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Hepatoprotective Effect of *Flacourtia inermis* Roxb Aqueous Leaves Extract Against Paracetamol Induced Liver Toxicity in Rats

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ABSTRACT

This study aim to evaluate the Hepatoprotective effect of *Flacourtia inermis* Roxb (FI) aqueous leaves extract against Paracetamol induced liver toxicity in rats. FI leaves were extracted with aqueous solvent by maceration procedure. The hepatoprotective activity was evaluated through an in vivo study using Wistar albino rats. The study involved four groups of rats. **Group I** was administered with 0.5% CMC solution + dietary supplement served as solvent control (Control), **Group II** was administered with 0.5% CMC solution orally + dietary supplement served for seven days + on the 8th day Paracetamol was administered as disease-induced control (Paracetamol negative control), **Group III** was administered with 50 mg/kg BW of Silymarin orally + dietary supplement served for seven days + on the 8th day Paracetamol was administered (Silymarin Positive Control), **Group IV** was administered with 200 mg/kg BW single dose of FI leaves extraction orally + dietary supplement served for seven days + on the 8th day Paracetamol was administered. On the tenth day of the study, the animals were humanely euthanized to minimize distress, and blood samples were collected via cardiac puncture. The collected serum was then processed for further analysis. The isolated serum was subjected to a comprehensive analysis, which included the evaluation of key liver enzymes such as SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamate Pyruvate Transaminase), as well as Alkaline Phosphatase (ALP). Additionally, the levels of Total Serum Bilirubin (TB) and Total Albumin (TA) were assessed to gain a deeper understanding of the serum's composition and potential implications. The study found that administering 200 mg/kg/day of FI's aqueous extract orally significantly reduced the increase in serum SGOT, SGPT, ALP, and total bilirubin levels compared to the Paracetamol-treated group ($p < 0.05$). Histopathological examination of the liver tissue revealed that FI extract helped restore normal hepatic architecture and reduced centrilobular necrosis in treated rats. These findings suggest that FI's aqueous extract may possess hepatoprotective properties, making it a potentially safe and effective oral treatment option with no observed side effects.

Keywords: Carbon methyl cellulose (CMC), *Flacourtia inermis* Roxb, Hepatoprotective activity, Liver, Paracetamol, Silymarin

1 INTRODUCTION

Flacourtia inermis Roxb (Salicaceae) commonly known as lobi-lobi. The term "lovi-lovi" was introduced from Sumatra, Indonesia by Dr. Roberto Coronel in 1987[1]. In previous studies proved that the plant has antimicrobial activity, anti-diabetic activity and used for prevention and treatment of obesity[2]. This investigation aimed to explore the potential hepatoprotective effects of the aqueous extract of *Flacourtia inermis* (FI) leaves against liver damage experimentally induced by Paracetamol.

2 MATERIALS AND METHODS

2.1 Plant Collection and Authentication:

Fresh leaves of *Flacourtia inermis* (FI) were harvested from the Puliyanam region in Ernakulam district, Kerala, India, during the post-monsoon period. The authenticity of the plant material was confirmed by Prof. Dr. L. Mullainathan at the Department of Botany, Annamalai University, Annamalai Nagar. The plant material has an authentication reference number: 591.

Then the leaves are washed and dried under sunshade in dark room. The leaves were sieved through No. 40 and No. 60 meshes for size reduction and the leaves were stored in a tightly sealed, airtight container to preserve its integrity and facilitate future use.

2.2 Extraction of the plant material

In the maceration method, 100 g of coarse powdered leaves parts FI was soaked in 500 ml of distilled water for 72 hours. Following soaking, the mixture was subjected to filtration to remove insoluble particles, and the resulting filtrate was then concentrated through rotary vacuum evaporator at 55 ˚ C. The aqueous extract was further concentrated using a water bath at 50°C, until a thick paste formed, yielding the dry crude extract. The obtained crude extract was weighed and stored at 4ºC in a refrigerator for the further analysis[3].The filtrate was dried by vacuum rotary vacuum evaporation to yield a solid residue of 17.5g (Percentage yield 17.5%).

