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Research Article

# HEPATOPROTECTIVE ACTIVITY OF *KIRGANELIA RETICULATA* POIR. (BAILL) ROOT AGAINST PARACETAMOL INDUCED HEPATO-TOXICITY IN WISTAR RATS

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**ABSTRACT**

Objective: To demonstrate the *in-vivo* hepatoprotective effect of the ethanolic extracts of root of *Kirganelia reticulata* Poir (Baill) against paracetamol induced hepatotoxicity in Wistar rats.

Methods: Wistar rats of either sex were divided into six groups with six in each group. Group 1-Normal control: The animals were maintained under normal control, which were given 0.5% Tween 80 used as vehicle. Group 2-Induction of hepatotoxicity: The animals received paracetamol (2gm/kg, *p.o.*) every 72 h for 7 days. Group 3: The animals were treated with Silymarin (100 mg/kg, *p.o*) which served as standard. Groups 4 to 6: Animals received ethanolic root extract of *K. reticulata* (EEKR) at (100, 200 & 300 mg/kg, *p.o.*) everyday for 7 days. Groups 3 to 6 were intoxicated with paracetamol (2gm/kg, *p.o.*) 1 h before the administration of extract or Silymarin for 7 days. Histopathological findings, different hepatic biochemical parameters viz. Serum glutamic-oxaloacetic transaminase (sGOT), serum glutamic pyruvic transaminase (sGPT), serum alkaline Phosphatase (sALP), Total bilirubin, Total albumin & blood urea were evaluated to investigate the hepatoprotective activity.

Results: Paracetamol induced a significant rise in sGOT, sGPT, sALP, Total bilirubin, Total albumin, & blood urea estimation. Administration of 300 mg/kg, *p.o.* ethanolic root extract of *K. reticulata* less effectively reduced these pathological damages caused by paracetamol intoxication. The ethanolic root extract of *K. reticulata* also promoted the body weight in Wistar rats as shown in Figure 1. Histopathological changes of the liver were compared with the normal control as shown in Figures 2-6 respectively.

Conclusions: The ethanolic roots extract at dose of 300mg/kg, *p.o.* of *K. reticulata* have significant effect on hepatoprotective activity against paracetamol induced hepatotoxicity in Wistar rats.

**Keywords**: *Kirganelia reticulata*, Paracetamol, Silymarin, sGPT, sGOT, sALP & Histopathology.

## INTRODUCTION

Liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification of the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism[1]. Diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences. About 20, 000 deaths occur every year due to liver diseases. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases each year[2]. During the metabolism, excessive free radicals are generated & may cause liver damage. In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell[3]. There are, however, a number of drugs employed in traditional system of medicine for liver afflictions[4]. Therefore drugs from natural source are being adopted to treat hepatitis/ liver diseases. It has great capacity to detoxicate toxic substances & synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences[5]. *Kirganelia reticulata* (Poir.) Baill. is a large, often scandent, belonging to the family euphorbiaceae popularly known as "potato plant or potato bush" and is variously named in different parts of the world. Synonymously, it is also named as *Phyllanthus reticulatus* Poir.[6,7]. The plant grows throughout tropical areas of India, Malay Island, Bangladesh, and China[8]. The leaves, bark and root bark are used as astringent, attenuant and diuretic. Juice of leaves is used for the treatment of diarrhoea in children[7]. *K. reticulata* elaborates different class of organic compounds of medicinal importance including alkaloids, flavonoids lignans, sitosterol, polyphenols, triterpenoids, saponins, coumarin and phytosterols[9,10]. The maximum number of phytochemical compounds are present in leaves then stem and root[11]. The phytochemicals, stigmasterol, β-sitosterol, tricin, fridelin, 21α-hydroxyfridelin-3-one, betulin, glochidonol, sorghumol and kokoonol have been reported to be in the root[12,13].The plant is reported to have antidiabetic[14], antibacterial[15,16], antioxidant[17-21], antiplasmodial[22], antinociceptive[23], hyperglycaemic[23], analgesic[24], anti-inflammatory[24,25], antiviral[26], cytotoxic and insectisidal activity[27]. The ethanol extract of aerial part of plant has been observed to demonstrate hepatoprotective activity against $CCl_4$ induced liver damages in rats[28].

