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Evaluation of Hepatoprotective Activity of Hydro Alcoholic Extract of *Croton bonplandianum.* Baill Leaves in Paracetamol Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Aim of the study: The main aim of the study is to evaluate the hepatoprotective activity of hydro alcoholic extract of *Croton bonplandianum.* Baill (HECB) leaves in paracetamol induced hepatotoxicity in Wistar rats.***Materials and methods:*** The powdered leaf material is extracted by maceration method for 72 hours. Initially, the phytochemical investigations of HECB leaves were performed. The study involved five groups, Group I (Normal control group), Group II (paracetamol induced hepatotoxicity), Group III (silymarin), Group IV and Group V (test drug 1 and test drug 2) were treated upto 7 days, on the 8th day all the groups except Group I received paracetamol and After 10 hours of paracetamol induction, rats from the each of the group were anesthetized; blood collected in a sterile centrifuge tube by intra cardiac puncture method. ***Results:*** The HECB leaves that demonstrated the presence of alkaloids, glycosides, flavonoids, carbohydrates, saponins, tannins and proteins. The results showed that HECB leaves significantly reduced Paracetamol-induced elevated serum enzyme levels and improved histopathological damage. The extract prevented hepatonecrotic changes induced by the toxic dose of paracetamol.***Conclusions:*** The results of this study strongly indicate the protective effect of HECB leaves against acute liver injury. Overall, the study provides scientific evidence for the traditional use of *Croton bonplandianum*. Baill leaves in the treatment of liver disorders.

Keywords: *Croton bonplandianum*. Baill, histopathological damage, Carboxy Methyl Cellulose (CMC), Hepatoprotective activity, Silymarin and paracetamol.

1. INTRODUCTION:

The liver is a vital organ involved in metabolism, detoxification, and storage, constantly exposed to toxins through blood from the digestive tract [1]. It metabolizes drugs and chemicals, generating reactive oxygen species (ROS), which at high levels cause oxidative stress and liver damage [2]. The liver, because of its role in metabolism, is exposed to many kinds of xenobiotics and therapeutic agents [3]. Hepatitis is a common disease in the world especially in the developing countries [4]. *Croton bonplandianum.* Baill is used in traditional medicine for various ailments. It's used to treat jaundice, constipation, abdominal dropsy, and dysentery. The plant is also used for external wounds, hypercholesterolemia, and hypertension. *Croton bonplandianum.* Baill is traditionally used in Asia and South America. Research has isolated bioactive compounds like diterpenes, alkaloids, and flavonoids [5]. The plant exhibits hepatoprotective, anti-inflammatory, and antimicrobial activities. It also shows antioxidant and anti-tumor properties. *Croton bonplandianum.* Baill is a rich source of traditional medicine. In vitro and in vivo studies validate its traditional medicinal uses. The plant's extracts and bioactive constituents have potential therapeutic applications [6]. The present investigation describes the hepatoprotective effect of HECB leaves. The hepatoprotective effect was assessed in paracetamol induced liver damage. To select, identify and authentication of *Croton bonplandianum.* Baill leaves [7]. The objectives of this study is to prepare the hydro alcoholic extract of *Croton bonplandianum.* Baill leaves by using maceration method and evaluation of the hepatoprotective activity against paracetamol induced hepatic damage in Wistar rats and to compare the protective effect of the extract with the standard drug silymarin and observe the biochemical parameters such as total bilirubin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), direct bilirubin, indirect bilirubin, albumin, total protein and triglycerides content including histopathological study [8].

2. MATERIALS AND METHODS

2.1. Plant collection

Fresh *Croton bonplandianum*. Baill leaves were gathered from the Bhuvanagiri area in the Cuddalore district of Tamilnadu, India. 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: 621.

2.2. Preparation and Extraction of the plant material

The collected leaves are cleaned, washed with distilled water, dried under sunshade in dark room, and course powdered by using mechanical mixer. Upon size reduction, the leaves are sieved through No. 40 and No. 60 meshes. 100g of the coarsely powdered leaf material of *Croton bonplandianum*. Baill leaves are subjected to extraction with 500 ml of hydro alcohol for 72 hours. The extract are filtered through Whatmann filter paper No: 1, distilled and stored at -20°C until use [9]. The extraction of pharmaceuticals from their natural sources has been an essential component of the drug discovery process [10].

