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Evaluation of Antioxidant and Antiulcer Potentials of *Prunus domestica* Fruit Methanolic and Extract on Wistar Albino Rats

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ABSTRACT

Medicinal plants have been traditionally used for the treatment and prevention of peptic ulcer. The present study was designed to investigate antioxidant and antiulcer potential of methanolic fruit extract of *Prunus domestica*. Antioxidant activity was measured by 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide free radical scavenging method. The extract showed maximum scavenging activity i.e., 82.12 ± 0.654 and 79.43 ± 0.876 at $200 \mu\text{g mL}^{-1}$. Further the extract was evaluated for its anti-ulcerogenic activity by pyloric ligation model. The extract showed significant inhibition of ulcer i.e., 70.58% at 200 mg kg^{-1} . The present study concludes that *Prunus domestica* fruit possesses potent antioxidant and anti-ulcerogenic effect.

Key words: *Prunus domestica*, antioxidant, antiulcer, pyloric ligation, free radical

INTRODUCTION

Recently there is huge increase in the focus of plants all over the world (Ansari *et al.*, 2011). There is a enormous range of natural plants used to treat various diseases and injuries (Abbasi *et al.*, 2009). Constituent of plants are rich in nutritive and therapeutic potential (Gazdik *et al.*, 2008). The medicinal components from plants were used from the ancient time of human evolution. There are lots of plants to be explored and to evaluate their pharmacological activity (Archana *et al.*, 2011).

Nature has enormous variety of wild plants which grows as such in different parts of India. (Joseph and Raj, 2011). *Prunus domestica* belongs to rosaceae family which is one of the largest family includes 100 genera and 200 species, researchers have paid attention towards rosaceae family because many plant of the family possess immense therapeutic potential (Trease, 2002). Many of its species are sources of fruits, oils, timber, ornamentals, jams, jellies, pickle etc. (Lee and Wen, 2001). In India (Kashmir), Persia, China Japan and Afghanistan, it has been used in homeopathy, Ayurveda and natural medicine (Ahmed *et al.*, 2010).

Many theories suggest that substances which exhibit free radical scavenging activity can be employed as protective agent for gastric mucosal membrane. Destruction of gastric mucosa results in peptic ulcer. Peptic ulcer occurs due to imbalance between the mucosal defense versus luminal acid peptic attack (Srikanth and Muralidharan, 2009). Peptic ulcer disease is common

gastrointestinal disorder that requires a well defined and specific therapeutic treatment (De Sousa Falcao *et al.*, 2008). To treat peptic ulcer researchers extended their approach to natural remedies for the development of new drugs and novel molecules which provide better protection and decreased toxicity (Mishra *et al.*, 2009). The objective of the present study was to evaluate *Prunus domestica* fruit extract for their antioxidant and anti-ulcer potential.

MATERIALS AND METHODS

Plant material: *Prunus domestica* fruit was purchased in the month of August 2010 from the local grain market of sector -26 Chandigarh (India). It was authenticated in the National Institute of Science Communication and Information Resources, New Delhi and the voucher specimen no.1494/9 has been deposited in the department. The healthy looking fruits were dried and carefully powdered in a grinder at room temperature.

Extraction procedure: The powdered fruit material was extracted by simple maceration for 72 h using methanol as solvent at room temperature and was defatted using hexane. The solvent was filtered off using muslin cloth and the residue macerated again with the fresh solvent. Both the solvents were combined and filtered using Whatman filter paper and concentrated under reduced pressure on rotary evaporator (Heidolph Laborota. model No. 517-0100-06-0). The concentrated extract was stored in refrigerator at 20°C throughout the investigation.

Phytochemical screening of the extract: Phytochemical screening was carried out for various constituents such as: flavonoids, tannins, alkaloids, terpenoids, carbohydrates, anthraquinone glycosides, coumarin glycosides, proteins according to standard procedure (Harborne, 1973).

Drugs and chemical: 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide were obtained from Sigma Chemical. Ranitidine was obtained as free sample from Ranbaxy Laboratories, Gurgaon. Methanol and sodium hydroxide of analytical grade were purchased from Merck limited.

Animals: Wistar albino rats of either sex weighing 150-250 g were procured from the animal house of Agricultural University, Ludhiana (Punjab). The animals were kept in polypropylene cages at standard laboratory conditions. The animals were fed with standard rodent pellet diet (Hindustan Lever Ltd.) and water. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal) registration no. 874/ac/05/CPCSEA.

Hydrogen peroxide free radical scavenging assay: Hydrogen peroxide free radical scavenging activity was determined according to the method of Isfahlan *et al.* (2010).

1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) scavenging activity: Free radical scavenging activity was determined according to the method of Gill *et al.* (2010a).