2.3 PHARMACOLOGICAL STUDY

2.3.1 Animal Models:

In this study, we used 8-10 week old Wistar rats (150-250 g) and 6-8 week old Albino mice (25-30 g) were used for the study. The animals were sourced from mass biotech private limited, Chennai. All the animals were housed and maintained by central animal house, Cuddalore Medical College and Hospital. The animals were randomly assigned to treatment groups and housed in well-ventilated polypropylene cages with paddy husk bedding, which was changed twice a week. The animal housing conditions were strictly controlled, with a temperature range of 24±2°C, relative humidity of 30-70%, and a 12-hour light/dark cycle. All animals had free access to water and were fed standard commercial pelleted rat chow. Prior to the experiment, the animals underwent a one-week acclimatization period to adapt to the laboratory environment. The study protocol received approval from the Institute's Animal Ethics Committee (IAEC) (Proposal No: GMCHC-IAEC/1384/3/24), which is registered with the Committee for the Control and Supervision of Experiments on Animals (CCSEA).).

2.3.2 Acute oral toxicity

The study was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals, section 423, Acute Oral Toxicity - Acute Toxic Class Method. Mice were fasted overnight and provided with water *ad libitum.* Four groups of three animals each received oral ingestion of the aqueous extract of FI leaves at varying dose levels: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight. Observations were made periodically over 30 minutes during the first 24 hours and daily for 14 days, with specific attention given during the first 4 hours. Parameters monitored included morbidity, mortality, toxic symptoms (behavioral changes, locomotor activity, convulsions), and direct observation parameters (tremors, salivation, diarrhea, sleep, coma, changes in skin, fur, eyes, and mucous membranes, as well as respiratory, circulatory, autonomic, and CNS effects)[4,5].

Table 1: Acute oral toxicity study design

Group	Group specification	Treatment
Group I	Three Albino mice	Treated with aqueous extract of FI leaves of 5mg/kg,p.o.,
Group II	Three Albino mice	Treated with aqueous extract of FI leaves of 50mg/kg,p.o.,
Group III	Three Albino mice	Treated with aqueous extract of FI leaves of 300mg/kg,p.o.,
Group IV	Three Albino mice	Treated with aqueous extract of FI leaves of 2000mg/kg,p.o.,

2.3.3 Preparation of Plant Extract, Hepatotoxin and Standard drug

Preparation of Extract Solution: Aqueous leaves extract was dissolved in 0.5% carboxymethyl cellulose (CMC) to prepare an oral administration solution. The resulting solution was then diluted to achieve a dose of 200 mg/kg body weight, ready for administration.

Hepatic toxin: Paracetamol was dissolved in 0.5% CMC (1:1; 1 ml/kg, p.o.,) body weight orally.

Standard Drug: Silymarin are also suspended in 0.5% CMC and the substance was given orally at a dose of 50 mg/kg body weight[6].

2.3.4 Experimental study design using Paracetamol induced hepatic damage in animal model

A total of 24 male rats were randomly assigned to four groups, each consisting of six rats. The treatment regimen for each group is described as follows. Silymarin and FI leaves extract extraction was dissolved in 0.5% CMC solution. Silymarin (50 mg/kg) and single concentrations (200 mg/kg) of FI leaves aqueous extraction were administered orally for seven days each morning, using a 16 G gavage needle. Meanwhile, the control group received 0.5% CMC solution as a vehicle control. On the 8th day, before euthanizing, group II, group III and group IV were administered with Paracetamol (2g/kg, p.o)[7]. Body weights were regularly monitored and recorded throughout the duration of the experiment[8].

Group I was administered with 0.5% CMC solution + dietary supplement served as solvent control (Control).

Group II was administered with 0.5% CMC solution orally + dietary supplement served for seven days + on the 8th day Paracetamol was administered as disease-induced control (Paracetamol negative control).

Group III was administered with 50 mg/kg BW of Silymarin orally + dietary supplement for seven days + on the 8th day Paracetamol was administered (Silymarin Positive Control).

Group IV was administered with 200 mg/kg BW single dose of FI leaves extracts orally + dietary supplement for seven days + on the 8th day Paracetamol was administered.

