

# Hepatoprotective Activity of Ethanolic Extract of *Caesalpinia bonduc* (L.) in Paracetamol Intoxicated Albino Rats

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**Abstract:** Hepatoprotective activity of aqueous ethanolic leaf extract of *Caesalpinia bonduc* (L.) against paracetamol induced hepatic damage in albino rats was observed. In the present study the effect of ethanolic extract of *Caesalpinia bonduc* (L.) on blood and liver glutathione, Na<sup>+</sup> K<sup>+</sup>-ATPase activity, serum marker enzymes, serum bilirubin, glycogen and thiobarbituric acid reactive substances against paracetamol induced damage in rats have been studied to find out the possible mechanism of hepatoprotection. It was observed that extract of chamomile has reversal effects on the levels of above-mentioned parameters in paracetamol hepatotoxicity. The extract of *Caesalpinia bonduc* (L.) functions as a hepatoprotective agent and this hepatoprotective activity of extract may be due to normalization of impaired membrane function activity.

**Key words:** *Caesalpinia bonduc*, hepatoprotection, lipid peroxidation, Na<sup>+</sup>K<sup>+</sup>-ATPase, paracetamol.

## Introduction

Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione (GSH) depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses. [1] Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome. [2] Or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. [3], [4] Liver is the vital organ of metabolism and excretion. India, about 40 polyherbal commercial formulations reputed to have hepatoprotective action is being used. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders. [5] 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. [6] There are however, members of drugs employed in traditional system of medicine for liver affections. [7] *Caesalpinia bonduc* (L.) family Fabaceae popularly known as sagar goti, also known as nicker nut, wait-a-bit, hold back, fever nut is scrambling shrub to 1.5 m in height (unsupported) and 6 m or more in extension, and has stems up to 5 cm in diameter or more. is a reputed medicinal used in both traditional and modern system of medicine. The essential oil of *caesalpinia bonduc* has anti-inflammatory and softening effect and is useful in the treatment of gastric colic enteralgia, gastritis, bloating, inflammation and respiratory tract.

## Materials and Methods

**Collection and Extraction of plant material:** Fresh natural mature leaves of *Caesalpinia bonduc* (L.) were collected from the medicinal garden of S.N.Institute of Pharmacy; Pusad Shade-dried powder (1kg of leaves of *Caesalpinia bonduc* (L.)) was extracted by percolation at R.T. with 70% ETOH. Ethanolic extract of was concentrated under reduced pressure and dried in a vacuum desiccators (50°C). The residue (30.9 gm) was dissolved in 70% ETOH and filtered.

**Animals:** Albino rats of either sex (120-150 gm) were maintained under control conditions of light and temperature (25°C+1°C) in animal house of S.N. Institute of Pharmacy, Pusad. Food pellets and tap water were provided ad libitum. For experimental, animals were kept fasting overnight but were allowed free access to water.

**Paracetamol toxicity:** The ethanolic extract, paracetamol, saline were given with the help of feeding cannels. Three groups (Group I, Group II and III) of rats, six rats in each group were taken. The *Caesalpinia bonduca* (L.) extract at a fixed dose (400 mg kg<sup>-1</sup>, P.O.) that was daily fed for seven days to one group (Group III) of rats and paracetamol (200 mg kg<sup>-1</sup> P.O.) was administered on 5th day after 5th administration of the extract. The paracetamol treated group (Group II) received normal saline in place of *Caesalpinia bonduca* (L.) extract. After 48h of paracetamol feeding rats were sacrificed by cervical dislocation for estimation of blood glutathione, reduced liver glutathione, liver Na<sup>+</sup>K<sup>+</sup>-ATPase activity, serum marker enzymes, serum bilirubin and liver thiobarbituric acid reactive substances using standard methods.

