

## HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF AERIAL PARTS OF *ALBIZIA PROCERA* ROXB (*BENTH.*) AGAINST PARACETAMOL INDUCED LIVER TOXICITY ON WISTAR RATS

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### ABSTRACT

**Objective:** The aim of this study was to evaluate the hepatoprotective effect of *Albizia procera* against Paracetamol-induced liver injury in rats.

**Methods:** In the present study, hepatoprotective effect of ethanolic extract of aerial parts of *Albizia procera* was investigated against Paracetamol-induced hepatotoxicity and compared with Silymarin, a standard hepatoprotective reference drug. Liver marker enzymes (ALT, ALP, AST and GGT) and Serum (Total Bilirubin, Total Protein, Total Cholesterol, Triglycerides, Albumin, Urea and Creatinine) were evaluated and histopathological analysis was done for the control and experimental rats.

**Results:** Paracetamol treatment leads to elevated levels of liver marker enzymes and histological observations. However, treatment with *Albizia procera* significantly reversed the above changes compared to the control group as observed in the paracetamol-challenged rats.

**Conclusion:** The results clearly demonstrate that *Albizia procera* possesses promising hepatoprotective effects and hence suggests its use as a potential therapeutic agent for protection from paracetamol overdose.

**Keywords:** Hepatotoxicity, Paracetamol, *Albizia procera*, Serum parameters.

### INTRODUCTION

Liver is an important organ in the body as it provides protection from potentially injurious exogenous and endogenous compounds and in this process it gets affected[1]. The liver is our greatest chemical factory, it builds complex molecules from simple substances absorbed from the digestive tract, it neutralises toxins, it manufactures bile which aids fat digestion and removes toxins through the bowels[2]. But the ability of the liver to perform these functions is often compromised by numerous substances we are exposed to on a daily basis; these substances include certain medicinal agents which were taken in over doses and sometimes when introduced within therapeutic ranges injures the organ[3]. Liver disease is worldwide problem. Conventional, drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety[4]. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders.

*Albizia procera* is a tree with an open canopy, up to 30 m tall and trunk of 35 (60 max.) cm in diameter. It is widely distributed from India and Myanmar through Southeast Asia to Papua New Guinea and northern Australia. This plant is used traditionally in anticancer[5], pain, convulsions, delirium, and septicemia[6]. The decoction of bark is given for rheumatism and haemorrhage and is considered useful in treating problems of pregnancy and for stomach-ache, sinus. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotoxic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc[8]. Even though, traditionally, leaves of *Albizia procera* were extensively used for the treatment of variety of wounds[7]. Seeds are powdered and used in amoebiasis. It cures urinary tract infections including glycosuria, haemorrhoids, fistula and worm infestation. It also suppresses skin diseases. Fruits of *Albizia procera* acts as astringent and diminishes Kapha and Sukra[16]. In India, leaves are poulticed onto ulcers[10]. Our literature survey revealed that the hepatoprotective activity of ethanolic extract from aerial parts of *Albizia procera* was not investigated; hence these activities have been investigated in the present study.

### MATERIAL AND METHODS

#### Collection and Identification of Plant materials

The aerial parts of *Albizia procera* were collected from Tularai, Thirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India, Palayamkottai. The aerial parts of *Albizia procera* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

#### Preparation of Extracts

The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus[9] for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

#### Animals

Male albino wistar rats each weighing 150-200 gm was procured from the central animal house, RMMCH in Annamalai University at Chidambaram. The animals were maintained on their respective diets and water *ad libitum*, and rats were maintained on a 12 hour light / dark cycle in a temperature regulated room (20-25°C) during the experimental procedures. The experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University (Approved number: 160/1999/CPCSEA/777). The animals were cared for according to the guiding principles in the care & use of animals.

#### Acute toxicity test

Acute toxicity tests were performed according to OECD - 423 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 ethanolic extract of *Albizia procera* suspended in normal saline: 0.5% carboxy methyl cellulose was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. The mortality was observed in 5/6 or 6/6 animals, and then the dose administered was considered as toxic dose. However, the mortality was observed in less than four

rats, out of six animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was repeated for higher doses i.e. 2000mg/kg.

