Original Article
Toxicology International, *May-Aug 2016 / Vol-23 / Issue-2, 112-121*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i2/146685

Evaluation of hepatoprotective activity of ethanolic extract of aerial parts of *Premna tomentosa* against paracetamol-induced liver toxicity on wistar rats

Mano Jervin M*, Somasundaram G., Sudarcodi R., Manimekalai K.

Dept. of Pharmacology, Mahatma Gandhi Medical College & Research Institute, Puducherry
*drjervin@live.com

Abstract

Hepatoprotective activity of ethanolic extract of aerial parts of *Premna tomentosa* were evaluated against paracetamol-induced liver toxicity and comparison to the standard hepatoprotectant, silymarin. Thirty male wistar albino rats were randomised into five groups with six rats in each group. Group A & B sereved as normal control and toxic control respectively, and were administered normal saline. Group C& D were treated with ethanolic extract of Premna tomentosa at doses 100mg/kg and 200mg/kg respectively. Group E was treated with the standard drug Silymarin 25mg/kg. Toxicity was induced in all the groups except the first by giving Paracetamol at 2g/kg on the 5th day. All the rats were treated for 7 days and after the last dosing the rats were fasted for 24hrs and sacrificed. Serum was sent for biochemical analysis for assaying liver profile. Histopathology and antioxidant studies were done on liver. In our study ethanolic extract Premna tomentosa showed beneficial effect on paracetamol induced liver toxicity which was evident by the significant improvement in liver profile consisting of AST, ALT, ALP, GGT, total cholesterol, total protein, triglycerides and total bilirubin in a dose dependent manner, which is consistent with the histopathological findings. Increase in GSH and Catalase and decrease in TBARS validates the antioxidant property of the extract. The study has proved that Premna tomentosa posses a significant hepatoprotective activity which was comparable to the standard drug silymarin. It warrants further study in humans before including it in the prevention of hepatotoxicity.

Keywords: *Premna tomentosa*, Paracetamol, hepatoprotective, antioxidant

Received: 27-01-2016 **Revised:** 25-03-2016 **Accepted:** 05-07-2016

1. Introduction

Drug induced liver diseases (DILD) has an estimated incidence of 1:10,000 cases in developed countries but this can only be taken as the tip of the iceberg due to the widespread problem of underreporting of cases.[1] This problem is exponentially aggravated in developing countries due to multiple issues like over the counter drugs, use of alternative system of medicine and practice of polypharmacy leading to a gross underestimation of this condition. The leading cause of DILD is Paracetamol in developed countries, while in India it is antitubercular drugs.[2] Though the incidence of DILD associated with Paracetamol is less in India, certain epidemiological factors like malnutrition and alcoholism may act as high risk factors.[3]

The progression of liver disease is associated with major adverse metabolic derangements, while the therapeutic options to halt the disease process are limited. The mainstay of management is supportive care with immediate cessation of the offending drug and administration of drug specific antidote. Further, drugs with hepatoprotective effect like ursodeoxycholic acid, silymarin and steroids can be administered.[1] Ultimately, the unhalted progression of the disease leads to liver transplant as the last resort of management. This makes prevention and hepatoprotection, a better option for treating liver damage.

The focus of international research has been gradually shifting towards plant sources of drugs due to the limited

* *Author for correspondence*

efficacy of the currently available drugs and cost incurred in new drug development. However, there is a paucity of scientific research regarding their potential efficacy. Moreover, several plants with claimed hepatoprotection have themselves been demonstrated to be highly toxic to the liver.[4] In the field of hepatoprotective therapy, this focused research has revealed the therapeutic potential of Silymarin which now constitutes one of the most commonly used drugs in treatment of liver disease.

Many formulations are claimed by Indian indigenous systems of medicine to possess beneficial effects on liver. Pidangunarikudineer is one such polyherbal formulation used widely in Siddha system of medicine used to treat liver diseases. *Premna tomentosa,* a plant native to South India is the major constituent of this formulation. Several beneficial properties of this plant have been researched including anti-inflammatory activity, antidiabetic activity, immunomodulatory activity, but the most widely used property in alternate system of medicine is hepatoprotective activity.

Careful search on relevant literature revealed a paucity of studies done on this beneficial effect on liver and an absence of studies comparing its efficacy to the standard drug silymarin. Hence we planned a study to evaluate the hepatoprotective activity of *Premna tomentosa* on paracetamol induced liver toxicity in male wistar albino rats and comparison of the hepatoprotective activity to Silymarin.

2. Subjects and Methods

2.1 Preparation of plant extract

The aerial parts of *Premna tomentosa* were collected from Kallar area of Nilgiris district, Tamilnadu during the months of April-May 2014. The plant was identified and authenticated by experts of Horticultural College & Research Institute, TNAU, Coimbatore. The collected plant materials weighing 7kg approximately were cleaned, cut into small pieces and shade dried. The dried parts were then segregated and pulverized by a mechanical grinder and passed through a 40 mesh sieve.

