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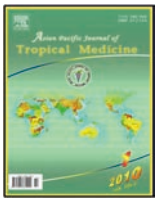

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Hepatoprotective effect of *Premna corymbosa* (Burm. f.) Rottl. & Willd. leaves extract on CCl_4 induced hepatic damage in Wistar albino rats

Karthikeyan M[1*], Deepa K[2]

[1]*Department of Pharmacology, Al Shifa College of Pharmacy, Poonthavanam,Kizhattur(PO),Perintalmanna, Malappuram district, Kerala State – 679329, India*

[2]*Department of Pharmaceutics, Nehru college of Pharmacy, Pambady,Triruvilwamala , Thrissur district, Kerala State – 680 597, India*

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ABSTRACT

Objective: To investigate the hepetoprotective activity of *Premna corymbosa* leaves against carbon tetrachloride (CCl_4) induced hepatic damage. **Methods:** Hepatotoxicity was induced in wistar rats of both sexes by intraperitoneal injection of CCl_4, 1 mL/kg body weight for every 72 h. The ethanolic extract of *Premna corymbosa* leaves were administrated at doses of 200 & 400 mL/kg body weight, p. o., daily for 14 days. The hepatotoxicity and its prevention was assessed by serum markers like serum alkaline phosphatase (SALP), serum triglycerides (STG), serum total protein (STP), serum cholesterol (SC), and liver wet weight and histopathological studies of the liver. **Results:** In treatment with the ethanolic extract, the toxic effect of CCl_4 was controlled significantly ($P<0.01$) by restoration of the levels of biochemical parameters as compared to normal and standard drug silymarin treated groups. The liver weight was reduced by the ethanolic extract treated groups. The histopathology of the liver sections evidenced the hepatoprotective activity. **Conclusion:** The ethanolic extract of the leaves of *Premna corymbosa* possess significant acute hepatoprotective activity. *Premna corymbosa* can be recommended for the liver disorders.

1. Introduction

Premna corymbosa (Burm.f.) Rottl. & Willd. Syn *Cornutia corymbosa* (Burm.f.) (Verbenaceae) is widely distributed through out India in the plains. It is commonly known as munna in ayurvedic system of medicine in kerala [1]. The useful parts of the plants are whole plant, roots, root bark, stem and leaves [2]. The leaves are used in ayurveda, siddha, and other traditional system of medicine in India. The roots decoction are used as, antiinflammatory, cardiotonic, antibacterial. The leaves are stomachic carminative, and galactagge, and are useful in dyspepsia, colic, flatulence, agalactiae, cough, fever, rheumatalgia, neuralgia, heamorrhoids and tumours [3]. The phytochemical studies revealed the presence of stem bark contains premnine, ganiarine, resin, spermine, alphandrine, tannins , betulin and β-sitosterol; leaves contains β-sitosterol and luteolin;stem contains betulin and β-sitosterol[1-5]. In the prevous study, the hydroalcaholic extract of the roots of *Premna corymbosa* Rottl. possess antihyperglycemic effect on both normoglycemic and alloxan induced hyperglycemic rats [6] and the aqueous extract of the leaves of *Premna corymbosa* showed sedative activity and anti-hyperlipidemic activity [7,8]. The Premna corymbosa is used in the ayurvedic system of medicine for treatment of hepatopathy. Therefore the aim of the present study was to evaluate the protective effect of *Premna corymbosa* which was investigated in CCl_4 induced liver damage in rats. The literature survey revealed that there is a lack of scientific report regarding hepatoprotective activity of the leaves of *Premna corymbosa*. Hence the present study was designed to investigate the hepatoprotective effect of ethanolic extract of *Premna corymbosa* against CCl_4 induced liver damage in experimental rats.

2. Materials and methods

2.1. Plant material

Premna corymbosa (Burm.f.) Rottl. and Willd. were collected from Plant Anatomy Research Center (PARC), Chennai in December 2006. It was identified by Prof. P. Jayaraman, Director, PARC, Institute, West Tambaram, Chennai, Tamil nadu, India. A voucher specimen (No: PARC/07/SRM/31) was deposited in the herbarium of the institute.