### Experimental Design

Rats were divided randomly into five groups of six animals each and treated for one week (7 days) as follows.

**Group-I** Animals served as normal control, treated with vehicle (0.5% carboxy methyl cellulose).1ml/kg once daily for 7 days orally.

**Group-II** Animals served as toxic control, will receive 1ml vehicle for 7 days and on the 5<sup>th</sup> day paracetamol 2g/kg b.wt will be given per orally,

**Group III** Animals received Ethanolic extract of *Albizia Procera* (Roxb.)Benth. 200mg/kg b.wt, orally daily for 7 days. A single dose of paracetamol 2g/kg body weight will be administered per orally on 5<sup>th</sup>day .

**Group-IV** Received Ethanolic extract of *Albizia Procera* (Roxb.)Benth. 400mg/kg b.wt, by orally daily for 7 days. A single dose of paracetamol 2g/kg b.wt will be administered p.o on 5<sup>th</sup>day.

**Group-V** Received *Silymarin* 25mg/kg b.wt by orally daily for 7 days and a single dose of paracetamol 2g/kg b.wt will be administered p.o on 5<sup>th</sup>day.

### Biochemical Analysis

#### Dissection and Homogenization

On the 8<sup>th</sup> day all animals were sacrificed by cervical dislocation. Blood sample was collected in previously labelled centrifuging tubes and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 minutes [14,15].

#### Biochemical Estimation

The separated serum was used for the estimation of some biochemical parameters like SGOT and SGPT were measured according to the method of Rietman and Frankel[11]. ALP was measured according to the method of Kind and King[12], and The Kjeldhal method is used for estimation of gamma glutamyl transferase ( $\gamma$ -GT) according to Szaszi G (1969)[13]. Also, Total protein (TP) levels were determined by the method of Lowry *et al.*,(1951)[38]. Measurement of Urea was done according to the method of Patton and Crouch (1977)[35]. The Jaffe's method (1986)[34] was used to evaluate the Creatinine (CRT) levels. Total cholesterol (TC) was performed according to Henry *et al.*(1974)[33]. Estimation of Total bilirubin (TB) was followed by modified DMSO method (Mallay and Evelyn, 1936)[36] and Triglyceride (TG) were estimated by the method of Foster *et al.*,(1973)[37].

#### Histopathological Observation

Immediately after sacrifice, a portion of the liver was fixed in 10% formalin, then washed, dehydrated in descending grades of isopropanol and finally rinsed with xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 mm thickness, stained with haematoxylin and eosin was observed microscopically for histopathological changes.

#### Statistical Analysis

The values were expressed as mean  $\pm$  SEM. (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test by using

Graph Pad Instat Software. P value less than 0.05 was considered to be statistically significant. \*P<0.05, \*\*<0.01 and \*\*\*<0.001, when compared with control and toxicant group as applicable.

### RESULTS

#### Acute Toxicity

The acute toxicity of ethanolic extract of aerial parts of *Albizia Procera* was carried out as per OECD-423 guidelines for safe dose administration to animals and the study was carried out as described in experimental section. The results of acute toxicity study revealed that LD<sub>50</sub> values of ethanolic extract of aerial parts of *Albizia Procera* were high and apparently showed the safety of extract. The treatment of rat with ethanolic extract of aerial parts of *Albizia Procera* did not change any autonomic or behavioural response in rats. The zero percent mortality for ethanolic extract of aerial parts of *Albizia Procera* was found at the doses of 2000mg/kg. Overall results suggested the LD<sub>50</sub> value of 2000mg/kg. Hence the therapeutic dose was calculated as 1/10<sup>th</sup>(200mg/kg) of the lethal dose for hepatoprotective and *in-vivo* antioxidant activity.