Anti-ulcer activity

Experimental design for pyloric ligation induced ulcer model: The study was carried out from 3rd Feb to 16th May 2011. Animals are divided into six groups, each group consist of 6 rats:

Group 1: Administered vehicle (normal saline 0.9% w/v p.o.) 1 h before pyloric ligation

Group 2: Control group subjected to surgical procedure with out pyloric ligation

Group 3: Administered ranitidine (50 mg kg⁻¹, p.o.) 1 h before pyloric ligation

Group 4: Administered extract (100 mg kg⁻¹, p.o.) 1 h before pyloric ligation

Group 5: Administered extract (150 mg kg⁻¹, p.o.) 1 h before pyloric ligation

Group 6: Administered extract (200 mg kg⁻¹, p.o.) 1 h before pyloric ligation

Pyloric ligation induced peptic ulcer: Methanolic fruit extract of *Prunus domestica* (100, 150 and 200 mg kg⁻¹) was administered for a period of 7 days. On day 7, after the last dose the rats were kept for 24 h fasting with free access to water. The abdomen was opened and pylorus was ligated without causing any damage to its blood vessels, under pentobarbitone anesthesia (Shay *et al.*, 1945). Stomach is replaced carefully and the abdominal wall was closed with interrupted sutures. The animals were deprived of water during the postoperative period. After 4 h of ligation, stomachs were dissected out and contents were collected into clean tubes (Sood *et al.*, 2009).

Anti-ulcer activity parameters: Estimation of gastric volume, free acidity and total acidity. Four hours after ligation, stomachs were dissected out and contents were collected into measuring cylinder to measure the volume of gastric content.

Free and total acidity: The gastric contents were centrifuged at 1000 rpm and subjected to titration for estimation of free and total acidity. One milliliter of the supernatant liquid was pipette out and diluted to 10 mL with distilled water. The solution was titrated against 0.01 N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH was taken as corresponding to the free acidity. Titration was further continued by adding 1% solution of phenolphthalein till the solution gained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. The sum of the two titrations was total acidity (Gill *et al.*, 2009):

$$\text{Acidity (meq/L/100g)} = \frac{\text{Volume of NaOH} \times \text{Normality}}{0.1} \times 100$$

Estimation of gastric ulcerative index changes in pyloric ligation model: The stomach was opened along the greater curvature and washed with running tap water (Gill *et al.*, 2011a). Then it was placed on a flat wooden plate to count the ulcerative area (Gill *et al.*, 2009). The ulcer index was determined by using the formula:

$$\text{Ulcer index} = \frac{10}{X}$$

Whereas X is total mucosal area/total ulcerated area.

Percentage ulcer protection was calculated using the formula:

$$\text{Ulcer protection \%} = \frac{100 - U_t}{U_c} \times 100$$

Whereas U_t is ulcer index of treated group, U_c is ulcer index of control group.

Statistical analysis: All the biochemical results were expressed as Mean±Standard Error of Means (SEM). Data were analyzed by Tukey's multiple test range using Sigma Stat Version-3.5 software. A probability value of $p < 0.05$ was considered to be statistical significant.

RESULTS

The phytochemical screening of fruit extract of *Prunus domestica* indicates the presence of triterpenoids, carbohydrates, coumarin glycoside and phenolic compound phytoconstituents. These results have been presented in Table 1.

Antioxidant potential of methanolic fruit extract of *Prunus domestica* was evaluated by 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide radical scavenging activity. The reduction capability of 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide radical was determined by the decrease in its absorbance at 517 and 230 nm, respectively. Maximum free radical scavenging activity of methanolic fruit extract of *Prunus domestica* was shown at a dose of 200 $\mu\text{g mL}^{-1}$ is 82.12 ± 0.654 by 1,1-diphenyl-2-picrylhydrazyl model as shown in Table 2 and 79.43 ± 0.876 by hydrogen peroxide model as shown in Table 3. Ascorbic acid was used as standard to compare the free radical scavenging activity of methanolic fruit extract of *Prunus domestica*.

Gastric secretion accumulated in the disease control group due to pyloric ligation. Free and total acidity estimated in the disease control group were 61.29 ± 3.914 and 109.15 ± 2.452 meq L^{-1} , respectively. Animals which are pretreated with methanolic fruit extract of *Prunus domestica* at the dose of 200 mg kg^{-1} has shown significant reduction in gastric volume 1.96 mL, free acidity 29.64 ± 0.934 meq L^{-1} , total acidity 62.61 ± 2.011 meq mL^{-1} and ulcerative index 1.7, which were comparable to the ranitidine treated group. These results have been presented in Table 4. At a concentration of 200 mg kg^{-1} methanolic fruit extract of *Prunus domestica* has shown significant reduction in ulcer i.e., 70.58% as compared with standard (ranitidine).