2.3. Experimental animals:

Male Albino Wistar rats (150 – 250 g) were used for the study. The animals were obtained from MASS BIOTECH private limited, Chengalpet (REG. NO: 2084/PO/RcBt/S/19/CPCSEA). All the animals were housed and maintained by central animal house, Cuddalore Medical College and Hospital. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals will be housed at a temperature of 24±20C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat pellets. The animal were acclimatized to the laboratory condition for a week before starting the experiment. Paddy husk were used as bedding and it were changed twice in a week. The study protocol received approval from the Institutional Animal Ethics Committee (IAEC) (Proposal No: GMCHC –IAEC/1404/4/25), which is registered with the Committee for the Control and Supervision of Experiments on Animals (CCSEA).

2.4. Experimental study design

Table 1: Experimental study

GROUPS		GROUP SPECIFICATION	TREATMENT
Group I (Normal control)		6 Wistar rats	Animals received 0.5% of CMC daily for eight days.
Group II (Negative control)		6 Wistar rats	Animals received 0.5% of CMC daily for seven days. On the 8th day paracetamol (2g/kg, p.o.) were administered.
Group III (Standard)		6 Wistar rats	Silymarin (50 mg/kg, p.o.) were administered for consecutively seven days. On the 8th day paracetamol (2g/kg, p.o.,) were administered.
Group IV (Test 1)		6 Wistar rats	Low dose of hydro alcoholic extract of *Croton bonplandianum.* Baill leaves (200 mg/kg,p.o) were administered for consecutively seven days. On the 8th day paracetamol (2g/kg, p.o.,) were administered.
Group V (Test 2)		6 Wistar rats	High dose of hydro alcoholic extract of *Croton bonplandianum.* Baill leaves (400 mg/kg,p.o) were administered for consecutively seven days. On the 8th day paracetamol (2g/kg, p.o.,) were administered.

2.5. Methodology

The animals were divided into five groups of six animals each [11]. The grouping of animals is shown in table 1. On 8th day of induction, all the animals except group I were induced with paracetamol (2g /kg/p.o)., After 10 hours of paracetamol induction, rats from the each of the group were sacrificed; blood were collected in a sterile centrifuge tube by cardiac puncture [12]. Serum were separated by centrifuging at 3000 rpm at 4℃ for 20 min and used for the biochemical assays.

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2.6. Effects on Biochemical parameters

Biochemical parameters i.e., aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubin (TB), Direct bilirubin (DB), Indirect bilirubin (IB), total protein (TP), Serum albumin and Triglycerides (TGL) were analyzed [13,14,15].

2.7. Histopathological studies

Before histopathological examination and for the preservation of isolated livers of rats of different groups, 10% formalin solution were used. The liver were mounted by embedding it in paraffin; it were cut into size of 6 mm sections, put on slide and stained consequently with the dyes eosin and hematoxylin and observed in the light microscope intended hepatoprotection [16].

2.8. Statistical analysis

Data were expressed as Mean ±SEM. Comparison between different groups was performed using One-way ANOVA, followed by Dunnett's multiple comparison tests [GraphPad Prism 10.4.2 (633)]. $P<0.05$ was considered to be statistically significant [17].

3. RESULTS

3.1. Effects on Biochemical parameters

In this experiment, the HECB leaves appears to be effective in reducing the paracetamol caused injury as observed from a significant reduction of paracetamol induced elevated serum enzyme levels.

Table 2: Effects on Biochemical parameters

GROUPS	TOTAL PROTEIN (g/dL)	SERUM ALBUMIN (g/dL)
GROUP-I	6.5±0.16	3.4±0.27
GROUP-II	4.9±0.14**	2.5± 0.18ns
GROUP-III	5.9±0.14ns	3.2±0.21ns
GROUP-IV	5.0± 0.57ns	3.0±0.25ns
GROUP-V	5.3±0.12**	3.1±0.27ns

n=6 animals per group; Values are expressed as mean ±SEM. One way ANOVA followed by Dunnett's test. ns = non-significant,*p < 0.05, **p < 0.01, ***p< 0.001 and ****p< 0.0001 denotes significant difference compared with control group.