On the 10th day, i.e., after 24 hours of Paracetamol induction, rats from the each of the group were anaesthetized under ketamine (87 mg/kg i.p) + xylazine (13 mg/kg i.p.,)[9].

2.3.4.1 Biochemical parameters

In this in-vivo study, biochemical parameters serve as the most reliable indicators. These parameters encompass the measurement of various enzymes, including: SGOT, SGPT and Serum alkaline phosphatase (SALP). Additionally, this section includes the estimation of Serum bilirubin (SBLN) and Protein content in liver tissue. These biochemical parameters provide valuable insights into liver function and are essential for assessing the efficacy and safety of treatments[10].

2.3.4.2 Histological parameters

These include the necrosis induced, type and the extent of degradation and fibrosis in Liver tissue[11].

2.3.5 Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's post-hoc multiple comparison tests to compare differences between groups[GraphPad Prism 10.2.3(403)][12].

3. RESULTS

The results of acute toxicity study revealed that LD_{50} values of aqueous extract of FI leaves was high and apparently showed the safety of aqueous extract. The zero percent mortality for aqueous extract of FI leaves was found at the dose of 2000mg/kg.

In addition to these the animals were observed continuously for one hours at first instance, and then at an interval of two hours for 24 hours to examine any change occurs (or) observed in the mice behavior such skin and fur, eye, Sense of touch and sound, salivation, lethargy, sleep, convulsion, tremors, diarrhea and mortality.

There is no significant toxicity was observed for 14 days. The aqueous extract of FI was found to be safe for mice and pre-screening investigation with 1/10th of 2000 mg/kg, i.e. 200 mg was done. The dose 200 was selected against hepatoprotective activities.

Rats pre-treated with FI aqueous extract reported significant protection against hepatotoxicity induced by Paracetamol and the results of the serum biochemical parameter analysis are summarized in Table 2.

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Oral administration of paracetamol to rats resulted in liver injury, as evidenced by a significant elevation in serum levels of ALT, AST, ALP enzymes, and bilirubin, compared to the control group and also significant reduction in total protein and serum albumin compared with control group. The elevated enzyme levels are a marker of hepatocellular injury, indicating that the cell membrane's functional integrity has been compromised, resulting in the release of intracellular enzymes into the bloodstream. The concurrent administration of aqueous extracts of FI resulted in a remarkable reduction ($P < 0.05$) in the elevated serum levels induced by paracetamol, indicating a protective effect against hepatotoxicity.

Table 2: Effect of FI leaf extract on Paracetamol induced hepatic damage in rats (n=6)

GROUPS	TREATMENT	Total **Protein** (g/dL)	**Serum Albumin** (g/dL)	Total Bilirubin (µmol/L)	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)
Group-I	Normal control group	6.383±0.162	3.767± .294	1± 0.28	60.62±1.34	71.13±0.90	128±2.82
Group-II	Paracetamol treated group	5.5±0.23***	3.1±0.18***	2.21± 0.29***	116.6±2.83***	225.7±4.81***	167.1±1.69***
Group-III	Silymarin treated group	6.1 ±0.23^{ns}	3.50± 0.20^{ns}	1.31±0.52**	73.50±1.71***	119.5±2.30***	158.5±1.99***
Group-IV	*F.inermis* treated group	5.9±0.14***	3.3±0.14**	1.23±1.2***	86 ± 2.01***	109.3±2.16***	163.4±1.01***

Values are mean ± SEM. Significance was assessed by one-way ANOVA with Dunnett's test.

ns = non-significant, **p < 0.05, ***p < 0.01 denotes significant difference compared with group I.

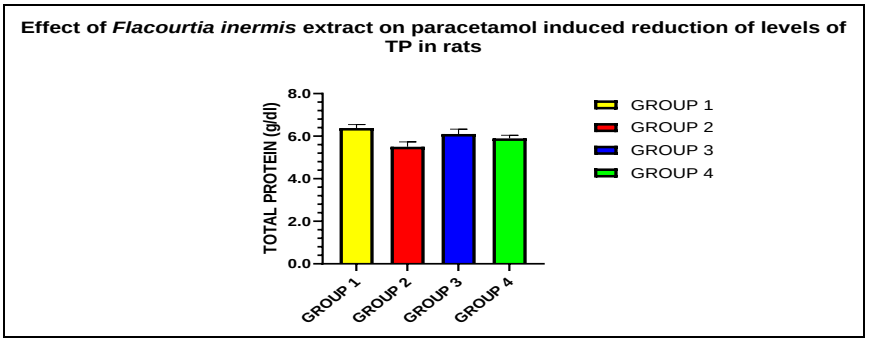

Figure 1: Effect of FI extract on Paracetamol induced reduction of levels of total protein in rats

