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Hepatoprotective Constituents from the Leaves of *Pisonia grandis* R.Br

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ABSTRACT

Objective: *Pisonia grandis* R.Br is a plant with a diversity of ethnic medicinal uses along with antioxidant activity. Hence we have intended to screen hepatoprotective activity with ethanolic (EEPG) and aqueous (AEPG) extracts of leaves of *Pisonia grandis* R.Br. Powder of leaves successively extracted with ethanol and aqueous solvents and it was subjected for phytochemical screening to categorize the different phytoconstituents. **Materials and Methods:** Hepatoprotective activity of both the extracts was studied against the liver injury induced by carbon tetrachloride, paracetamol or thioacetamide and chronic liver damage induced by carbon tetrachloride in rats. **Results:** Result showed that the extracts significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin. EEPG at the dose of 250 mg kg^{-1}, p.o.) prevented the increase in liver weight when compared to hepatoxin treated control, while the AEPG at the dose 250 mg kg^{-1} was ineffective except in the paracetamol induced liver damage. In the chronic liver injury induced by carbon tetrachloride, EEPG at the dose of 250 mg kg^{-1}, p.o.) was found to be more effective than the AEPG 250 mg kg^{-1}, p.o. Histological examination of the liver tissues supported the hepatoprotection. **Conclusion:** It is concluded that both extracts of leaves of *Pisonia grandis* R.Br possesses good hepatoprotective activity.

Key words: *Pisonia grandis* R.Br, hepatoprotective activity, carbon tetrachloride, paracetamol, thioacetamide

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INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction[1]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol and prescribed and over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease[2,3]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects[4]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders[5]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Pisonia grandis (Synmyn: *Pisonia alba*, *Pisonia morindifolia*) commonly known as Leechikottai kerai in Tamil, Velati salet in Hindi[6]. The plant *Pisonia grandis* R.Br., belonging to the family Nyctaginaceae, is an evergreen glaborous garden tree with young shoots are minutely puberulous. It is native of Hawai island and naturalized throughout India. In the alternative system of medicine *Pisonia grandis* leaves are used as analgesic, antiinflammatory, diuretic[7,8], hypoglycemic agent[9], antifungal[10]. It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing[11], rheumatism and arthritis[12]. Leaves also consumed as vegetable and salad, fed to cattle[13].

MATERIALS AND METHODS

Preparation of extracts: The leaves of *Pisonia grandis* R.Br. were collected in the month of July 2008 from the

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karripatti, Salem District, Tamilnadu, India. The plant material was taxonomically identified by the botanist Mr. A. Balasubramanian (consultant central siddha research) Executive Director ABS botanical garden, Salem, Tamilnadu. The dried powdered leaves of *Pisonia grandis* R.Br. were defatted with petroleum ether (60-80°C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted with ethanol. Aqueous extract was prepared by cold maceration process. The solvent removed by distillation under low pressure and the resulting semisolid mass was vacuum dried using rotary evaporator and used for this study.

Animals: Wister rats (100-150 g) used in the present studies were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd, Bangalore) and water ad libitum. All the animals were acclimatized for a week before use.

Acute hepatitis models

Evaluation if acute and chronic hepatotoxic activity

Carbon tetrachloride (CCl_4) induced acute hepatotoxicity: The CCl_4 was diluted with liquid paraffin (1:1) before administration. The animals were divided into 5 groups of 6 each. The animals were then subjected to either one of the following treatments for 9 days:

Group 1: Distilled water (1 mL kg^{-1}, p.o.)
Group 2: Distilled water for 9 days + CCl_4 (1 mL kg^{-1}, p.o.) on ninth day
Group 3: Silymarin (100 mg kg^{-1} day^{-1}, p.o.) for 9 days+CCl_4 (1 mL kg^{-1}, p.o.) on ninth day
Group 4: EEPG (250 mg kg^{-1} day^{-1}, p.o.) for 9 days+CCl_4 (1 mL kg^{-1}, p.o.) on ninth day
Group 5: AEPG (250 mg kg^{-1} day^{-1}, p.o.) for 9 days+CCl_4 (1 mL kg^{-1}, p.o.) on ninth day

Food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 h after the administration of CCl_4. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartateaminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to histopathological examination[14].