#### Hepatoprotective Activity

The effect of ethanolic extract of aerial parts of *Albizia Procera* on biochemical parameters such as AST, ALT, ALP, and GGT is summarized in Table (1). There was a significant increase in AST, ALT, ALP and GGT levels in serum was increased in paracetamol treated rats when compared to control. The ethanolic extract of aerial parts of *Albizia Procera* treatments reversed the level of AST, ALT, ALP and GGT when compared to paracetamol alone treated rats. Silymarin treated animals also showed significant decrease in AST, ALT, ALP and GGT levels when compared to paracetamol alone treated rats.

Table 2 shows the effect of pretreatment of rats with ethanolic extract of aerial parts of *Albizia Procera* on non enzyme markers of tissue damage in paracetamol induced tissue toxicity. Administration of paracetamol significantly increased the levels of Total Bilirubin, urea and creatinine when compared to control group of rats. The serum Total Bilirubin, Urea, Creatinine were significantly decrease in paracetamol with ethanolic extract of aerial parts of *Albizia Procera* treated rats (Group III & IV) and as well as standard drug (Group V) compared with paracetamol treated rats (Group II).

Table 3 was summarized the effect of the effect of the ethanolic extract of aerial parts of *Albizia Procera* on serum Total Cholesterol and Triglycerides in paracetamol treated rats. The paracetamol administered rats were significantly increased the levels of Total Cholesterol and Triglycerides when compared to control group of rats. The serum Total Cholesterol and Triglycerides were significantly decrease in paracetamol with ethanolic extract of aerial parts of *Albizia Procera* treated rats (Group III & IV) and as well as standard drug (Group V) compared with paracetamol treated rats (Group II).

Table 4 shows that the effect of the ethanolic extract of aerial parts of *Albizia Procera* on Total Protein and Albumin in paracetamol treated rats. The paracetamol treated rats were significantly decrease in the level of Total Protein and Albumin (Group II) when compared to control group of rats (Group I). Treatment of rats with ethanolic extract of aerial parts of *Albizia Procera* treated rats (Group III & IV) and as well as standard drug (Silymarin (Group V)) significantly increase the level of Total Protein and Albumin compared with paracetamol treated rats (Group II).

**Table 1: Effect of ethanolic extract of aerial parts of *Albizia Procera* on serum enzymes (AST, ALT, ALP, GGTP) in paracetamol treated rats.**

Treatment	AST (IU.L <sup>-1</sup> )	ALT (IU.L <sup>-1</sup> )	ALP (IU.L <sup>-1</sup> )	GGT(IU.L <sup>-1</sup> )
Group-I	73.45 $\pm$ 3.29	99.43 $\pm$ 3.78	189.65 $\pm$ 4.82	1.37 $\pm$ 0.12
Group-II	187.09 $\pm$ 19.24 <sup>a***</sup>	198.87 $\pm$ 9.32 <sup>a***</sup>	265.98 $\pm$ 15.43 <sup>a***</sup>	1.98 $\pm$ 0.19 <sup>a**</sup>
Group-III	124.76 $\pm$ 11.32 <sup>b**</sup>	165.13 $\pm$ 6.55 <sup>b**</sup>	213.14 $\pm$ 12.03 <sup>b**</sup>	1.58 $\pm$ 0.08 <sup>ns</sup>
Group-IV	98.87 $\pm$ 5.83 <sup>b***</sup>	117.54 $\pm$ 5.38 <sup>b***</sup>	204.85 $\pm$ 9.08 <sup>b**</sup>	1.49 $\pm$ 0.07 <sup>b*</sup>
Group-V	76.56 $\pm$ 4.15 <sup>b***</sup>	106.74 $\pm$ 4.12 <sup>b***</sup>	197.39 $\pm$ 5.83 <sup>b***</sup>	1.41 $\pm$ 0.10 <sup>b**</sup>

Data are expressed as mean $\pm$ SEM, n = 6 rats per group. P values, \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; ns= not significant; compared to Paracetamol group. One way ANOVA followed by Dunnett's test. **a**→ Group II compared to Group I; **b**→ Group II compared to Group III, IV and V.

