World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF SEEDS OF PRUNUS DULCIS

***Shalima N. K., Ayswarya K., Shitha G., Ayisha Nitha P., Aiswarya G. K., Aparna P., Dr. Biju C. R., Dr. Byju K., Dr. G. Babu and Nimmi M.**

Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Chelembra 673634, Malappuram District, Kerala.

***Corresponding Author: Shalima N. K.**
Department of Pharmaceutical Chemistry, Devaki Amma Memorial College of Pharmacy, Chelembra 673634, Malappuram District, Kerala.

Article Received on 27/11/2024 **Article Revised on 17/12/2024** **Article Accepted on 07/01/2025**

ABSTRACT
The present study was conducted to evaluate the hepatoprotective activity of ethyl acetate extract of seeds of *prunus dulcis* The extract at the dose of 200 mg/kg and 400 mg/kg b. wt. was tested for its hepatoprotection, by inducing hepatotoxicity with CCl_4 in Wistar albino rats and using silymarin (100 mg/kg) as the reference standard. Biochemical parameters like, SGOT, SGPT, SALP and serum bilirubin were determined to assess the hepatoprotective effect. The extract has shown significant hepatoprotection in albino rats in reducing SGOT, SGPT, SALP and serum bilirubin levels.

KEYWORDS: Hepatoprotection, Prunus dulcis, Aging, Cirrhosis.

INTRODUCTION

Plants and their secondary metabolites have a long history of use in modern 'western' medicine and in certain systems of traditional medicine. Plants are used in traditional system of medicine for the management of liver disorders. However many of them have not investigated for their described effects. *Prunus dulcis*, belonging to the family Rosaceae, is one such medicinal plant used in the treatment of liver disorders in folk medicine. The active compounds that have been reported are flavones, glycosides, steroids, saponins, triterpenoids and other secondary metabolites.[1] It also has anticancer, antibacterial, antihistaminic, antidiabetic activitie[2], etc. This has triggered the authors to evaluate the hepatoprotective activity of ethyl acetate extract of *prunus dulcis* against liver intoxication by CCl_4 in wistar albino rats. Biochemical parameters like, SGOT, SGPT, SALP and serum bilirubin were determined to assess the hepatoprotective effect. The study revealed that ethyl acetate extract significantly reduced SGOT, SGPT, SALP and serum bilirubin levels. The preliminary findings suggest that the plant *prunus dulcis* possess potential hepatoprotective activity. The present study scientifically validated the traditional use of *prunus dulcis* for liver disorders.

MATERIALS AND METHODS

Plant material

The seeds of *prunus dulcis* was collected in the month of December and was authenticated by Dr. M Sabu, Professor and Head, Department of Botany, Calicut University, Malappuram, Kerala, and a voucher specimen was deposited in the Department (Specimen No: 107892).

The powdered plant material (1 Kg) was extracted with chloroform, ethyl acetate and distilled water separately, in a Soxhlet apparatus, by continuous hot extraction method. Each extracts were then concentrated under reduced pressure on a rotary evaporator to dryness to give the crude residue. The crude residues were employed for further investigation.[3] The extracts were subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, proteins and amino acids, fixed oils and fats, gums and mucilage, alkaloids, phytosterols, flavanoids, tannins and phenolic compounds, saponins, triterpenoids[4], etc.

Animals

Albino mice of Swiss strain and albino rats of Wistar strain were used for toxicological studies and pharmacological studies respectively. Female mice selected were nulliparus and non-pregnant. Female mice weighing 25 to 30 g and rats of either sex weighing 125 to 150 g were used for the study. Each animal, at the commencement of its dosing, was between 8 and 12 weeks old and their weight variation was within ± 20% of the mean weight of any previously dosed animals. The temperature in the experimental animal room was

22ºC (± 3ºC) and the relative humidity was between 50-60%. These animals were fed with pellet diet manufactured by Amrut laboratory, Animal Feed Company, Sangli, Maharashtra and drinking water ad libitum. They were kept in 12 h/12 h light/dark cycle and maintained for at least 5 d prior to dosing to allow for acclimatization to the laboratory conditions. The animal experimental protocol has been approved by OACE of U win life science, Malappuram, where the animal studies were carried out.

