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Antihepatotoxic effect of *Premna spinosa* Leaf in Acetaminophen-Induced Liver Damage to Rats by Restoring Hepatic Structure, Functions and Oxidative Stress

Biswakanth Kar[1*], Sanjib Bhattacharya[2]

[1]School of Pharmaceutical Sciences, Siksha O Anusandhan Deemed to be University, Bhubaneswar 751003, Odisha, India
[2]West Bengal Medical Services Corporation Ltd., Salt Lake City, Kolkata 700091, West Bengal, India
***Corresponding author**: biswa_kanth2005@yahoo.co.in

Abstract

Premna spinosa L. (Lamiaceae), is a small shrub/tree and applied traditionally in India for different medicinal purposes. The present work screened the antihepatotoxic effect of methanol extract from *P. spinosa* leaf (MEPS) against acetaminophen-evoked hepatic lesions in rats. Hepatic toxicity was elicited in Wistar albino rats via single oral administration of acetaminophen at the dose of 640 mg/kg body weight. Then MEPS was given to rats at the doses of 200 and 400 mg/kg body weight orally for 16 consecutive days. Silymarin (200 mg/kg body weight) similarly was employed as reference drug. Hepatoprotective effect was assessed by determination of hepatic functions viz. SGPT, SGOT, ALP, billirubin, total protein; liver oxidative stress biomarkers viz. reduced glutathione, lipid peroxidation, catalase, superoxide dismutase and histopathological examination of liver. In MEPS treated rats, the foregoing liver functions and oxidative stress parameters were significantly restored, when compared to acetaminophen control. Histopathological study of the liver tissue demonstrated maintenance of normal hepatocellular architecture in MEPS treated rats as compared with acetaminophen control, thus affirming the protective role of MEPS in acetaminophen-induced hepatic toxicity in rats. From the current work in can be inferred that, *P. spinosa* leaf possessed marked antihepatotoxic potential in acetaminophen-invoked hepatic damage in rats by virtue of its preserving liver structure, functions and antioxidant capacity.

Keywords: *Premna spinosa*, acetaminophen, antihepatotoxic, silymarin, leaf.

Introduction

The higher plants have traditionally been used in the treatment of several diseases including hepatic disorders. Liver is the major organ of metabolism of nutrients and toxicants. Liver damage elicited by toxic drugs and chemicals has been established as very common complication leading to a series of grave aftermath with major metabolic dysfunctions resulting in morbidity. Till now, there is no effective synthetic medicine to safeguard liver (1). Due to their wide spectrum of health-promoting properties, such as antioxidant and hepatoprotective effects, dietary/medicinal and aromatic plants and plant-obtained natural compounds (phytochemicals) have been utilized for a long time and have attracted significant attention recently (2,3).

Acetaminophen, also chemically known as N-acetyl-para-aminophenol or paracetamol in generic, is a frequently used non-steroidal painkiller and antipyretic that causes acute liver damage in mammals when taken in excess. Acetaminophen undergoes hepatic metabolism via sulphate and glucuronide conjugations. Acetaminophen causes hepatotoxic metabolites in the liver, and liver cytochrome P-450 enzymes transform acetaminophen into the high reactive metabolite viz. N-acetyl-P-benzoquinone imine (NAPQI). Typically, the mercapturic acid pathway conjugates reduced glutathione (GSH) to eliminate NAPQI. However, when NAPQI generation surpasses the corresponding detoxification by GSH in the liver during a toxic overdose, it causes oxidative stress that results in the oxidative degradation of tissue macromolecules such as lipids or sulphydryl (-SH) groups of proteins or enzymes and disrupts the homeostasis of the tissue redox system which in turn, triggers inflammatory tissue damage (2,4,5).

Modern medicine has greatly benefited from the use of phytochemicals, which are components of dietary, medicinal and aromatic plants. The main advantages of plant-based pharmaceuticals seem to be their established effectiveness, decreased incidence of serious adverse events and reduced expenses. As a result, the majority of people on the planet can now afford to use medicinal plants and their traditional medicinal products to address their fundamental healthcare needs. The undiscovered treasure of the plant kingdom can still be an interesting viable source for the development of effective therapeutic products (6,7).