*Corresponding author: Karthikeyan M, M. Pharm., D.S.M., Senior Lecturer Department of Pharmacology,Al Shifa College of Pharmacy, Poonthavanam, Kizhattur(PO), Perintalmanna, Malappuram district, Kerala State-679329, India.
Tel: +91 9656111669
E-mail: karthikeyanpgt@gmail.com

2.2. Preparation of the ethanolic extract

The leaves of the tree were washed well with water, dried under shade and powdered to fine grade by using laboratory scale mill. A batch of 500 g of powdered material was subjected to extraction in a soxhlet apparatus at 50 - 60 ℃ for 36 hours of 6 cycles (6 hours per cycles) in 99 % (v/v) of 2 liters of absolute ethyl alcohol. The extracted material was concentrated over a heating mantel maintained at 50 ℃ until greenish semi solid masses were obtained. The yield of the product was approximately 18.39 % (w/w) of the dry leaves of *Premna corymbosa* (Burm.f.) Rottl. and Willd. The final product was stored in vacuum desiccators at room temperature until analysis. For administration, the extract was suspended in 2 % of tween 80, to required concentrations.

2.3. Animals

The rodents used in the experiments were Wistar albino rats of both sex (150–220 g) which were procured from the inbred stock of the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai, Tamil Nadu, India. They were housed in well ventilated polypropylene cages at controlled temperature of (24 ± 1) ℃, with a 12 h light/12 h dark cycle and they are given standard pellet diet and water ad libitum. The mice were assimilated to laboratory conditions for 7 days. Animals were kept under fasting for over night, but allowed free access of water before commencement of experiments. The experiments were conducted according to the ethical norms approved by ministry of social justice and empowerment, Government of India and the study was got approved from the Institutional Animal Ethical Committee (IAEC) (Approval No.MPL/09/IAEC.9/2007) of Committee for the Purpose ad Control and Supervision of Experiments on Animal (CPCSEA).

2.4. Drugs, chemicals and instruments

The following drugs and chemicals were used for the study: Drugs and Chemicals; Silymarim (Serum International Ltd, Pune, India.), Sodium Chloride (Loba chemicals, Mumbai, India.), Ethyl Alcohol (Changu&Hu, China.), Liquid Paraffin (Rankem,Mumbai, India.), Tween 80 (Ranbaxy Fine Chemicals, Mumbai, India.), Formalin(Rankem, Mumbai,India.). Kits; Total protein (Span Diagnostics Ltd. Sachin, India.), Cholesterol Kit (Ranbaxy Fine Chemicals, Mumbai, India.), Alkaline PhosphataseKit(Merck SpecialtiesPvt.Ltd., Mumbai,India.), TriglyceridesKit (RanbaxyFine Chemicals, Mumbai, India.). Instruments; Cooling centrifuge (REMI, India.), Auto analyzer (Mayson 500e, India.).

2.5. Acute toxicity studies

Acute toxicity studies were carried out as per the guidelines of Organization for Economic Co-operation and Development (OECD). Acute toxicity of the plant extract was carried out using groups of Swiss albino mice by administering orally, while control group received normal saline. The toxicological effects were assessed on the basis of mortality and behavioral changes [9].

2.6. CCl_4-Induced liver damage

Rats weighing 150–220 g were divided into five groups of six animals in each group. Group I (Normal Control) animals were administered a single daily dose of liquid paraffin or tween 80 (1mL/kg body weight, p. o.). Group II (Negative Control) received Carbon tetrachloride (1 mL/kg body weight, i. p.). Group III–IV (Test groups) were administered *Premna corymbosa* ethanolic extract of 200 mg/kg & 400 mg/kg body weight, p. o., for administration, the extract was suspended in 2 % of tween 80, daily once a day, simultaneously the animals were treated with CCl_4. Group V (Positive Control) received Silymarin at a dose of 100 mg/kg body weight, p. o. [10–12], along with the CCl_4. Treatment duration was 14 days. Dosage of carbon tetra chloride was administered as 1:1 dilution with liquid paraffin[13] for every 72 hours. Animals were sacrificed 48 hours after the last injection. Blood was collected, allowed to clot and serum separated. Liver was dissected out, weighed and used for biochemical and histopathological studies [14,15].

2.7. Assessment of hepatoprotective activity

The rats were sacrificed after 48 hours of last dose by cervical decapitation. The blood samples were collected separately by cardiac puncture and allowed to clot coagulates for 30 min at room temperature. The clear serum was separated at 2 500 rpm for 10 min. The biochemical parameters like serum constituents: serum alkaline phosphatase(SALP), serum triglycerides (STG), serum total protein (STP), serum cholesterol (SC), were determined by standard kits by using auto analyzer and liver weight wet weight was taken.

2.8. Histopathological studies

The liver tissues was collected and immediately fixed in 10 % formalin saline for proper fixation. These tissues were processed and embedded in paraffin wax sections of 4 to 6 microns in thickness were cut stained with hematoxylin and eosin (H–E) dye for photomicrocscopic observations.