The survey of literature reveals that the root of *K. reticulata* is found to be used in traditional system of Indian medicine as folkeric use. However, hepatoprotective activity of root of *Kirganelia reticulata* Poir. (Baill). has not been scientifically investigated. Therefore, in the present study hepatoprotective effect of ethanolic extract of root of *Kirganelia reticulata* Poir. (Baill). have been evaluated against paracetamol induced hepatotoxicity in Wistar rats.

## MATERIALS AND METHODS

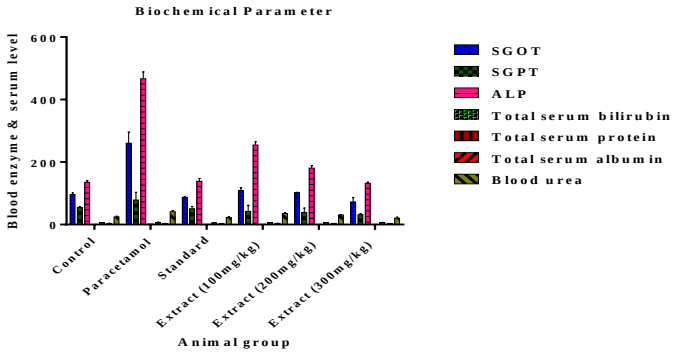
### Collection and authentication of plant materials

The root of *K. reticulata* was collected in the month of September from Kukrail forest near Central Institute of Medicinal and Aromatic Plants, Lucknow (U.P), India and authenticated by Dr. Tariq Hussain, Senior Principal Scientist & Head of Plant Diversity, Systematics and Herbarium Division National Botanical Research Institute, Lucknow (U.P), India. A voucher specimen has been deposited at department of systematics and herbarium, NBRI Lucknow, (U.P), India. (Accession No. **LWG-007**).

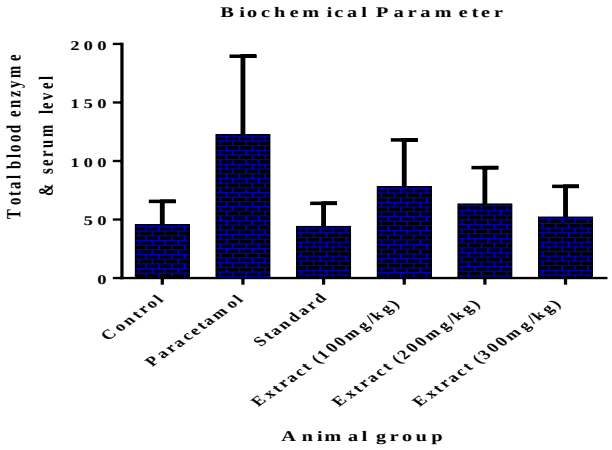
### Drugs and chemicals

Silymarin was procured as a gift sample from Krypton Pharma Ltd. India. Paracetamol was procured as gift sample from Pharmasynth Formulations Ltd, Haridwar (Uttrakhand) India.

All other chemicals and reagents used for the experiment were of high analytical grade.




**Graph 1: Bar diagram representation of Enzyme & serum level in blood.**




**Graph 2: Bar diagram showing total average of enzyme and serum level in blood.**

**Preparation of plant extracts**

The fresh roots were cleaned, shade dried and then powdered using grinder. The dried coarse powder of the roots (200gm) was packed in the Soxhlet apparatus and continuously extracted by ethanol at the temperature 70±2 °C.

The extracts were pooled together and concentrated by rotary evaporator. The yield was 7.5% w/w. Suspensions of extract were prepared using 0.5% Tween 80, Paracetamol (2gm/kg) suspension prepared by using 0.5% Tween 80 and subjected for hepatoprotective activity against paracetamol-induced hepatotoxicity.

**Preliminary Phytochemical investigation**

Preliminary phytochemical investigation of ethanol extract of root of *K. reticulata* was carried out by standard procedure given by Kokate[29] and Khandelwal[30].