The powdered plant materials were extracted with ethanol by hot continuous percolation method as elaborated by Mahanta and Mukherjee.[5] 50g of dried powder was packed in the thimble of soxhlet apparatus and was continuously extracted with 95% ethanol refluxing at 50-70°c which yielded a dark green syrupy mass. The extract was concentrated using a rotary evaporator and dried using a lyophilizer until a dry powder is obtained. The yield obtained was about 17%. The stock powder was stored in a glass desiccator at 4°c.

2.2 DRUGS AND CHEMICALS

Tablets of Silymarin and Paracetamol, Microlabs, Bangalore was procured from local medical store. 2-thiobarbituric acid and Ellman's reagent was purchased from Sigma Aldrich, Bangalore. All other solvents and chemicals of analytical grade were used and obtained from SD fine – chem, Mumbai.

2.3 ETHICAL CLEARANCE

Ethical clearance was taken from Institutional Animal Ethics Committee (IAEC) of the institute before commencement of the study. (LETTER NO: 02/IAEC/MG /2014)

2.4 ANIMALS

Experimental animal used in this study were 30 healthy male albino Wistar rats weighing 180 ± 20g and 10 to 12 weeks of age. They were procured from King's Institute, Guindy, Chennai and housed in central animal house, MGMCRI. The animals were kept in polypropylene cages, with dry paddy husk bedding and covered with stainless steel mesh lid. The environment of the room was maintained on a 12-hour light/dark cycle at a constant room temperature of 26±2° and relative humidity of 45-55%. The rats had free access to standard rat chow diet and water ad libitum. The rats were acclimatised to the surroundings for 2 weeks prior to the experiment. The animals were cared for as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.[6]

2.5 Acute toxicity test

Acute toxicity tests were performed according to OECD - 425 guidelines. Albino rats (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hr. with free access to water only. The extract was suspended in normal saline and was initially given at a dose of 2000mg/kg orally to one animal. As there was no lethality; four more animals were administered with the extract sequentially. Animals were observed individually during the first 30 minutes and

periodically during the first 24hours (special attention given during the first 4 hours). During this period the animals were observed for tremors, clonic convulsions, tonic extensions, catatonia, spasticity, opisthotonus, ataxia, sedation, ptosis, respiration.[7] No mortality was observed and the LD50 value was fixed to be more than 2000mg/kg. As the limit dose at 2000mg/kg did not exhibit any signs of toxicity, the doses were selected as 1/20th and 1/10th of LD50 i.e. 100mg/kg and 200mg/kg.

2.6 Experimental Design

A total number of 30 rats were used for the study. After acclimatization for a period of one week, the rats were numbered and were randomised into five groups of six rats each. Randomisation was done by internet based randomisation tool based on the numbers given to individual rats.

The study was conducted according to below mentioned protocol

Groups		Drugs to be administered for 7 days	Induction of hepatotoxicity on 5th day (Single dose)
A	Normal Control	Normal saline 1ml/kg	-
B	Toxic Control	Normal saline 1ml/kg	Paracetamol 2g/kg po
C	Test Group 1	E.E. Premna tomentosa 100mg/kg	Paracetamol 2g/kg po
D	Test Group 2	E.E. Premna tomentosa 200mg/kg	Paracetamol 2g/kg po
E	Standard group	Silymarin 25mg/kg	Paracetamol 2g/kg po

The doses of drugs and plant extract was calculated based on the body weight of each animal, suspended in normal saline and the respective volume was administered orally with the help of tuberculin syringe and gavage needle. The doses of the Paracetamol and Silymarin and duration of the treatment were calculated from previous studies.[8]

2.7 Euthanasia

The rats were fasted overnight after the last dosing. On the 8th day the rats were sacrificed by overdose of sodium pentobarbital 150mg/kg i.p.[6]

2.8 Blood sample collection

The blood samples were collected by cardiac puncture using a 20-gauge needle as described by McClure et al.[9] in labelled centrifuging tubes and allowed to clot for 45 min at room temperature. The samples were centrifuged at 2500rpm for 15 min. The separated serum was immediately sent for biochemical analysis.

2.9 Specimen collection

The liver was harvested and washed with normal saline and blotted with a filter paper. A small portion of the liver was transferred to individually labelled containers with 1.15% KCl and stored in a deep freezer at -18^{0}C. The rest of the liver was carefully transferred to containers with 10% formalin and marked for histopathology.

