PHARMACOLOGICAL PROFILE OF *SALVADORA PERSICA*

MANSOOR AHMAD[1], HINA IMRAN[2], ZAHRA YAQEEN[2], ZAKIR-UR REHMAN[2], ATIQ-UR-RAHMAN[2], NUDRAT FATIMA[2] AND TEHMINA SOHAIL[2]

[1]*Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi*
[2]*Pharmaceutical Research Centre PCSIR Laboratories Complex, Karachi*

ABSTRACT

This work was conducted to investigate the various pharmacological activities of *Salvadora. persica* family Salvadoracea 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. Carraganeen 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 indomethacine, 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 (Adedapo *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 Salvadoracea (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 Department of Pharmacognosy, University of Karachi with voucher No. SP-06-9102006. Plant sample was cut into small pieces and then finally grinded to make powder. The extract was prepared by mixing 2kg plant powder and 8lit ethanol in dry screw caped 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 (Reckitt Benckiser, Pakistan), Carrageenan (Sigma, USA), Indomethacine (Sigma, USA), Diazepam (Efroze Chemical, Pakistan), Vitamin K and Transamin (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 Dawely 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 illness was replaced by healthy animals.

**Corresponding author*: e-mail: herbalist53@yahoo.com

Acute oral toxicity

The acute oral toxicity of extract of *S. persica* was determined in mice (Loomis, 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-1 and Group-II received extract of *S. persica* at 300 and 500mg/kg body weight. Group-III received Indomethacine 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 administrated 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.I, 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-1 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-1 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*., 2008).

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 compare to standard drug indomethcine. 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 indomethcine was progressively increased and reached a maximum 75.3% at three hours (table 2; fig. 1). The results were found significant up to $p \leq 0.001$ at both doses.

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

Behavior type	Extract (mg/kg)		Diazepam	GP IV Normal saline
	GPI 300(mg/kg)	GPII 500(mg/kg)	GPIII 5(mg/kg)	
Alertness/ awareness	-	+	+++	-
Sound response	-	-	++	-
Touch response	-	-	++++	-
Pain response	-	+	+++	-
Pinna reflex	+	-	+++	-
Grip strength	-	-	++++	-

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

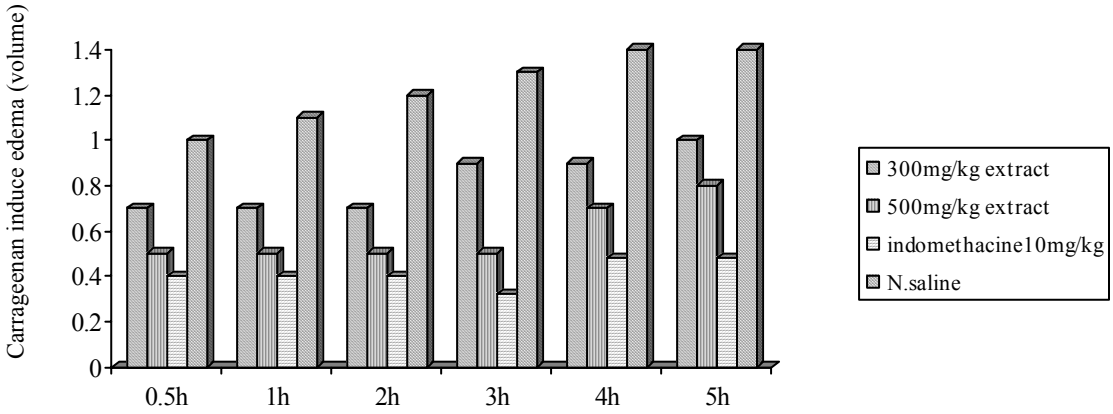


Fig. 1: Effect of extract of *S. persica* on Paw edema induced by Carrageenan in rats

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. presica* on hind paw edema induced by Carrageenan in rats

Treatments		Carrageenan induce edema (volume in ml)					
		3min	1hr	2hr	3hr	4hr	5hr
S. persica	Group I 300mg/kg	0.7±0.27	0.7±0.27	0.7±0.27	0.9±0.22	0.9±0.22	1±0.35
	Group II 500mg/kg	0.5±0	0.5±1	0.5±1	0.5±1	0.7±0.25	0.8±0.21
Indomethacine	Group III 10mg/kg	0.4±0.14	0.4±0.3	0.4±0.26	0.32±0.20	0.48±0.04	0.48±0.044
N. saline	Group IV	1±0.08	1.1±0.22	1.2±0.46	1.3±0.44	1.4±0.41	1.4±0.36

