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Hepatoprotective activity of *Prunus domestica* fruit extract against paracetamol-induced liver damage in Albino rats

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Abstract:
There is a lack of reliable hepatoprotective drugs in modern medicine to prevent and treat drug-induced liver damage. Fruits of Plum (P. domestica), belonging to family Rosaceae are used traditionally for their hepatoprotective effect. We wanted to evaluate the hepatoprotective activity of *Prunus domestica* synergistic hepatoprotection exists with silymarin.
Materials and Methods:
Albino rats (150–200 g) were divided into seven groups. Groups 1 and 2 were normal and experiental controls, respectively. Groups 3, 4, 5, 6 received the ethanol, methanol, chloroformand petroleum ether extract of P. domestica fruits 100 mg/kg BW/day, and Group 7 positive control silymarin (25 mg/kg, p.o.) respectively, for 10 days. Hepatotoxicity was induced in all on the eighth day with paracetamol 2 g/kg BW/day. The hepatoprotective effect was evaluated by performing an assay of the serum proteins, Serum bilirubin, SGPT, SGOT and ALP. The assay results were presented as mean and standard error of mean (SEM) for each group. The study group was compared with the control group by one-way ANOVA, followed by Dunnett's Multiple Comparison Test. A *P*-value of <0.01 was considered significant.
Results:
In groups 3, 4, 5, 6 and 7, liver enzymes and bilirubin were significantly ($P < 0.01$) closer to normal than in group 2. In extract treated groups; methanolic exteact of fruits of P. domestica treated rats showed significant decrease in SGOT level, SGPT level and ALP level which was less than disease control non-treated rats. In extract treated groups; methanolic exteact of fruits of P. domestica treated rats showed significant decrease in Serum bilirubin level and significant increased in serum protein level which was inversed than disease control non-treated rats
Conclusion:The P. domestica ethanolic fruit extract shows significant hepatoprotective activity and synergism with silymarin.

Keywords: Hepatoprotective, hepatotoxicity, P. domestica, paracetamol, silymarin

INTRODUCTION

The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug-induced hepatotoxicity is a major cause of iatrogenic diseases, accounting for all hospital admissions.[1]
Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health as well as to prevent, diagnose, improve or treat physical and mental illnesses. Herbal treatments are the most popular form of traditional medicine. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients.[2] However, no scientific data regarding the identity and effectiveness of these herbal products were available, except in the treatise of Ayurveda and Unani medicine.[3] The World Health Organization (WHO) has laid emphasis on promoting the use of traditional medicine for health care.[4] Hence, we see

afocus on research on traditional and herbal medicine, especially in developing countries, with individual as well as collaborative efforts by national research organizations.[4]

There is an acute necessity of reliable hepatoprotective drugs in modern medical practice.

Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced from the voluminous work on their hepatoprotective potentials.[5] More than 700 mono- and polyherbal formulations from over a hundred different plants are available for use.[6]

Prunus domestica (Plum), is a well known medicinal plant, which grows wild as well as in households and farms in India. It has been traditionally regarded as possessing rejuvenating, tonic and vitalizing properties that contribute to longevity and a healthy life.[7] Fruits of *P. domestica* possess expectorant, diaphoretic, antiseptic, spasmolytic, stimulant and anticatarrhal properties and are used as cold and cough remedies, for fever, pain,[8] gastrointestinal disorders (like dyspepsia, vomiting), worm infestations, skin diseases, snakebite and scorpion sting.[9,10,11]

we wanted to build on these findings in relation to paracetamol-induced hepatotoxicity and observe for a synergistic or an additive effect with the combination of Prunus domestica and a standard hepatoprotectant.

Thus, the aim of our study was to:Evaluate the hepatoprotective activity of Prunus domestica fruits on paracetamol-induced hepatotoxicity in albino rats as compared with silymarin.

MATERIALS AND METHODS

Experimental Animals

Healthy albino rats of Wistar strain (both male and female), weighing 100–200 g each (obtained from Central Animal House, BN College of Pharmacy, Udaipur) were given the standard diet with water *ad libitum* during the entire period of the experiment as per the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal facilities.[12]

Drugs

All drug suspensions were prepared for the different groups with 3% (W/V) aqueous suspension of gum acacia as vehicle.

