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Hepatoprotective Effects of Selected Plant Extracts on Paracetamol-Induced Hepatotoxicity in a Rat Model

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Abstract

This study sought to assess the protective effects of prunus dulcis shell skin ethanol extract in relation to paracetamol-induced hepatotoxicity in rats. The rats were divided into five groups, namely the control, paracetamol (2g/kg), low dose, high dose PDEE (100, 200 mg/kg) and silymarin with paracetamol groups. Results showed that acute intraperitoneal injection of of paracetamol induced significant (p0.05) alterations in the serum levels of lipids Results showed that acute intraperitoneal injection of 800 mg/kg of acetaminophen induced significant (p0.05) alterations in the serum levels of AST ALT. In the paracetamol overdose group, the liver architecture showed necrotic changes, hydropic degeneration, congestion, and dilatation of central veins. This hepatocellular damage was confirmed by a significant increase of AST, ALT levels. However, pretreatments with 100 and 200 mg/kg significantly (p0.05) alterations in the serum levels of AST ALT and improved the antioxidant activity.

Keywords: Paracetamol, Liver toxicity, Prunus Dulcis, lipid oxidation, and antioxidant activity.

Introduction

According to Asrani et al. (Citation 2019), pharmaceuticals are one possible cause of liver disease, which causes over 2 million deaths annually worldwide. Drug-induced liver disease is uncommon, but because of its unpredictable nature and potentially lethal course, it nonetheless poses a significant clinical concern. Up to 20% of acute liver failure in children and a comparable proportion of acute liver failure in adults are caused by drug-induced liver illness. Although the precise number of cases is hard to estimate, between 40,000 and 45,000 persons may suffer from drug-induced liver damage annually [1-3]. Through a process called bioactivation, the liver frequently turns a medication hazardous through its typically operating enzymes and processes.

Paracetamol (PCM) is a clinically significant medication that has been linked to liver damage. Despite being the leading cause of acute liver failure in Western nations, paracetamol (PCM), also known as Tylenol in the USA, is the most widely used over-the-counter painkiller and antipyretic and is available without a prescription in the majority of countries (Du et al., Citation 2016). In many nations, paracetamol-induced hepatotoxicity continues to be the leading cause of abrupt liver failure. In both humans and animals, a paracetamol overdose can result in severe liver disease, liver necrosis, and kidney damage [4-7]. Much work has been done to comprehend the processes underlying paracetamol's

harmful effects because of the public's concern over its hepatotoxicity. Numerous studies show that oxidative stress plays a role in a number of paracetamol-related toxicity issues, such as liver illness brought on by PCM.

For paracetamol-overdosed patients, the US Food and Drug Administration (USFDA) advises N-acetylcysteine (NAC), a well-known antioxidant, as the sole treatment option; however, this drug has drawbacks, such as side effects and a limited therapeutic window.

In order to support clinical efficacy, the 21st century has witnessed a paradigm shift towards the therapeutic evaluation of herbal products in liver disease models by carefully combining the advantages of the traditional medical system with the contemporary idea of evidence-based therapeutic screening, authentication, and randomized placebo-controlled clinical trials. Despite the enormous progress, there is still no significant and secure hepatoprotective medication on the market [8-11]. As a result, the development of hepatoprotective drugs that are mostly plant-based and effective against a variety of liver illnesses has received the attention it deserves on a global scale.

Almonds, or Prunus dulcis, are origonally from western Asia (Pakistan to the eastern Mediterranean), although they are now cultivated in every continent. The almond nut is cultivated commercially for its edible qualities. Almonds contain many nutrients that are beneficial to cardiovascular health, including vitamin E, monounsaturated fatty acids, polyunsaturated fatty acids (PUFA), arginine, potassium, and magnesium. Amonds are a great source of vitamin E, specifically RRR-tocopherol [12-15]l. Flavonols (isorhamnetin), flavanones (naringenin), anthocyanins (cyanidins and delphinidins), procyanidins, and phenolic acids (caffeic acid, ferulic acid, P-coumaric acid, and vanillic acid) are just some of the phenolic compounds found in abundance in almond skin. Almonds contain several useful substances, but their primary components are proteins like amandin and albumin and amino acids including arginine, histidine, lysine, phenylalanine, leucine, valine, tryptophan, methionine, and cysteine.