2.10 Biochemical Estimation

The separated serum was used for the estimation of biochemical parameters. The liver transaminases, AST and ALT were measured according to the method of Rietman and Frankel[10]. ALP was measured according to the method of Kind and King[11], and the Kjeldhal method were used for estimation of gamma glutamyltransferase (GGT)[12]. Total protein (TP) levels were determined by Lowry protein assay.[13] Total cholesterol (TC) were performed according to Allain et al.[14] Estimation of Total bilirubin (TB) were followed by modified DMSO method[15] and Triglyceride (TG) were estimated by the method of Foster et al.[16]

2.11 In-vivo antioxidant activity

The antioxidant potential of the plant extract was tested using three tests: TBARS assay, Reduced GSH and Catalase activity. The test was done in Research Lab of Department of Biochemistry, MGMCRI, Puducherry.

2.12 Preparation of liver homogenate

1gm of the liver tissue was weighed and homogenised in 9ml of 1.15%KCl using a Homogenizer. After homogenisation, the liver homogenate was kept in a freezer until it got frozen, then brought to room temperature until it liquefied. This procedure was repeated for two freeze-thaw cycles. Then the liver homogenate was centrifuged at 3000rpm at 4°C for 30 minutes. The supernatant of the liver homogenate was separated and used for assays.[17]

Table 1. Effect of *Premna tomentosa* ethanolic extract(PTEE) on liver enzymes

Groups		ALT	AST	GGT	ALP
A	Normal	35.83±2.36	49.33±1.91	12.17±0.7	97.33±3.43
B	PCT	251.17±6.09	156.17±2.5	62±2.53	215.83±6.44
C	PTEE 1	182±3.12**,#	128.17±2.21**,#	50.17±1.92*,#	177.5±3.56**,#
D	PTEE 2	140.5±2.74**,ns	101.5±4.21**,ns	30.17±2.39**,ns	145.5±3.99**,ns
E	Silymarin	141.5±2.69**	93.67±2.22**	29±1.63**	140±4.5**

*Values are expressed as Mean±SEM; One way ANOVA followed by Tukey's post hoc test.*p<0.05 as compared to PCT; **p<0.001 as compared to PCT; #p<0.05 as compared to standard; ns: not significant as compared to Standard;*

Table 2. Effect of *Premna tomentosa* ethanolic extract(PTEE) on Total Protein, Total Bilirubin. Total Cholesterol, Triglycerides

Groups		TP	TB	TC	TG
A	Normal	6.9±0.21	0.25±0.03	95.5±3.53	80.17±3.3
B	PCT	3.78±0.15	2.27±0.08	215.5±6.04	151.17±6.81
C	PTEE 1	5.32±0.17*,#	1.88±0.07#	192.17±2.74*,#	128.17±3.18*
D	PTEE 2	6.07±0.16**,ns	1.02±0.12**,ns	127.5±2.46**,ns	106.17±4.99**,ns
E	Silymarin	6.22±0.09**	1.07±0.15**	118.33±4.2**	97.67±3.42**

*Values are expressed as Mean±SEM; One way ANOVA followed by Tukey's post hoc test.*p<0.05 as compared to PCT; **p<0.001 as compared to PCT; #p<0.05 as compared to standard; ns: not significant as compared to Standard;*

2.13 TBARS

To test the antioxidant potential of the extract, lipid peroxidation was measured. Thiobarbituric acid reactive substances (TBARS) is the marker for lipid peroxidation and the same was assayed by the method of Ohkawa et al.[17]

2.14 GSH

0.1ml of supernatant (liver tissue homogenate) with 2.4ml of 0.02M EDTA solution was mixed and kept in ice bath for 10 minutes. Then 2ml of distilled water and 0.5 ml of 50%TCA were added and kept in ice bath for another 10 minutes and then centrifuged at 3000rpm for 15 minutes. 1ml of supernatant was mixed with 2ml of Tris buffer and 0.05ml of DTNB solution (Ellman's reagent). Optical Density of the sample was measured at 412nm in spectrophotometer against the blank. Appropriate standards were run simultaneously.[18]

2.15 Catalase

Catalase activity (CAT) was measured based on the ability of the enzyme to break down H_2O_2. 10µl of supernatant (liver tissue homogenate) was mixed with 3ml of H_2O_2 in Phosphate buffer. Time required for 0.05 optical density changes was observed at240nm against a blank containing the enzyme source in H_2O_2 free phosphate buffer. A unit catalase activity is the amount of enzyme that liberates half the peroxide oxygen from H_2O_2 solution of any concentration in 100seconds at 25°C.[19]

2.16 HISTOPATHOLOGICAL OBSERVATION

Conventional techniques of paraffin-wax sectioning and haematoxylin-eosin staining were used for histological studies.[20]

3. Results

The above table shows the effect of *Premna tomentosa* ethanolic extract (PTEE) on the liver profile in paracetamol (PCT) induced hepatotoxicity model. There was a significant increase in ALT, AST, GGT, ALP, TB, TC levels and a significant decrease in total protein levels in paracetamol toxic control group (Group B) when compared with normal control (Group A). Silymarin administered rats (Group E) showed significant reduction ($p<0.001$) of liver enzymes, Total bilirubin, Total cholesterol and significant increase in total protein levels as compared to toxic control group (Group B).

