Pharmacy Butana, (Sonepat) Haryana, India (CPCSEA-667/02/c/CPCSEA) approved the studies.

**Determination of acute toxicity of the drug**

Acute toxicity was determined in fasting mice. Animals were divided into groups of 6 each and the extract was administrated orally with 1\% CMC or 10, 30, 100, 300, 1000 and
1200mg/kg body weight of *Salvadora persica* root-HA. The mice were observed continuously for 2\textsuperscript{nd}, 4\textsuperscript{th}, 6\textsuperscript{th}, 12\textsuperscript{th}, 24\textsuperscript{th} and up to 48 hours.\[12\]

**Analgesic activities**

**Eddy’s hot plate method in mice**

This study was carried out in albino mice of either sex, weighing 20-25g. Animals were divided in different groups, including standard and control animals. Thirty minutes prior to hot plate exposure, the mice were treated with test drug compound. The time of reaction to pain stimulus (interval between placing the mice on the Eddy’s hot plate and the lick or jump response) of the mice placed on the hot plate heated at 55°C ± 0.5°C, was recorded every thirty minutes for duration of 3 hours after drug administration. To avoid the variance in temperature extent animal was kept always in the centred of hot plate. The efficacy of analgesic activity was determined by comparing the delay in pain stimulus in control and drug treated animals.\[13\]

**Tail immersion in hot water method**

The present study was carried out in overnight fasted rats of either sex weighing from 200-250gm. Antinociceptive effect of the test substances was determined according to the initial (control) reaction time, noted in all the animals. Preliminary screening of animals were done to select animals having reflex stimuli lesser than five seconds at temperate 50\textdegree{}C. The selected animal will be acclimatizing for experimental conditions prior to study. Rats were randomized in desired group and were treated with test compound (extracts), 30 minute prior to noxious stimuli. One to two cm of tail of rat was immersed in warm water kept constant at 55°C. The reaction time taken by the animals to deflect their tails were accounted. The latent period of the tail-flick response was taken as the index of anti-nociception and was determined before and at 30, 60, 90, 120 and 150 min. after the administration of drugs. The maximum reaction time was fixed: 15 seconds. Tail flick reaction time in control and drug treated groups were compared. The extant of analgesia was calculated by percentage reduction latent period of the tail-flick response.\[14\]

**Statistical analysis**

The data were expressed as standard error mean (SEM). The significance of differences among the groups was assessed by using a one-way and multiple-way analysis of variance (ANOVA). The test was followed by Dunnet’s test and p values less than 0.05 were considered as significant.
RESULTS

Acute toxicity studies

Acute toxicity studies revealed the non-toxic nature of the HA-root extract at doses up to 1200 mg/kg of SP. There were no lethality or toxic reactions found at any of the doses selected until the end of the study period. All the animals were alive, healthy and active during the observation period.

Analgesic activity of root-HA of *Salvadora persica* in Eddy’s hot plate model in albino mice.

In this model, the reaction time in root-HA treated group increased significantly (P<0.05) in comparison to the control group. The maximum effect was observed at the highest dose viz.400 mg/kg p.o. at 90 min. which showed a reaction time of 10.52 sec., whereas the standard drug pantazocine (17.5 mg/kg i.p.) showed a reaction time of 11.15 sec., at 60 min. The extract also showed dose and time dependent activity (Table: 1).

Table: 1 Analgesic activity of root-HA of *Salvadora persica* in Eddy’s hot plate model in albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose mg/kg</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>3.74±0.23</td>
</tr>
<tr>
<td>II</td>
<td>Pantazocine</td>
<td>17.5mg/kg</td>
<td>3.62±0.21</td>
</tr>
<tr>
<td>III</td>
<td>Root-HA</td>
<td>100mg/kg</td>
<td>4.34±0.16</td>
</tr>
<tr>
<td>IV</td>
<td>Root-HA</td>
<td>200mg/kg</td>
<td>3.86±0.21</td>
</tr>
<tr>
<td>V</td>
<td>Root-HA</td>
<td>400mg/kg</td>
<td>3.80±0.11</td>
</tr>
</tbody>
</table>

Values are expressed in terms of mean ± SEM, n = 6 in each group. *P < 0.05 statistically highly significant as compared with control group. Root-HA = *Salvadora persica* hydroalcoholic extract root.

Analgesic activity of root-HA of *Salvadora persica* in albino rat by tail immersion method.

In the tail immersion test, increase in the reaction time was significant (P<0.05) as compared to the control group. Maximum effect was 12.60 sec., at 90 min. post treatment with 400 mg/kg p.o. of root-HA, whereas in the vehicle treated control group the reaction time was 3.83 sec., at 90 min., clearly indicating the analgesic property of the extract (Table: 2).
Table: 2. Analgesic activity of root-HA of *Salvadora persica* in Albino rats by tail immersion method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drugs</th>
<th>Dose mg/kg</th>
<th>Reaction time in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>3.88±0.24</td>
</tr>
<tr>
<td>II</td>
<td>Pentazocine</td>
<td>17.5mg/kg</td>
<td>3.59±0.15</td>
</tr>
<tr>
<td>III</td>
<td>Root-HA</td>
<td>100mg/kg</td>
<td>3.86±0.11</td>
</tr>
<tr>
<td>IV</td>
<td>Root-HA</td>
<td>200mg/kg</td>
<td>3.82±0.11</td>
</tr>
<tr>
<td>V</td>
<td>Root-HA</td>
<td>400mg/kg</td>
<td>3.47±0.19</td>
</tr>
</tbody>
</table>

Values are expressed in terms of mean ± SEM, n = 6 in each group. *P < 0.05 statistically highly significant as compared with control group. Root-HA = *Salvadora persica* hydroalcoholic extract root.

DISCUSSION

Various phytochemical studies on SP reported the presence of alkaloids salvadoreine, trimethylaine, and salvadoricine[5], flavonoids (quercitin), triterpenes, phytosterols, and trace of vitamin C.[6] Essential oil from the roots of SP contains benzyl isothiocyanate (70%) with other components such as a-pinene, camphene, benzaldehyde, b-pinene, myrcene, d-3-carene, limonene, terpinolene, benzyl nitrile, umbellulone, b-elemene, g-muurolene, myristicin, b-caryophyllene, and longifolene.[7] Preliminary phytochemical screening showed the presence of tannins, alkaloids, flavonoids and vitamin C in the *Salvadora persica* root hydro-alcoholic extract, so the observed analgesic activity may be attributed due to these compounds. Moreover, recent studies suggest that the inflammatory tissue damage is due to the liberation of reactive oxygen species form phagocytes invading the inflammation sites.[15] There are also reports on the role of flavonoid, a powerful antioxidant[16,17], in analgesic activity primarily by targeting prostaglandins.[18,19] Again the root-HA extract demonstrated good antioxidant action in tested models. So it can be assumed that cyclo-oxygenase (COX) inhibitory activity, together with antioxidant activity may reduce the production of free arachidonic acid from phospholipids or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relieve pain-sensation.

Eddy’s hot plate and tail immersion test are considered to be selective to examine compounds acting through OPIOID receptors, the extract increases mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain.[20,21]
CONCLUSION
From the above investigation it is quite apparent that a crude hydro-alcoholic extract of *Salvadora persica* root possesses the analgesic effect against different stimuli. This is evidenced by a significant increase in the reaction time by stimuli in different experimental models.

ACKNOWLEDGEMENTS
The authors are grateful to extend special thanks to Dr. Janardhan Singh, Professor, Pt. B.D. Sharma PGIMS, Rohtak, Haryana, for his constant encouragement and support throughout the work.

REFERENCES