Materials and methodology

CMC, Flavonoid, Liv.52 tablets, Gries reagent, sodium potassium phosphate buffer (pH-8), NaoH, Amonium molybdate, Ellman's reagent procured from UV Scientifics.

Experimental animals:

Male Wister rats wereighing 150-200g we used and procured from national centre for lab animal sciences, National Institution of Nutrition, Hyderabad, India. They were housed in groups of six under environmentally controlled conditions with 12-h light/dark cycle and had free access to food and water. After seven days of acclimatization period, they were randomly selected for different experimental groups.

All experimental procedures were carried out accordance with committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines. All the experimental procedures were approved by the institutional animal ethical committee.

Collection of plant

Prunus dulcis nut shells were collected from various areas. The plant material was identified by a Botanist, a voucher herbarium specimen is deposited at the herbarium of the Department of Pharmacy.

Preparation of Extracts

Drying *Prunus dulcis* plant for 2 days at room temperature in the shade, then drying it at [40–50 °C] for 3–4 hours, yielded a coarse powder that was then ground. The Soxhlet extraction method was used to get extracts from 980 grams of *Prunus dulcis* powder [16].

Methodology

- The animals are randomly divided into six groups of 6 rats each.
- Group-I: CMC 2ml/kg (p.o)
- Group-II: Paracetamol 2g/kg (p.o)
- Group-III: PDEE 100mg/kg(p.o)+ Paracetamol 2g/kg(p.o)
- Group-IV: PDEE 200mg/kg(p.o)+ Paracetamol 2g/kg(p.o)

- Group-V: SILIMARYN 3mg/kg+ Paracetamol 2g/kg(p.o)

Wistar rats of either sex weighing 150-200g are used. Paracetamol 2g/kg body weight is administered orally as a single dose. The animals are given the test drug for 6 days prior to paracetamol administration and on the seventh day along with paracetamol. The animals are sacrificed after 24hrs and the blood/serum is used for biochemical analysis, and the liver for histopathological studies.

Biochemical analysis

After recording the ECG, blood samples were collected, and the animals were sacrificed. The serum was separated by centrifugation at 2500 rpm at 30 ºC for 15 minutes and used for the biochemical analysis. Immediately after sacrifice, liver tissues were excised,washed in ice-cold saline, blotted free of blood and tissue fluids, Then the liver tissue was weighed accurately, and homogenized in 5 mL of 0.1 M Tris-HCl buffer (p^H7.4) in ice-cold condition. The homogenate was centrifuged at 2500 rpm and the clear supernatant solution was taken for the estimation of biochemical parameters [17].

Results

Plant Extraction:

The weight of the extract was 13.8 grams. Its percentage yield was found to be 11.50%

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of the ethanolic extract of the whole plant indicated the presence of flavonoids, glycosides, and saponins. The results have been tabulated in Table no. 1.

Table 1 concentration of Phytochemicals in the Extracts of Prunus dulcis

S.No	Chemical Test+	PDEE
1	Alkaloids	++
2	Amino Acids	++
3	Flavonoids	++
4	Glycosides	++
5	Gums and Mucilage	+
6	Proteins	-
7	Carbohydrates	++
8	Steroids	++
9	Tannins	--
10	Terpenoids and Saponins	-
11	Polyphenolic Compounds	+++

The PDEE (Prunus dulcis ethanol extract) effects on liver weight in rats with paracetamol-induced hepatotoxicity are presented in table .Significant differences in the liver weight were obsereved after hepatotoxicity induced by pretreatment with the PDEE, in both groups. The liver index was increased after paracetamol treatment but restored to normal values after PDEE pretreatment. It also have shown effect on different organs as shown in the below table 2.

Effect of Prunus dulcis on paracetamol induced hepatitis regarding body weights of different samples of wister rats. Negative control has increased body weight. Whereas high dose and low dose have equivalent effect to that of control group.