Rats administered with PTEE at dose 100mg/kg(Group C) showed a marked decrease in AST,ALT, ALP ($p<0.001$) and TC, GGT levels ($p<0.05$) with a significant

increase in Total Protein levels ($p<0.05$) whereas there was no significant changes in Total Bilirubin levels as compared to PCT toxic control group.(Group B).

PTEE 2 (200mg/kg) group showed significant decrease ($p<0.001$) in liver enzymes, Total bilirubin, Total cholesterol and significant increase ($p<0.001$) in total protein levels as compared to toxic control group (Group B).

The rats administered with PTEE 1 (Group C) demonstrated a significant difference($p<0.05$) in the liver parameters when compared to that of standard control group (Group E). This shows that the activity of PTEE at lower doses was not comparable to silymarin. The higher dose of PTEE (Group4) showed alterations of liver parameters which were not significant ($p> 0.05$) when compared to standard control group(Group E). This implies that the alterations in liver parameters demonstrated by PTEE 2 was comparable to that of standard control.

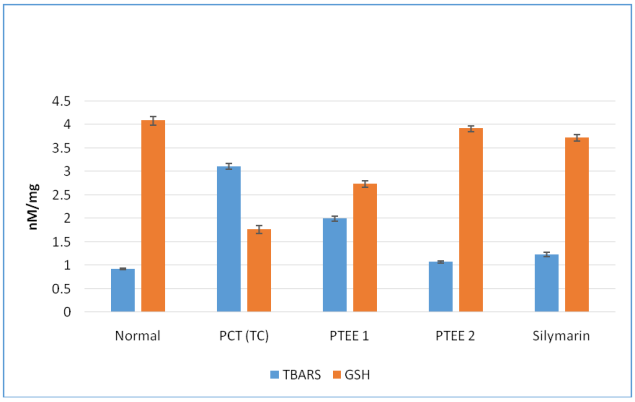


Figure 1. Effect of *Premna tomentosa* ethanolic extract on TBARS and GSH.

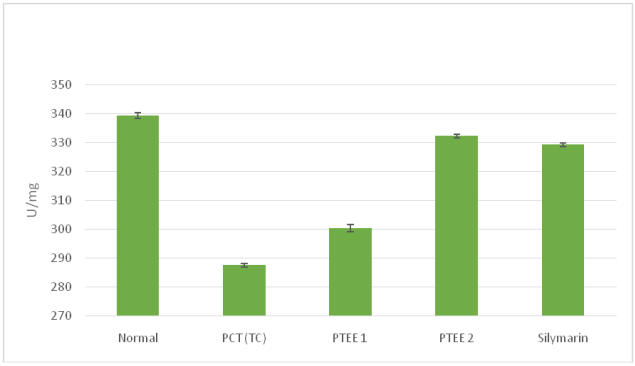


Figure 2. Effect of *Premna tomentosa* ethanolic extract (PTEE) on Catalase.

The above graphs show the antioxidant effect of *Premna tomentosa* ethanolic extract (PTEE) on paracetamol induced hepatotoxicity model. There was a significant increase in TBARS level and a significant decrease in GSH and CAT levels in paracetamol (PCT) toxic control group (Group B) when compared with normal control (Group A).

The other three groups which were treated with PTEE 100mg/kg (Group C), PTEE 200mg/kg (Group D) and Silymarin (Group E) showed significant reduction ($p<0.001$) of TBARS and significant increase ($p<0.001$) in GSH and CAT levels as compared to toxic control group (Group B).

The rats administered with PTEE 1 (Group C) demonstrated a significant difference($p<0.05$) in the levels of TBARS, GSH and CAT when compared to that of standard control group (Group E) whereas the higher dose of PTEE (Group4) showed the levels of TBARS, GSH and CAT to be not significant ($p> 0.05$) when compared to standard control group(Group E). This implies the change in the antioxidant markers demonstrated by PTEE 2 was comparable to that of standard control (Group E).

3.1 HISTOPATHOLOGY

Group 1: Section from liver of normal control group shows normal liver architecture with cords of hepatocytes radiating from centre to periphery.

Group 2: Section studied from liver with paracetamol treated group shows central venous congestion with periportal chronic lymphocytic infiltration. There is also congestion of hepatic sinusoids with mild distortion in the hepatocyte architecture.

Group 3: Section studied from liver of test group which was pre-treated with PTEE 100mg/kg shows central venous congestion with mild periportal chronic lymphocytic infiltration.

Group 4: Section studied from liver with PTEE 200mg/kg pre-treated group shows reduced periportal inflammation with mild hepatic venous congestion.

Group 5: Section studied from liver with PTEE 200mg/kg pre-treated group shows absence of periportal inflammation. Mild congestion in very few central veins.

