

Protective Effect of Methanolic Leaf Extract of *Caesalpinia Bonduc* (L.) on Gentamicin-Induced Hepatotoxicity and Nephrotoxicity in Rats

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ABSTRACT

The present study was conducted to evaluate the protective effect of methanolic leaf extract of *Caesalpinia bonduc* (L.) on gentamicin-induced hepatotoxicity and nephrotoxicity in rats. A control group (saline, group I, n = 6) was compared with rats administrated 80 mg/kg gentamicin, once daily for 7 days (groups II, III and IV). The effect of methanolic leaf extract of *Caesalpinia bonduc* (group III and IV) at a dose level of 250 mg/kg and 500 mg/kg was compared in gentamicin-induced hepatotoxicity and nephrotoxicity. The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), bilirubin, and total protein the values of urea, sodium, potassium and chloride were significantly increased in rats exposed to gentamicin. Moreover, administration of gentamicin resulted in damage to liver and kidney structures. Administration of methanolic extract of *C. bonduc* before gentamicin exposure prevented severe alterations of biochemical parameters and disruptions of liver and kidney structures. In conclusion, this study obviously demonstrated that pretreatment with methanolic extract of *C. bonduc* significantly attenuated the physiological and histopathological alterations induced by gentamicin. Also, the present study identifies new areas of research for development of better therapeutic agents for liver, kidney, and other organs dysfunctions and diseases.

Keywords: *Caesalpinia Bonduc*, *Gentamicin*, *Hepatotoxicity*, *Nephrotoxicity*

Aminoglycoside antibiotics have long been used in antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic and ototoxic side effects. It has been reported that gentamicin induce free radical generation which implicates a variety of pathological processes [1]. Gentamicin generates free oxygen radicals, leading to tissue injury such as nephrotoxicity and ototoxicity [2]. It is one of the aminoglycosides that also causes liver toxicity [3]. The toxicity of aminoglycosides has been widely studied [4]. It has been reported that renal damage can in turn leads to liver injury due to aminoglycosides [5]. Aminoglycosides (gentamicin) are potentially toxic at levels only slightly above therapeutic range [1,6]. Approximately, 5-10% of the

people, who are treated with aminoglycosides experience such toxic effects. The association of aminoglycosides with negatively-charged phospholipids and their accumulation in the lysosomes of tubular cells leads to phospholipidosis by inhibition of lysosomal phospholipases [7], which may trigger necrosis [8]. It has been reported that at cellular level, aminoglycosides interferes with protein synthesis, especially by inhibition of translocation [9].

Caesalpinia bonduc (L) Roxb (Caesalpinaceae) is a large scandant prickly shrub found throughout the interior part of India, Sri Lanka and West Indies. It is common in southern parts of India and is often grown as a hedge plant (Wealth of India) This plant has profound medicinal use and is a proved anti-inflammatory [10],

Table 1. Effect of methanolic extract of *C. bonduc* and gentamicin on liver and kidney weight

| | Body weight (gm) | Liver weight (gm) | Kidney weight (gm) |
|----------------------------------|---------------------|----------------------|-----------------------|
| Control | 245 ± 1.4 | 6 ± 0.12 | 0.6 ± 0.12 |
| Gentamicin (80mg/kg)-Treated | 250 ± 2.3 | 10 ± 0.41 | 1.1 ± 0.14 |
| Extract (250 mg/kg) + Gentamicin | 243 ± 1.7 | 9 ± 0.19 | 0.8 ± 0.28 |
| Extract (500 mg/kg) + Gentamicin | 235 ± 1.2 | 7 ± 0.15 | 0.7 ± 0.13 |

anthelmintic, antimalarial[11], antitumour [12], adaptogenic [13], antidiabetic [14] and antioxidant [15]. No detail report was found in literature to evaluate hepatic and renal damage experimentally in rats.