Table: 2. Effect of Prunus dulcis on organ weights

Values are expressed as Mean ± S.E., n=6

Group	**Body weight**	**Liver weight**	**Heart weight**	**Kidney weight**	**Lungs weight**
Control	210 ±2.70	6.275±0.17	2.350±0.23	6.850±0.12	4.32±0.12
Negative control	246±2.00	7.750±0.31	2.200±0.11	6.950±0.13	4.40±0.20
Low dose ()	233±2.30	7.125±0.12	2.325±0.22	6.850±0.57	4.310±0.23
High Dose ()	222±2.40	6.510±0.08	2.2125±0.21	6.925±0.35	4.330±0.30
Standard	208±3.10	6.3425±0.11	2.400±0.36	6.875±0.50	4.310±0.32

Effect of Prunus dulcis on paracetamol induced hepatitis regarding organ weights of different samples of wister rats. Negative control has increased organ weight only in case of liver. Whereas high dose and low dose have equivalent effect to that of control group in case of liver only. All other organ weights remained constant. The detailed numerical related to different organ weights given in table no: 8

Fig no: 1 shows the effect of Prunus dulcis on SGOT, SGPT (AST ALT) LDH activities in wister rats subjected to paracetamol induced hepatotoxicity. The negative control group, significantly differ from all other groups in each cases. The detailed numerical related to different parameters (SGOT, SGPT, LDH)