4. Discussion

In the present study the aim was to determine the hepatoprotective activity of *Premna tomentosa* in Paracetamol induced hepatotoxicity model, compare the hepatoprotective effects to the standard drug Silymarin and also to determine the antioxidant potential of *Premna tomentosa*.

4.1 Liver function tests:

Serum markers like ALT, AST, ALP, GGT, TB and TP are commonly used in assessing the liver diseases. ALT is a transaminase present in the mitochondria and cytoplasm of liver cells, thus rise in its levels is highly indicative of liver injury.[21] On the other hand, AST is not a specific marker for liver diseases since it is present in heart, muscle and kidney other than liver.[22] Therefore, the two markers are taken together for diagnosis and to differentiate the causes of liver diseases. Increase in levels of both transaminases ALT and AST is highly indicative of hepatotoxicity.[23] Marked rise in GGT as well as ALP is highly specific of hepatobiliary injury which is a prominent feature in paracetamol induced toxicity.[24]

The total protein level is indicative of the functional capacity of the liver specifically protein synthesis.[25] In hepatic injury the ability of hepatocytes to excrete bilirubin is affected leading to its accumulation in blood. Hence the total bilirubin can be used as an indication of hepatic clearance.[22]

In our study, the group which received only Paracetamol (Group B or the toxic control), an abnormal increase in liver enzymes namely AST, ALT, GGT and ALP was observed. There was also a notable elevation in total bilirubin levels and lowered total protein levels. The toxic metabolite NAPQI (N-acetyl-p-benzoquinone imine) formed during paracetamol metabolism is usually detoxified by conjugation reactions in liver. But in paracetamol toxicity, there is a saturation of conjugation reactions leading to accumulation of NAPQI causing hepatocellular damage. The free radical generated causes hepatocyte membrane damage leading to leakage and increased concentrations of liver enzymes in the plasma.[26]The decrease in TP in paracetamol treated group may be due to the decrease in number of healthy hepatocytes. This in turn leads to decrease in hepatic capacity to synthesise proteins. It is also attributed to the formation of protein adducts.[27]

The rats pre-treated with *Premna tomentosa* attenuated the increase in liver enzymes caused by Paracetamol toxicity. Significant reversal of total bilirubin and total protein levels to near normal values were observed with higher dose of the plant extract. This implies that *Premna tomentosa* at higher doses has a significant hepatoprotective activity in Paracetamol induced hepatotoxicity models. Our observations were comparable to a study by Devi, *et al* where pre-treatment with methanolic extract of *Premna tomentosa* significantly improved the liver profile in acetaminophen induced hepatitis in rat model thus denoting its hepatoprotective property.[28]This beneficial effects on liver functions were comparable to the effects of Silymarin.

The improvement in the liver function tests clearly implicates the ability of *Premna tomentosa* to maintain the liver function during acute paracetamol toxicity. The mode by which the extract acts may be a complex one and can be attributed to the following mechanisms. *Premna tomentosa* prevents hepatocellular membrane damage thus maintaining the functional as well as structural integrity of the liver.[29]Also, the extract upholds the calcium homeostasis preventing hepatocellular damage.[29] The plant extract also has anti-inflammatory activity and inhibits the activation of stellate cells.[30] Another mode of action may be due to its antioxidant potential neutralising the oxidative stress on the liver thus preventing the catastrophic sequel. Early researchers have attributed the hepatoprotective potential of *Premna tomentosa* to be due to the major phytoconstituent limonene. b-caryophyllene, another major phytoconstituent of *Premna tomentosa* has also been proved to have hepatoprotective activity.[31]

4.2 Total Cholesterol & Triglycerides

Cholestrolemia in hepatotoxicity is related to the abnormal functioning of the liver in removing cholesterol from blood. Therefore, increase in levels of total cholesterol is indicative of liver diseases.[32]Hypertriglyceridemia can also occur secondary to liver diseases.[33]

In the toxic control group, total cholesterol and TG levels were significantly increased when compared to the normal group. This is indicative of hepatic injury specifically drug induced hepatitis and may be attributed to the interference with the transport of TGs and cholesterol. In our study, groups which were pre-treated with *Premna tomentosa,* a dose dependent decrease in total cholesterol and TG levels was seen. This is similar

to findings by Devi *et al* and is postulated to be due to hepatoprotective effect of *Premna tomentosa* extract on inhibition of lipid accumulation.[34]

4.3 TBARS

TBARS (Thiobarbituric acid reactive substances) are substances which is formed as a by-product of lipid peroxidation which can be detected by TBARS assay. TBARS assay chiefly measures Malondialdehyde (MDA) in the sample which is formed when reactive oxygen species (ROS) degrades polyunsaturated lipids. MDA causes toxic stress in the cells. The production of MDA is used as a biomarker to measure the level of oxidative stress.[35]