Premna spinosa L. (Lamiaceae), locally called as Gandana Saga in Odia, is a small shrub or tree traditionally applied in Odisha, India for the treatment of various disorders. All parts of this plant have traditionally been used in Odisha, India to treat inflammation and diabetic complications (8). Reported chemical constituents from this plant include flavonoids like luteolin, alkaloids like premnine, triterpenes and sterols like lanosterol (9-11). Previous researchers reported hypolipidemic, immuno-modulatory, anti-inflammatory and antidiabetic activities of *Premna spinosa* (12-15). Nonetheless, this plant has still not been scientifically investigated for its hepatoprotective activities. Therefore, the current work attempts to determine the anti-hepatotoxic effect of methanol extract of the leaf of *P. spinosa* in acetaminophen-induced albino rats along with pertinent biochemical and histological parameters.

Materials and methods

Plant material

In May 2021, the fresh mature leaves were plucked from *Premna spinosa* L. (Lamiaceae) grown at the Utkal University campus. The species was authenticated by Dr. Puspanjali Misra, Department of Botany, Utkal University, Odisha, India and a reference specimen (Ref. No. RC/CNU-069) was deposited at the same place for preservation. The leaves collected were washed thoroughly with tap water, followed by distilled water then the plant material was air-dried. The shade-dried leaves were ground into a coarse powder which was preserved in an air-tight container for use in the study.

Extraction

The ground leaves (200 g) was extracted successively with n-hexane and methanol by means of a Soxhlet apparatus. The marc was similarly extracted twice with methanol. Then the methanol extracts were combined, filtered and then evaporated to dryness in vacuo at 45°C. The dried extract thus collected (MEPS, yield: 7.33%) was placed within a refrigerator for use in the experiments.

Preliminary phytochemical assessment

Preliminary phytochemical experiments were performed on MEPS for detecting the presence of respective phytoconstituents (15).

Chemicals and drugs

Acetaminophen was procured from M/s Intas Pharma Ltd., Ahmedabad, India and silymarin was obtained from M/s Natural

Remedies, Bengaluru, India. The rest of the reagents and chemicals used in the present study were of analytical mark commercially purchased. The biochemical test kits were procured from Autospan (Arkray), Gujarat, India.

Animals

Throughout the hepatoprotecive investigation, Wistar male albino rats of 150–180 g weight were employed. With no more than six rats per cage, the animals were housed in polypropelene cages under conventional laboratory settings, which included a temperature of 25±2°C and a 14/10-hour cycle of light and dark. They were given unrestricted access to food and water. Before the trial started, the rats were given a week to get used to the lab environment. The experimental protocol for usage of animals in the current study was ratified by the Institutional Animal Ethics Committee, School of Pharmaceutical Sciences, Siksha O Anusandhan University, Bhubaneswar, Odisha 751003, India (Reg. No. 1171/PO/Re/S/08/CPCSEA).

Acute toxicity study

The albino Swiss mice were employed in an acute oral toxicity evaluation of MEPS in accordance with the Organization for Economic Co-operation and Development standards 425 (16).

In vivo experimental design

The investigational rats were classified into five groups (n = 6). The group 1 served as normal control (got *p.o*. 0.9% w/v NaCl). The groups 2-5 received a single dose of acetaminophen (640 mg/kg, *p.o*.), the group 2 served as toxin (acetaminophen) control. On 45 minutes of acetaminophen receiving, the group 3 and 4 received MEPS *p.o*., at both doses of 200 and 400 mg/kg body weight, respectively on each day for 16 successive days. The group 5 was administered with the reference drug silymarin at the dose of 200 mg/kg body weight; *p.o*. similarly for 16 consecutive days (17). Following 24 h of the last treatment and 18 h of starving, all rats were immolated by cervical dislocation. Their blood and livers were obtained for the biomarker estimations and histo-pathological studies.

Determination of liver function parameters

Serum glutamic pyruvic transaminase (SGPT), glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), bilirubin and total protein were determined from the collected blood, by using commercially available kits mentioned under the drugs and chemical subheadings.

Determination of liver oxidative stress parameters

Rats that had been sacrificed had their livers removed right away, cleansed with ice-cold isotonic saline, blotted dry, and weighed. A 1.15% KCl solution was used to create a 25% (w/v) tissue homogenate, which was subsequently centrifuged for one hour at 3000 g. Using the commercially available kits listed under the drugs and chemicals subheading, the supernatant liquid was used to estimate antioxidative parameters such as reduced glutathione (GSH), lipid peroxidation (LPO, expressed as malondialdehyde MDA), catalase (CAT) and superoxide dismutase (SOD).