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

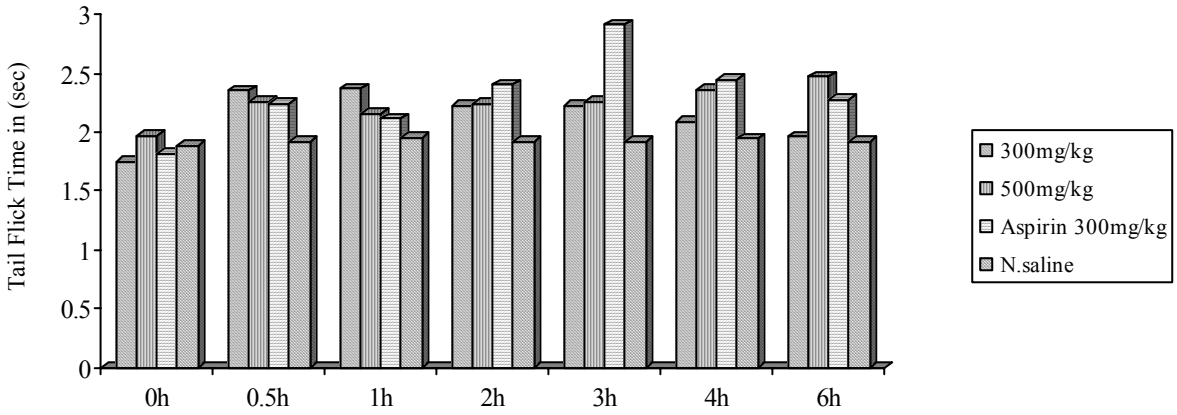


Fig. 2: Analgesic activity of extract of *S. presica* in rats

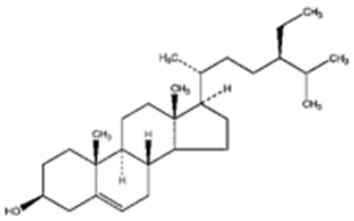
Table 3: Analgesic activity test of *S. persica* and standard drug by tail immersion test.

Treatments	Tail Flick Time before and after drug administration						
	0min	30min	1h	2h	3h	4h	6h
Group I 300mg/kg	1.75±0.32	2.35±0.55	2.37±0.43	2.22±0.45	2.22±0.49	2.09±0.35	1.96±0.16
Group II 500mg/kg	1.97±0.14	2.26±0.29	2.16±0.17	2.24±0.15	2.26±0.19	2.36±0.36	2.47±0.38
Group III Asprin 300mg/kg	1.82±0.09	2.24±0.14	2.11±0.27	2.40±0.41	2.91±0.49	2.44±0.19	2.27±0.20
Group IV N. saline	1.88±0.22	1.92±0.20	1.95±0.20	1.92±0.20	1.92±0.22	1.94±0.22	1.92±0.20

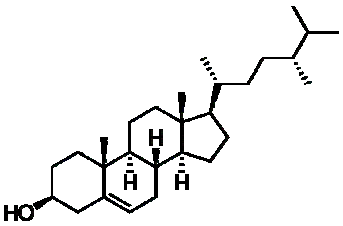
300mg/kg = t = 4.30; df = 10; p < 0.001; 500mg/kg = t = 8.24; df = 10; p < 0.001

capillaries and prevents gum inflammation (Poureslami *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*.

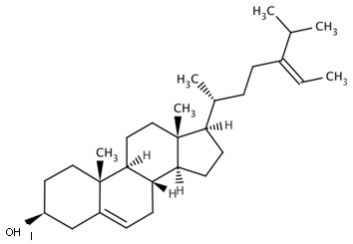
It is reported that these phytochemicals possesses anti-inflammatory and analgesic activities (Meena *et al.,* 2009; Adeolu *et al.,* 2008). It is also reported in another study that the enzyme prostaglandins are involved in pain perception and its synthetase is inhibited by flavonoids so it might be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



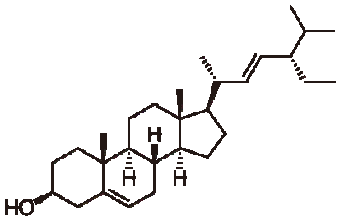
β- Sitosterol



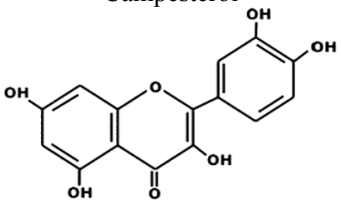
Campesterol



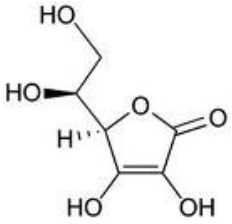
Avenasterol



Stigmasterol



Flavonoid



Ascorbic acid

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

Treatments	Dose mg/kg	Time spend on Rota rod (sec.)				
		30min	160min	90min	120min	150min
S. persica	Group I 300mg/kg	180.4±0.1	180.4±0.1	180.3±0.1	180.4±0.1	180.3±0.1
	Group II 500mg/kg	180.4±0.1	180.4±0.1	180.4±0.1	180.4±0.1	180.4±0.1
Diazepam	Group III 10mg/kg	53.4±71.2	68.8±70.5	77.2±61.4	125.6±75.8	143.8±54.6
N. saline	Group IV	180.4±0.1	180.4±0.1	180.3±0.1	180.4±0.1	180.3±0.1