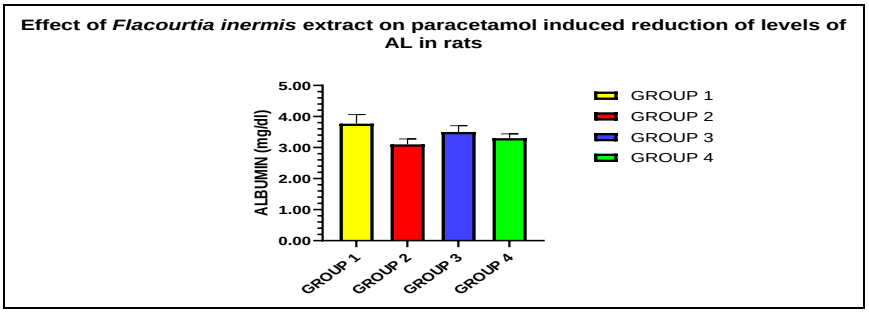

Figure 2: Effect of FI extract on Paracetamol induced reduction of levels of serum albumin in rats

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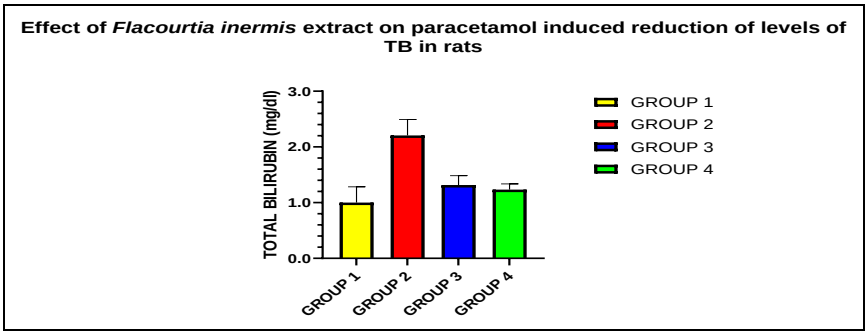

Figure 3: Effect of FI extract on Paracetamol induced elevated of levels of total bilirubin in rats

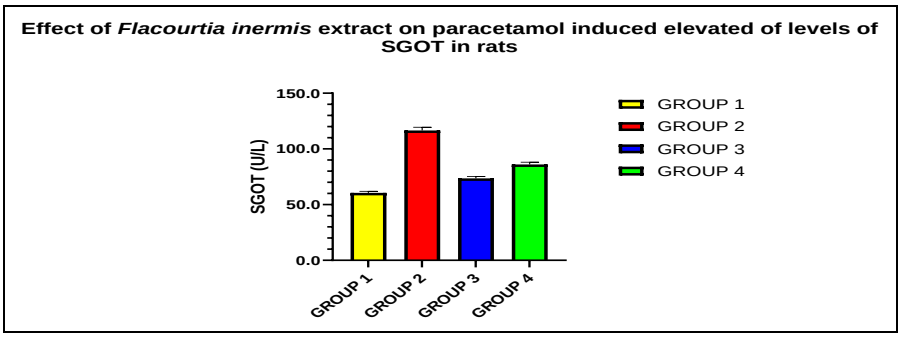

Figure 4: Effect of FI extract on Paracetamol induced elevated of levels of SGOT in rats