Histopathological observation

Each group's rats' livers were removed and cleansed with isotonic saline. Then, 10% buffered neutral formalin and bovine serum albumin solutions were used to fix the tissues. After that, these underwent the paraffin embedding process in order to be sectioned using a rotary microtome. The sections were rigorously produced using alcohol-xylene solutions, acquired at a thickness of 50 µm, and doubly stained with alum-haematoxylin and eosin. Good sections were mounted after that and examined under a compound microscope to determine the histological changes.

Statistical analysis

The values are given as mean ± SEM (standard error of mean). The data were assessed statistically as per one-way analysis of

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variance (ANOVA) with Dunnett's post hoc test. The P < 0.001 were considered as statistically significant.

Results and discussion

Preliminary phytochemical assessment

The preliminary phytochemical studies demonstrated that MEPS contained flavonoids, alkaloids, triterpenoids and steroids.

Acute toxicity

The mice administered up to the oral dose of 4000 mg/kg b.w. of MEPS showed no signs of toxicity. For the current investigation, therefore, two MEPS dosages 200 mg/kg and 400 mg/kg b.w. were used.

Liver function parameters

When rats were given a single high dosage of acetaminophen, their serum total protein content decreased in comparison to animals in the normal control group, but their serum transaminase activities (SGPT and SGOT) and alkaline phosphatase (ALP) activity with bilirubin level increased. The animals given MEPS and reference silymarin showed a significant ($p < 0.001$) increase in total protein content and a significant ($p < 0.001$) decrease in higher blood hepatic function markers such as ALP, SGPT, SGOT and bilirubin (Table 1). This showed that the liver function had returned to normal.

Table 1. Influence of MEPS on enzymatic activities, total bilirubin and protein contents of normal and acetaminophen-intoxicated rats.

Treatments	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/100 ml)	Total protein (mg/dL)
Normal control	54.06 ± 1.11	24.31 ± 1.42	10.06 ± 1.26	1.12 ± 0.19	7.73 ± 0.66
Acetaminophen (640 mg/kg)	140.19 ± 7.32#	125.33 ± 5.61#	44.89 ± 3.64#	3.58 ± 0.22#	3.85 ± 0.21#
MEPS (200 mg/kg)	105.32 ± 1.55*	77.89 ± 3.58*	27.33 ± 3.17*	2.56 ± 0.22*	5.30 ± 0.62*
MEPS (400 mg/kg)	77.69 ± 2.14*	52.05 ± 3.21*	16.19 ± 2.21*	1.61 ± 0.17*	6.02 ± 0.40*
Silymarin (25 mg/kg)	58.88 ± 1.25*	29.02 ± 1.96*	12.05 ± 1.22*	1.17 ± 0.21*	6.49 ± 0.80*

The data are expressed as mean ± SEM (n = 6), # acetaminophen control group *vs*. normal control group ($p < 0.001$), * all treated groups *vs*. acetaminophen control group ($p < 0.001$).

Liver oxidative stress parameters

Toxic overdose of acetaminophen markedly lowered the activities of endogenous antioxidative enzymes namely SOD, CAT and non-enzymatic antioxidant (GSH) with elevated lipid peroxidation (LPO/MDA) in the induced hepatic tissue, as noted in acetaminophen (toxin) control rats. MEPS treatment and significantly ($p < 0.001$) restored all the foregoing parameters in dose related way, as demonstrated in silymarin also (Table 2).

Table 2. Influence of MEPS on lipid peroxidation, GSH and CAT of normal and acetaminophen-intoxicated rats.

Treatments	Lipid peroxidation (MDA, nano m/mg wet tissue)	GSH (g/mg wet tissue)	CAT (M of H2O2 decomposed/min/ mg wet tissue)	SOD (U/mg wet tissue)
Normal control	21.13 ± 1.58	5.40±0.24	1.13±0.07	10.6±0.74
Acetaminophen (640 mg/kg)	97.22±4.34#	2.06±0.14#	0.45±0.08#	6.1±0.49#
MEPS (200 mg/kg)	55.78±2.53*	3.31±0.19*	0.82±0.07*	8.6±0.41*
MEPS (400 mg/kg)	43.09±1.38*	4.11±0.28*	0.98±0.11*	9.5±0.54*
Silymarin 25 mg/kg	30.64±3.50*	5.10±0.34*	0.99±0.03*	10.2±0.73*

The data are expressed as mean ± SEM (n = 6), # acetaminophen control group *vs*. normal control group ($p < 0.001$), * all treated groups *vs*. acetaminophen control group ($p < 0.001$).

