PHARMACOLOGICAL PROFILE OF SALVADORA PERSICA

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ABSTRACT
This work was conducted to investigate the various pharmacological activities of 
Salvadora persica family Salvadoraceae and that includes anti inflammatory, analgesic, CNS, bleeding and clotting time activity by oral 
administration at the dose of 300 and 500mg/kg of body weight in animal models. Acute oral toxicity results 
showed that crude extract of S. persica is safe up to the dose of 5g/kg body weight of animals. Carragenan 
induced hind paw edema method for anti inflammatory activity, tail immersion test method for analgesic 
activity, Rota rod and grip strength test for CNS activity were carried out in animal models. The analgesic 
activity was compared with aspirin, 300mg/kg body weight, anti inflammatory activity was compared with 
indomethacin, 10mg/kg body weight, Transamin 250mg/kg and Vitamin K 10mg were used for bleeding and 
clotting time activity respectively while diazepam 5mg/kg were used as standard for behavior and CNS 
activities. In all activities S. persica showed prolonged and dose dependent effects. Phytochemical analysis was 
also carried out which showed the presence of certain phytoconstituents which possesses these properties. 
Therefore the results justified the traditional use of the plant.

Keywords: Salvadora persica, phytochemical analysis, acute oral toxicity, gross behavior, anti inflammatory, 
analgesic activity, CNS activity, bleeding and clotting time activity.

INTRODUCTION
A number of plants are having medicinal properties therefore an attention is being focused on the 
investigation of the efficacy of plant-based drugs used in the traditional medicine. As per WHO, report about 80%
 of the world population still rely mainly on herbal remedies (Adebajo et al., 2008). The disease of mouth 
and teeth are main problem of human life therefore every 
time research is carried out on finding the solution of 
mouth diseases. Salvadora persica belongs to family 
Salvadoraceae (Marwat et al., 2008) also known as 
Miswak or tooth brush tree. It is used for the cure and 
care of mouth and teeth (Kassas et al., 1965; Wu C. D. 
et al., 2001; Rajish et al., 2009). Its different parts contain 
chemical compounds that show the plaque inhibiting and 
antimicrobial activities against oral pathogens (Abdel- 
Rahman et al., 2002; Eid et al., 1990; Kamel et al., 1992) 
It also inhibit dental carries and plaque formation (Almas 
et al., 2005; Naumi et al., 2010; Almas, 1993) regulates 
peristaltic movements of GIT (Chawla, 1983) and has 
analgesic activity (Sulaiman et al., 1996). Review of 
literature reveals that no previous investigator has assessed its different pharmacological actions.

MATERIAL AND METHODS

Plant material
Salvadora persica twigs were purchased from local 
market. Plant sample was deposited in the herbarium of

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Department of Pharmacognosy, University of Karachi 
with voucher No. SP-06-9102006. Plant sample was cut 
into small pieces and then finally ground to make 
powder. The extract was prepared by mixing 2kg plant 
powder and 8lit ethanol in dry screw capped bottles for 6
weeks then filtered and evaporated the solvent under 
reduced pressure in a rotary evaporator.

Chemicals and drugs
The chemicals used for this study include analytical grade 
of ethanol, sodium chloride (Merck, Germany), Aspirin 
(Rckitt Benckiser, Pakistan), Carrageenan (Sigma, 
USA), Indomethacin (Sigma, USA), Diazepam (Efozo 
Chemical, Pakistan), Vitamin K and Tranäamin (Hilton 
Pharma, Pakistan).

Phytochemical screening
The ethanolic extract was subjected for preliminary 
phytochemical analysis as reported by Fatima, 2008 and 
Venkatesan et al., 2009.

