Protective Effect of Methanolic Extract of *Caesalpinia Bonduc (L.)* on Artesunate -Induced Hepatotoxicity in Rats

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Summary

The present study was conducted to evaluate the protective effect of methanolic extract of leaves of *Caesalpinia bonduc (L.)* on artesunate-induced hepatotoxicity in rats. Adult male wistar rats used in the study were divided into 4 groups: Group I were given only distilled water and served as control; Group II received 4mg/kg of artesunate daily for 7 days; Group III received 40mg/kg of Liv.52 daily for 7 days and Group IV received 500mg/kg of methanolic extract of leaves of *Caesalpinia bonduc* daily for 7 days. The activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG), bilirubin, and total protein were statistically increased in rats exposed to artesunate. Moreover, administration of artesunate for resulted in damage of liver structures. Administration of methanolic extract of *C.bonduc* before artesunate exposure to rat can prevent severe alterations of biochemical parameters and disruptions of liver structures. The activity of the *C.bonduc* was comparable to the standard drug, Liv 52 (40mg/kg, p.o.). In conclusion, this study obviously demonstrated that pretreatment with methanolic extract of *C.bonduc* significantly attenuated the physiological and histopathological alterations induced by artesunate. Also, the present study identifies new areas of research for development of better therapeutic agents for liver and other organs dysfunctions and diseases.

Key words: *Caesalpinia bonduc*, Artesunate, Hepatotoxicity, Biochemical parameters

Introduction

Artesunate is a drug used to treat malaria, especially chloroquine resistant malaria..t is a semi-synthetic derivative of artemisinin, the active compound of the Chinese herb *Artemisia annua* which consists of the sodium succinyl salt of dehydroartemisinin[1]. Artesunate may have acted indirectly through generation of high levels of reactive oxygen species (ROS) or directly as toxin to the cells of the liver, affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis [2]. Moreover, there are also reports that cadmium toxicity in liver may be mediated by the production of reactive oxygen species known to induce necrosis in various rat organs [3, 4], lipid peroxidation [5] and a decrease in antioxidant enzymes [6].

Caesalpinia bonduc (L) Roxb (Caesalpiniaceae) 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 [7], anthelminitic, antimalarial [8], Antitumour activity [9], adaptogenic activity [10], antidiabetic activity [11] and antioxidant activity [12]. No detail report was found in literature to evaluate hepatic damage experimentally in rats.

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

Materials and Method

Collection and authentication of plant: The plant materials were 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. The voucher specimen (MIP/P'cology/VSN-TS-18) was deposited at Department of Pharmacology, 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 60ºC. The dried plant materials were then pulverized into coarse powder in a grinding machine. 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 [13].

Experimental animals: Three months old wistar albino rats of either sex weighing 150- 250g were used for the study. The animals were procured from Nagar Nigam Animal House, Indore. 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 ^{0}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/IU/1019/6/06/007

Toxicity Study [14]: Acute oral toxicity was conducted for extract on albino mice according to OECD 425 and median effective dose (ED_{50}) of extract was selected based on LD_{50} obtained from acute toxicity studies.

Artesunate induced liver damage [15]: Albino wistar rats of either sex (200-250g) were used. All the animals were divided into the four groups each group consists of 6 animals and they received the treatment as follows.

Group I: Control (Distilled water p.o.)

Group II: Artesunate (4mg/kg p.o.) for 7 days

Group III: Standard drug (Liv.52, 40mg/kg p.o.) + Artesunate for 7 days

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

Biochemical estimation: At the end of experimental period, rats were anaesthetized with ether. Blood samples were collected from orbital venous plexus in nonheparinized tubes, centrifuged at 3000 rpm for 20 minutes, and blood sera were then collected and stored at 4◦C prior immediate determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), direct bilirubin (DB), total bilirubin (TB) and total protein. 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 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 compare with respective control group. A value was of $P<0.05$ was considered significant.

Results

Phytochemical investigation of methanol extract of *C. bonduc* revealed presence of various active constituents Alkaloids, Glycosides and Amino acids.

The LD_{50} of plant methanolic extract was found to be 2,000 mg/kg. The effective dose 250 mg/kg was selected based on LD_{50} of plant.

Oral administration of artesunate at a dose of 4mg/kg in caused a significant (P<0.01) increased in liver 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 and total protein were statistically increased group II. Liv.52 significantly (P<0.01) reduced these levels near to normal. A moderately significant (P<0.01) decrease was observed in the AST, ALT, ALP, LDH, TB, DB and Total protein (Table 2) in the animals treated with methanolic extract of *C. bonduc* (500mg/kg) and showed dose dependant activity.

Lightmicroscopic examination of the liver of control rats showed the normal structure in Figure 1(a). Histopathological effects of artesunate on liver of treated rats are presented in Figure1 (b). Rats treated with artesunate showed many severe histopathological alterations. Administration of artesunate for seven days resulted in the damage of liver structure along with disarrangement of hepatic strands. Several cells also show 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 artesunate (group 2). Treatment with Liv.52 and methanolic extract of *C. bonduc* brought back the cellular arrangement around the central vein and reduced necrosis (Figures 1 (c) and (d)). Also,it helped to bring the blood vessels to normal condition.

Table 1: Effect of methanolic extract of *C. bonduc*, artesunate and Liv.52 on liver weight and liver volume.

	Body weight (gm)	Liver weight (gm)	Liver volume (ml)
Control	245±1.3	4±0.9	7±2.3
Artesunate Treated	250±1.6	7±0.2	9±1.6
Liv 52 (40mg/kg) +Artesunate	235±2.2	5.5±1.1	7±1.3
Methanolic extract (500mg/kg) +Artesunate	243±2.9	5±1.6	7.5±2.1

Table 2: Effect of methanolic extract of *C.bonduc*, artesunate and Liv.52 on different biochemical parameters of liver.