In the group which received only Paracetamol, the TBARS value was higher when compared to other groups. This showed that Paracetamol causes oxidative stress due to its toxic metabolite NAPQI resulting in increased lipid peroxidation of hepatocytes leading to increased levels of MDA thus the high TBARS value. The hepatotoxicity associated with Paracetamol has been linked to the NAPQI induced oxidation of protein molecules and cell membrane constituents and also the subsequent increased levels of lipid peroxide which may cause alterations in plasma membrane of hepatocytes.[36]

Pre-treatment with *Premna tomentosa* extract had lesser TBARS value than toxic control group. Thus *Premna tomentosa* was able to reduce the lipid peroxidation by its free radical scavenging effect. The higher dose of *Premna tomentosa*-200mg/kg had a better antioxidant potential evident by the lower TBARS value and was comparable to that of Silymarin pre-treated group. This is in accordance with the studies by Devi *et al*, in which pre-treatment with *Premna tomentosa* extract showed lower values of TBARS as compared to toxic paracetamol group indicating its antioxidant potential.[37] This protective activity can be attributed to the Limonene present in the *Premna tomentosa* which scavenges the agents responsible for lipid peroxidation & helps in hepatoprotection.

4.4 GSH

Glutathione is a non enzymatic antioxidant present abundantly in the liver. It is involved in the phase 2 biotransformation reactions.

In our study Group B which received paracetamol only, we observed decreased levels of reduced Glutathione (GSH). This is due to the local generation of reactive drug metabolites and utilisation of reduced Glutathione (GSH) in detoxification of NAPQI. The depleted GSH stores augment liver damage since NAPQI escapes glutathione conjugation and binds covalently to cellular macromolecules leading to cellular death.

In the study group E which was pre-treated with Silymarin showed increased levels of GSH, which is postulated to be due to the prevention of lipid peroxidation by silymarin which was in accordance to previous studies.[38]

Groups C & D which received pre-treatment with *Premna tomentosa* at doses 100mg/kg and 200mg/kg also had reduced levels of GSH, the latter showed better improvement with near normal levels of GSH. This can be due to two mechanisms. The first being similar to silymarin by preventing lipid peroxidation, the other is the ability of the extract to maintain GSH concentrations even during paracetamol toxicity.

This is comparable to results obtained from similar study by Devi, *et al* which demonstrated attenuation of the paracetamol induced changes in GSH levels to near normal values thus demonstrating its antioxidant activity. This has been attributed to stabilisation of mitochondrial Calcium sequestering ability thus providing protection against toxic substance NAPQI.[37]

Previous studies attribute the maintenance of GSH levels to the presence of D-Limonene in the plant since dietary limonene is proved to maintain liver GSH concentrations in paracetamol challenged rats and also prevent the SH group loss[39] This activity is comparable to that of Silymarin.

4.5 CATALASE

Catalase(CAT) is an enzymatic antioxidant which is chiefly concentrated in liver and erythrocytes. Catalase scavenges the dangerous hydrogen peroxide and converts it into oxygen and water thus protecting the cells from highly reactive hydroxyl radicals.[40]Therefore, in conditions of oxidative damage the levels of catalase will be reduced as an indication of hepatotoxicity.

In our study group B which was paracetamol only treated group, catalase levels were significantly reduced when compared to the normal group A. This may be due to the consumption of CAT for neutralizing the hydrogen peroxide caused as a result of oxidative stress induced by paracetamol toxicity.

Group E which was pretreated with silymarin showed

a marked rise in CAT levels indicating free radical scavenging activity of Silymarin. These results are similar to the study conducted by Maity, *et al* in which antioxidant activity was demonstrated by increased levels of catalase in the standard control group pretreated with Silymarin.

The groups C, D which was pre-treated with *Premna tomentosa* 100mg/kg, 200mg/kg had increased levels of CAT and the CAT levels of group D which was given *Premna tomentosa* 200mg/kg was comparable to that of silymarin. The effect of *Premna tomentosa* on catalase activity can be compared to the previous studies by Devi *et al*. This antioxidant effect may be similar to that of Silymarin and related to the free radical scavenging activity of catalase thereby attenuating the oxidative stress induced by paracetamol.[37]

The administration of Paracetamol 2g/kg did not cause death of any the animals nor alerted the general behaviour. No other adverse event was observed.

4.6 HISTOPATHOLOGY

In paracetamol toxic group histopathology showed central venous congestion, dilatation of sinusoids with periportal lymphocytic infiltration. No marked changes in nuclei were seen. Cell integrity was maintained. Pathological changes were mainly located in the centrilobular region.

The congestion of central vein and hepatic sinusoids are typical features of paracetamol toxicity as per Zhang *et al*. The toxic metabolite NAPQI induces oxidative damage to the hepatocytes leading to pathological changes in centrilobular area.[41]

Moreover, the marked periportal inflammatory changes indicate the paracetamol induced toxicity. This is in accordance with Blazka*et al*., demonstrating the role of Kupffer cells and inflammatory mediators in the pathogenesis of paracetamol induced liver toxicity.[42]

The group C which was pre-treated with *Premna tomentosa*100mg/kg showed marked central vein and hepatic sinusoidal congestion with periportal lymphocytic infiltration in majority of the histopathological slides. There was only a mild decrease in inflammation in two of the slides indicating that *Premna tomentosa* at lower doses of 100mg/kg failed to produce significant ameliorative effects on paracetamol induced hepatotoxicity. Lack of beneficial effects may be due to the lesser dose of *Premna tomentosa*.