Paracetamol (PCM) induced hepatotoxicity: The liver was damaged using PCM (1 g kg^{-1}, po) diluted with sucrose solution (40% w/v).

The animals were divided into 5 groups of 6 each. The animals were then subjected to either one of the following treatments for 9 days.

Group 1: Distilled water (1 mL kg^{-1}, p.o.)
Group 2: Distilled water for 9 days+PCM (1 g kg^{-1}, p.o.) on ninth day
Group 3: Silymarin (100 mg kg^{-1} day^{-1}, p.o.) for 9 days+PCM (1 g kg^{-1}, p.o.) on ninth day
Group 4: EEPG (250 mg kg^{-1} day^{-1}, p.o.) for 9 days+PCM (1 g kg^{-1}, p.o.) on ninth day
Group 5: AEPG (250 mg kg^{-1} day^{-1}, p.o.) for 9 days+PCM (1 g kg^{-1}, p.o.) on ninth day

Food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 h after the administration of PCM. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartateaminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to histopathological examination[15].

Thioacetamide (TAA) induced liver necrosis: The Liver Damage was induced by using TAA (100 mg kg^{-1}, sc), which was prepared in distilled water (2% solution) 12 the animals were divided into 5 groups of 6 each. The animals were then subjected to either one of the following treatments for 9 days.

Group 1: Distilled water (1 mL kg^{-1}, p.o.)
Group 2: Distilled water for 9 days+TAA (100 mg kg^{-1}, p.o.) on ninth day
Group 3: Silymarin (100 mg kg^{-1} day^{-1}, p.o.) for 9 days+TAA (100 mg kg^{-1}, sc) on ninth day
Group 4: EEPG (250 mg kg^{-1} day^{-1}, p.o.) for 9 days+TAA (100 mg kg^{-1}, sc) on ninth day
Group 5: AEPG (250 mg kg^{-1} day^{-1}, p.o.) for 9 days+TAA (100 mg kg^{-1}, sc) on ninth day

Food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 hr after the administration of TAA. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartateaminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was immediately isolated and washed with normal saline, blotted with filter paper and

weighed. The liver was then subjected to histopathological examination[16].

Chronic toxicity induced by CCl_4: The animals were divided into 5 groups of 6 rats each and treated as follows[17].

Group 1: Distilled water (1 mL kg^{-1}, p.o.) for 8 weeks (control)
Group 2: CCl_4 (1 mL kg^{-1}, p.o.) weekly twice for the 8 weeks
Group 3: Silymarin 100 mg kg^{-1} day^{-1}, p.o., for 8 weeks + CCl_4 (1 mL kg^{-1}, p.o.) weekly twice for 8 weeks
Group 4: EEPG (250 mg kg^{-1}, p.o.) for 8 weeks + CCl_4 (1 mL kg^{-1}, p.o.) weekly twice for 8 weeks
Group 5: AEPG (250 mg kg^{-1}, p.o.) for 8 weeks + CCl_4 (1 mL kg^{-1}, p.o.) weekly twice for 8 weeks

Food was withdrawn 12 h before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4 and 5. The animals were sacrificed 24 h after the administration of CCl_4. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartateaminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to histopathological examination.

Statistical analysis: The statistical significance was assessed using one way Analysis of Variance (ANOVA) followed by Bonferroni's multiple comparison test. The values are expressed as Mean±SE and $p \leq 0.05$ were considered significant.

RESULTS

Preliminary phytochemical investigation: The preliminary phytochemical investigation of the both extracts showed that it contains carbohydrates, tannins, flavanoids, saponins, steroids, proteins and amino acids.