**Experimental animals**

Wistar rats of (180-220gm) were procured from Institute of Pharmacy, Bundelkhand University Jhansi, India. The animals were maintained under standard hygienic conditions. The animals were given food and water and were exposed to proper light and dark cycle (12 hours each of light and darkness) and were housed in standard laboratory conditions of temperature [(25±2) $^{o}$C]. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Bundelkhand University, India.(Refrence number **BU/Pharm/IAEC/12/026**).

**Evaluation of hepatoprotective activity**

The method used for evaluation of hepatoprotectivity was according to Nirmala *et al*[31], Malar *et al*[32] and Chaudhari *et al*[33] with some minor modifications. The animals were divided into six groups of six animals in each group. Group 1 served as normal control which received 0.5% Tween 80. Group 2 served as paracetamol control & received paracetamol at a dose of 2gm/kg, *p.o*. at every 72 h for 7 Days. Groups 4 to 6 received ethanolic extract of the root of *K. reticulata* at (100, 200 & 300 mg/kg, *p.o.*) for 7 days. Group 3 served as standard control & received Silymarin (100 mg/kg, *p.o*). Groups 4 to 6 were intoxicated with paracetamol (2gm/kg, *p.o.*) 1 h before the administration of extract or Silymarin for 7 days. The animals were sacrificed 48 h after paracetamol administration under mild anaesthetic chloroform. Blood from each rat was withdrawn by heart punctured under chloroform anaesthesia for biochemical investigation *i.e.* sGPT, sGOT, sALP, Total serum bilirubin, Total serum protein and blood urea estimation. Blood was allowed to coagulate at 37 $^{o}$C for 30 min and the serum was separated by centrifugation at 2500 rpm for 10 min. The liver of all the experimental animals were removed and processed immediately for histopathological investigation[34].

**Histopathological studies**

After collecting the blood, liver samples were excised, washed with normal saline and processed separately for histopathological examinations. Initially, the materials were fixed in 10% buffered formalin for 48 h. Paraffin sections were taken at 5 mm thickness, were stained with haematoxylin and eosin. The sections were examined photo microscopically for histopathological changes.

**Statistical analysis**

Statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Dunnett's test. Results were expressed as mean±SEM from six rats in each group & differences among the control and treatment group were

determined by using statistical software (graph pad prism) version 6.03. P values < 0.05 were considered significant. See Graph 1 and 2.

**RESULTS AND DISCUSSION**

**Preliminary phytochemical investigation**

Preliminary phytochemical invstigation revealed the presence of carbohydrates, proteins, amino acids, phenolic compounds & tannins, flavonoids, saponins, and phytosterols in ethanolic extract.

**Hepatoprotective activity**

Administration of paracetamol (2.0gm/kg, p.o.) induced a marked increase in the serum hepatic enzyme levels, sGOT, sGPT, sALP, serum bilirubin, serum total protein, total serum albumin, & Blood Urea as compared to normal controls indicating hepatotoxicity (centri- lobular necrosis). Pre-treatment of the rats with ethanol extract prior to paracetamol administration caused a significant reduction in the value of sGOT, sGPT, sALP, serum bilirubin, serum total protein, total serum albumin, & Blood Urea almost comparable to the silymarin (standard group).

**Histopathological results**

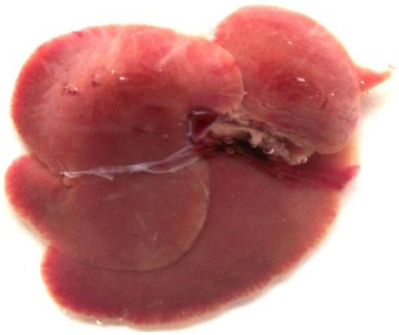
The hepatoprotective effect of *Kirganelia reticulata* root was confirmed by histopathological examination of the liver tissue of normal control, standard and ethanolic extract treated animals. Gross necropsy and histopathological study were performed on the liver to observe any irregularities or abnormalities on the structure. Gross necropsy of normal liver demonstrated normal appearances (i.e., dark maroon in-colour liver with smooth surfaces (Photograph 1A). Mean while, the liver intoxicated with PCM showed major changes of the colour of the lobes from maroon to brown (Photograph 1B). Pre-treatment with 100mg/kg silymarin (Photograph1C) or the EEKR (Photograph 1D–1F) reversed the toxic effect of PCM with only mild brown colour changes observed.