Animal selection
Before proceeding to study animals i.e. albino mice (20- 
30g) and Sprague Dawley strain rats (140-250g) reared at 
animal house of PCSIR Labs Complex Karachi, were 
selected and grouped accordingly. All these animals were 
housed separately in plastic cages with sliding perforated 
stainless steel covers under strict observation for the 
period of two weeks before start of experiment with 
allowing free access to food and water. Any animal 
showing laziness, sluggish movements or any sign of 
ilness was replaced by healthy animals.
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Acute oral toxicity
The acute oral toxicity of extract of S. persica was determined in mice (Loonis, 1978). Mice (25-30g) fasted for 12h were randomly divided into groups comprising of six animals per group. Graded doses of the extract dissolved in normal saline (500-5000 mg/kg p.o.) were separately administered to the mice while control group received normal saline only in the same quantity by means of feeding cannula. All animals were then allowed free access to food and water and observed over a period of first six hours and then for the period of 72 hours for signs of acute toxicity. Daily observation on general health, growth, gross physical and behavioral activities and also the morbidity and mortality if any was noted and recorded.

Behavioral effects
The effects of S. persica extract (300 and 500 mg/kg, p.o.) on alertness/awareness, sound response, touch response, pain response, pinna reflex and grip strength were observed in mice (n = 8) by the method describe by Kumar et al., 2008. Diazepam (5 mg/kg, p.o.) was used as a reference drug. The animals were kept under observation for their behavioral changes if any, at 30 min intervals in the first one hour and at the hourly intervals for the next 4 hour after drug administration (table 1).

Anti-inflammatory activity
Albino rats of 140-190g (Male & Female) were divided into four groups (n = 5) Group I and Group II received extract of S. persica at 300 and 500mg/kg body weight. Group-III received Indomethacin 10mg/kg body weight as standard while Group-IV was given normal saline and serve as control group. Paw edema was induced by reported method (Dimo et al., 2006). Test and standard drugs were orally administered an hour prior to carrageenan injection. Paw volume was measured by mercury displacement at 0, 0.5, 1, 2, 3, 4 and 5 hours after the injection.

Analgesic activity
Analgesia was evaluated by using Tail immersion method (Vogel, 2002; Pendota et al., 2009). Young female rats (170-210g) were divided into 4 groups (n = 5). Group I and II received test drug in dose of 300mg/kg and 500mg/kg respectively. Group III received Aspirin as standard in dose of 300mg/kg while Group IV served as control and received normal saline only in the same quantity by feeding cannula orally. The initial readings were taken immediately before administration of drug and then analgesia was measured at 0.5, 1, 2, 3, 4 and 6 hours after drug administration.

CNS activity
Rota rod Test
Motor coordination or fatigue resistance was assessed on mice of both sexes (20-25g) by using Rota rod (Panlab S.L, Spain). The selected animals were divided into four groups (n = 5) Group I and II received test drug in dose of 300mg/kg and 500mg/kg respectively. Group III received Diazepam (5mg/Kg) as standard while Group IV served as control and received normal saline by feeding cannula orally. The performance of each mouse was evaluated at 30, 60, 90, 120, and 150 min after drug administration by placed mouse on rota rod at the speed of 5 rpm (Kumar et al., 2008).

Grip strength test
The effect of test and standard drugs on muscle strength of male and female rats (150-180g) was assessed by using the grip strength meter (UGO Basile biological research apparatus, Italy). The animals were divided into four groups (n = 5). Group I and II received test drug in dose of 300mg/kg and 500mg/kg respectively. Group III received Diazepam (10mg/Kg) as standard while Group IV served as control and received normal saline. Assessment of muscle strength was started at the interval of 15, 45, 75, 105 and 135 minutes after the last oral administration of tested and standard drugs (Rojecky et al., 2005).

Bleeding time
Albino rats (200-250g) were divided into four groups (n = 5). Group-I and II received test drug in dose of 300mg/kg and 500mg/kg respectively. Group-III received Transamin 250mg/kg as standard while Group IV served as control and received normal saline. The bleeding time of each animal was recorded according to the reported method (Kung et al., 1998; Sugidachi et al., 2000).

Clotting time
Albino rats (200-250g) were divided into four groups (n = 5). Group-I and II received test drug in dose of 300mg/kg and 500mg/kg respectively. Group-III received vitamin K 10mg/kg as standard while Group IV served as control and received normal saline. The clotting time of each animal was then recorded according to the reported method (Salawu et al., 2005).