Carbon tetrachloride induced acute hepatotoxicity: The dose of EEPG (250 mg kg^{-1} p.o.) and silymarin (100 mg kg^{-1}; p.o.) produced a significant reduction in serum marker enzymes ($p \leq 0.001$). AEPG AT THE dose of (250 mg kg^{-1}, p.o.) also produced a significant reduction in ALT, AST, ALP and serum bilirubin when compared to CCl_4 treated group, but it was less effective. Administration of CCl_4 produced a non-significant increase in liver weight. Silymarin and the AEPG at dose of 250 mg kg^{-1}, p.o. did not affect the liver weight, when compared to CCl_4 treated control, whereas EEPG at the dose of 250 mg kg^{-1}, p.o.) showed a significant reduction in the liver weight ($p \leq 0.05$) when compared with CCl_4 treated group (Table 1). Histological examination of the liver tissue from CCl_4 treated animals revealed that CCl_4 had produced profound inflammation and congestion especially in the sinusoids. Hydropic degeneration and steatosis in the periportal region was also observed. Pretreatment of animals with silymarin, AEPG (250 mg kg^{-1}, p.o.) and EEPG (500 mg kg^{-1}, p.o.) reduced the inflammation, degenerative changes and steatosis (Fig. 1).

Paracetamol induced hepatotoxicity: After 48 h of administration of PCM, the serum levels of ALT, AST, ALP and bilirubin were markedly increased. Pretreatment with EEPG (250 mg kg^{-1}, p.o.) and silymarin significantly reduced the levels of biochemical markers when compared to PCM treated group ($p \leq 0.001$). Pretreated with AEPG (250 mg kg^{-1}, p.o.) did not show significant effect when compared with the PCM control. Pretreatment with AEPG (250 mg kg^{-1}, p.o.) and silymarin significantly reduced the increase in the liver weight seen after PCM intoxication (Table 2). PCM produced severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis. Silymarin reduced the pyknosis of hepatocytes when compared to PCM treated control. Animals treated with both lower and higher dose of PGJ showed mild hydropic degeneration and there was no pyknosis or congestion (Fig. 2).

Thioacetamide induced liver necrosis: A significant difference in serum biochemical markers was observed between normal and TAA treated group ($p \leq 0.001$). Pretreatment of animals with EEPG 250 mg kg^{-1}, p.o. and AEPG 250 mg kg^{-1}, p.o. and silymarin significantly reduced the levels of AST, ALT and ALP ($p \leq 0.001$). EEPG and AEPG at both the dose of 250 mg kg^{-1}, p.o. did not affect serum bilirubin levels. TAA induced acute toxicity increased the weight of liver significantly ($p \leq 0.01$). EEPG at the dose of 250 mg kg^{-1}, po and silymarin prevented the increase in liver weight that was observed in TAA treated group, AEPG at the dose of 250 mg kg^{-1}, p.o. did not produce any significant decrease in liver weight (Table 3). Histological examination showed perilobular hepatocyte necrosis, inflammation and congestion with cytoplasmic vacuolation in TAA treated control animals. In silymarin treated animals, mild inflammation and mild necrosis of hepatocytes with cytoplasmic vacuolation was noted. Animals treated with lower dose showed periportal necrosis and those treated with higher dose showed mild inflammation and no necrosis (Fig. 3).

Table 1: Effect of silymarin, EEPG and AEPG on serum ALT, AST, ALP, bilirubin levels and liver wet weight in CCl_4 induced acute liver injury in rats

Parameters	Vehicle control	CCl_4 control	CCl_4+Silymarin (100 mg kg^{-1})	CCl_4+EEPG (250 mg kg^{-1})	CCl_4+AEPG (250 mg kg^{-1})
ALT (U L^{-1})	72.34±5.02	379.00±21.07a	57.41±2.77***	60.14±7.64*	16.68±4.22 **
ASP (U L^{-1})	186.29±7.72	642.41±30.21a	205.39±6.23***	572.61±43.11ns	151.70±13.85***
ALP (U L^{-1})	398.11±6.49	749.44±36.32a	407.99±5.23***	540.23±79.24***	488.79±9.99**
Serum Bilirubine mg dL^{-1}	0.26±0.43	1.55±0.12a	0.29±0.01***	0.27±0.41***	0.27±0.01***
Liver weight g/100 g of b.w	3.3±0.04	3.76±0.283b	3.9±0.04ns	3.17±0.01 ns	2.99±0.12*