**Table 2: Effects of ethanolic extract of aerial parts of *Albizia procera* on serum enzymes (Creatinine, Urea and Total Bilirubin) in paracetamol treated rats.**

Treatment	Creatinine (milimol <sup>-1</sup> )	UREA (milimol <sup>-1</sup> )	TB (mg/dl)
Group - I	54.5±2.63	7.52±0.21	1.29±0.14
Group - II	74.4±4.13 <sup>a***</sup>	18.76±3.09 <sup>a***</sup>	4.67±1.20 <sup>a***</sup>
Group - III	62.05±2.65 <sup>b*</sup>	10.63±0.82 <sup>b**</sup>	2.08±0.19 <sup>b*</sup>
Group - IV	59.0±1.57 <sup>b**</sup>	8.92±0.32 <sup>b***</sup>	1.41±0.13 <sup>b**</sup>
Group - V	57.6±2.31 <sup>b***</sup>	7.94±0.35 <sup>b***</sup>	1.32±0.17 <sup>b***</sup>

. Statistical data and Details of group I-V are same as in Table 1

**Table 3: Effects of ethanolic extract of aerial parts of *Albizia procera* on serum enzymes (TG,TC) in paracetamol treated rats.**

Treatment	TG (mg/dl)	TC (mg/dl)
Group - I	49.3±2.63	93.4±4.85
Group - II	78.9±5.37 <sup>a***</sup>	195.3±12.02 <sup>a***</sup>
Group - III	61.4±3.94 <sup>b*</sup>	153.5±7.32 <sup>b**</sup>
Group - IV	55.8±3.67 <sup>b**</sup>	149.3±5.88 <sup>b***</sup>
Group - V	52.7±3.65 <sup>b***</sup>	99.1±4.93 <sup>b***</sup>

.Statistical data and Details of group I-V are same as in Table 1

**Table 4: Effects of ethanolic extract of aerial parts of *Albizia procera* on serum enzymes (TP, and ALB) in paracetamol treated rats.**

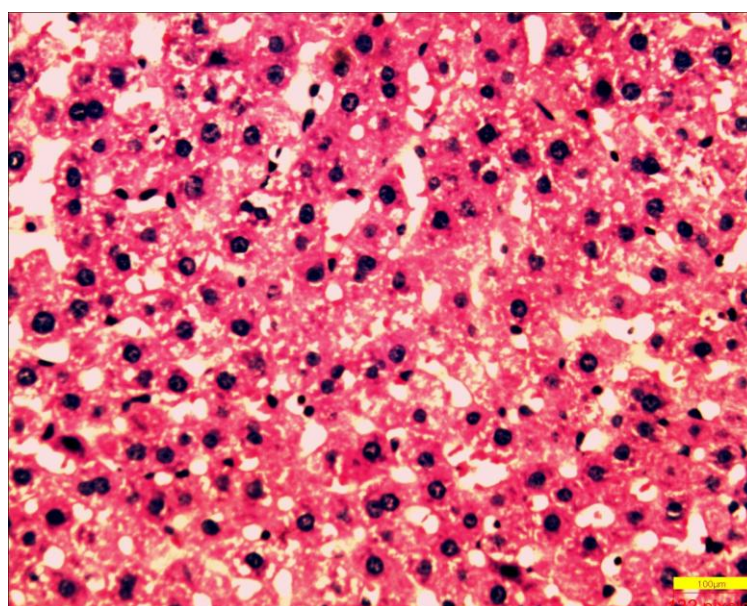
Treatment	TP (mg/dl)	ALB (g/dl)
Group - I	7.53±0.32	6.12±0.12
Group - II	5.83±0.21 <sup>a***</sup>	5.17±0.15 <sup>a***</sup>
Group - III	6.53±0.25 <sup>NS</sup>	5.82±0.18 <sup>b*</sup>
Group - IV	6.97±0.15 <sup>b**</sup>	5.96±0.16 <sup>b**</sup>
Group - V	7.13±0.12 <sup>b**</sup>	6.07±0.08 <sup>b***</sup>