STATISTICAL ANALYSIS
The data were analyzed by student t-test and p value was found for all activities which are shown at the end of each table (Walpole et al., 1998).

RESULTS
The phytochemical screening reveals that the alcoholic extract of S. persica contains alkaloids, tannins, saponins, flavonoids, sterols, terpenoids, protein and carbohydrates.

The ethanolic extract of S. persica did not show any untoward effect up to the dose of 5000mg/kg body weight and did not cause death of any tested animal. The results of experiments carried out on gross behavioral changes.
induced by the oral introduction of ethanolic extract of *S. persica* are given in table 1.

The results of anti-inflammatory activity by carrageenan induced paw edema method revealed that the extract of *S. persica* possesses dose dependent anti-inflammatory activity i.e. more activity at higher dose (fig. 1).

The results of analgesic activity showed that the extract of *S. persica* possesses prolonged activity at higher dose than lower dose by showing increase in reaction time (fig. 2). The results of CNS activity indicate that the extract of *S. persica* has enhancing effects on muscle coordination and grip of animals at all the test doses (figs. 3 and 4).

The bleeding and clotting time obtained for the treated animals was significantly lower than that of control and standard groups. The activity was dose dependent it was significantly decreased at higher dose (fig. 5).

DISCUSSION

Generally, the plants possess many pharmacological activities as they contain numerous constituents of active chemicals in it. The phytochemical screening of *S. persica* constituents has also been confirmed by another research study (Ahmed *et al.*, 2008; Rajesh *et al.*, 2009). The ethanolic extract of *S. persica* did not show any untoward effect up to the dose of 5000mg/kg body weight and did not cause death of any tested animal. According to the results obtained from various experiments for behavioral effects showed that the extract of *S. persica* induced only slight depression or not significant at both doses and all animals found quite normal during whole observation period while standard drug diazepam causes significant depression as compared with test group (table 1).

The results of anti-inflammatory activity by carrageenan induced paw edema method revealed that the extract of *S. persica* possesses dose dependent anti-inflammatory activity i.e. more activity at higher dose. But the test drug at both doses showed delayed and less potent anti-inflammatory effects in terms of intensity and duration as compared to standard drug indomethicine. Both doses of test drug showed a maximum anti-inflammatory effect of about 44.4% at 300mg/kg and 61.5% at 500mg/kg at 3hours after drug given while the anti-inflammatory effect induced by standard drug indomethicine was progressively increased and reached a maximum 75.3% at three hours (table 2; fig. 1). The results were found significant up to *p* ≤ 0.001 at both doses.

Table 1: Effect of extract of *Salvadora persica* on general behavior in mice

<table>
<thead>
<tr>
<th>Behavior type</th>
<th>Extract (mg/kg)</th>
<th>Diazepam</th>
<th>GP IV Normal saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPI 300(mg/kg)</td>
<td>GPII 500(mg/kg)</td>
<td>GPIII 5(mg/kg)</td>
</tr>
<tr>
<td>Alertness/ awareness</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Sound response</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Touch response</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Pain response</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Pinna reflex</td>
<td>+</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Grip strength</td>
<td>-</td>
<td>-</td>
<td>++++</td>
</tr>
</tbody>
</table>

No effect (-), Slight depression (+), Moderate depression (++), Strong depression (+++), Very strong depression (++++)

Fig. 1: Effect of extract of *S. persica* on Paw edema induced by Carrageenan in rats
Pharmacological profile of *Salvadora persica*

The anti-inflammatory activity of *S. persica* was also reported in literature (Ezmirly *et al.*, 1979) but it did not show at which dose anti-inflammatory activity is more.

The results of analgesic activity by tail immersion method showed that the extract of *S. persica* possesses prolonged analgesic activity at higher dose than lower dose by showing increase in reaction time. The test drug showed slow onset of analgesic action at 500mg/kg dose level but the duration of analgesic effect was more as compare to lower dose and the duration was almost equal to standard drug after 6hr. The test drug showed 11.3% analgesic effect at 300mg/kg, 25.3% at 500mg/kg while standard drug showed 24.7% analgesic effect at 6 hours after drug given. In spite of the prolonged action of test drug, the test drug showed less potent analgesia in terms of intensity as compare to standard drug aspirin (table 3; fig. 2). The results were found significant up to $p \leq 0.001$ level at 300mg/kg dose and 500mg/kg dose.

