

Evaluation of analgesic activity of *P. domestica* L.

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Abstract: This research study was conducted to investigate acute oral toxicity and analgesic activity of ethanol extract of *P. domestica* fruit by using tail flick analgesiometer at 300 and 500mg/kg doses in animal models. Acute oral toxicity results showed that crude extract is safe up to the dose of 5g/kg body weight of animals. The analgesic activity revealed that *P. domestica* extract at 500mg/kg dose possesses highest significant and prolonged analgesic activity in dose dependent manner as compared to standard and control groups. Aspirin 300mg/kg body weight was used as standard drug. Phytochemical analysis was also carried out which showed the presence of certain phytochemicals constituents in test drug that are responsible for analgesic activity. Therefore the results are justified.

Keywords: *P. domestica* fruit, acute oral toxicity, analgesic activity, rats and mice.

INTRODUCTION

From earliest period herbal medicines have been used in mankind as medicines for the treatment of series of diseases. Although there is great advances observed in the field of synthetic medicine in recent decades but plants still make an important contribution to health care because these commonly use synthetic drugs are not only expensive but also have side effects therefore new drugs of plant origin are still necessary because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant based drugs (Tambe *et al.*, 2010; Besra *et al.*, 1996; Zulfiker *et al.*, 2010).

Prunus domestica (Rosaceae) commonly known as Plum, Alu-Bukhara, Alucha found commonly in India, Pakistan, Afghanistan and Persia (Nadkarni 1976 and Narayan 2003) Many pharmacological activities are reported for blood circulation, measles, digestive problems, anticancer, anti-diabetes, anti-obesity, cardiovascular problems, dyspepsia, nausea, vomiting, thirst, in bilious fevers, headache, jaundice and hepatitis, leucorrhea, miscarriage, antioxidant, antihyperlipidemic, anxiolytic, asthma and laxative (Ferrel, 1998; Jabeen and Aslam, 2011; Qureshi *et al.*, 1988; Soni *et al.*, 2011).

The major chemical constituents present in *P. domestica* are carbohydrates, amino acids, vitamin A, vitamin B complex, vitamin K, potassium, calcium, magnesium, zinc, copper, manganese, selenium, boron and dietary fibers, pectin, hemicellulose, cellulose, lignins, sorbitol, glucose, fructose and sucrose, malic, citric, tartaric, benzoic and boric acids, benzaldehyde, linalool, ethyl nonanoate, methyl cinnamate and γ -decalactone, benzaldehyde, 2-furancarboxyaldehyde, ethyl cinnamate,

chlorogenic acid, neochlorogenic acid, caffeic acid, coumaric acid, rutin, proanthocyanidin and melanodins (Jabeen and Aslam, 2011; Lombardi-Boccia *et al.*, 2004; Parmar *et al.*, 1992).

Inspite of number of reported pharmacological activities on *P. domestica* no research studies were conducted on its analgesic activity therefore, we have reported analgesic activity of *P. domestica* very first time.

MATERIAL AND METHOD

Plant material

P. domestica dried fruit was purchased from local market. The extract was prepared by mixing 5kg material and 10liter ethanol in dry screw capped bottles for 6 weeks then filtered and evaporated under reduced pressure in rotary evaporator.

Chemicals and drugs

The chemicals used for this study include analytical grade of ethanol, Sodium chloride (Merck, Germany), Aspirin (Reckitt Benckiser, Pakistan).

Phytochemical screening

The ethanol extracts of *P. domestica* fruit was subjected for preliminary phytochemical analysis as reported elsewhere (Fatima 2008; Venkatesan *et al.*, 2009).

Animal selection

Before proceeding for toxicity and analgesic studies animals i.e. Swiss albino mice (20-30g) and Wistar strain rats (140-200g) reared at animal house of PCSIR Labs Complex Karachi, were selected and housed separately and kept under strict observation for a period of 3 weeks with free access to food and water. Any animal showing sluggish movement or any sign of illness was rejected.

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Principles of laboratory animal care guidelines were followed and prior permission was sought from the Ethical Committee PCSIR for conducting the study.

Acute oral toxicity

The acute oral toxicity of extract of *P. domestica* was determined in mice (20-30g) extract dissolved in 0.5 ml of physiological saline was administered in a single dose by means of a gavage in graded doses (2.5 and 5.0 g/kg body weight) to different animal groups while control group received normal saline only in the same quantity to each animal (n=5). Observations with reference to physico-behavioral changes and mortality rate within 24 hours noted. Animals were further observed for a period of 72 hours for changes in behavioral pattern and delayed mortalities. Macroscopic findings were made on autopsied animals and recorded (Loomis, 1978).

Analgesic activity by Tail flick Test

Analgesic activity was carried out by Tail flick method (Salawu *et al.*, 2008) on analgesiometer (UGO Basile, Italy). Wistar Albino rats were divided into four groups comprising of five animals each. Group-I and Group-II was treated as test groups received *P. domestica* extract in two different doses i.e. 300 and 500mg/kg *p.o.* Group-III was given Aspirin (300mg/kg, *p.o.*) treated as standard group. The test and standard drugs were dissolved in normal saline while group IV received normal saline only, which served as control group. The results were noted initially before giving the drug i.e. at 0 min and then at 30 min, 1 hr, 2 hr, 3 hr and at 4 hours after the administration of the test and standard drugs.