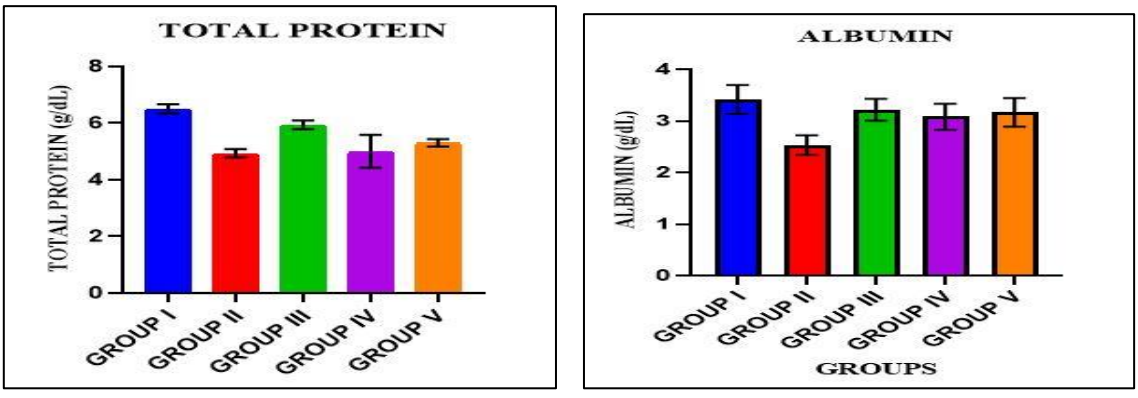

Figure 1: Effects on Biochemical parameters such as Total protein and Albumin

Administration of paracetamol (2g/kg) toxin in rats (Group II) shows significant depletion of serum TP and serum albumin than control (Group I). Treatment with silymarin (Group III) and plant extract (Group IV and Group V) significantly inhibited paracetamol induced depletion of serum TP and serum albumin at the dose 50 mg/kg, 200mg/kg and 400mg/kg as compared to paracetamol treated group.

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Table 3: Effects on Biochemical parameters

GROUPS	SGOT(IU/L)	SGPT(IU/L)	Total Bilirubin (mg/dL)	Triglycerides (IU/L)
GROUP-I	255.4±2.21	281.5±1.64	0.6±0.07	149.0±4.04
GROUP-II	397.3±1.94****	406.1±1.93****	0.9±0.13ns	196.0±3.89**
GROUP-III	263.1±1.55ns	302.6±1.34****	0.7±0.08ns	150.0±3.35ns
GROUP-IV	324.0±1.63****	310.9±1.86**	0.8±0.09ns	156.0± 3.44ns
GROUP-V	300.6±1.78***	304.2±1.63****	0.8±0.08ns	152.0±4.65ns

n=6 animals per group; Values are expressed as mean ±SEM. One way ANOVA followed by Dunnett's test. ns = non-significant,*p < 0.05, **p < 0.01, ***p< 0.001 and ****p< 0.0001 denotes significant difference compared with control group.