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

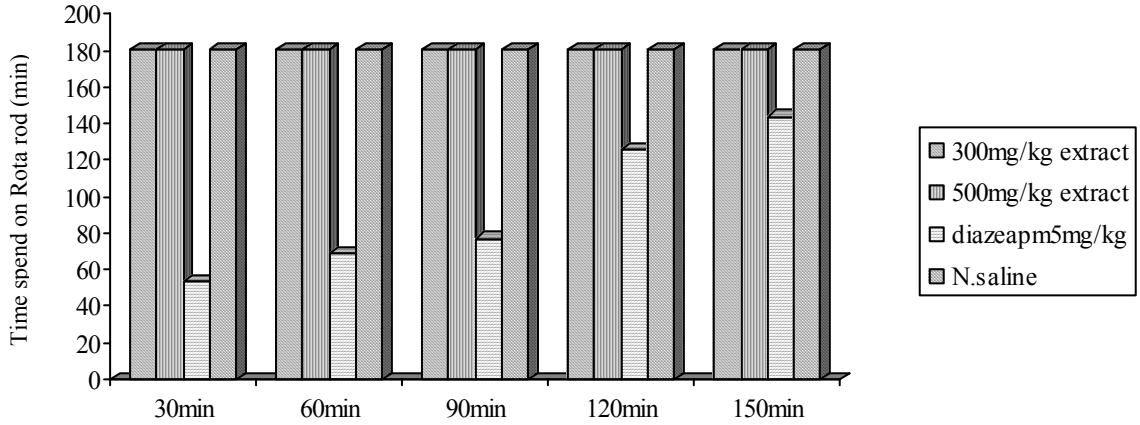


Fig. 3: Motor coordination of extract of *S. presica* in mice

Table 5: Muscles strength activity (grip strength) test

Treatments		Grip Strength (g)				
		0 min	15 min	45min	75 min	105min
S. persica	Group I 300mg/kg	35.4±11.17	101.2±33.11	89±64.80	68.2±23.87	54.8±14.35
	Group II 500mg/kg	36.6±13.16	42.2±24.87	56.4±56.31	73.2±30.20	84.8±63.77
Diazepam	Group III 10mg/kg	36.4±10.06	56.2±14.93	44±15.44	47.8±18.71	35.6±6.26
N. saline	Group IV	35.6±10.69	37.2±8.25	46.2±6.49	52.8±12.07	53.6±10.87

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

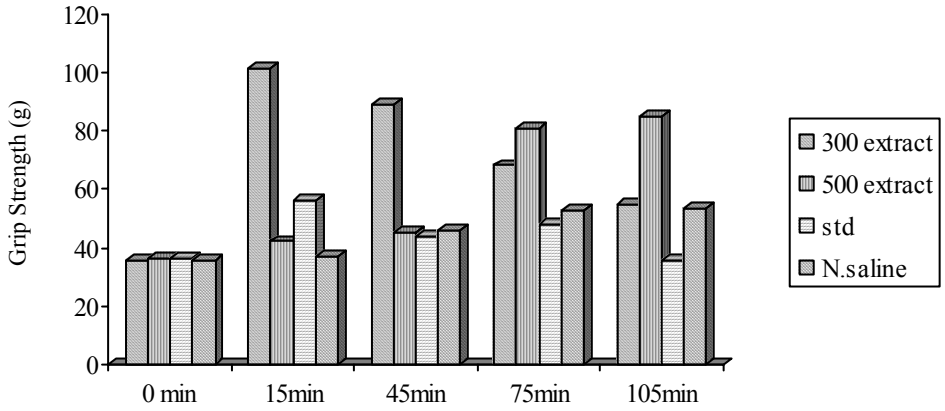


Fig. 4: Effects on grip strength activity of extract of *S. presica.*

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 $p \leq 0.1$ 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 $p \leq 0.1$ 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). Transamin 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. presica*

Treatments	Dose (mg/kg)	Bleeding time (sec)
S. persica	Group I 300mg/kg	468±0.908
	Group II 500mg/kg	282±1.524
Transamin	Group III 50mg/kg	708±1.151
Normal saline	Group IV	804±1.949

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

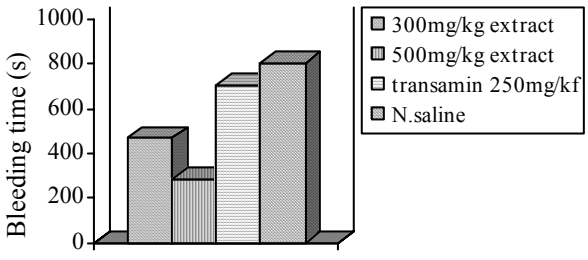


Fig. 5: Bleeding time activity of extract of *S. presica*

Table 7: Clotting time activity of extract of *S. presica*

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

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

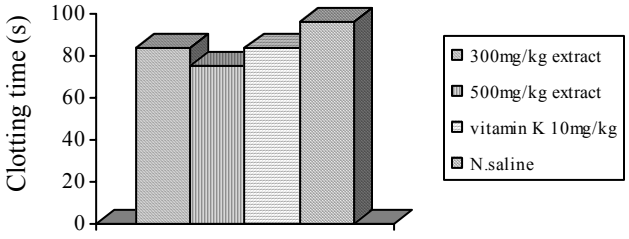


Fig. 6: Clotting time activity of extract of *S. presica.*

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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