Group D which was pre-treated with *Premna tomentosa*200mg/kg showed mild hepatic venous congestion as well as reduced infiltration of lymphocytes. There was no congestion noted in hepatic sinusoids. Majority of the slides demonstrated a marked ameliorative action of *Premna tomentosa* at higher doses 200mg/kg. These changes are comparable to the findings by Guguloth, *et al* demonstrating beneficial effects of *Vitexnegundo*in paracetamol induced liver toxicity.[43] Studies focusing on histopathological studies of hepatoprotective effects of *Premna tomentosa* are lacking.

The beneficial effects of *Premna tomentosa* can be attributed to its neutralising effect on NAPQI by maintenance of glutathione levels and also due to the free radical scavenging property of *Premna tomentosa*.

In group E which was pre-treated with Silymarin, there was absence of periportal inflammation. Mild congestion was observed in very few central veins. Therefore, the Silymarin treated group demonstrated an overall beneficial effect in liver histopathology with an almost complete reversal to normal liver histology. These findings are in accordance with Girish*et al*., and further consolidate the hepatoprotective effects of Silymarin.[44]The membrane stabilizing activity of Silymarin can be the reason behind the decrease in inflammation, and the free radical scavenging activity and maintenance of glutathione levels adds to the hepatoprotective function.

5. Acknowledgement

I would like to thank Dr.Dhananjay S. Kotasthane, Professor, Department of Pathology and Dr. Srinivasan A.R., Professor, Department of Biochemistry,Dr. Shanmugasamy K., Assistant professor, Department of Pathology and Mr. Kulanthaivel, Lecturer, Department of Biochemistry and for their valuable help and suggestions.

Source(s) of support: NIL
Conflicting Interest: NIL

6. References

1. Devarbhavi H. An Update on Drug-induced Liver Injury. J ClinExpHepatol. 2012 Sep;2(3):247–59.
2. Devarbhavi H, Dierkhising R, Kremers WK, Sandeep MS, Karanth D, Adarsh CK. Single-center experience with drug-induced liver injury from India: causes, outcome, prognosis, and predictors of mortality. Am J Gastroenterol. 2010;105(11):2396–404.
3. Myers RP, Shaheen AAM, Li B, Dean S, Quan H. Impact of liver disease, alcohol abuse, and unintentional ingestions on the outcomes of acetaminophen overdose. ClinGastro-