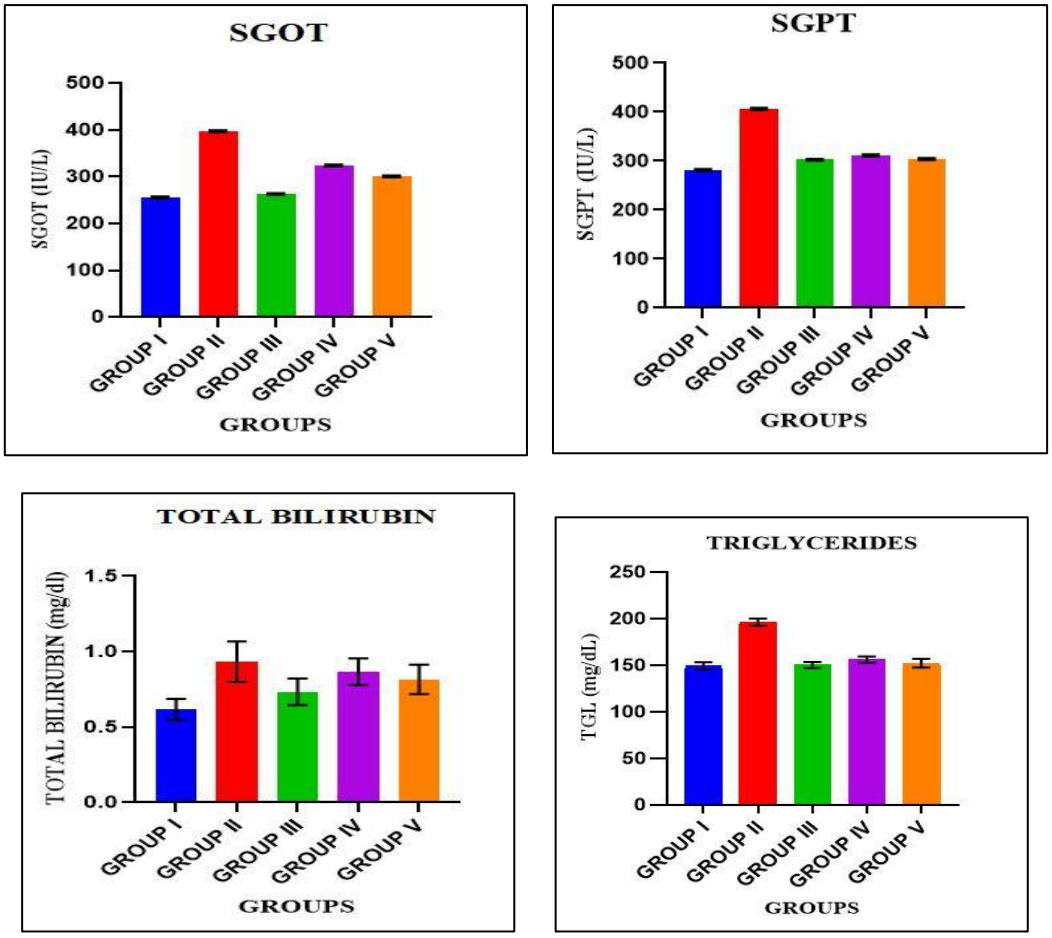

Figure 2: Effects on Biochemical parameters such as SGOT, SGPT, Total bilirubin and Triglycerides

Administration of paracetamol (2g/kg) toxin in rats (Group II) shows significant elevation of serum SGOT, SGPT, Total bilirubin and triglycerides than control (Group I). Treatment with silymarin (Group III), plant extract (Group IV and Group V) significantly inhibited paracetamol induced elevation of serum SGOT, SGPT, Total bilirubin and Triglycerides at the dose 50 mg/kg, 200 mg/kg and 400mg/kg as compared to paracetamol control group.

3.2. Histopathological studies

The histopathological study was performed using light microscopy, 40 X resolution using hematoxylin-eosin stain. The histopathological damage induced by paracetamol was improved in rat liver treated with plant extract. This implies that concomitant administration of HECB leaves prevented hepatonecrotic changes induced by the toxic dose of paracetamol. Histopathological

studies of the liver showed fatty changes, swelling and necrosis with loss of hepatocytes in paracetamol treated rats. HECB leaves treated groups showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver at the dose 200 mg/kg and 400 mg/kg. The histopathological observations of the liver of rats treated with HECB leaves showed a more or less normal architecture of the liver having reversed to a large extent, the hepatic lesions produced by paracetamol, almost comparable to the normal control groups.

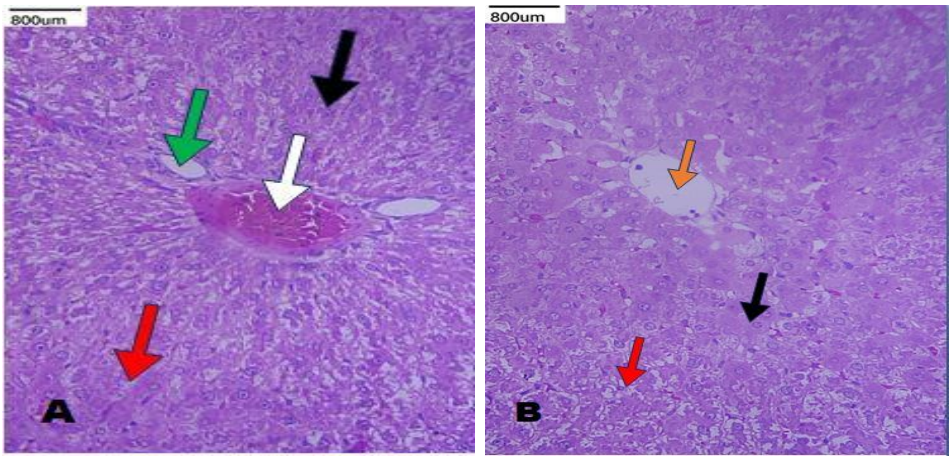

Figure 3: A and B- Histopathology of liver tissue in normal control group (Group I)

Normal control group (Figure 3) showed normal hepatocytes (black arrow), portal triad structures (white arrow) are normal, sinusoids (red arrow) and central vein (orange arrow) appears normal. No significant pathology noted.