The present study was hence designed to determine protective effect of methanolic leaf extract of *Caesalpinia bonduc* (L.) on gentamicin-induced hepatotoxicity and nephrotoxicity in rats. In addition, we attempted to test and compare the possible action of methanolic extract of *Caesalpinia bonduc* on gentamicin induced nephrotoxicity and hepatotoxicity in rats.

MATERIALS AND METHODS

Collection and authentication of plant

The plant was collected from local area of Shirpur, Dhule Maharashtra, India. The plant was authenticated by Dr. B.S. Baghel, Department of Botany, Krishi Vigyan Kendra Horti culture College, Mandsaur, Madhya Pradesh, India.

Extraction methodology

The plant materials were washed with water, cut into pieces, sun dried for 5 days and then dried in an oven below 60 °C. The dried plant materials were then pulverized into coarse powder in a grinding machine. About 10 gm of plant sample was extracted separately in cold methanol. Solvent from each sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract and the phytochemical investigation was performed [16].

Experimental animals

Three-months old Wistar albino rats of either sex weighing 150-250 g were used for the study. The animals were procured from B.R. Nahata College of Pharmacy, Mandsaur. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2 °C and relative humidity of 30-70%. A 12/12 h light and dark cycle was followed. All animals were fed on standard balanced diet and provided with water ad libitum.

All the experimental procedures and protocols used in the study were reviewed and approved by the (IAEC) Institutional Animal Ethical Committee of Mandsaur Institute of Pharmacy, Mandsaur and were in accordance with the guidelines of the Committee for the

purpose of Control and Supervision of Experiments on Animals (CPCSEA). Registration No.MIP/IAEC/2010/002

Toxicity Study [17]

Acute oral toxicity was conducted for extract on albino mice according to OECD 425 and median effective dose (ED₅₀) of extract was selected based on LD₅₀ obtained from acute toxicity studies.

Gentamicin induced liver and kidney damage [18]

Albino wistar rats of either sex (200-250g) were used. All the animals were divided into the four groups; each group consisted of 6 animals and they received the treatment as follows:

Group I: Control (1 ml/kg Saline p.o.)

Group II: Gentamicin (80 mg/kg i.p.) for 7 days

Group III: Methanolic extract of *C.bonduc*. (250 mg/kg p.o.) + Gentamicin for 7 days

Group IV: Methanolic extract of *C.bonduc* (500 mg/kg p.o.) + Gentamicin for 7 days

Biochemical estimation

At the end of experimental period, rats were anaesthetized with ether. Blood samples were collected from retro orbital venous plexus in nonheparinized tubes, centrifuged at 3000 rpm for 20 minutes, and blood sera were collected and stored at 4 °C prior immediate determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), direct bilirubin (DB), total bilirubin (TB) total protein, urea, sodium, potassium and chloride. All of these parameters were measured using Automated Clinical Chemistry Analysis System, Dimension type RXL Max (Dade Behring Delaware, DE 19714, U.S.A.).

Histopathological Examination

For light microscopic examination, liver and kidney tissues from each groups were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4 μm thickness and stained with haematoxylin and eosin.

Statistical Analysis

All the data expressed as mean ± S.E.M and analyzed statistically using ANOVA followed by Dunnett test and compared with respective control group. A value of $p < 0.05$ was considered significant.

Table 2. Effect of methanolic extract of *C.bonduc* and gentamicin on different biochemical parameters of liver

| Group | AST (I.U./L) | ALT (I.U./L) | T.G. (mg/dl) | TotalProtein (mg/dl) | T.B. (mg/dl) | D.B. (mg/dl) |
|---------------------------------|-----------------|-----------------|-----------------|-------------------------|-----------------|-----------------|
| Control | 49.34± 1.1 | 125.05± 0.4 | 117.1± 0.4 | 2.9 ± 0.8 | 0.16± 0.005 | 0.16± 0.008 |
| Gentamicin (80mg/kg) Treated | 87.3± 1.9** | 208.9 ±0.4 | 145.1± 1.5* | 4.2 ± 0.2* | 0.90± 0.9* | 0.43± 0.01 |
| extract (250mg/kg) +Gentamicin | 70.2±0.2* | 180.2 ±0.7 | 130.8± 0.2* | 3.91± 1.3* | 0.83± 0.01** | 0.36± 0.03* |
| Extract (500mg/kg) + Gentamicin | 55.15±0.6 | 165.8±0.3** | 120.2± 1.1* | 3.71± 1.7* | 0.72 ±0.06 | 0.31± 0.05* |