.Statistical data and Details of group I-V are same as in Table 1

### Histopathological Results

The hepatoprotective effect of ethanolic extract of aerial parts of *Albizia Procera* was confirmed by histopathological examination of the liver tissue of control and treated animals: **Fig1:** Liver section of normal control rats (Group I) showing normal liver lobular architecture with well brought out central vein and prominent nucleus and nucleolus. **Fig 2:** Toxic control (Paracetamol 2g/kg) treated rats (Group II) showing severe toxicity with congested blood

vessels with inflammatory cell collection and endothelial cell swelling. **Fig 3:** Ethanolic extract of aerial parts of *Albizia Procera* (200 mg/kg) treated rats (Group III) showing only moderate inflammation around portal tract. **Fig 4:** Ethanolic extract of aerial parts of *Albizia Procera* (400mg/kg) treated rats (Group IV) produced mild degenerative changes and absence of necrosis, sinusoidal dilation, and central vein congestion. **Fig 5:** Standard drug (Silymarin 25mg/kg) treated rats (Group V) showed almost normal hepatic architecture.



**Fig. 1: Control Rat Liver**

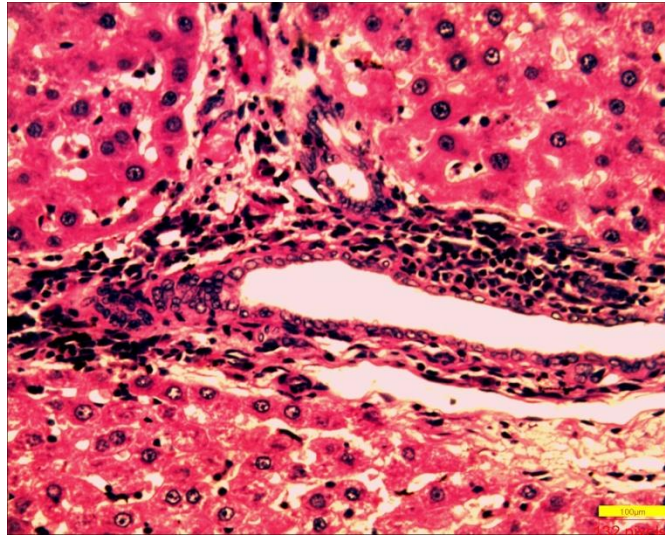


Fig. 2: Paracetamol (2g/kg) Treated Rat Liver

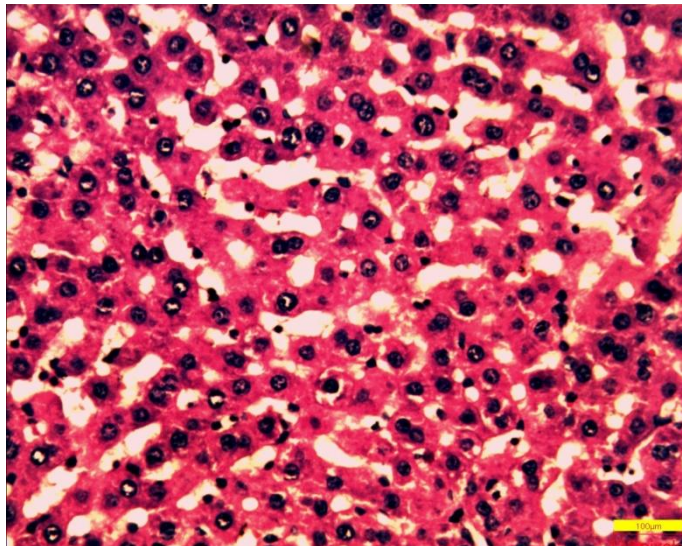


Fig. 3: Paracetamol (2g/kg) + Treated group 200mg/kg of Albizia Procera

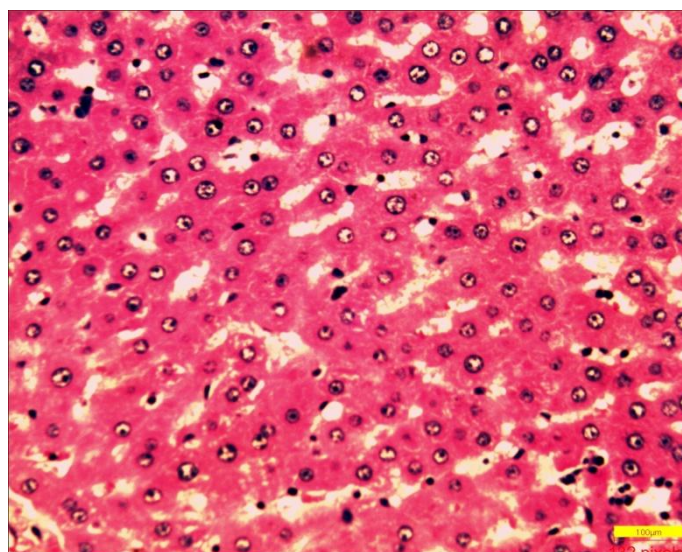


Fig. 4: Treated group 400mg/kg of Albizia Procera + Paracetamol (2g/kg)

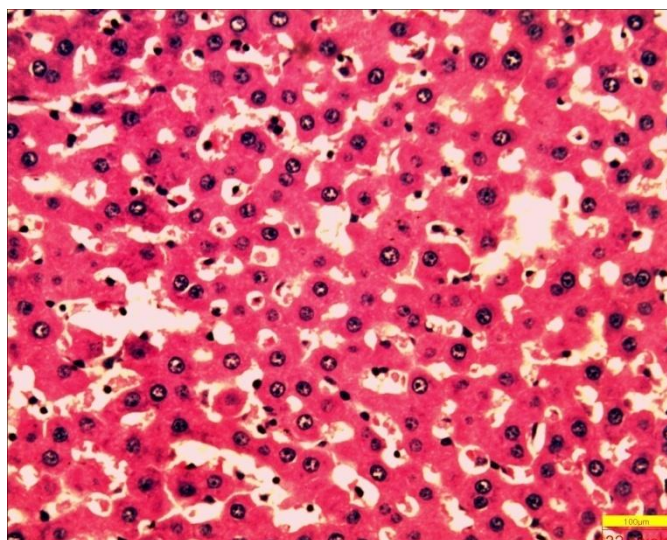


Fig. 5: Standard (Silymarin 25mg/kg) + Paracetamol (2g/kg) Rat Liver

## DISCUSSION

Paracetamol is a common analgesic and antipyretic drug. Several studies have demonstrated the induction of hepatocellular damage or necrosis by acetaminophen higher doses in experimental animals and humans [17]. For screening of hepatoprotective agents, paracetamol-induced hepatotoxicity has been used as a reliable method. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted by the kidney. Paracetamol in larger doses produces liver necrosis after undergoing bio-activation to a toxic electrophile, N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P-450 monooxygenase [19]. NAPQI binds to macromolecules and cellular proteins. Liver enzymes, ALT, AST, and ALP are usually low in normal control. Injury to the liver results in the release of these enzymes into the blood. An elevated level of these enzymes markers of hepatic necrosis in animals treated with only acetaminophen indicates tissue damage. Injury to the hepatocytes changes their transport function and membrane permeability, causing leakage of enzymes from the cells [21]. In the assessment of liver damage by paracetamol the determination of enzyme levels such as aspartate transaminase and alanine transaminase is largely used. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [30]. Hepatocellular necrosis leads to high level of serum markers in the blood, among these, aspartate transaminase, alanine transaminase represents 90% of total enzyme and high level of alanine transaminase in the blood is better index of liver injury,

Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of alkaline phosphatase in the blood serum is related to the increased synthesis of it by cells lining bile canaliculi usually in response to cholestasis and increased biliary pressure [31,32].  $\gamma$ -glutamyl transferase is a microsomal enzyme, which is widely distributed in tissue including liver. The activity of serum  $\gamma$ -glutamyl transferase is generally elevated as a result of liver disease, since  $\gamma$ -glutamyltransferase is a hepatic microsomal enzyme. Serum  $\gamma$ -glutamyl transferase is most useful in the diagnosis of liver diseases. Changes in  $\gamma$ -glutamyl transferase is parallel to those of amino transferases.