Values are Mean±SE from 6 animals in each group, p values: a =0.001 vs. vehicle control, b =0.05 vs. vehicle control, ns >0.05, *≤0.05, **≤0.01, ***≤0.001 vs. CCl_4 treated control

Table 2: Effect of silymarin, EEPG and AEPG on serum ALT, AST, ALP, bilirubin levels and liver wet weight in paracetamol (PCM) induced acute liver injury in rats

Parameters	Vehicle control	CCl_4 control	CCl_4+Silymarin (100 mg kg^{-1})	CCl_4+EEPG (250 mg kg^{-1})	CCl_4+AEPG (250 mg kg^{-1})
ALT (U L^{-1})	72.85±6.194	383.00±75.02a	10074.99±4.20***	98.26±2.51***	86.93±18.99***
ASP (U L^{-1})	36±7.801	642.19±31.04a	167.33±5.49***	170.5±5.11***	178.94±8.04***
ALP (U L^{-1})	18±6.729	749.54±36.36a	322.11±6.95***	624.27±89.7ns	338.32±39.9***
Serum bilirubine mg dL^{-1}	26±0.085	1.56±0.21a	0.24±0.049***	0.48±0.01***	30.54±0.12***
Liver weight g/100 g of b.w	12±0.10	4.40±0.139a	3.11±0.04***	18±0.069*	2.88±0.019ns

Values are Mean±SE from 6 animals in each group, p values: a≤0.001 vs. vehicle control, ns >0.05, *≤0.05, **≤0.01, ***≤0.001 vs. PCM treated control

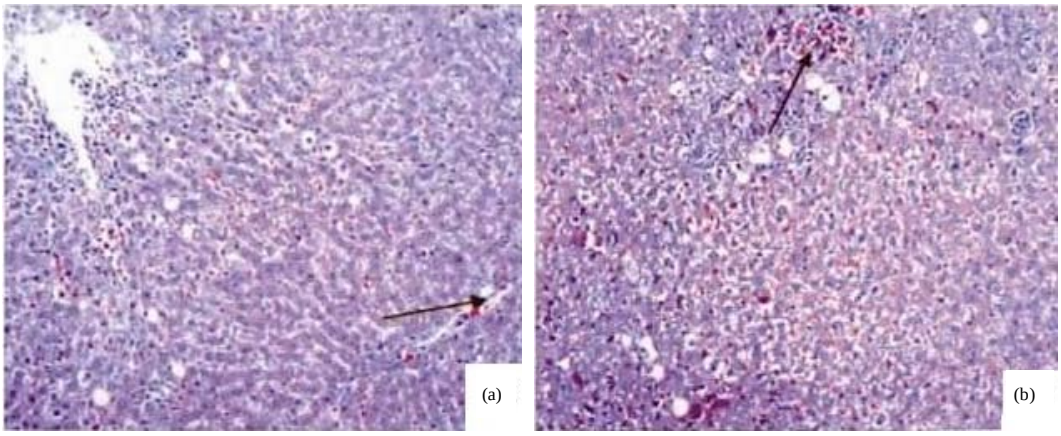

Fig. 1(a-b): Effect of *Pisonia grandis* R.Br leave extract on acute liver injury induced by CCl_4, (a) CCl_4 treated control: profound inflammation and congestion and (b) CCl_4+extract: reduced inflammation degenerative changes and steatosis