**Assay of liver glutathione and blood glutathione:** Blood was collected, allowed to clot and serum separated. Liver was dissected out and used for biochemical studies. Freshly collected livers were washed with 0.9% NaCl, weighed and homogenates were made in a ratio of 1g of wet tissue to 9ml of

1.25% KCl by using motor driven Teflon-pestle. Reduced glutathione (GSH) was estimated using DTNB.<sup>[8]</sup> The blood glutathione was estimated by the method of Beutler.<sup>[9]</sup> The absorbance was read at 412 nm.

**Liver Na<sup>+</sup> K<sup>+</sup>-ATPase activity:** To measure the liver Na<sup>+</sup>K<sup>+</sup>-ATPase activity the liver was dissected out quickly, rinsed with cold phosphate buffer, liver plasma membranes were isolated and subjected for the estimation of Na<sup>+</sup> K<sup>+</sup>-ATPase activity.<sup>[10]</sup>

**Thiobarbituric acid reactive substances (TBARS):** The concentration of TBARS was measured in liver using the method of Ohkawa *et al.*<sup>[11]</sup> the concentration of TBARS was expressed as “moles of malondialdehyde per mg of protein using 1,1,3,3, -tetraethoxypropane as the standard.

**Serum marker enzymes:** The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)<sup>[12], [13]</sup> total serum protein (TSP)<sup>[14]</sup> and bilirubin<sup>[15]</sup> were measured. Glycogen (GLY)<sup>[16]</sup> and protein were estimated in liver homogenate.

**Statistical analysis:** The data were expressed as mean ± S.E.M. and statistically assessed by one-way analysis of variance (ANOVA). The difference between drug treated animals and controls was evaluated by student's t-test.

**Table 1: Effect of *Caesalpinia bonduca* extract on blood and liver glutathione (GSH), liver Na<sup>+</sup>K<sup>+</sup>-ATPase, serum marker enzymes (ALT, AST, ALP) and liver thiobarbituric acid reactive substances (TBARS) in Paracetamol intoxicated albino rats**

Parameters	Group I	Group II	Group III
Blood GSH (mg%)	1.56± 0.06a	0.37 ± 0.05	1.37 ± 0.04 c
Na <sup>+</sup> K <sup>+</sup> -ATPase (U mg <sup>-1</sup> protein)	7.16 ± 0.52a	6.11 ± 0.17	8.01±0.16c
Liver GSH (μmoles g <sup>-1</sup> liver)	10.98± 0.32c	6.02 ± 0.32	9.92± .18b
TBARS (n mol of MDA g <sup>-1</sup> of wet tissue h <sup>-1</sup> )	282.7 ± 9.36	564.4a ± 9.1	279.7c± 7.20
ALP (KA unit)	59.3±7.2b	99.2±6.75	55.9±2.2
ALT (U mg <sup>-1</sup> protein)	61.2±1.06c	237.4±10.9	43.61±1.89c
AST (U mg <sup>-1</sup> protein)	69.4±2.01a	172.8±18.1	44.5±1.01b
Bilirubin (mg %) (Total)	1.43±0.12a	2.89±0.10	1.33±0.16b
TSP (mg protein mL <sup>-1</sup> serum)	57.89±2.6a	58.12±2.16	62.5±1.99c
GLY (mg g <sup>-1</sup> wet tissue)	30.10 ± 2.84a	22.45 ± 1.18	30.49±2.34c
Body weight			
Before treatment (g)	147±3a	148.6±2	146±4c
After treatment (g)	158±5b	149.3±3	161±7b
Liver weight (g)	6.89±0.3	5.78±0.6	6.45±0.5b

Results are mean of six observations ± S.E.M.

a p<0.001 when compared with normal control (Group I), b p<0.001 when compared with normal control (Group I).

c p<0.001 when compared with paracetamol treated control (Group II).