Various constituents are found in *S. persica* which possess medicinal values for the treatment of different diseases. It is also claimed that Ascorbic acid and Sitosterol content of this plant strengthens the gum.

**Table 2:** Effects of extract of *S. persica* on hind paw edema induced by Carrageenan in rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Carrageenan induce edema (volume in ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3min</td>
</tr>
<tr>
<td><em>S. persica</em></td>
<td></td>
</tr>
<tr>
<td>Group I 300mg/kg</td>
<td>0.7±0.27</td>
</tr>
<tr>
<td>Group II 500mg/kg</td>
<td>0.5±0</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
</tr>
<tr>
<td>Group III 10mg/kg</td>
<td>0.4±0.14</td>
</tr>
<tr>
<td>N. saline</td>
<td></td>
</tr>
<tr>
<td>Group IV 10mg/kg</td>
<td>1±0.08</td>
</tr>
</tbody>
</table>

300mg/kg = $t = 5.45; df = 8; p < 0.001$; 500mg/kg = $t = 7.16; df = 8; p < 0.001$

![Graph showing tail flick time in (sec)](image)

**Fig. 2:** Analgesic activity of extract of *S. persica* in rats

**Table 3:** Analgesic activity test of *S. persica* and standard drug by tail immersion test.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tail Flick Time before and after drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0min</td>
</tr>
<tr>
<td>Group I 300mg/kg</td>
<td>1.75±0.32</td>
</tr>
<tr>
<td>Group II 500mg/kg</td>
<td>1.97±0.14</td>
</tr>
<tr>
<td>Group III Aspirin 300mg/kg</td>
<td>1.82±0.09</td>
</tr>
<tr>
<td>Group IV N. saline</td>
<td>1.88±0.22</td>
</tr>
</tbody>
</table>

300mg/kg = $t = 4.30; df = 10; p < 0.001$; 500mg/kg = $t = 8.24; df = 10; p < 0.001
capillaries and prevents gum inflammation (Pourestami et al., 2007). Mansour et al. (1996) also studied S. persica and reported that its decoction showed more analgesic effects against thermal stimuli than the chemical stimuli. The anti-inflammatory and analgesic activities of S. persica extract may be due to the presence of flavonoids and sterols. According to literature search β-Sitosterol, Campesterol, Avenasterol, Stigmasterol and flavonoids are found in S. persica.

It is reported that these phytochemicals possesses anti-inflammatory and analgesic activities (Meena et al., 2009; Adeolu et al., 2008). It is also reported in another study that the enzyme prostaglandins are involved in pain perception and its synthetase is inhibited by flavonoids so it might be possible that the reduce availability of prostaglandins produce analgesic effects (Hajare et al., 2000).

Table 4: Motor coordination test of S. persica and standard drugs

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose mg/kg</th>
<th>Time spend on Rota rod (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30min</td>
</tr>
<tr>
<td>S. persica</td>
<td>Group I 300mg/kg</td>
<td>180.4±0.1</td>
</tr>
<tr>
<td></td>
<td>Group II 500mg/kg</td>
<td>180.4±0.1</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Group III 10mg/kg</td>
<td>53.4±71.2</td>
</tr>
<tr>
<td>N. saline</td>
<td>Group IV 100mg/kg</td>
<td>180.4±0.1</td>
</tr>
</tbody>
</table>