Acute toxicity study

Acute toxicity study was carried out as per OECD 423 (Organization of Economic Cooperation and Development) guidelines. Swiss albino mice weighing 20-25g, were selected and fastened overnight. The selected animals were grouped as three in one group. The animals were and the test sample PD01 was given orally at a starting dose of 5mg/Kg body weight and observed for a period of 2 h and occasionally for 4 h to detect any toxic signs and mortality. Since no mortality was observed, same dose was repeated with another group of animals. The procedure was repeated for doses of 50, 300 and 2000 mg/kg in separate group of animals. The experiment was repeated for seven more days and also for fourteen days, no change was observed from the experiment.[5] So the maximum dose of 2000 mg/Kg can be used as safe dose. From this result $1/10^{th}$ and $1/5^{th}$ values of 2000 mg/Kg were taken for further studies.[6]

Hepatoprotective activity

Albino rats of Wistar strain weighing 125-150 g of either sex were used for the study. They were housed in polypropylene cages with not more than six animals per cage and maintained under standard conditions. Thirty rats were divided into five groups of six animals each. Group I was given a single daily dose of Carboxy Methyl Cellulose (1 ml of 1% w/v, p.o. b. wt.).[7] Group II received carbon tetrachloride (1 ml/kg b. wt., sc.1:1 v/v mixture of CCl_4 and liquid paraffin) was given for every 72 h for 14 days.[8] Group III received Silymarin, a known hepatoprotective compound, at a dose of 100 mg/Kg .p.o., along with carbon tetrachloride for every 72 h for 14 d.[9] While group IV and V received orally, 200 and 400 mg/kg b. wt. of PD 01 and PD 02 in 1 %w/v, CMC respectively[10], along with carbon tetrachloride as in group II.

Replenishing a known quantity of fresh food daily at 8.00 a.m. and thereby measuring the food intake of the previous day and carried out measurement of daily food consumption. Body weight of rats was recorded weekly to assess percentage of weight gain of each animal. Animals were kept starved overnight on the last day. On the next day, after recording the weight of each animal, they were euthanized by decapitation under ether anesthesia, by making an incision on jugular vein; blood was collected in sterile centrifuge tubes and allowed to clot. The liver was dissected out immediately, rinsed with ice cold phosphate buffer and homogenized with 5% formalin solution immediately after removal from the animal to avoid decomposition. Embedding in paraffin wax was carried out by removal of water using alcohol from 30-100% and then stained with hemotoxylin, which has an aqueous base. The sections were dehydrated using increasing concentration of alcohol and then stained with eosin. They were treated with diphenylxylene (DPX) and examined under the microscope.[10]

Serum was separated from the collected blood by centrifugation and subjected to various biochemical estimations like serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), and serum bilirubin.

Statistical analysis

Statistical analysis was performed by one way (ANOVA) followed by using results were expressed as mean ± the standard error of the mean (SEM) for 6 rats in each group. $P< 0.05$ was considered significant.

RESULTS AND DISCUSSION

Carbohydrates, tannins, flavonoids, saponins, and coumarins were found to be present in the ethyl acetate extract of *Prunus Dulcis* Seeds. CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases and proves highly useful as an experimental model for the study of acute hepatic injury.[11] In this study, CCl_4 administration to rats lead to marked elevation in the levels of serum enzymes like, SGOT, SGPT, SALP and serum bilirubin level. The Table 1 shows that CCl_4 causes significant increase in SGOT value from control 188.99 ± 9.36 to 640.25 ± 11.24, SGPT value from 74.25 ± 4.32 to 388 ± 12.25, SALP value from 402.54 ± 3.65 to 645.25 ± 11.25, and serum bilirubin value from 0.28 ± 0.01 to 1.45 ± 0.03. This might be due to release of these enzymes from the cytoplasm, into the blood stream rapidly after rupture of the plasma membrane and cellular damage[12]. Treatments with ethyl acetate extract of seeds of *Prunus Dulcis*(200 mg/kg and 400 mg/kg) significantly reduced the levels of these marker enzymes in CCl_4 treated rats, when compared with standard drug Silymarin.