Histopathological observation

The histopathological study of normal control group rat's livers exhibited typical hepato-cellular architecture i.e., definite hepatic cells (hepatocytes), sinusoidal spaces and central vein (Fig. 1A). The acetaminophen-induced rat livers showed non-arrangement of normal hepatocytes with centrilobular and interlobular necrosis, inflammatory and fatty alterations detected with cytoplasmic vacuolization (Fig. 1B). The rats treated with both the doses of MEPS (Figs. 1.C and D) and reference silymarin (Fig. 1.E) showed signs of recovery from acetaminophen toxicity in their livers, including a predominance of typical hepatocellular architecture, a decrease and lack of necrosis and little inflammatory changes close to the central vein.

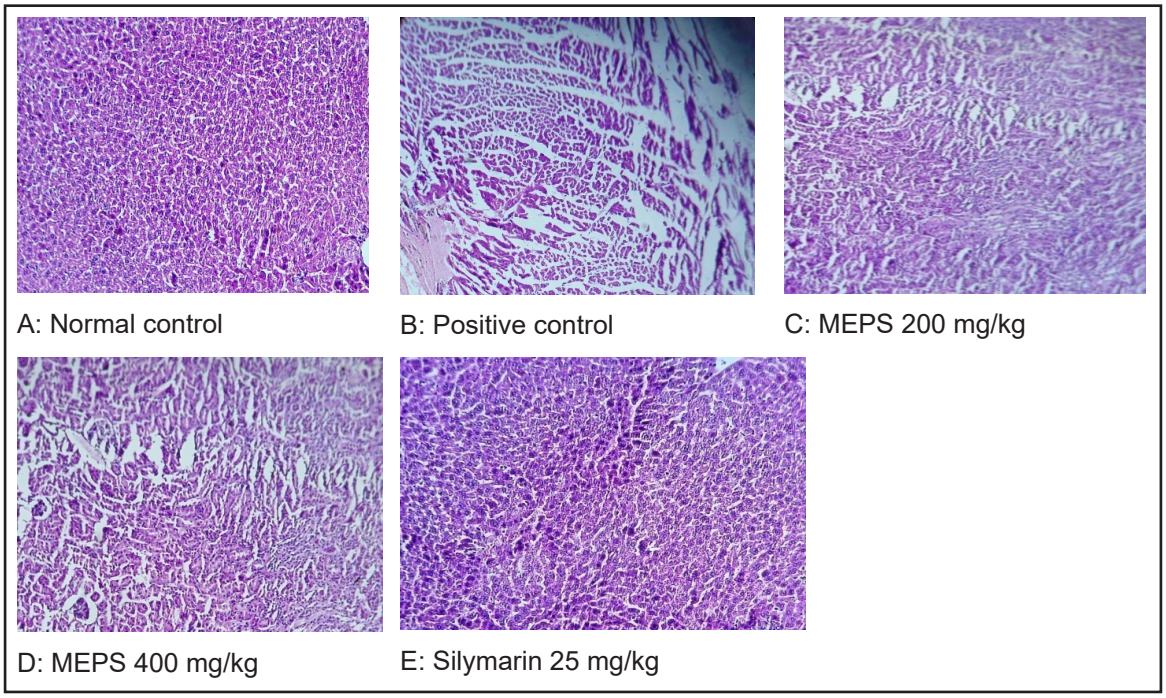

Fig. 1. Liver sections A; normal control, B; Liver section of acetaminophen-induced rat with big necrosis, C; Liver section of MEPS (200 mg/kg) treated rat, with decrease in necrosis, D; Liver section of MEPS (400 mg/kg) treated rat with signs of recuperation and E; Liver section of reference silymarin (200 mg/kg) treated rat with the signs of recovery.