300mg/kg = t = 1.89; df = 8; p < 0.1; 500mg/kg = t = 2.12; df = 8; p < 0.1

Fig. 3: Motor coordination of extract of S. persica in mice

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**Table 5: Muscles strength activity (grip strength) test**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Grip Strength (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td><em>S. persica</em></td>
<td></td>
</tr>
<tr>
<td>Group I 300mg/kg</td>
<td>35.4±11.17</td>
</tr>
<tr>
<td>Group II 500mg/kg</td>
<td>36.6±13.16</td>
</tr>
<tr>
<td>Diazepam</td>
<td></td>
</tr>
<tr>
<td>Group III 10mg/kg</td>
<td>36.4±10.06</td>
</tr>
<tr>
<td>N. saline</td>
<td></td>
</tr>
<tr>
<td>Group IV</td>
<td>35.6±10.69</td>
</tr>
</tbody>
</table>

300mg/kg = t = 1.87; df = 8; p < 0.1; 500mg/kg = t = 2.14; df = 8; p < 0.1

![Graph showing grip strength activity for each group](image)

Fig. 4: Effects on grip strength activity of extract of *S. persica*.

The results of Rota rod activity indicated that the extract of *S. persica* has enhancing effects on muscle coordination of mice at all the test doses, as all the animals maintained their balance on Rota rod bar by increase in the time on the bar and decrease in no. of falls. While the results of standard drug Diazepam indicate that it possesses significantly higher sedative effects than that produce by extract as the animals were unable to maintain their balance on bar and there was shortening the time of animals spent on Rota rod bar (table 4; fig. 3). The results were found significant up to p ≤ 0.1 level. Similarly, in Grip Strength Activity test, the results showed that the grip of animals treated with test drugs was strengthened as compare to those of standard and control group animals (table 5; fig. 4). The results were found significant up to p ≤ 0.1 level.

It is reported that triterpenoids are responsible for CNS depressant action (Srikanth et al., 2009). Therefore, the absence of triterpenoids in *S. persica* extract confirmed that the extract possesses CNS stimulant activity.

The bleeding time obtained for rats treated with extract was significantly lower than that of control and standard group. The activity was dose dependent it was significantly decreased at higher dose. The clotting time obtained for rats treated with extract was lower than that of control group. The activity was also dose dependent (tables 6 and 7; figs. 5 and 6). Transamín and vitamin K were used as standard for bleeding and clotting activity respectively because these drugs are commonly used for treating these problems in clinical practice. Statistical analysis showed that the results of both bleeding and clotting time activities are significant (p < 0.05).

**Table 6: Bleeding time activity of extract of *S. persica***

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>Bleeding time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. persica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I 300mg/kg</td>
<td>468±0.908</td>
<td></td>
</tr>
<tr>
<td>Group II 500mg/kg</td>
<td>282±1.524</td>
<td></td>
</tr>
<tr>
<td>Transamin</td>
<td>Group III 50mg/kg</td>
<td>708±1.151</td>
</tr>
<tr>
<td>Normal saline</td>
<td>Group IV</td>
<td>804±1.949</td>
</tr>
</tbody>
</table>

300mg/kg = t = 5.85; df = 8; p < 0.001; 500mg/kg = t = 7.89; df = 8; p < 0.001

![Graph showing bleeding time activity for each group](image)

Fig. 5: Bleeding time activity of extract of *S. persica*.
Table 7: Clotting time activity of extract of S. presica

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>Clotting time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. persica</td>
<td>Group I 300mg/kg</td>
<td>84±13.41</td>
</tr>
<tr>
<td></td>
<td>Group II 500mg/kg</td>
<td>75±15.00</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>Group III 250mg/kg</td>
<td>84±17.102</td>
</tr>
<tr>
<td>Normal saline</td>
<td>Group IV</td>
<td>96±8.215</td>
</tr>
</tbody>
</table>

300mg/kg = t = 2.88; df = 8; p < 0.05; 500mg/kg = t = 2.74; df = 8; p < 0.05

Fig. 6: Clotting time activity of extract of S. presica.

CONCLUSION

Over all the pharmacological data of S. persica extract indicates that this plant is a good source of active compounds capable of exerting potential therapeutic activity in organisms. The research work of present study demonstrates that the orally administered extract displayed significant analgesic, anti-inflammatory, bleeding and clotting time activities and side by side no CNS depressant action. Therefore, on the basis of the results and the safe pharmacological profile it confirms the traditional use of this plant.

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REFERENCES


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