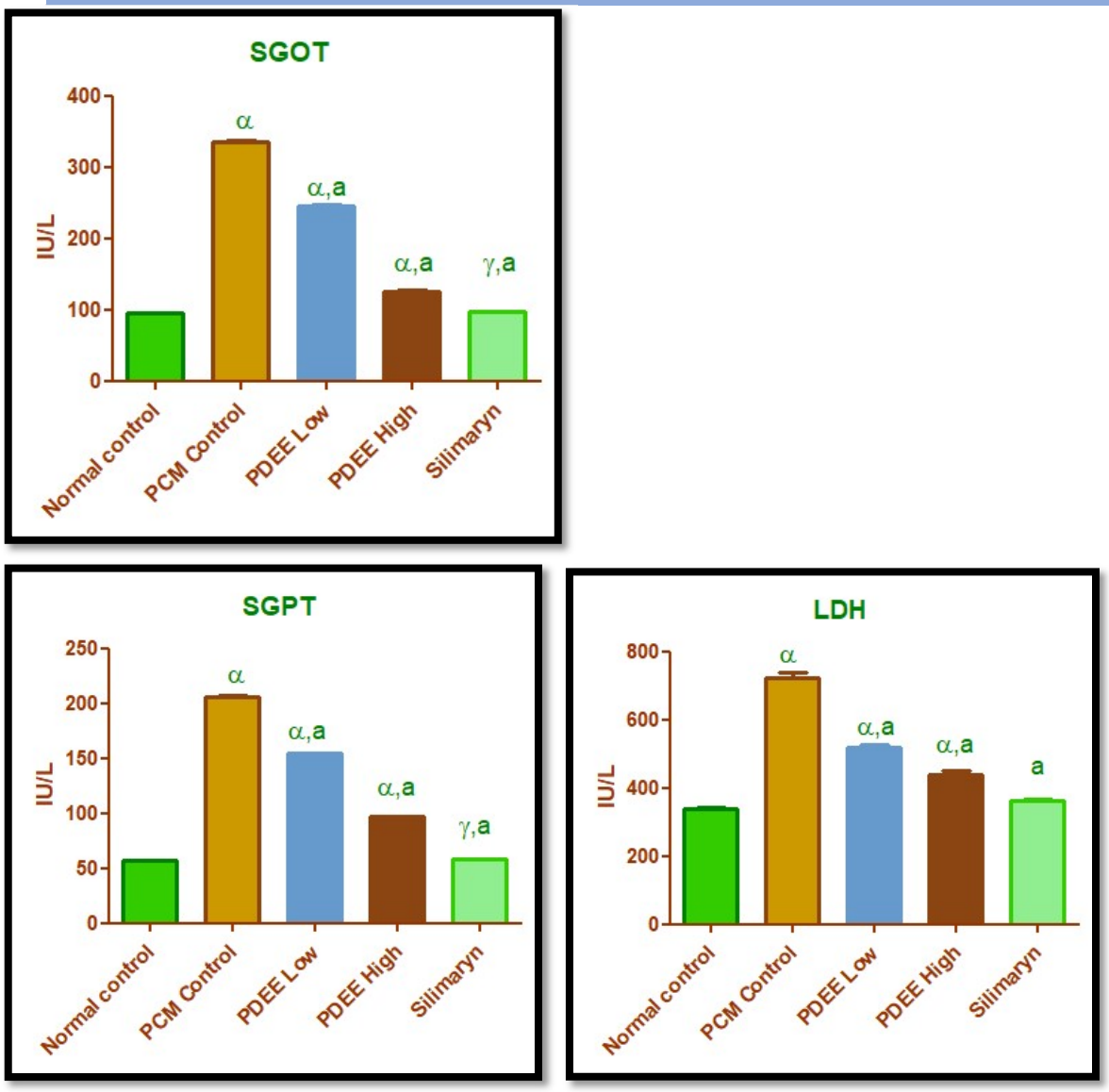

Fig no:1 the effect of Prunus dulcis on SGOT, SGPT, LDH activities in Wistar rats subjected to paracetamol-induced hepatotoxicity.

The effect of Prunus dulcis on total protein, tissue nitrate, GSH, catalase in Wistar rats subjected to paracetamol-induced hepatotoxicity. The negative control group significantly differs from all other groups in each case. The detailed numerical data related to different parameters is given in Table 10

Table no. 2 the effect of Prunus dulcis on total protein, tissue nitrate, GSH, catalase in Wistar rats subjected to paracetamol-induced hepatotoxicity.

Group	**Total Protein (g/dl)**	**Tissue Nitrate (mg/mg protein)**	**GSH (µg/mg protein)**	**Catalase (µmole of H_2O_2 consumed/mg protein)**
Control	6.46±0.23	9.20±0.05	12.51±0.14	36.32±1.68

Negative control	3.16±0.14	14.0±0.22	4.75±0.25	14.96±0.17
Low dose ()	4.56±0.06	13.1±0.12	6.79±0.19	23.83±0.08
High Dose ()	5.73±0.12	11.76±0.20	8.52±0.26	27.80±0.60
Standar d	5.96±0.03	9.95±0.15	9.96±0.03	31.59±0.83

Values are expressed as Mean ± S.E., n=6,HISTOPATHOLOGY:

The liver tissue was dissected out and fixed in 10% formalin, dehydrated in a graded ethanol series (50-100%),cleared in xylene, and embedded in paraffin wax.

The sections, which were 5-6 mm thick, were then prepared using a rotary microtome and stained with hematoxylin and eosin dye for microscopic observation of histopathological changes in the liver. Next, the liver sections were scored and evaluated**.**

Light microphotographs of hematoxylin and eosin stained sections of the formalin fixed liver cells of normal rats. The liver cells of Group 1 rats (normal) have well-preserved hepatic cells with a prominent nucleus and nucleolus. The Group II rats (exposed to paracetamol) exhibited extensive fatty changes, characterized by disruption of the hepatocyte lattice structure, damaged hepatic sinusoids, and necrosis. Presences of reticular strands is visible and nucleuses of two to three are joined together. Liver cells of Group III rats (exposed to paracetamol and low dose of test drug), have shown minimal disruption of the hepatic cellular structure compare with only paracetamol-treated Group II.**Group** IV rats (exposed to paracetamol and high dose of test drug), have shown very less disruption of the hepatic cellular structure compare with only paracetamol-treated Group II and maintained a nearly similar structure to the control Group I. Group V rat (exposed to paracetamol and the standard drug) have hepatic cells with good cytoplasm, prominent nuclei, and nucleoli, as shown in figure 2.