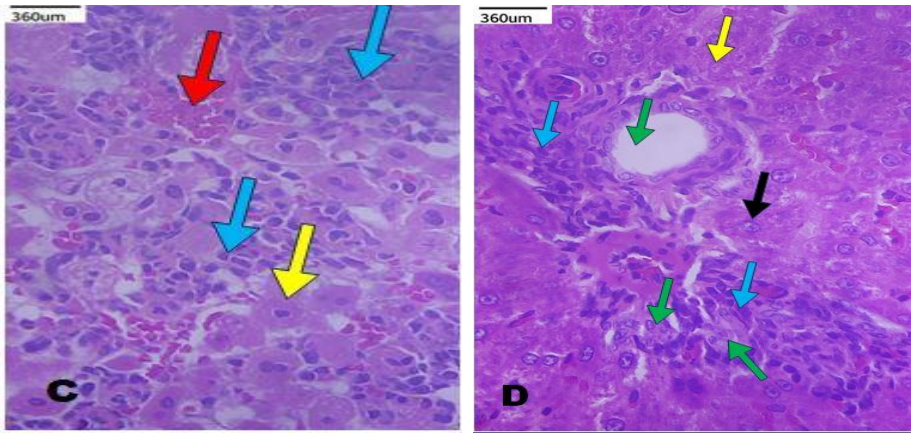

Figure 4: C and D- Histopathology of liver tissue in negative control group (Group II)

Negative control group (Figure 4) showed degenerated hepatocytes (yellow arrow) with increased cytoplasmic colour (eosinophilic- pink) intensity with pyknosis and karyorrhexis. Surrounded by dense inflammatory infitrates. Also noted the extensive bile duct proliferation (green arrow), interface hepatitis (blue arrow), dilated sinusoids (red arrow) and congested central vein (orange arrow).

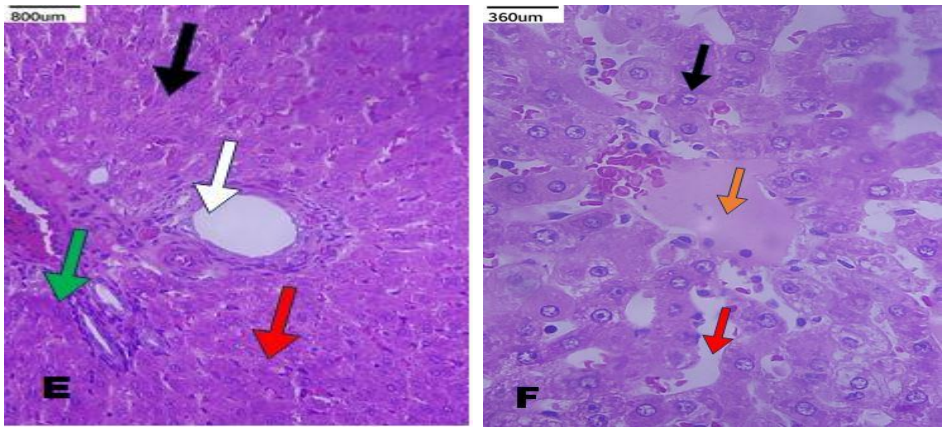

Figure 5: E and F- Histopathology of liver tissue in standard group (Group III)

Standard group (Figure 5) showed no inflammation and degeneration showed normal hepatocytes (black arrow), portal triad structures (white arrow) are normal, sinusoids (red arrow) and central vein (orange arrow) appears normal.

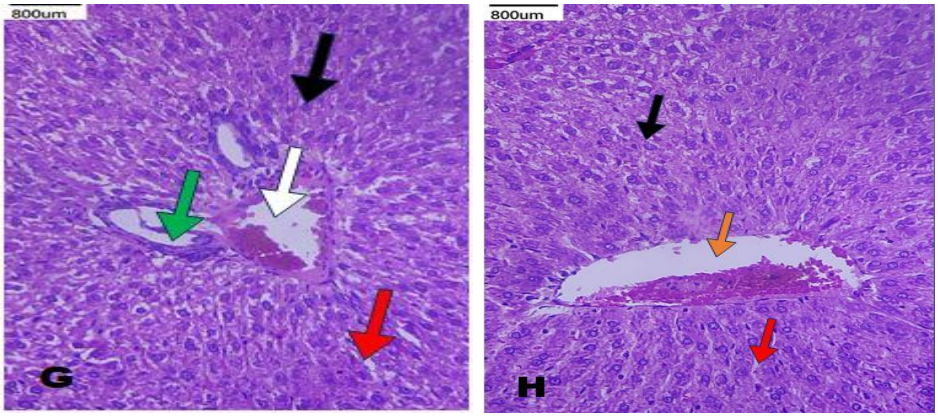

Figure 6: G and H- Histopathology of liver tissue in Test drug 1 (Group IV)

Test drug 1 (Figure 6) showed normal hepatocytes (black arrow), portal triad structures (white arrow) are normal, sinusoids (red arrow) and central vein (orange arrow) appears normal.