Values are expressed as mean ± S.E.M. (n=6)

*P<0.05, when compared with the gentamicin treated groups (one-way ANOVA followed by Dunnetts test)

**P<0.01, when compared with the gentamicin treated groups (one-way ANOVA followed by Dunnetts test)

Table 3. Effect of methanolic extract of *C.bonduc* and gentamicin on different biochemical parameters of kidney

| Group | Urea (mg/dl) | Sodium (m/l) | potassium (m/l) | chloride (m/l) |
|--|-----------------|-----------------|--------------------|-------------------|
| Control | 23± 1.5 | 143.05± 0.4 | 5.1± 0.2 | 81.3± 0.8 |
| Gentamicin (80mg/kg) Treated | 81.3± 1.9** | 224.9±0.4 | 7.7± 0.1* | 115.2± 1.9* |
| Methanolic extract (250mg/kg)+Gentamicin | 52.2±0.2* | 185.2±0.7 | 6.7± 0.2* | 102.7± 1.3* |
| Methanolic extract (500mg/kg) + Gentamicin | 36.15±0.6 | 166.8±0.3** | 6.2± 0.15* | 96.5± 1.7* |

Values are expressed as mean ± S.E.M. (n=6)

*P<0.05, when compared with the gentamicin treated groups (one-way ANOVA followed by Dunnetts test)

**P<0.01, when compared with the gentamicin treated groups (one-way ANOVA followed by Dunnetts test)

RESULTS

Phytochemical investigation of methanol extract of *C. bonduc* revealed presence of various active constituents such as alkaloids, glycosides and amino acids. The LD₅₀ of plant methanolic extract was found to be 2,000 mg/kg. The effective dose 250 mg/kg was selected based on LD₅₀ of plant.

Intraperitoneal administration of gentamicin at a dose of 80 mg/kg caused a significant ($p < 0.01$) increase in liver and kidney weight but no significant change in body weight was observed (Table 1). In comparison with control values, level of serum marker enzymes such as ALT, AST, ALP, TG, TB, DB, the

total protein and the values of urea, sodium, potassium and chloride were statistically increased in group II. A moderately significant ($p < 0.01$) decrease was observed in the AST, ALT, ALP, LDH, TB, DB, Total protein, urea, sodium, potassium and chloride in the animals treated with different doses (250 mg/kg and 500 mg/kg) of methanolic extract of *C. bonduc* (Tables 2-3).

Light microscopic examination of the liver in control rats showed the normal structure (Fig 1A). Histopathological effects of gentamicin on liver of treated rats are presented in Fig 1B. Rats treated with gentamicin showed many severe histopathological alterations. Administration of gentamicin for seven days