enterolHepatol Off ClinPract J Am Gastroenterol Assoc. 2008;6(8):918–25.
4. Chan K. Some aspects of toxic contaminants in herbal medicines. Chemosphere. 2003;52(9):1361–71.
5. Mahanta M, Mukherjee AK. Neutralisation of lethality, myotoxicity and toxic enzymes of Najakaouthia venom by Mimosa pudica root extracts. J Ethnopharmacol. 2001;75(1):55–60.
6. CPCSEA. CPCSEA Guidelines for laboratory animal facility. Indian J Pharmacol. 2003;35(4):257–74.
7. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure [Internet]. Paris: Organisation for Economic Co-operation and Development; 2008 [cited 2015 Sep 24]. Available from: http://www.oecd-ilibrary.org/content/book/9789264071049-en
8. Chattopadhyay RR. Possible mechanism of hepatoprotective activity of Azadirachtaindica leaf extract: part II. J Ethnopharmacol. 2003;89(2-3):217–9.
9. McClure DE. Clinical pathology and sample collection in the laboratory rodent. Vet Clin North Am ExotAnim Pr. 1999;2(3):565–90.
10. Reitman S, Frankel S. In vitro determination of transaminase activity in serum. Am J ClinPathol. 1957;28:56–64.
11. Kind PRN, King EJ. Estimation of Plasma Phosphatase by Determination of Hydrolysed Phenol with Amino-antipyrine. J ClinPathol. 1954;7(4):322–6.
12. Szasz G. A kinetic photometric method for serum gamma-glutamyltranspeptidase. Clin Chem. 1969;15(2):124–36.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265–75.
14. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem. 1974 Apr;20(4):470–5.
15. Helga Tait Malloy KAE. The Determination of Bilirubin with the Photoelectric Colorimeter. J Biol Chem. 1937;119(2):481–90.
16. L B Foster RTD. Stable reagents for determination of serum triglycerides by colorimetric Hatzsch condensation method. Clin Chem. 1973;19(3):338–40.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351–8.
18. Ellman GL. Tissue sulfhydryl groups. Arch BiochemBiophys. 1959;82(1):70–7.
19. Luck H. Catalase. Methods Enzym Anal. 1965;885–8.
20. Carleton HM, Drury RAB, Wallington EA. General staining procedures. In: Carleton's Histological technique. 5th ed. New York: Oxford University Press; 1980. p. 147–8.
21. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring. Clin Chem. 2000 Dec;46(12):2050–68.
22. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008;245(3):194–205.
23. Reuben A. Hy's law. Hepatology. 2004 Feb 1;39(2):574–8.
24. Sheehan M, Haythorn P. Predictive values of various liver function tests with respect to the diagnosis of liver disease. ClinBiochem. 1979;12(6):262–3.
25. Thapa BR, Walia A. Liver function tests and their interpretation. Indian J Pediatr. 2007;74(7):663–71.
26. Potter WZ, Thorgeirsson SS, Jollow DJ, Mitchell JR. Acetaminophen-induced hepatic necrosis V. Correlation of hepatic necrosis, covalent binding and glutathione depletion in hamsters. Pharmacology. 1974;12(3):129–43.
27. Zaher H, Buters JTM, Ward JM, Bruno MK, Lucas AM, Stern ST, et al. Protection against Acetaminophen Toxicity in CYP1A2 and CYP2E1 Double-Null Mice. ToxicolApplPharmacol. 1998;152(1):193–9.
28. Pandima Devi K, Devaki T. Protective effect of Premna tomentosa on acetaminophen-induced acute hepatitis in rats. Med Sci Res. 1998;26(11):785–7.
29. Pandima Devi K, Sreepriya M, Balakrishna K, Devaki T. Protective effect of Premna tomentosa (L. Verbenaceae) extract on membrane-bound phosphatases and inorganic cations transport in acetaminophen-induced hepatotoxicity rats. J Ethnopharmacol. 2004;93(2–3):371–5.
30. Hari Prasad K, Jayalakshmi R, Chandrakasan G. Inhibitory effect of premna tomentosa (l. Verbenaceae) on stellate cell activation and collagen accumulation in dmn induced hepatifibrosis in rats. Biomedicine. 2006;26(3, 4):34–42.
31. Calleja MA, Vieites JM, Montero-Meterdez T, Torres MI, Faus MJ, Gil A, et al. The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. Br J Nutr. 2013;109(03):394–401.
32. McIntyre N. Plasma lipids and lipoproteins in liver disease. Gut. 1978;19(6):526–30.
33. Müller P, Fellin R, Lambrecht J, Agostini B, Wieland H, Rost W, et al. Hypertriglyceridaemia Secondary to Liver Disease. Eur J Clin Invest. 1974 Feb 1;4(1):419–28.
34. Devi KP, Sreepriya M, Balakrishna K, Veluchamy G, Devaki T. Assessment of the Protective Potential of Premna tomentosa (L. Verbenaceae) Extract on Lipid Profile and Lipid-Metabolizing Enzymes in Acetaminophen-Intoxicated Rats. J Altern Complement Med. 2004;10(3):540–6.
35. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free RadicBiol Med. 1990;9(6):515–40.
36. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J PharmacolExpTher. 1973;187(1):211–7.
37. Devi KP, Sreepriya M, Balakrishna K, Devaki T. Protective effect of Premna tomentosa extract (L. verbanacae) on acetaminophen-induced mitochondrial dysfunction in rats. Mol Cell Biochem. 2005;272(1-2):171–7.
38. Muriel P, Garciapiña T, Perez-Alvarez V, Mourelle M. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J ApplToxicol. 1992;12(6):439–42.
39. Reicks MM, Crankshaw D. Effects of D-limonene on hepatic microsomal monooxygenase activity and parac-

etamol-induced glutathione depletion in mouse. Xenobiotica. 1993;23(7):809–17.
40. Chance B, Greenstein DS, Roughton FJW. The mechanism of catalase action. I. Steady-state analysis. Arch BiochemBiophys. 1952;37(2):301–21.
41. Zhang X, Ouyang J, Thung SN. Histopathologic manifestations of drug-induced hepatotoxicity. Clin Liver Dis. 2013 Nov;17(4):547–64.
42. Blazka ME, Elwell MR, Holladay SD, Wilson RE, Luster MI. Histopathology of acetaminophen-induced liver changes: role of interleukin 1 alpha and tumor necrosis factor alpha. ToxicolPathol. 1996 Apr;24(2):181–9.
43. Guguloth S, Vivekanandan L, Singaravel S, Sheik HS, Thangavel S. Hepatoprotective activity of Vitexnegundo Linn bark against chemical induced toxicity in experimental rats. Pharmanest. 2011;2(5-6):534–41.
44. Girish C, Koner BC, Jayanthi S, Ramachandra Rao K, Rajesh B, Pradhan SC. Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. FundamClinPharmacol. 2009;23(6):735–45.