Table 1: Phytochemical screening of *Prunus domestica* fruit extract

| Plant constituent | Methanolic extract |
|--------------------|--------------------|
| Alkaloids | - |
| Carbohydrates | ++ |
| Triterpenoids | ++ |
| Coumarin glycoside | ++ |
| Sterols | - |
| Proteins | + |
| Flavonoids | + |
| Phenolic compound | ++ |

-: Absence, +: Minor presence, ++: High presence of the corresponding constituent

Table 2: Percentage inhibition of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) by extract and ascorbic acid

| Concentration ($\mu\text{g mL}^{-1}$) | Inhibition of DPPH radical (%) | |
|---|--------------------------------|-------------------|
| | Extract | Ascorbic acid |
| 100 | 32.85 ± 0.895 | 62.40 ± 0.327 |
| 150 | 58.00 ± 0.463 | 74.00 ± 0.856 |
| 200 | 82.12 ± 0.654 | 85.00 ± 0.476 |

Values are Mean±SEM of triplicate experiments

Table 3: Percentage inhibition of H₂O₂ (hydrogen peroxide) radical by extract and ascorbic acid

| Concentration (µg mL ⁻¹) | Inhibition of H ₂ O ₂ radical (%) | |
|--------------------------------------|---|---------------|
| | Extract | Ascorbic acid |
| 100 | 30.01±0.837 | 54.00±0.648 |
| 150 | 52.98±0.397 | 67.00±0.196 |
| 200 | 79.43±0.876 | 84.00±0.584 |

Values are Mean±SEM of triplicate experiments

Table 4: Effect of methanolic extract of *Prunus domestica* fruit pulp on ulcerative parameter in pyloric ligation model

| Groups | Gastric volume (mL/100 g) | Free acidity (meq L ⁻¹) | Total acidity (meq L ⁻¹) | Ulcerative index | Inhibition (%) |
|--------|---------------------------|-------------------------------------|--------------------------------------|------------------------|----------------|
| I | 1.68±0.092 | 31.42±0.646 | 60.92±1.949 | 0.0±0.000 | 0.00 |
| II | 3.61±0.046 | 61.29±3.914 | 109.15±2.452 | 5.1±0.000 | 0.00 |
| III | 1.42±0.012 | 29.42±1.963 ^a | 58.32±1.646 ^a | 1.1±0.381 ^a | 78.40 |
| IV | 2.42±0.014 | 38.96±2.941 ^b | 76.16±2.464 ^b | 2.4±0.934 ^b | 52.04 |
| V | 2.01±0.046 | 31.45±1.616 ^b | 69.41±1.963 ^b | 2.2±0.758 ^b | 56.86 |
| VI | 1.96±0.064 | 29.64±0.934 ^a | 62.61±2.011 ^a | 1.7±0.316 ^a | 70.58 |

Values are Mean±SEM, Statistical analysis was done by one-way ANOVA followed by Tukey's test to PL control group, ^{a,b}p<0.05 as compared to control and ranitidine treated group, respectively

DISCUSSION

In the present study, methanolic fruit extract of *Prunus domestica* was evaluated for its antioxidant activity followed by *in vivo* antiulcer activity. Many plant of the family such as *Prunus armeniaca*, *Prunus cerasus* and *Prunus dulcis* and *Prunus mume* etc. had shown to possess significant antioxidant potential, this review supports the presence of antioxidant potential in methanolic fruit extract of *Prunus domestica* (Yigit *et al.*, 2009; Wang *et al.*, 1999; Xia *et al.*, 2010). Prune juice from *Prunus domestica* had also shown significant antioxidant activity by inhibiting the oxidation of low density lipoprotein, but in present study antioxidant potential of methanolic fruit extract of *Prunus domestica* was evaluated by 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide radical scavenging activity (Donovan *et al.*, 1998). DPPH free radical after accepting an electron or hydrogen radical becomes stable molecule (Gusdinar *et al.*, 2011). Various human neurological and other disorders are due to free radicals or oxidative injury (Saha *et al.*, 2008). Many plant sources like vegetable, fruits, seeds flowers etc. are explored and have potent antioxidant activity (Sood and Muthuraman, 2009). Natural antioxidants are widely used because synthetic antioxidants are toxic and carcinogenic (Rahman *et al.*, 2011). Free radicals are responsible for stomach and duodenal ulcers. Thus, free radical scavengers are mostly emphasized these days to protect mucosal membranes. (Rachmilewitz *et al.*, 1994) Severe gastric mucosal lesions occurs in pyloric ligated animals due to accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa (Gill *et al.*, 2011b). Peptic-ulcer occurs due to imbalance between protective and aggressive factors (Gill *et al.*, 2010b). In contrast to synthetic drug the natural products are safer to mankind (Sood *et al.*, 2010). The phytochemical screening indicates the presence of flavonoids and phenolic compounds in methanolic fruit extract of *Prunus domestica*. The presence of flavonoids give basis for the evaluation of *Prunus domestica* fruit as antioxidant which is further responsible for its antiulcerogenic effect through free radical scavenging mechanism. Thus, the extract of *Prunus domestica* fruit can be employed as antioxidant and antiulcerogenic activity. Further, the fruit can be studied for its toxic and non toxic nature.

CONCLUSION

The methanolic fruit extract of *Prunus domestica* shows potent antioxidant and antiulcer activity in dose dependent manner and can be used as natural antioxidant and anti-ulcerogenic agent for human welfare.

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