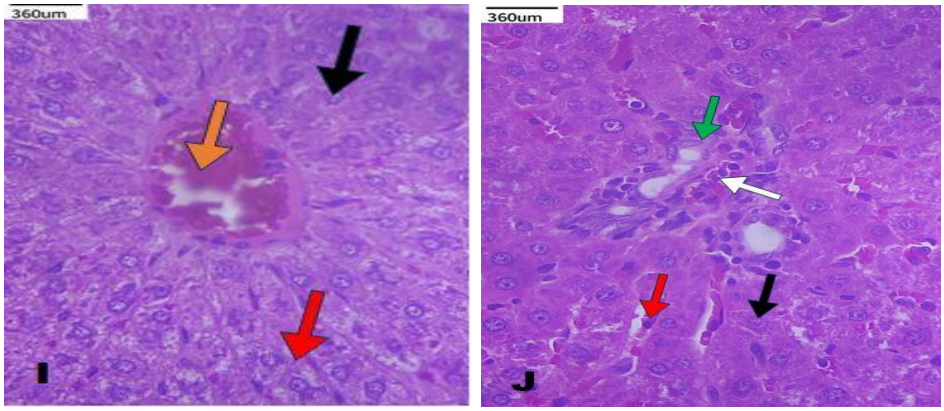

Figure 7: I and J- Histopathology of liver tissue in Test drug 2 (Group V)

Test drug 2 (Figure 7) showed normal hepatocytes (black arrow), portal triad structures (white arrow) are normal, sinusoids (red arrow) and central vein (orange arrow) appears normal.

4. DISCUSSION

The present investigation describes the hepatoprotective effect of HECB leaves. The hepatoprotective effect was assessed in paracetamol induced liver damage. paracetamol induced hepatotoxicity model, Group III (silymarin), Group IV and Group V (test drug 1 and test drug 2) were treated upto 7 days, on the 8th day all the group except Group I received paracetamol and the blood samples were collected on 10th day for various treatments as depicted in experimental design. These blood samples were analyzed to determine the different serum enzymes which are indicators of the liver damage and total protein content. Moreover, liver tissue was dissected after decapitation and liver tissues were examined histopathologically to assess liver damage.

In this experiment, the HECB leaves appears to be effective in reducing the paracetamol caused injury as observed from a significant reduction of paracetamol induced elevated serum enzyme levels [18]. It was also noted that the histopathological damage induced by paracetamol was improved in rat liver treated with plant extract. This implies that concomitant administration of HECB leaves prevented hepatonecrotic changes induced by the toxic dose of paracetamol.

Hepatoprotective effect of HECB leaves was further confirmed by histopathological studies of the liver, which basically supported the results from the serum assays [19]. Histopathological studies of the liver showed fatty changes, swelling and necrosis with loss of hepatocytes in paracetamol treated rats [20]. The HECB leaves treated groups showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver at the dose 200 mg/kg and 400 mg/kg. The histopathological observations of the liver of rats treated with HECB leaves showed a more or less normal architecture of the liver having reversed to a large extent, the hepatic lesions produced by paracetamol, almost comparable to the normal control groups [21].

5. CONCLUSIONS

The results of this study strongly indicate the protective effect of HECB leaves against acute liver injury. Further research is needed to isolate and characterize the bioactive compounds responsible for the hepatoprotective activity and to explore the underlying mechanisms of action. The present study may confirm the ability of HECB leaves to produce hepatoprotective activity due to phytoconstituents presence in the leaves such as glycoside, alkaloids and flavonoids responsible for significant hepatoprotective activity and may have future scope to replace allopathic medications, hence reduces side effects, cost and enhance patient compliance. Overall, the study provides scientific evidence for the traditional use of *Croton bonplandianum*. Baill leaves in the treatment of liver disorders.

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Conflict of Interest Statement: All authors have nothing else to disclose.