The liver section of normal liver showed central vein and cord of hepatocytes (Figure 1); and paracetamol intoxicated group (Figure 2), rat liver section showed hepatocellular degeneration with fatty changes, inflammation and more necrosis occurred. Silymarin treated group (Figure 3), rat liver section showed normal vein with mild hepatocytic changes and absence of necrosis without inflammation. Ethanolic extract treated group such as in case of low dose of 100 mg/kg (Figure 4), moderate protection has observed degenerative changes, necrosis, haemorrhage; Ethanolic extract 200 mg/kg (Figure 5), treated group exhibited mild hepatoprotective activity as evidenced by the presence of less necrosis with minimum inflammation; and 300 mg/kg (Figure 6) treated rat liver section exhibits significant protection against paracetamol intoxication as evident by presence of hepatic cord and absence of necrosis with minimal inflammatory condition around central vein

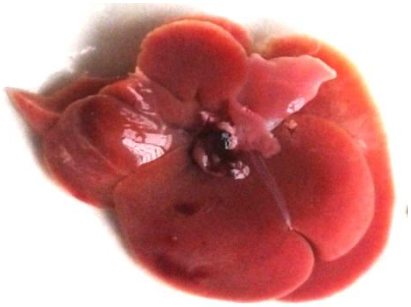
Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug induced liver disorders which occurred frequently can be life threatening. Paracetamol being a drug capable of causing liver disorders if over doses are consumed. The covalent binding of N-acetyl-P-benzoquinoneimine(NAPQI), an oxidation product of paracetamol, to sulphydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier[35].

The extent of toxicity was estimated by histopathological studies and biochemical parameters like sGOT, sGPT, sALP, serum bilirubin, serum total protein, total serum albumin & blood urea can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol, are released into the blood stream.

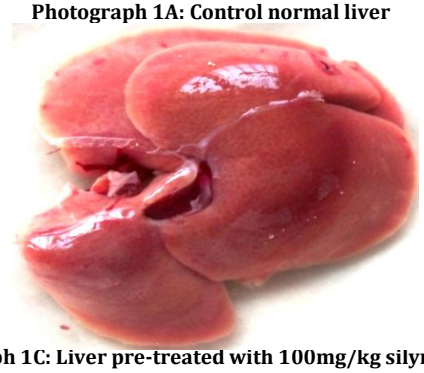
Administration of paracetamol at a dose of (1-3g/kg/day), p.o. results in hepatic toxicities. The toxic metabolic N-acetyl-p-benzoquineimine is an oxidative product of paracetamol formed by the action of cytochrome P-450 and it reacts with reduced glutathione (GSH) to yield non-toxic 3-GS-yl-paracetamol. Depletion of GSH causes the remaining quinine to undergo covalent bonding with cellular sulphydryl groups of protein and leads to cell death. Histopathology of the liver shows necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis, eosinophilic cytoplasm and large excessive hepatic lesions[36].



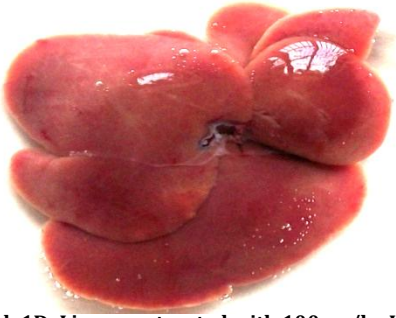
**Photograph 1A: Control normal liver**



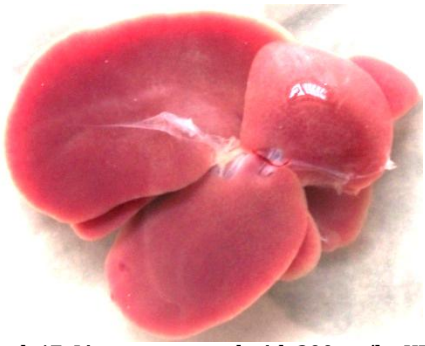
**Photograph 1B: Liver intoxicated with 2 g/kg PCM: gross image shows major colour changes of liver lobes**