This implies that the extract tends to prevent liver damage, suppresses the leakage of enzymes through cellular membranes, preserves the integrity of the plasma membranes and hence restores these enzymes levels. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes.[13]

Effective control of SALP, and serum bilirubin levels point toward an early improvement in the secretary mechanism of the hepatic cells. Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver.[14] So, the result of present

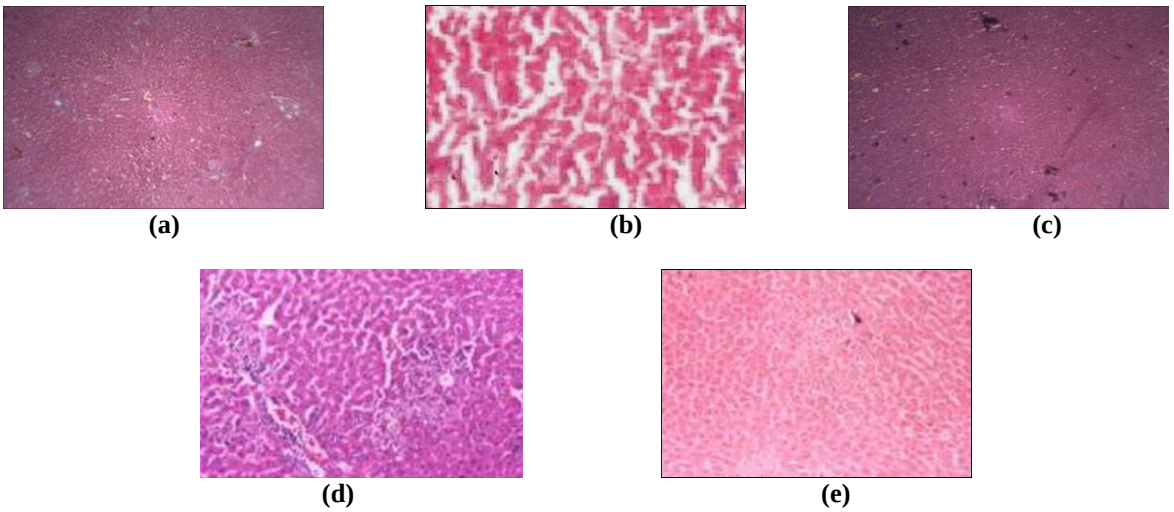
investigation indicates that the ethyl acetate extract of *Prunus Dulcis* possess good hepatoprotective activity. Serum total bilirubin and total protein levels on other hand are related to the function of hepatic cell.

The above fact is supported by histopathological studies. The Fig 1 indicates that the normal architecture of liver was completely lost in rats treated with CCl_4 with the appearance of vacuolated hepatocytes and degenerated nuclei. Section of liver in Silymarin treated group shows liver parenchyma with intact architecture. Section of liver in test drug treated groups (200 and 400 mg/Kg) shows intact architecture, few regenerative hepatocytes, and scattered mononuclear inflammatory cells which is similar to Silymarin treated group. The livers of rats treated with PD01 and PD02 showed a significant attenuation from CCl_4 induced liver damage as evident from normal hepatocytes with well defined nuclei. This study also supported the hepatoprotective activity of liver.

Table 1: Biochemical changes in *albino rats* treated with ethyl acetate extract of rhizomes of *Curculigo orchioides* against CCl_4 induced hepatic injury.

S. No.	Groups	SGOT	SGPT	ALP	Bilirubin
1	Control	188.99 ± 9.36	74.25 ± 4.32	402.54 ± 3.65	0.28 ± 0.01
2	CCl_4	640.25 ± 11.24	388 ± 12.25	645.25 ± 11.25	1.45 ± 0.03
3	CCl_4 + Silymarin	195.68 ± 6.25***	60.26 ± 2.56***	305.26 ± 5.65***	0.30 ± 0.01***
4	CCl_4 + PD01 (200 mg/Kg)	360.25 ± 2.55***	210.44 ± 4.45***	458.44 ± 1.15***	0.48 ± 0.50**
5	CCl_4 + PD02 (400 mg/Kg)	185.26 ± 8.08***	70.44 ± 2.05***	300.25 ± 2.50***	0.22 ± 0.01***

Values are the mean ± S.E.M of six rats/treatment, *P<0.05, **P<0.01 and ***P<0.001 significance compared to CCl_4



(a) **(b)** **(c)** **(d)** **(e)**

Fig 1: Histopathological changes in liver of albino rats. Hematoxylin and Eosin (x100).