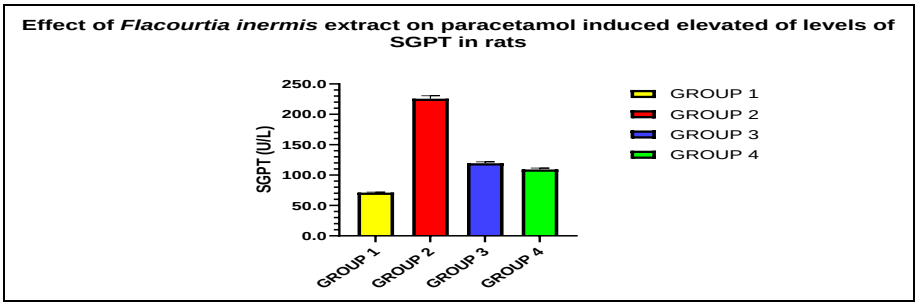

Figure 5: Effect of FI extract on Paracetamol induced elevated of levels of SGPT in rats

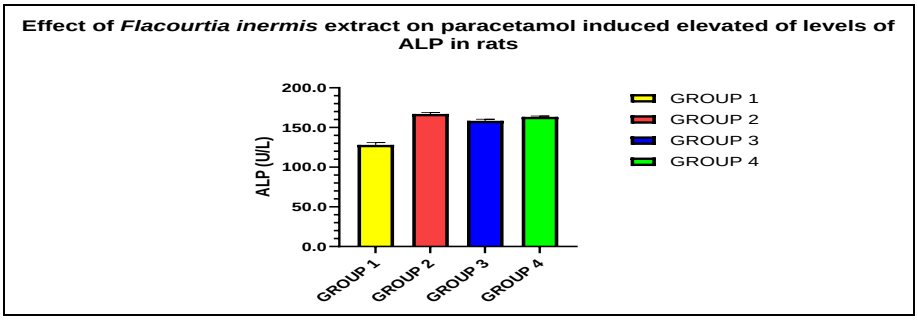

Figure 6: Effect of FI extract on Paracetamol induced elevation of levels of ALP in rats

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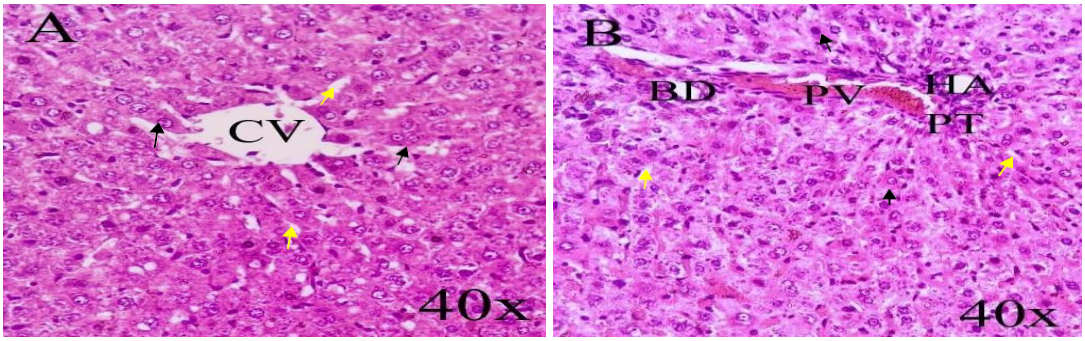

Figure 7: Histological section of Group I

(A) Photomicrograph shows normal intact rat liver parenchyma of hepatocytes (black arrows) radiating away from the CV (Central Vein) with sinusoids (yellow arrows). (B) Shows PT (Portal triad) comprised of PV (Portal Vein), HA (Hepatic Artery) and BD (Bile duct) with hepatocytes (black arrows) and sinusoids (yellow arrows).