Test drug

Prunus domestica ethanol, methanol, chloroform and petroleum ether fruit extract (PDE). This was prepared as follows:

The fruits of Prunus Domestica were collected, shade dried for 3 weeks, powdered mechanically and sieved through No. 20 mesh sieve. The fruits of Prunus domestica were extracted by successive solvent extraction method with the help of soxhlet apparatus. The plant material first extracted with petroleum ether for defatted then chloroform, methanol, ethanol. Rotary vacuum evaporator and dried at room temperature. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The percentage yield of methanolic extract was 12.75%W/W.

Standard hepatoprotective

Silymarin (SILY) powder (obtained from Micro Labs Ltd., Bangalore, India) was used to make the suspension in doses of 25 mg/kg BW for the respective groups following the method of Mankani *et al*. and Mansour *et al*.[13,14,15]

Hepatotoxin

Paracetamol (PCM) powder (I.P.) (obtained from Bharat Chemicals, Tarapur, Gujarat, India) was used to make the suspension in a dose of 2 g/kg BW for the respective groups.

Methods

The experiment was carried out on 30 healthy albino rats for 10 days. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week.

Grouping and Treatment Schedule

The rats were randomly divided into seven groups of six animals each after weighing, recording and numbering. Each group received treatment as follows:

Group-1(NC): Received water (5 ml/kg, p.o.) for 7 days once daily, and served as normal control.
Group-2 (DC): Received received distilled water 5ml/kg b.w. p.o. for 8 days. A single dose of paracetamol (2g/kg body weight, i.p.) was given after one hour of vehicle on 7th day, served as disease control.
Group-3 (PD-ETOH): Received methanolic extract (100 mg/kg) b.w. p.o. for 8 days. A single dose of paracetamol (2g/kg body weight, i.p.) was given after one hour of vehicle on 7th day, served as ethanolic extract treaded.
Group-4 (PD-MEOH): Received ethanolic extract (100 mg/kg) b.w. p.o. for 8 days. A single dose of paracetamol (2g/kg body weight, i.p.) was given after one hour of vehicle on 7th day, served as methanolic extract treaded.
Group-5 (PD-CHCl3): Received Chloroform extract (100 mg/kg) .w. p.o. for 8 days. A single dose of paracetamol (2g/kg body weight, i.p.) was given after one hour of vehicle on 7th day, served as chloroform extract treaded.
Group-6 (PD-PET): Received Petroleum ether extract (100 mg/kg) b.w. p.o. for 8 days. A single dose of paracetamol (2g/kg body weight, i.p.) was given after one hour of vehicle on 7th day, served as petroleum ether extract treaded.
Group-7 (PD-PC): Received standard drug silymarin (25 mg/kg, p.o.) for 8 days. A single dose of paracetamol (2g/kg body weight, i.p.) was given after one hour of vehicle on 7th day, served as positive control

Dosing and Administration of Drugs
The drug suspensions and the vehicle were administered per orally by an intra-gastric feeding tube at a uniform volume of 5 ml/kg BW.
Induction of Hepatic Injury
A single dose of paracetamol 2 g/kg BW/day was given to groups on the eighth day of the experiment. It was administered after overnight fasting of the animals, i.e. the diet was restricted 12 h prior to the administration of paracetamol. However, free access to water was permitted.[16]
Laboratory Assessments
On the 10th day, blood was collected from the hearts of the animals under light ether anesthesia. The blood was kept undisturbed for 30 min and the clot was dispersed with a glass rod. The samples were centrifuged for 15–20 min at 2000 rpm to separate the serum and then sent for liver function tests (LFT), namely serum proteins, Serum bilirubin, SGPT, SGOT and ALP.[17,18,19]
Statistical Analysis
Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P value considered as $P<0.05$ Significant (*), $P<0.01$ Very Significant (**), $P<0.001$ Highly Significant (***).

RESULTS
As per Table:1 in Paracetamol induced hepatotoxic model the extract treated rats showed significant hepatoprotective activity as comppaerd to nontreated hepatotoxic rats. In extract treated groups; methanolic exteact of fruits of P. domestica treated rats showed significant decrease in SGOT level (194.5 u/l), SGPT level (94.46 u/l) and ALP level (208.3 u/l) which was less than disease control non-treated rats 248.0 u/l, 157.7 u/l and 358.5 u/l respectively. In extract treated groups; methanolic exteact of fruits of P.domestica treated rats showed significant decrease in Serum bilirubin level (0.9697mg/dl) and significant increased in serum protein level (7.343gm/dl) which was inversed than disease control non-treated rats 2.857mg/dl and 4.547gm/dl respectively.
Table 1: Effect of methanolic extract of P. domestica and Silymarin pre-treatment on biochemical parameters of the rats intoxicated with Paracetamol.