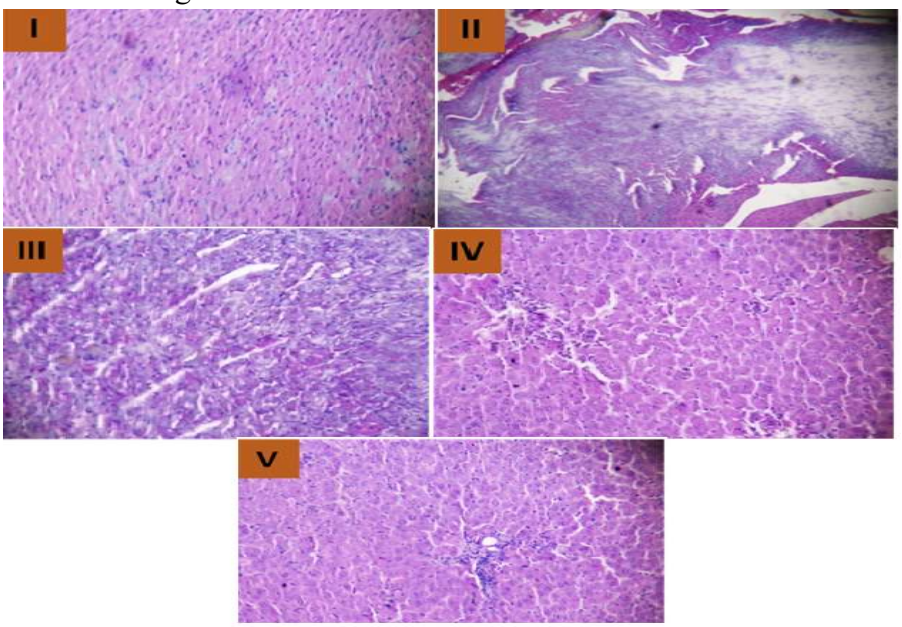

Fig no:2 histopathology of liver

DISCUSSION

In the present study, administration of paracetamol (2 g/kg, p.o.) to fasting rats resulted in massive increases in both serum ALT and AST levels. Several researchers have reported elevations in serum transaminases following administration of toxic doses of paracetamol in rats. Owing to their high concentrations and ease of liberation from the hepatocyte cytoplasm, ALT and AST are sensitive indicators of necrotic lesions within the liver.18,20 Hence, the marked release of transaminases into the circulation is indicative of severe damage to hepatic tissue membranes during paracetamol intoxication. Paracetamol induced hepatic injury is considered one of the most commonly used models and reliable method for screening of hepatoprotective agents [18,19].

In the present study, it was found that PDEE exerted a strong protective effect against paracetamol-induced hepatotoxicity in Wistar rats. It was noted that the test drug PDEE has shown very little disruption of hepatic cellular structure when given at a high dose when it was compared with that of the paracetamol-treated group. However, at the low dose of PDEE it was found to have minimal disruption of hepatic cellular structure when it was compared to the paracetamol treated group. The present study results shown that the test drug had a hepatoprotective effect against paracetamol induced hepatotoxicity [20,21].

The observed significant decrease in serum AST ALT in the PDEE and silymarin-treated groups demonstrated their hepatoprotective effects against paracetamol damage. Similar results have been reported by other investigators. The protective effects of silymarin and PDEE could be explained via the preservation of membrane integrity mediated by their observed antioxidants effects [22]

Histological examinations of liver sections of rats subjected to paracetamol hepatotoxicity revealed degenerative changes that involved the hepatocytes and cells lining the blood sinusoids. The damage extended to the majority of the hepatic lobule, with a marked loss of its normal pattern. These changes positively correlated with the noted increases in transaminase activities.

CONCLUSION

In the present study, the nutritional value, biological activities, and phytochemical profile of almonds, a popular and important medicinal and dietary nut with a long history of use, are examined. The abundant micronutrient polyphenols play an important role in protection against chronic degenerative diseases. Hence these all give evidence to our present study where the extract had showed the hepatoprotective action against Paracetamol induced hepatotoxicity Such effects can be correlated directly with its ability to reduce lipid peroxidation and enhance the antioxidant defense status.

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