**Photograph 1C: Liver pre-treated with 100mg/kg silymarin and induced with PCM: colour changes was noted**



**Photograph 1D: Liver pre-treated with 100mg/kg KREE and induced by PCM**



**Photograph 1E: Liver pre-treated with 300mg/kg KREE and induced by PCM**

**Photograph 1F: Liver pre-treated with 200mg/kg KREE and induced by PCM**

**Photograph 1: Effect of EEKR on the body weight and liver weight after induction with PCM**

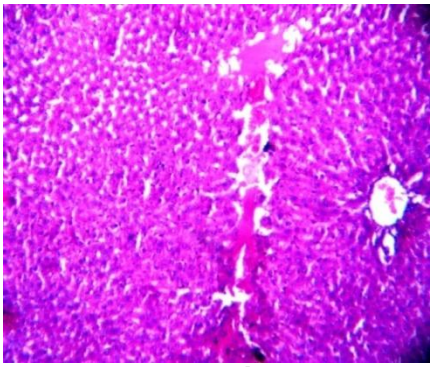


**Fig.1: Normal Liver**

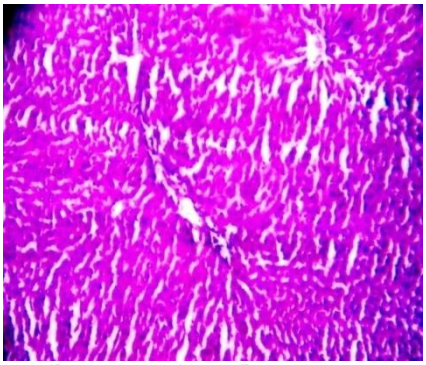


**Fig. 2: Section of liver tissue of (2gm/kg, *p.o.*) paracetamol - treated group**

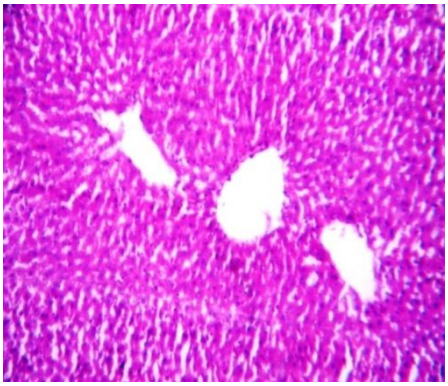


**Fig.3: Section of (100mg/kg, *p.o.*) silymarin liver tissue pre-treated on the liver followed by paracetamol**

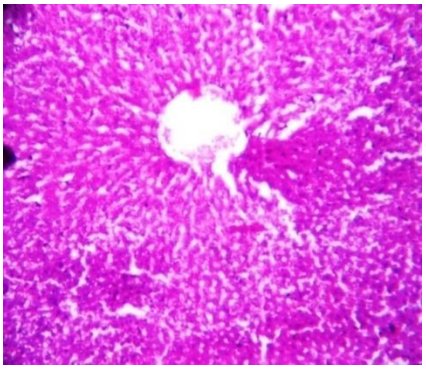


**Fig.4: Section of pre-treated (100 mg/kg, *p.o.*) EEKR liver tissue followed by paracetamol**

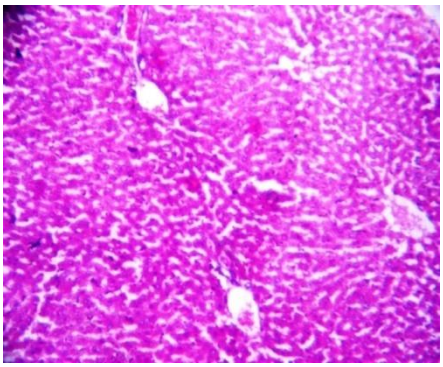


**Fig.5: Section of pre-treated (200 mg/kg, *p.o.*) EEKR liver tissue followed by paracetamol**

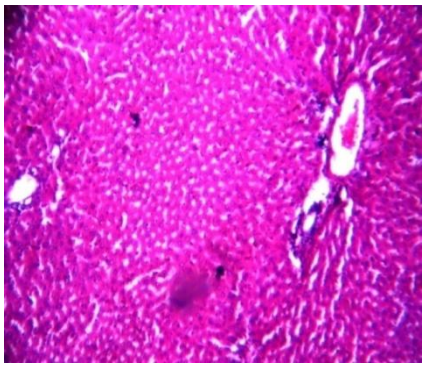


**Fig.6: Section of pre-treated (300 mg/kg, *p.o.*) EEKR liver tissue followed by paracetamol**

The present study reports the possible hepatoprotective activity of ethanolic extract of root of *K. reticulata* against hepatotoxicity produced by paracetamol in Wistar rats. Paracetamol is well known analgesic and antipyretic agent, which is safe to therapeutic doses. But can produce fatal hepatic necrosis in man, rats and mice with over doses. It is employed as an hepatotoxic agent[37].

The ethanolic extract showed more significant result and report shows flavonoids and steroids may be responsible for hepatoprotective effect[38-41]. Perhaps steroids and flavonoids present in the root of *K. reticulata* may be responsible for the marked hepatoprotective activity, observed in the present study.

Pre-treatment with silymarin (100mg/kg, *p.o.*); ethanolic extract (100, 200 and 300 mg/kg, *p.o.*) of root of *K. reticulata* for 7 days has significantly reduced the elevated serum enzyme level.