The supplementation of ethanolic extract of aerial parts of *Albizia Procera* extract brought down to elevated levels of AST, ALT, ALP, and GGT. These biochemical restorations may be due to the inhibitory effects on cytochrome P-450 or /and promotion of its glucuronidation [18]. Consequently, the significant release of these enzymes and their increased activity in animals treated with acetaminophen can be interpreted to be as a result of liver cell destruction and alteration in the membrane permeability [22]. Increased bilirubin content reflects the pathophysiology of the liver

and one of the most sensitive and useful test to substantiate the functional integrity of the liver and severity of necrosis which measures the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocytes degradation rate [23]. Increased levels of bilirubin reflect the depth of jaundice and increased aminotransferases and alkaline phosphatase was the clear manifestation of cellular leakage and loss of functional integrity of the cell [24].

The ethanolic extract of *Albizia Procera* and silymarin showed a dose dependent activity in reducing the levels of these enzymes. The reversal of increased serum enzymes in acetaminophen-induced liver damage by the extract may be due to its membrane stabilizing activity thus preventing the leakage of intracellular enzymes. The effective control of total protein and total bilirubin can be attributed to an improvement in the hepatic cells' secretory mechanisms. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin [25]. Both silymarin and the *Albizia Procera* extract decreased acetaminophen induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytes, its cell membrane or regeneration of damaged liver cells.

Several metabolic disorders including urea and creatinine derangements are possible in the presence of acetaminophen over dosage [26]. Increased concentration of serum urea and creatinine are considered for investigating drug induced nephrotoxicity in animals and man [27]. Acetaminophen treatment obviously interfered with kidney filtration functions as seen by its elevated values in rats. Urea, a waste product of protein catabolism can rise when the kidney is defective. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of its clearance [28]. The ethanolic extract of *Albizia Procera* had a dose dependent reversal of the effects on this parameter. The present study showed the hepatoprotective effects of the ethanolic extract of aerial parts of *Albizia Procera* in acetaminophen-induced toxicity.

There was an appreciable increase in the cholesterol levels of the acetaminophen treated group when compared with the normal group. The liver is central to the regulation of cholesterol levels in the body, not only does it synthesize cholesterol for export to other cells but it also removes cholesterol from the body by converting it to bile salts and excreting it into the bile. Levels of triglyceride in the serum or liver could increase due to several processes, including increased availability of free fatty acid, glycerophosphate, decreased VLDL in the serum and decreased removal of triglyceride and cholesterol from serum due to diminished lipoprotein activity [29]. The extracts showed a dose dependent reversal of such

increased. The ethanolic extract of aerial parts of *Albizia Procera*, being able to show more efficacy than the individual makes a case for the use of *Albizia Procera* extracts. Also, the triglyceride levels obtained from the study showed a significant decrease across the group.

Histopathological analysis of the liver sections is in good agreement with biochemical changes[20], Liver sections also revealed that the normal liver architecture was disturbed by hepatotoxin in Paracetamol group, whereas in the liver sections of the rat treated with the ethanolic extract and intoxicated with Paracetamol the normal cellular architecture was retained and it is comparable with the standard Silymarin group. Therefore, on the basis of our results, the possible mechanism of hepatoprotective activity of *Albizia Procera* might be due to the presence of flavonoids, saponin, terpenoids and other active constituents.

## CONCLUSION

In the present study, the administration of ethanolic extract of aerial parts of *Albizia Procera* shows a significant hepatoprotective activity in paracetamol induced liver damage in rats. However Further studies are in progress to isolate the active constituents of *Albizia Procera* and also to evaluate the exact mechanism of action for the hepatoprotective activity.

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