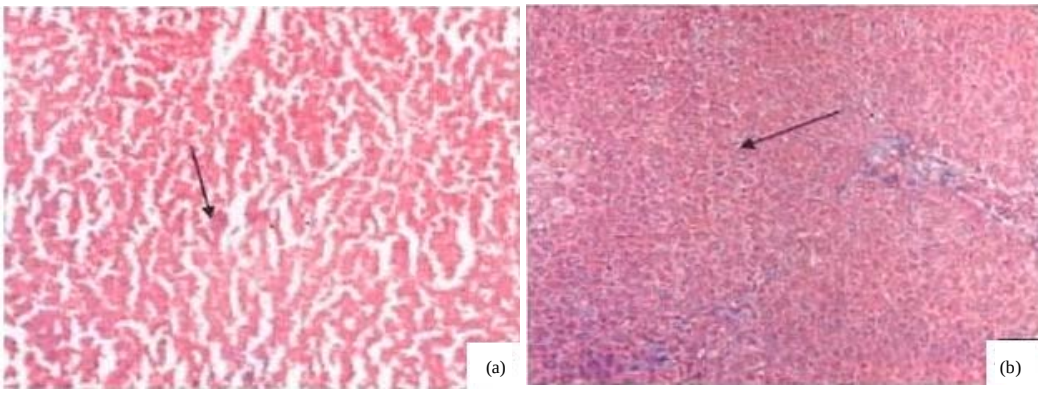

Fig. 2(a-b): Effect of *Pisonia grandis* R.Br leave extract on paracetamol induced acute liver injury, (a) Paracetamol treated control: severe congestions, hydropic degeneration pyknosis and occasional necrosis and (a) Paracetamol+extract: mild degeneration and no pyknosis

Table 3: Effect of silymarin, EEPG and AEPG on serum ALT, AST, ALP, bilirubin level and liver weight in thioacetamide (TAA) induced acute liver injury in rats

Parameters	Vehicle control	CCl_4 control	CCl_4+silymarin (100 mg kg^{-1})	CCl_4+EEPG (250 mg kg^{-1})	CCl_4+AEPG (250 mg kg^{-1})
ALT (U L^{-1})	72.25±5.26	336.74±32.44a	101.84±4.21***	132.35±7.71***	32.33±7.89***
ASP (U L^{-1})	187.16±5.41	438.34±10.69a	185.61±8.29***	175.78±7.25***	237.48±15.4***
ALP (U L^{-1})	400.20±5.84	769.77±23.87a	418.62±5.11***	380.65±13.5***	479.45±7.85***
Serum bilirubine mg dL^{-1}	0.28±0.06	0.45±0.11b	0.23±0.65ns	0.21±0.04ns	0.35±0.07ns
Liver weight g/100 g of b.w	3.12±0.12	4.07±0.96c	3.29±0.21*	3.63±0.04ns	2.76±0.12***

Values are Mean±SE from 6 animals in each group, p values: a≤0.001 vs. vehicle control, ns >0.05, *≤0.05, **≤0.01, ***≤0.001 vs.thioacetamide control

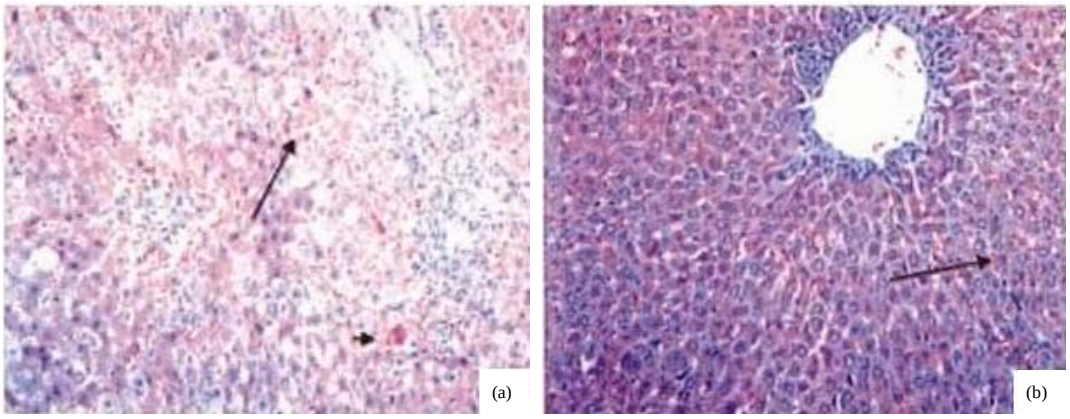

Fig. 3(a-b): Effect of *Pisonia grandis* R.Br leave extract on thioacetamide induced acute liver damage, (a) Thioacetamide treated control:arrow-Perilobular hepatocyte necrosis, inflammation and congestion; arrow head: cytoplasmic vaccoulation (b) Thioacetamide+extract:mild inflammation and no necrosis