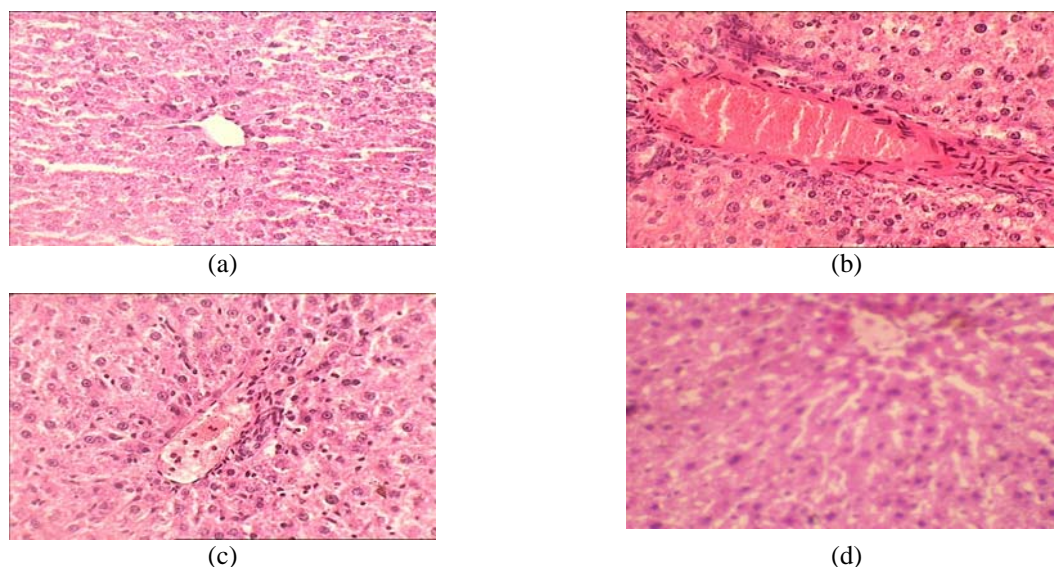


Fig 1. Liver micrographs of control (a), gentamicin (b), and methanolic extract of *C.bonduc* ((c) and (d)) treated rats. Original magnification X400.

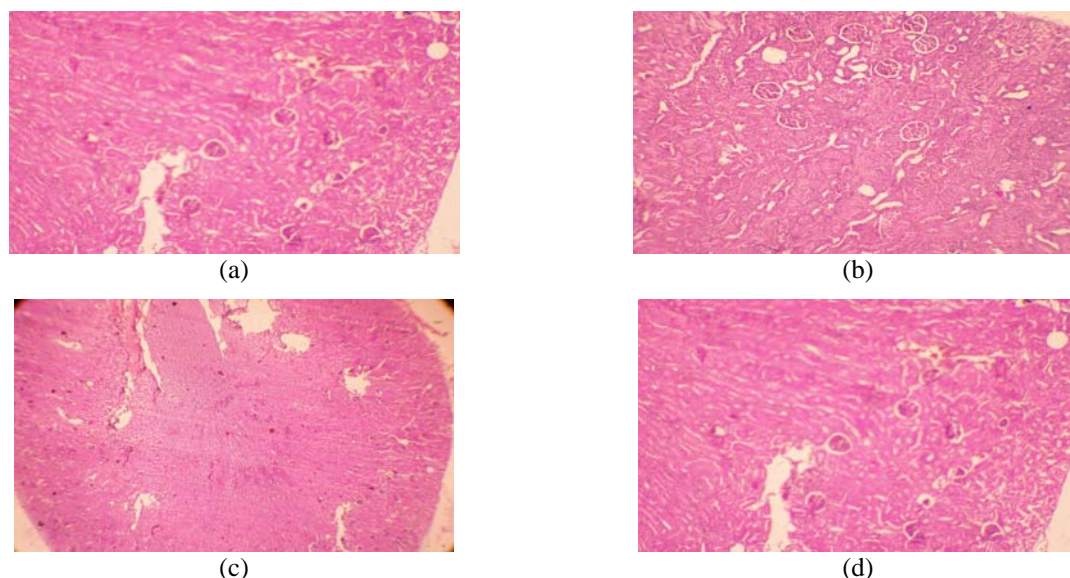


Fig 2. Renal corpuscle micrographs of control (a), gentamicin (b) and methanolic extract of *C. bonduc* ((c) and (d)) treated rats. Original magnification X400.