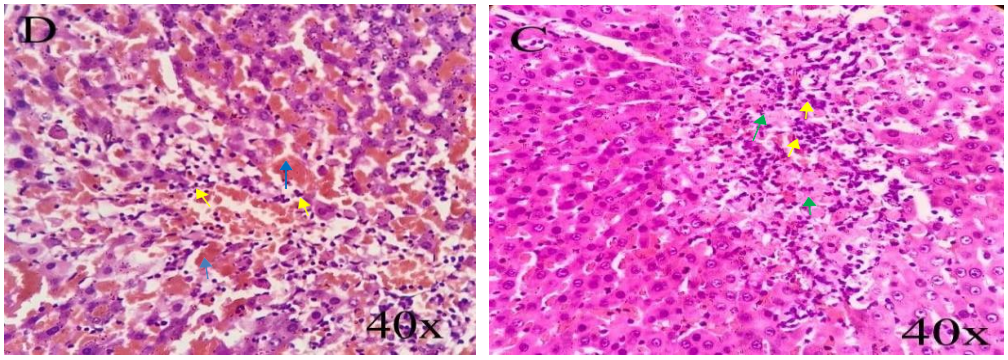

Figure 8: Histological section of Group II

(C) Yellow and green arrows denote severe diffuse infiltration of mononuclear inflammatory cells and hepatocytes necrosis respectively. (D) Blue arrows indicate severe sinusoidal hemorrhage's along with inflammation of mononuclear cells (yellow arrows)

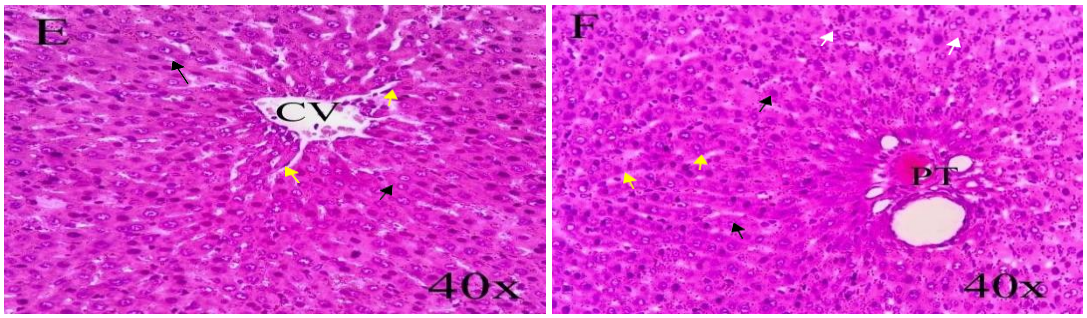

Figure 9: Histological section of Group III

(E) Show normal hepatocytes (black arrows) and sinusoids (yellow arrows) with CV (Central Vein). (F) Reveals normal PT (Portal Triad) with normal hepatocytes (black arrows) and sinusoids (yellow arrows). White arrows represent artifacts.

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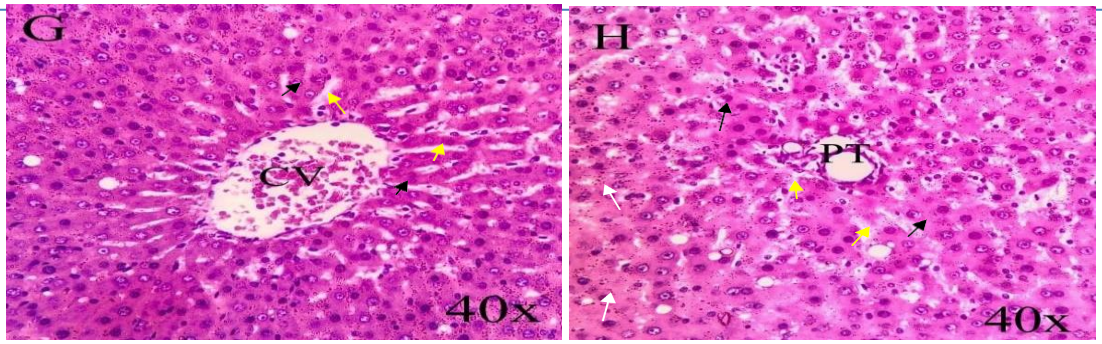

Figure 10: Histological section of Group IV

(G) Reveals normal cords of hepatocytes (black arrows), sinusoids (yellow arrows) and minimal number of RBCs present inside the CV (Central Vein). (H) Shows normal PT (Portal triad) with hepatocytes (black arrows), sinusoids (yellow arrows) and artifacts (white arrows).

4 DISCUSSION

The human body uses the liver as its second major organ for metabolizing foreign substances. In humans, the majority of chemicals and medications cause liver damage. A growing number of researchers are working to discover novel hepatitis medications by studying traditional treatments.