Ethanolic extract of root of *K. reticulata* reduced the histological changes caused by paracetamol, which further confirmed its hepatoprotective activity against paracetamol induced hepatic toxicity.

In conclusion, the result of this study demonstrated that ethanolic extract of root of *Kirganelia reticulata* (300mg/kg, p.o.); shows significant hepatoprotective activity against paracetamol induced hepatotoxicity in Wistar rats. Hence the present study justifies the traditional use of *K. reticulata* Poir. (Baill). in treatment of liver disease.

**Table 1: Biochemical parameter of *in vivo* effects of root extract of *K. reticulata* subjected to paracetamol induced hepatotoxicity.**

| Groups | SGOT (IU/L) (AST) | SGPT (IU/L) (ALT) | ALP (IU/L) | Total Serum bilirubin (μ mol/L) | Total serum protein (g/dL) | Serum Albumin (g/dL) | Blood Urea (mg/dL) |
|---|---|---|---|---|---|---|---|
| Control | 96.13±4.924 | 54.200±2.862 | 135.23±5.070 | 0.860±0.003 | 6.32±0.650 | 3.34±0.71 | 24.63±2.25 |
| Paracetamol | 260.308±35.250[a] | 78.77±24.760[a] | 466.5±21.763[a] | 1.863±0.007[a] | 6.98±0.54[a] | 3.57±0.39[a] | 41.30±3.23[a] |
| Silymarin (100mg/kg) | 87.14±3.354[ab] | 50.883±6.897[ab] | 138.872±8.971[ab] | 0.833±0.031[ab] | 5.98±0.30[ab] | 3.29±0.53[ab] | 22.76±1.870[ab] |
| Ethanolic extract (100mg/kg) | 108.56±8.760[ab] | 42.46±19.393[ab] | 254.833±9.799[ab] | 0.860±0.054[ab] | 6.89±0.060[ab] | 3.65±0.36[ab] | 35.54±1.76[ab] |
| Ethanolic extract (200mg/kg) | 102.065±1.01[ab] | 38.977±13.614[ab] | 180.760±7.430[ab] | 0.840±0.065[ab] | 6.07±0.130[ab] | 3.53±0.21[ab] | 30.76±1.43[ab] |
| Ethanolic extract (300mg/kg) | 92.98±13.385ab | 31.842±2.953[ab] | 132.185±3.810[ab] | 0.720±0.033[ab] | 6.61±0.540[ab] | 3.71±0.09[ab] | 20.89±2.86[ab] |

Values are expressed as (mean±SEM). of six replicates.

[a]Data differed significantly (P<0.05) when compared to the normal group within each respective column.

[b]Data differed significantly (P<0.05) when compared to the paracetamol group within each respective column.

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**CONFLICT OF INTEREST STATEMENTS**

We declare that we have no conflict of interest.

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