2.9. Statistical analysis

The statistical analysis of all the result was carried out using one– way ANOVA followed by Dennett´s multiple comparison using graph pad instat 3 software and all the results obtained in the study were compared with the control group. P value <0.05 was considered statistically significant.

3. Results

3.1. Acute toxicity studies

The behavior of the treated mice appeared to be normal. No toxic effect was observed to 10 times of the effective dose of the PCEE and there was no mortality of the animals and it was found to be safe.

3.2. Effect of ethanolic extracts on SALP, STG, STP, SC and liver weight

The results of hepatoprotective effect of the ethanolic extract on CCl_4 intoxicated rats was shown in Table 1. In CCl_4 treated negative control group, the SALP, SC and liver weight were increased to 85.01 IU/L, 111.48 mg/dL & 5.99 g and decreased in STG, STP to 82.03 mg/dL, 6.29 g/dL, meanwhile the values shown in the normal control group are 46.71 IU/L, 42.11 mg/dL, 4.16 g, 110.12 mg/dL and 10.21 g/dL, respectively, in contrast the test groups treated with ethanolic extract 200 & 400 mg/kg body weight decreased significantly (P<0.01) the elevated levels of SALP, SC and liver weight and increased significantly (P<0.01) the decreased levels of STG and STP towards normalization in dose dependent manner. The treatments with 400 mg/kg body weight of ethanolic extract showed high significant activity. This was almost compared to the groups treated with silymarin a potent hepatoprotective drug used as reference standard and to control group.

3.3. Histopathological observations

The histopathological studies of the liver showed fatty changes, swellings, necrosis with loss of heaptocytes in CCl_4 (negative control) group rats in comparison to normal control rats. The histology of the liver sections of normal control animals (Figure 1)

showed normal heptatic cells with preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. The liver sections of CCl_4 intoxicated rats (Figure 2) showed fatty changes, necrosis, infiltration of the lymphocytes and Kupffer cell around the central vein. CCl_4 – induced group was more server than other groups. The histopathological architecture of liver sections of rats treated with *Premna corymbosa* ethanolic extract of 200 & 400 mg kg/body weight (Figure 3–4) showed more or less normal lobular pattern with a mild degree of necrosis and lymphocyte infiltration almost comparable to the normal control and the silyamrin treated group (Figure 5) showed more or less to normal control with minimal inflammatory conditions.

Table 1
Effect of ethanolic extract of *Premna corymbosa* leaves on CCl_4 induced hepatotoxicity in Wistar albino rats.

Group	Treatments	SALP (IU/L)	SC (mg/dL)	STG (mg/dL)	STP (g/dL)	Liver weight (g)
Normal control	Liquid paraffin (1 mL/kg)	46.71 ± 0.44	42.11 ± 1.02	110.12 ± 1.77	10.21± 0.58	4.16 ± 0.16
Negative control	CCl_4(1 mL/kg)	85.01 ± 1.29*	111.48 ± 1.93*	82.03 ± 1.56*	6.29 ± 0.18*	5.99 ± 0.24**
Ethanolic extract	200 mg /kg	41.25 ± 0.59**	33.06 ± 1.79*	108.24 ± 0.94^{NS}	11.50 ± 0.31^{NS}	4.4 ± 0.40 ^{NS}
	400 mg /kg	32.64 ± 1.36*	62.40 ± 0.84*	91.06 ± 1.20*	10.23 ± 0.40^{NS}	4.8 ± 0.26 ^{NS}
Positive control	Silymarin(100 mg /kg)	40.44 ± 2.057*	32.86 ± 0.65*	95.56 ± 0.74*	9.15 ± 0.33^{NS}	4.39 ± 0.21^{NS}

[b]Values are expressed in Mean ± S.E.M.(n = 6); Difference between groups were statistically analyzed by one-way ANOVA; *P<0.01,**P<0.05, Dunnett´s test as compared to normal control.