resulted in the damage of liver structure along with disarrangement of hepatic strands. Several cells also showed histological features of necrosis. Moreover, an enlargement of the sinusoids and vacuole formations in hepatocytes, leucocytic infiltrations, dilation, and congestion of blood vessels with hemorrhage were noted in liver of rats exposed to gentamicin (group 2). Treatment with methanolic extract of *C. bonduc* brought back the cellular arrangement around the central vein and reduced necrosis (Figs 1C and 1D). Also, it helped to bring the blood vessels to normal condition. Fig 2 shows the histology structures of the kidney in control group (Fig 2A), gentamicin-treated rats (Fig 2B) and methanolic extract of *C. bonduc* treated rats (Figs 2C and 2D). Areas of renal cortex containing renal corpuscles and associated tubules showed more pronounced changes in treated animals compared with control. Therefore, these areas were selected for histological examination with the light microscope. The normal renal corpuscle consisted of a tuft of capillaries, the glomerulus, surrounded by a double-walled epithelial capsule called Bowman's capsule. Between the two layers of the capsule is the urinary or Bowman's space (Fig 2A). In seven days, gentamicin induced pronounced changes in the structure of renal corpuscle including swelling appearances, increasing of urinary spaces, high degeneration of glomeruli, Bowman's capsules and associated tubules structure (Figs 2B).

Methanolic extract of *C. bonduc* treatment reversed abnormal histology of renal cortex areas induced by malathion intoxication (Figs 2C and 2D).

DISCUSSION

Certain drugs may induce oxidative stress by forming drug-derived radicals that can not only deplete

the antioxidant defenses but can also react directly with biomolecules. Aminoglycosides can cause nephrotoxicity as well as hepatotoxicity. It has been proposed that the aminoglycosides disrupt the signal transduction pathway and increases the cellular permeability by acting on membrane phospholipids [19]. Aminoglycoside has been reported to alter activities of antioxidant enzymes such as superoxide dismutases (SOD), catalase, glutathione peroxidase (GSH-px), glutathione-S-transferase (GST) and glutathione reductase (GR) in various tissues [20]. The reduced enzyme activity in the gentamicin group is a generalized response, not specific to one enzyme indicating impaired function at several steps of the antioxidant pathway [4]. Bendush *et al.* reported that AST level increases in patients receiving aminoglycoside injection [21]. It was postulated that aminoglycoside-induced free radical generation and alteration in antioxidant enzyme activities may be the cause of tissue injury. Aminoglycosides alters liver glycogen phosphorylase activity leading to decrease in liver glycogen content [22].

Results of this study confirmed that gentamicin at a dose of 80 mg/kg produces significant hepatotoxicity and nephrotoxicity as evidenced by increase in serum AST, ALT, TG, DB, TB, total protein, urea, sodium, potassium and chloride level. In addition, gentamicin induced severe hepatic and renal damages as shown in histopathological examination which coupled with markedly elevated levels of liver biochemical markers (AST, ALP, TG, DB, TB and total protein) and significant changes of kidney biochemical indices including statistically increased levels of urea, sodium, potassium and chloride. In gentamicin-treated rats, there was a significant increase in oxidative stress suggesting the liver and kidney damage. Treatment with *Caesalpinia bonduc* methanol leaf extract recovered the

injured liver and kidney to normal after 24 hrs at a dose of 500 mg/kg which indicate that *Caesalpinia bonduc* has antihepatotoxic as well as antinephrotoxic effect. The possible antihepatotoxic and antinephrotoxic mechanism of *Caesalpinia bonduc* have not been reported yet. It is assumed that the effect of *Caesalpinia bonduc* extract on liver protection is related to glutathione-mediated detoxification as well as free radical suppressing activity.

In conclusion, the present findings show that oral administration of methanolic leaf extract of *Caesalpinia bonduc* produces significant hepatoprotective and nephroprotective effects in gentamicin-treated rats. Further investigations are required to explore exactly the mechanism action of *Caesalpinia bonduc* against gentamicin induced physiological disturbances and histopathological changes. Finally, the present study identifies new areas of research for development of better therapeutic agents for liver, kidney, and other organs' dysfunctions and diseases.

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