(a) Rats treated with normal saline 1 ml/kg b. wt.: Normal structure of hepatic lobes is seen; white spot indicates the presence of vacuoles, a prominent blood vein with normal appearance.

(b) Rats intoxicated with CCl_4 1 ml/kg b. wt.: Massive fatty changes are seen; necrosis, ballooning degeneration and broad infiltration of the lymphocytes are seen.

(c) Rats treated with CCl_4+silymarin 100 mg/kg b. wt.: Drastic recovery of hepatic parenchyma, mild congestion and micro vesicular changes are seen.

(d) Rats intoxicated with CCl_4 + 200 mg/kg b. wt.: Marked recovery of hepatic cells, mild congestion and micro vesicular changes are seen.

(e) Rats intoxicated with CCl_4 + 400 mg/kg b. wt.: Drastic recovery of hepatic parenchyma, mild congestion and micro vesicular changes are seen.

CONCLUSION

From the above results, it may be concluded that the ethyl acetate extract of seeds of *Prunus Dulcis* is non-toxic and is safe. As the preliminary studies indicated, the extract possesses significant hepatoprotection, and after carrying out a thorough study of clinical trials, the plant may be considered as a low cost, potent, herbal liver tonic.

ACKNOWLEGEMENT

The authors are sincerely thankful to the Manager, Devaki Amma Memorial College of Pharmacy, Chelembra, for providing the facilities for carrying out the work.

REFERENCES

1. Bafna AR and Mishra SH. Methanolic extract shows immunostimulatory effect on immunosuppressed mice. Journal of ethnopharmacology, 2006; 104: 1-4.

2. Anuj kumar and Sanjaya kumar panda. Phytochemical screening of root tubers of *Prunus dulcis*. Journal of Chemical and Pharmaceutical Research, 2010; 2(2): 107-11.
3. Hari Babu B et al. Phytochemical and Antimicrobial screening of leaves of Givotia rottleriformis Griff. Journal of Pharmacy Research, 2011; 47(7): 2146-2148.
4. Harborne JB. Phytochemical Methods, Chapman & Hall, London, 1983; 3rd Ed: pp. 1-3.
5. Paget GE and Barnes JM. Evaluation of Drug Activities, In: Laurence, D.R. and Bacharach, A.L. (Eds.), Pharmacometrics, Academic Press, London, 1983; 1: 115.
6. Sharada AC *et al*., Toxicity of *Withania somnifera* root extract in rats and mice. *International Journal of Pharmacognosy*, 1993; 31(3): 205-212.
7. Rajesh SV *et al*., Effect of *Clausen dentata* (Willd.) M. Roem. against paracetamol induced hepatotoxicity in rats. *Pakistan Journal of Pharmaceutical Sciences*, 2009; 22(1): 90-91.
8. Mishra SH and Sureshkumar SV. Hepatoprotective effect of *Pergularia daemia* (Forsk.) ethanol extract and its fraction. Indian Journal of Experimental Biology, 2008; 46: 447-452.
9. Biswadev B *et al*., Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl_4 intoxicated mature albino rats. Journal of Toxicological Sciences, 2002; 27(3): 139-146.
10. Dianzani MU. Biochemical aspects of fatty liver, In: Meeks, R.G., Harrison, S.D. and Bull, R.J. (Eds.). Hepatotoxicology, CRC Press, Boca Raton, FL, 1991; 327-399.
11. Clawson GA. Mechanisms of Carbon Tetrachloride Hepatotoxicity. Pathology and Immunopathology Research, 1989; 8(2): 104-112.
12. Sallie R et al., Drugs and the liver. Biopharmaceutics and Drug Disposition, 1991; 12: 251-259.
13. Thabrew M. and Joice P. A comparative study of the efficacy of Pavetta indica and Osbeckia octanda in the treatment of liver dysfunction. Planta Medica, 1987; 53(3): 239-241.
14. Moss DW and Butterworth PJ. Enzymology and Medicine, Pitman Medical Co., London, 1974; pp.139-141.