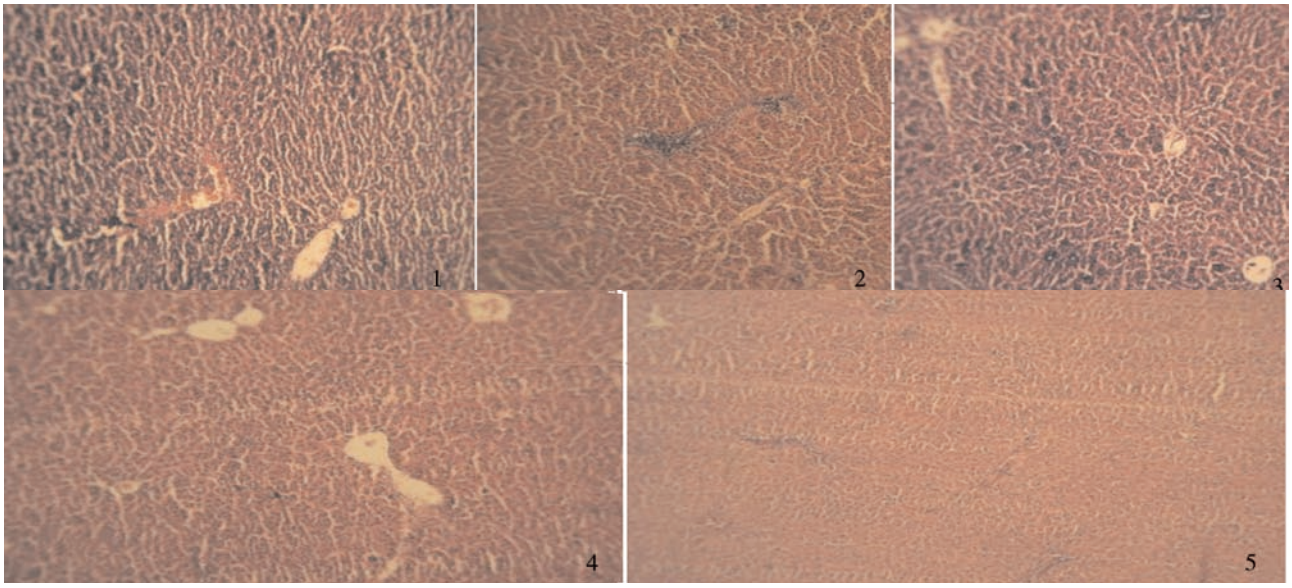

Figure1. Group I: Liver sections of normal control rats: Showing cords of hepatocytes around the central vein; prominent nucleus and nucleolus (H&E staining, 100 × expansion).

Figure 2. Group II: Liver section of the negative control: Showing liver with focal hepatocytic damage and inflammatory collection (H&E staining, 100 × expansion).

Figure 3. Group III: Liver section of rats treated with extract 200 mg/kg body weight,p.o.: Showing minimal inflammatory collection and damaged hepatocytes (H&E staining, 100 × expansion).

Figure 4. Group IV: Liver section of rats treated with extract 400 mg/kg body weight,p.o.: liver appearing normal no foci of damage or inflammation collection (H&E staining, 100 × expansion).

Figure 5. Group V: Liver section of rats treated with silymarin 100 mg/kg body weight,p.o.: Liver appearing near to normal (H&E staining, 100 × expansion).

4. Discussion

The present study showed *Premna corymbosa* ethanolic extract possess hepatoprotective activity against the CCl_4 induced liver damage in wistar albino rats. In the acute toxicity study the LD_{50} of the Premna corymbosa ethanolic extract is high (No death even with 10 times of the effective dose) indicating the high margin of safety. It is well established that CCl_4 induces hapatotoxicity by metabolic activation; there fore it selectively causes toxicity in liver cells maintaining semi normal metabolic functions. CCl_4 is bio transformed by the cytochrome P 450 system in endo plasmic reticulum to produce trichloromethyl free radical. Tri chloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than tricholoromethyl radical. Thus, trichloromethyl peroxyl radical leads to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis and finally, results in cell death. These results in changes of the strucutres of the endoplasic recticulm and membrane, loss of enzymes metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6- phosphatase activation, leading to liver damage [16]. In the present study, treatment with the ethanolic extract made the SALP, SC, STC, STP and the liver weight to more or less towards normalization indicates that ethanolic extract significantly (P<0.01) protects the liver injury induced by CCl_4 in dose dependent manner and the hisopathological results also support the hepatoprotective potency of the ethanolic extract.

In conclusion, the results of the present work clearly demonstrate with histopathological evidence the hepatoprotective property of Premna corymbosa ethanolic extract. The phytochemical study revealed the presence of β-sitosterol or luteolin in leaves. The β-sitosterol showed protective effect of the liver in liver injury induced by CCl_4 in rats [17]. Luteolin was reported to be a strong antioxidant against reactive oxygen species *in vitro* and hepetoprotective activity against hepatotoxicity induced by CCl_4 in rats [18]. The above class of compounds from the *Premna corymbosa* leaves may contribute the hepatoprotective activity. Further studies are required to isolate, characterize and find out the mechanism of action of the active compounds in ethanolic extract responsible for hepatoprotective activity.

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