Group No.	Treatment Dose	AST (SGOT)	ALT (SGPT)	ALP (IU/mL)	Total Billirubin	Total Protein

		(IU/mL)	(IU/mL)		(mg/dl)	(gm/dl)
1	NC (10mg/dl)	166.1± 0.5921***	63.11± 0.2515 ***	192.3± 1.504***	0.3683± 0.004773 ***	9.585± 0.07628***
2	DC (25mg/kg)	248.0± 0.5584***	157.7± 1.345***	358.5± 0.4955 ***	2.857± 0.02472***	4.547± 0.1388***
3	PD-ETOH (100 mg/Kg)	181.0± 0.3699***	88.68± 0.6425***	186.0± 1.174***	0.5467± 0.01256***	8.918± 0.05218***
4	PD-MEOH (100 mg/Kg)	194.5± 1.255***	94.46± 0.5866***	208.3± 0.3220***	0.9697± 0.005959***	7.343± 0.1573***
5	PD-$CHCl_3$ (100 mg/Kg)	205.8± 1.509***	108.9± 0.4162***	246.2± 1.481***	1.538± 0.06489***	6.342± 0.06009***
6	PD-Pet. Ether (100 mg/Kg)	229.2± 0.5712 ***	116.2± 1.485 ***	288.1± 0.4954***	2.120± 0.03733***	5.532± 0.1331***
7	PC (25mg/kg)	177.9± 0.3356***	87.09± 0.4723 ***	167.8± 1.079***	0.4583± 0.009458***	9.437± 0.06946 ***

Statistical Analysis:
Data are expressed as Mean ± SEM and analyzed statistically by One way ANOVA followed by Dunnett's Multiple Comparison Test, using Graph Pad Prism Software trial version. IN Dunnett's Multiple Comparison Test, Group DC was compared with NC and other treated groups were compared with DC. P value considered as $P<0.05$ Significant (*), $P<0.01$ Very Significant (**), $P<0.001$ Highly Significant (***).

DISCUSSION

The administration of paracetamol to the animals resulted in a significant fall in the levels of total serum proteins and albumin globulin ratio and a significant rise in serum ALP, AST and ALT. In groups 3, 4, 5, 6 and 7, the toxic effect of paracetamol was partly reversed in the animals. Compared with the paracetamol (experimental control) group, the 3 and 4 groups showed a significant increase in the serum ALP, AST and ALT levels. Also significant difference was observed in the total protein levels in these groups. Group 3 & 4 in comparison with silymarin (standard) showed a significant decrease in the serum AST and ALT alone. Thus, group 3 & 4 showed greater hepatoprotection than other groups, considering the results of the Liver Function Test.

PCM, used as a tool to induce hepatotoxicity in experimental animals, leads to covalent bonding of its toxic metabolite N-acetyl P bezoquinoneimine to sulfydryl groups of proteins. This causes exhaustion of reduced glutathione in the liver, resulting in cell necrosis and lipid peroxidation.[20] An increase in the level of transaminases and ALP is an indication of cellular leakage and loss of functional integrity of the hepatic cell membranes.[21,22,23]

Administration of the ethanoilc and methanoilc extract of *Prunus domestica* fruits showed significant hepatoprotective activity, as shown previously in other studies.[10] Plums are an excellent source of vitamin A, calcium, magnesium, iron, potassium and fiber. Plums are free of sodium and cholesterol. Like all fruit plums contain a substantial amount of vitamin C.[24,25,26]

The dried fruit, known as prunes, is a safe and effective laxative and stomachic. Plum has active constituents named amygdalin and prunasin, those having strong antioxidant and antimicrobial properties.[10] *Prunus domestica* contains many phenolic and dihydroflavonol compounds showed better antioxidant, antiseptic and cell regenerative potentials.[27,28,29] Moreover, the fixed oil of *Prunus domestica* contains chologenic acid, which is responsible for its anti-inflammatory activity.[30,31] Hence, chologenic acid may also be responsible for reversing the inflammatory features associated with hepatic injury thus adding to the hepatoprotective effect.

CONCLUSION

Thus, the fruits of Plum (Prunus domestica) have highly significant ($P < 0.01$) hepatoprotective activity. The *Prunus domestica* group showed better hepatoprotection than the disease control group.

Silymarin is a well-known standard hepatoprotective, whereas presence of impurities in the *Prunus domestica* extract may have caused a lower hepatoprotective effect.

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