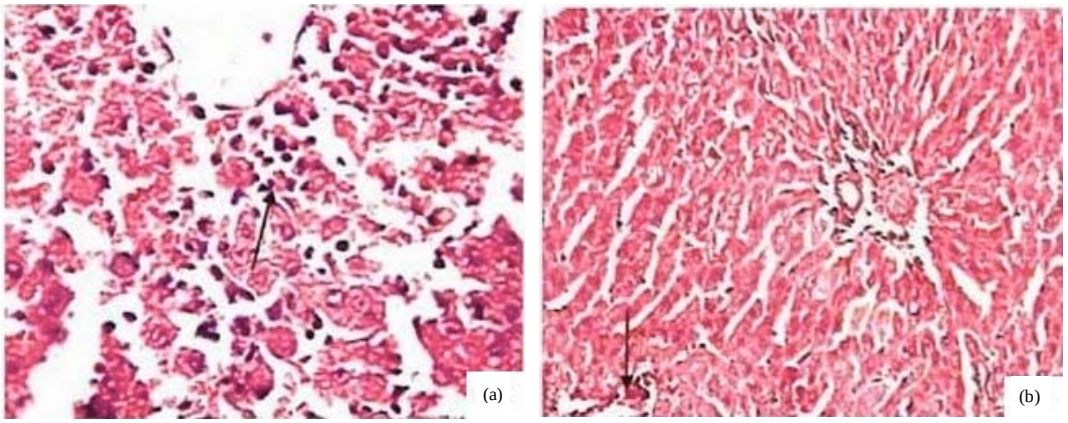

Fig. 4(a-b): Effect of *Pisonia grandis* R.Br leave extract on CCl_4 induced chronic liver damage, (a) CCl_4 treated control: fatty changes, mild congestion, connective tissue and cirrhosis and (b) CCl_4+extract: degenerative changes

Chronic hepatitis induced by CCl_4: A significant difference in biochemical markers, ALT, AST, ALP and bilirubin was observed between normal and CCl_4 treated group ($p \leq 0.001$). Comparative analysis between different groups revealed that EEPG (250 mg kg^{-1}, p.o.) and silymarin (100 mg kg^{-1}, p.o.) have similar activity ($p \leq 0.001$), whereas AEPG (250 mg kg^{-1}, p.o.) did not prevent the increase in biochemical markers. Pretreatment with EEPG (250 mg kg^{-1}; p.o.) and silymarin significantly prevented the increase in liver weight, observed after intoxication with CCl_4. AEPG (250 mg kg^{-1}; p.o.) did not produce any significant

Table 4: Effect of silymarin, EEPG and AEPG on serum ALT, AST, ALP, bilirubin levels and liver wet weight in CCl_4 induced chronic liver injury in rats

Parameters	Vehicle control	CCl_4 control	CCl_4+Silymarin (100 mg kg^{-1})	CCl_4+EEPG (250 mg kg^{-1})	CCl_4+AEPG (250 mg kg^{-1})
ALT (U L^{-1})	49.71±2.03	320.4±17.01a	137.14±6.99***	329.95±8.42ns	238.66±15.0***
ASP (U L^{-1})	119.90±4.52	617.82±15.5a	436.63±19.01***	504.61±26.1ns	422.22±15.5***
ALP (U L^{-1})	424.87±13.3	755.27±22.8a	575.47±18.01***	651.91±25.04*	613.16±13.9***
Serum bilirubine mg dL^{-1}	0.351±0.02	0.868±0.048a	0.530±0.02***	0.951±0.04ns	0.681±0.03***
Liver weight g/100 g of b.w	2.83±0.11	3.93±0.20a	2.94±0.28*	3.45±0.077ns	2.87±0.281