The acute oral toxicity test suggested that the aqueous extract of FI leaves is non-toxic and safe at doses up to 2000 mg/kg. Therefore, on the basis of toxicity study data, 200 mg/kg doses were selected to assess the hepatoprotective effect of the aqueous extract of FI leaves against Paracetamol induced liver damage.

Paracetamol-induced liver damage models are employed to evaluate the hepatoprotective effects of various agents. Paracetamol is primarily metabolized in the liver via conjugation with glucuronide and sulfate, followed by renal excretion[13]. However, acetaminophen also undergoes cytochrome P450-mediated metabolism to form N-acetyl-p-benzoquinone imine (NAPQI), a highly toxic metabolite. Normally, NAPQI is detoxified through conjugation with glutathione and excreted in the urine. Nevertheless, acetaminophen overdose depletes glutathione reserves, leading to NAPQI accumulation, mitochondrial dysfunction, and ultimately, acute hepatic necrosis[11].

The enzyme levels such as SGOT, SGPT and ALP are measured during the experiment that can be commonly used to assess Paracetamol induced liver damage. These enzymes are released into the bloodstream due to hepatocyte necrosis or membrane injury, allowing them to be detected in serum. SGOT is predominantly located in hepatocyte mitochondria. SGPT is considered as common marker for hepatic injury because it is primarily found in liver cells[14]. Additionally, damage to liver cells is associated with changes in serum ALP and bilirubin levels.

The extract potentially stabilizes membranes and hinders the leakage of intracellular enzymes, which otherwise would elevate serum enzyme levels in Paracetamol-induced liver damage. This aligns with the commonly accepted idea that restoring hepatic parenchymal restore and hepatocyte regeneration leads to normalization of transaminase levels in the bloodstream[15,16].

Elevated liver enzymes (SGOT, SGPT, and ALP) are a hallmark of hepatic injury. Treatment with *Flacourtia inermis* (FI) leaves extract (200 mg/kg) demonstrated comparable hepatoprotective effects to silymarin (50 mg/kg), a standard reference drug. The aqueous extract of FI leaves demonstrated a protective effect against Paracetamol-induced liver injury, evidenced by a significant decrease in elevated serum enzyme levels. Histopathological examination revealed improved liver tissue architecture in rats treated with the plant extract, suggesting that FI co-administration prevented Paracetamol-induced hepatonecrosis. The hepatoprotective properties of FI were further substantiated by histopathological studies, which corroborated the serum assay findings.

Histopathological examination of the liver tissue in Paracetamol-treated rats revealed severe damage, characterized by fatty changes, cellular swelling, and necrosis, leading to a loss of hepatocytes[17]. Administration of FI (200 mg/kg) facilitated hepatocellular regeneration, resulting in normalized hepatic steatosis and necrosis. Histopathological analysis revealed that FI treatment significantly reversed Paracetamol-induced hepatic lesions, with liver architecture approaching near-normal conditions, comparable to the control group. These findings suggest that FI exhibits hepatoprotective effects, warranting further investigation[18].

5 CONCLUSION

This study highlights the significant hepatoprotective effects of FI leaves against Paracetamol-induced liver damage. Administering an aqueous extract of FI leaves at a dose of 200 mg/kg effectively reduces elevated serum hepatic enzyme levels and bilirubin levels and restores total protein, albumin. The biochemical improvements were further supported by histopathological findings, which revealed a decrease in hepatocyte necrosis and fibrosis, accompanied by an increase in cellular regeneration in the FI-treated groups. These observations suggest that FI treatment not only improved liver function but also promoted cellular repair and regeneration, leading to a reduction in liver damage. These findings underscore FI's potential to enhance liver health. The multifaceted benefits of *Flacourtia inermis* (FI) underscore its potential for the liver function, justifying further clinical investigation to fully elucidate its hepatoprotective effects against drug- related liver injury. Further investigation is warranted to explore the optimal dosage, duration of treatment, and potential synergistic effects with existing hepatoprotective agents. From the present study, this revealed that the above data strongly recommended that the aqueous extract of FI could have hepatoprotective activity. This can be regarded as a safe, orally active, acceptable and also free from side effects.

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Conflict of Interest Statement:
The authors have no conflicts of interest to declare.

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