Discussion

Liver is the principal and biggest organ of metabolism of mammals and it is the earmark of toxicity because of its detoxifying part in metabolizing drugs/chemicals and xenobiotics. Drug-induced liver toxicity happening occasionally may be fatal and can precipitate other liver diseases. Acetaminophen in overdoses, can elicit liver toxicity. As previously stated in the introduction section, the hepatotoxicity of acetaminophen (paracetamol) is caused by the binding of NAPQI, the active metabolite of acetaminophen produced in the liver, to tissue sulphydryl groups (enzymes, proteins) leading to tissue leison (necrosis) and oxidative lipid peroxidation owing to a decline in the liver's protective reduced glutathione (GSH) content (18).

The definitive evaluation of normal liver functioning involves determining the activity of certain serum biomarker enzymes, namely SGPT, ALP and SGOT. These endoenzymes normally found in the cytoplasm, flow into the bloodstream when necrosis damages the hepatic cell membranes. Their measurement in blood serum is a very useful quantitative indicator for the kind and degree of hepatocellular injury (19,20). The hepatotoxin-induced liver damage in the acetaminophen-intoxicated rats in this study is responsible for the elevated activity of the blood biomarker enzymes. The antihepatotoxic efficacy of MEPS and reference silymarin is demonstrated by the normalization of certain liver function marker enzyme activity following treatment. MEPS's hepatoprotective action was further supported by the fact that, a rise in the bilirubin content of poison control rats indicated the incidence of jaundice, which was almost completely relieved in rats treated with it. Hepatopathy is typically implied by the decreased total protein level in rats given acetaminophen (21). The hepatoprotective potential of MEPS was also indicated by the normalization of total protein content in the rats treated with it.

The inhibition of the over production of reactive nitrogen/oxygen species (ROS) i.e., antioxidative activity of MEPS is the crucial mode of obviation from acetaminophen-invoked liver damage, as one of the critical events of acetaminophen-invoked liver lesions is the over generation of reactive lipid peroxides (TBARS) i.e., lipid peroxidation - invoked by the overproduced oxidative/nitrosative free radicals (2,17). The mammalian body normally possesses efficient antioxidative defense mechanisms, consisting of the endogenous antioxidant enzymes (first defense line) involving superoxide dismutase (SOD), catalase (CAT) and non-enzymatic antioxidants (second defense line), viz. reduced glutathione (GSH) (17-20). The in vitro studies conducted here also demonstrated marked free radical scavenging effect of MEPS thus further confirming its antioxidant effect against NAPQI-induced ROS, corroborating the in vivo role.

Acetaminophen-induced hepatotoxicity results in oxidative stress, lipid peroxidation, and ultimately hepatic tissue death because the tissue regulation of ROS overproduction and endogenous antioxidative defenses is disrupted or destroyed. When compared to the toxin control rats, MEPS therapy showed a notable increase in several antioxidant defense indices, confirming MEPS's *in vivo* antioxidant efficacy. The degree of hepatic lipid peroxidation (MDA production), is the signs of redox imbalance and oxidative liver damage that compromised the normal structure and functions of rat liver tissue and hepatocellular membranes (21-23). All of these harmful redox alterations to the liver were considerably and dose-dependently reversed by MEPS treatment.

Histopathological assessment of liver tissue unveils that; the typical liver tissue architecture was destroyed by the toxicity of acetaminophen. The livers from the acetaminophen-induced rats treated with MEPS or silymarin, the normal hepato-cellular architecture was demonstrated preserved, as compared with acetaminophen control rats, thereby confirming the preservative role of MEPS against acetaminophen-induced hepatic lesions in rats.

It hence becomes evident from the present experiments that, MEPS exerted significant antihepatotoxic potential in a dose-dependent way in acetaminophen-invoked experimental male albino rats. Previous phytochemical studies reveal that, Premna spinosa possesses flavonoids, alkaloids, triterpenes and sterol (9-11). All of these natural phytoconstituents are known to exhibit several health-giving effects including antioxidant and cytoprotective potential which could restore xenobiotic-induced oxidative/nitrosative organ/tissue damages (24, 25).

Conclusion

From the present investigation, it can be inferred that the methanol extract from *Premna spinosa* leaf is significantly effective against acetaminophen-kindled hepatotoxicity in Wistar albino rats by virtue of its preserving liver structure, liver functions with antioxidant mech-

anisms *in vivo*. To the best of the knowledge of the present authors, this is the first record of antihepatotoxic/hepatoprotecive effect of *Premna spinosa* leaf. Exploration on this plant part involving phytochemical isolation and molecular mechanism studies may further be required to harness its exact benefit to therapeutically manage drug-induced liver toxicity in humans.

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