RESULTS

The phytochemical screening reveals that the alcoholic extract of *P. domestica* contains tannins, saponins, flavonoids, sterols, protein and carbohydrates (table 1).

Table 1: Chemical constituents of *P. domestica*

S. No.	Chemical Constituents	Present	Absent
1	Triterpenes	-	+
2	Tannins	+	-
3	Saponins	+	-
4	Alkaloids	-	+
5	Carbohydrates	+	-
6	Proteins	+	-
7	Sterols	+	-
8	Flavonoids	+	-

Table 2: Acute oral toxicity test of *P. domestica*

Treatments	Dose	No. of animals treated	No. of animals survived	No. of animals died	% Mortality	% Survival
N. Saline	0.5 ml	05	05	Nil	0%	100%
<i>P. domestica</i>	300mg/kg	05	05	Nil	0%	100%
<i>P. domestica</i>	500mg/kg	05	05	Nil	0%	100%

The ethanol extract of *P. domestica* did not show any untoward effect up to the dose of 5g/kg body weight. No mortality was observed. Autopsy done revealed no gross pathological and physical changes. All vital organs i.e. heart, liver lungs and kidneys were found to be normal. No mortality was observed during the claimed 72 hours observation period (table 2).

The results of analgesic activity by using tail flick test showed that *P. domestica* possesses highest and prolonged activity in dose dependent manner i.e. highest at 500mg/kg dose as compared to standard and control groups (table 3).

Statistical calculation was carried out by student *t* test and the values were found to be significant at $p \leq 0.01$

DISCUSSION

It is well known that herbal materials have variety of pharmacological activities because of its numerous active chemical constituents. The phytochemical screening of *P. domestica* showed that it possesses active chemical constituents like tannins, saponins, carbohydrates, proteins, sterols and flavonoids, which were also confirmed by other research studies (Jabeen and Aslam, 2011).

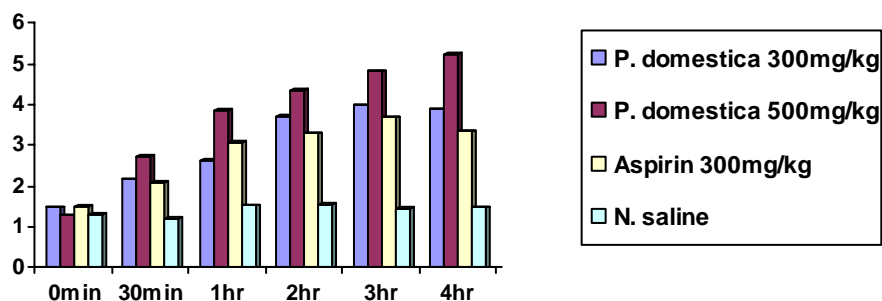
The results of analgesic activity using tail flick instrument revealed that the test drug i.e. ethanol extract of *Prunus domestica* possesses analgesic activity by boost in reaction time in a similar manner like standard drug Aspirin on analgesiometer, but an interesting thing to be noted is that *P. domestica* extract at 500mg/kg dose possesses highly significant and prolonged activity in dose dependent manner even better results than Aspirin, which is a remarkable observation (table 3 and fig. 1). The data was analyzed by student *t* test and the result was found to be significant at $p \leq 0.01$.

The presence of analgesic activity in the test drug may be due to the presence of some active chemical constituents like flavonoids, tannins, carbohydrates, and sterols which have been reported to have the ability to inhibit pain sensitivity (Zulfiker *et al.*, 2010; Okwu *et al.*, 2006; Rao *et al.*, 1998; Argal and Pathak, 2006; Malairajan *et al.*, 2006; Meena *et al.*, 2009; Vanu *et al.*, 2006). This statement was also confirmed by Hajare *et al.* (2000), according to that study the enzyme prostaglandins are involved in pain perception and its synthetase is inhibited

Table 3: Analgesic activity of test and standard drugs by tail flick test

Treatments	Dose	Tail flick time (sec.)					
		0min	30 min	1hr	2hr	3hr	4hr
<i>P. domestica</i>	Group I 300mg/kg	1.48±0.30	2.16±0.56	2.62±0.70	3.70±1.00	3.98±1.11	3.88±0.76
	Group II 500mg/kg	1.28±0.31	2.74±0.79	3.86±0.42	4.34±0.30	4.82±0.19	5.22±0.67
Aspirin	Group III 300mg/kg	1.5±0.33	2.08±0.75	3.06±1.58	3.3±0.51	3.68±0.71	3.34±0.75
Control	Group IV Normal saline	1.3±0.48	1.2±0.30	1.52±0.77	1.54±0.70	1.46±0.27	1.48±0.21

Statistical calculation by Student t test; values are found to be significant at $p \leq 0.01$

**Fig. 1:** Comparison of analgesic activity of test and standard drugs

by flavonoids so it might be possible that the reduced availability of prostaglandins produce analgesic effects.

CONCLUSION

It is concluded that *P. domestica* was found to have significant analgesic activity and it can play fruitful role in preparation of herbal drugs for analgesia and it can also be used in preparation of analgesic drugs which can be safely used during pregnancy and lactation, because very little allopathic analgesic medicines are allowed during pregnancy.

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