## Results and Discussion

The glutathione level in liver homogenate and in blood, liver Na<sup>+</sup>K<sup>+</sup>-ATPase, serum marker enzymes and liver thiobarbituric acid reactive substances are given in Table 1. The concentration of GSH in animals treated with paracetamol was significantly ( $p < 0.001$ ) reduced in homogenate of liver and so was the level of glutathione in blood and Na<sup>+</sup>K<sup>+</sup>-ATPase level ( $p < 0.001$ ) as compared with saline control animals. While thiobarbituric acid reactive substances of paracetamol treated animals was significantly higher ( $p < 0.001$ ) than the saline treated control animals. Administration of *C. bonduc* extract increased the concentration of glutathione in liver ( $p < 0.01$ ) and glutathione in blood ( $p < 0.001$ ) and liver Na<sup>+</sup>K<sup>+</sup>-ATPase activity significantly ( $p < 0.001$ ) when compared to its paracetamol treated control group. On the other hand, the increased level of liver thiobarbituric acid reactive substances of paracetamol treated animals was significantly ( $p < 0.001$ ) reduced in group of animals receiving both *C. bonduc* extract and paracetamol. The abnormal high level ( $p < 0.001$ ) of serum ALT, AST, ALP and bilirubin observed (Table 1) in paracetamol induced liver toxicity. Treatment with AET of *C. bonduc* reduced the enhanced level of serum ALT, AST, ALP and bilirubin. On the other hand, the level of liver glycogen of paracetamol treated animals was significantly ( $p < 0.001$ ) reduced. Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses [17],[18]. Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations [19],[20]. Liver is the most important and main part of the animal body. It is highly affected primarily by toxic agents and that why the above-mentioned parameters have been found to be of great importance in the assessment of liver damage. From our results, it can be speculated that decreasing effect GSH, blood glutathione, liver Na<sup>+</sup>K<sup>+</sup>-ATPase activity and increasing effect of liver thiobarbituric acid reactive substance level in rat treated with paracetamol were due to hepatocellular damage and *C. bonduc* extract afforded protection from such paracetamol induced liver damage. The extract significantly increased the hepatic and blood glutathione. Then results suggest that a

significantly higher content glutathione in blood and liver would offer the tissue a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol induced hepatotoxicity. (c) The activities of Na<sup>+</sup> K<sup>+</sup>-ATPase are decreased in paracetamol-induced animals; *C. bonduc* extract prevented this effect of paracetamol. Therefore, *C. bonduc* extract may be useful agent for the normalization of paracetamol induced impaired membrane function. The abnormal high level of serum ALT, AST, ALP and bilirubin observed in our study (Table 1) are the consequence of paracetamol induced liver dysfunction and denotes the damage to the hepatic cells. Treatment with *C. bonduc* extract reduced the enhanced level of serum ALT, AST, ALP and bilirubin, which seem to offer the protection and maintain the functional integrity of hepatic cells. A reduction in total serum protein (TSP) (Table 1) and liver glycogen (GLY) observed in the paracetamol treated rats may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and GLY and consequently decrease in the liver weight (Table 1). But, when the *C. bonduc* extract was given along with paracetamol, the significant increase in TSP and liver GLY was observed indicating the hepatoprotection activity of extract and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration. From our results, it can be concluded that decreased the levels of GSH, blood glutathione, serum marker enzymes, liver Na<sup>+</sup>K<sup>+</sup>-ATPase activity and increased liver thiobarbituric acid reactive substance level in albino rats treated- paracetamol was due to hepatocellular damage. Extract of *C. bonduc* afforded protection from such paracetamol induced liver damage. Possible mechanism that may be responsible for the protection of paracetamol induced liver damage by *C. bonduc* extract include the following, *C. bonduc* extract could act as a free radical scavenger intercepting those radicals involved in paracetamol metabolism by microsomal enzymes. A significantly higher content GSH in blood and liver would afford the tissue a better protection against antioxidative stress, thus contributing to the abolishment of paracetamol induced hepatotoxicity. Therefore, *C. bonduc* extract is a promising hepatoprotective agent.

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