Group	AST (I.U./L)	ALT (I.U./L)	T.G. (mg/dl)	D.B. (mg/dl)	T.B. (mg/dl)	Total Protein (mg/dl)
Control	72.33±1.45	75±2.88	122±1.52	0.16±0.08	0.64±0.02	6.80±.15
Artesunate(4mg/kg) Treated	152±1.45*	340±5.77*	142±1.45*	0.36±0.01*	0.88±0.02**	7.33±.88*
Methanolicextract (500mg/kg)+ Artesunate	118.3±4.41**	86.67±4.41*	124±2.08**	0.17±0.01*	0.63±0.02**	66.73±.12*
Liv52(40mg/kg)+ Artesunate	125.3±2.60*	88.67±4.10**	126±0.88*	0.18±0.01*	0.69±0.02**	6.96±.88*

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

* P<0.05, ** P<0.01, when compared with the artesunate treated groups (one-way ANOVA followed by Dunnetts test)

Histopahological Changes:

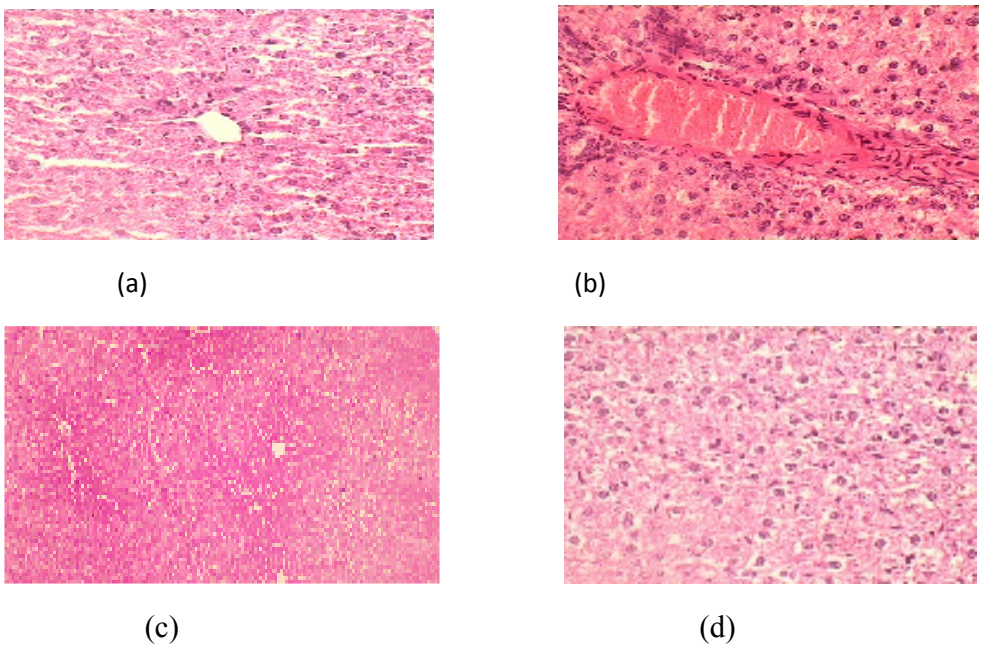

Figure 1: Liver micrographs of control (a), artesunate (b), Liv.52 (c) and methanolic extract of *C.bonduc* (d) treated rats. Original magnification X400.

Discussion

More than 1000 xenobiotic substances are potentially hepatotoxic [16]. The ability of the chemical to produce liver damage in vivo often results from the interaction of a series of complex cellular processes involved in the uptake, biotransformation and elimination of these potentially toxic compounds.

The observed increase in the relative weight of the liver in all the treated groups is in agreement with the work of Simons et al, 1995 who reported that increased organ weight (whether absolute or relative) is a sensitive indicator of organ toxicity [17].

Bilirubin and all the enzymes measured in this study were significantly increased in all the treated groups. Liver enzymes are usually raised in acute hepatotoxicity, but tend to decrease with prolonged intoxication due to damage to the liver cells [18]. Increases in liver enzymes such as ALT, AST, TG and Total protein are common findings in liver toxicity. The present study has shown that there was a significant increase in all the measured liver enzymes indicating liver damage [19].

Generally, artesunate exerts its anti-malarial activity by the generation of reactive oxygen species (ROS) from its endoperoxide bond [20] leading to lipid peroxidation [21]. The accumulation of lipid peroxides is toxic to the membrane structure, leading to a change in permeability and to disintegration of cellular organelles [22].Several situations, the rate of generation of ROS exceeds that of their removal and oxidative stress occurs [23]. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause liver necrosis [24].

Results of this study confirmed that artesunate at a dose of 4 mg/kg produces significant hepatotoxicity as evidenced by increase in serum AST, ALT, TG, DB, TB and total protein level. In addition, artesuate induced severe hepatic damage as shown in histopathological examination which coupled with markedly elevated levels of liver biochemical markers (AST, ALP, TG, DB, TB and total protein). In artesunate treated rats, there was a significant increase in reactive oxygen species (ROS) suggesting the liver damage. Treatment with *Caesalpinia bonduc* extract recovered the injured liver to normal after 24 hrs at a dose of 500 mg/kg which indicate that *Caesalpinia bonduc* has antihepatotoxic effect. The possible antihepatotoxic mechanism of *Caesalpinia bonduc* has 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.

Conclusion

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

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