Values are Mean±SE from 6 animals in each group, p values: a≤0.001 vs. vehicle control, ns >0.05, *≤0.05, **≤0.01, ***≤0.001 vs. CCl4 control

reduction in liver weight (Table 4). Liver sections from CCl_4 treated control animals showed moderate degree of fatty changes, mild congestion, connective tissues, proliferation and cirrhosis. It also showed focal areas of coagulating necrosis. Formation of pseudolobular with fibrosin was also observed. Further, there were evidences of regenerating hepatocytes. In silymarin treated animals, there were fewer amounts of necrosis and regeneration. There were mild congestions, mild fatty changes and mild connective tissue proliferation. Animals treated with AEPG (250 mg kg^{-1}; p.o.) showed congestion vessels and moderate degree of fatty changes, connective tissue and cirrhosis. EEPG at the dose of 250 mg kg^{-1}; p.o.) reduced the degenerative changes compared to CCl_4 treated animals (Fig. 4).

DISCUSSION

Liver cirrhosis, a critical stage in chronic liver diseases with high morbidity and mortality, may be caused by viral infection, tissue-immune-mediated damage, toxic agents, obstructive jaundice, gene abnormalities, or alcohol and non-alcohol steatohepatitis[18], one of the major functions of the liver is detoxification of xenobiotics and toxins[19]. In many cases reactive oxygen species are produced during detoxification[20].

The ethanolic and aqueous extract of *Pisonia grandis* R.Br leaves showed superior hepatoprotective activity when administered at dose of 250 mg kg^{-1} orally. AEPG at the dose of 250 mg kg^{-1}; p.o. did not show hepatoprotective result in chronic hepatic damage induced by CCl_4. The effect shaped by the ethanolic extract of *Pisonia grandis* R.Br was alike to silymarin (100 mg kg^{-1}; p.o.), a well known hepatoprotective agent. Liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs[21]. The CCl_4 is converted into reactive metabolite, halogenated free radical by hepatic cytochrome P450s[22]. Which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation with subsequent tissue injury[23,24]. Drugs posessing antioxidant activity is effectual in treating CCl_4 induced hepatotoxicity. The CCl_4 induced a significant raise in liver weight, which is due to blocking of secretion of hepatic triglycerides into the plasma[25]. Silymarin and AEPG (250 mg kg^{-1}; p.o.) did not avert the increase of liver weight, whereas EEPG (250 mg kg^{-1}; po) barred the increase of liver weight in rats.

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses[26]. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome[27] or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity[28,29]. Depletion of GSH causes the remaining quinone to bind to cellular macromolecules leading to cell death[30]. The anti-hepatotoxic actions of EEPG (250 mg kg^{-1}; p.o.) were substantiated by significant attenuation of the increased levels of serum enzymes in rats intoxicated with PCM. Drugs having antioxidant activity are also effective in treating paracetamol induced hepatotoxicity by scavenging the free radicals produced by PCM metabolism, thereby preventing the liver induced by both PCM metabolite and due to depletion of glutathione. Extracts of *Pisonia grandis* R.Br is a known antioxidant[31] and this activity may be responsible for its effect in PCM induced hepatotoxic model. The PCM induced a significant increase in liver weight, which is due to the blocking of secretion of hepatic triglyceridesintotheplasma[25]. EEPG and AEPG (250 mg kg^{-1}; p.o.) prevented the increase in liver weight of rats pretreated with PCM.

TAA interferes with the movement of RNA from the nucleus to the cytoplasm, which may cause membrane injury. A metabolite of TAA (S-oxide) is responsible for hepatic injury[32]. Pre treatment with EEPG and AEPG (250 mg kg^{-1}, p.o.) significantly reversed the elevated serum enzyme markers in animals treated with TAA. This effect may also be due to antioxidant effect of PGJ, which may neutralize the reactive metabolite of TAA.

CONCLUSION

The ethanolic and aqueous extract of *Pisonia grandis* R.Br. leaves showed significant hepatoprotective activity in CCl_4 induced acute and chronic liver damage, PCM induced liver damage and TAA induced liver necrosis. Activity may be due to the phaytoconstituents present in

the both extracts as well as the anti oxidant nature of the plant. Further studies to characterize the active principles and to elucidate the mechanism of action are in progress.

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