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Evaluation of Hepatoprotective Activity of *Caesalpinia Bonduc (L.) Roxb.* on Experimentally Induced Liver Damage in Animals

Hemant Vinayak Deore[1] *, Harshal S. Bhandari[1], Vinod S. Ahire[1], Swapnil B. Deshmukh[1], Jayashree A. Patil[2], Pravin V. Gomase[3], Touseef Begum[4], Shoeb Qazi[5], Rahil Meman[6], Ansari Yaasir[7], Ansari Vaseem Ahamad[8], Ansari Mohd. Razi[9]

[1]DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra, India, 424005
[2] Gangamai College of Pharmacy Nagaon Dhule, Maharashtra, India, 424005
[3] JES's College of Pharmacy, Nandurbar-425412, M.S., India.
[4] Ibn Sina National College for Medical Studies, Jeddah, KSA.
[5] AIKTC School of Pharmacy, New Panvel, Navi Mumbai-410206, Maharashtra, India.
[6] Ismail Mehta College of Pharmacy, Ambad, Dist-Jalna-431204, M.S., India.
[7] Ali Allana College of Pharmacy, Akkalkuwa, Dist-Nandurbar-425415, M.S., India.
[8] School of Pharmacy, LNCT University, Bhopal-462042, M.P., India.
[9] Torrent Pharmaceuticals, Baddi-173205, H.P., India.

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KEYWORDS

Hepatoprotective activity, *Caesalpinia bonduc (L.) Roxb.*, Liver damage, Animals.

ABSTRACT:

Hepatoprotective activity of the Ethanolic extract of *Caesalpinia bonduc* (Caesalpiniaceae) seed was investigated in rats by inducing toxicity with Carbon tetrachloride, Paracetamol, Ethanol. The extract has been shown to possess significant protective effect by lowering the level of AST, ALT, ALP, LDH, Cholesterol and bilirubin. The Ethanolic extract of *Caesalpinia bonduc* at a dose of 125mg/kg, 250mg/kg, and 500mg/kg showed significant hepatoprotective activity which was comparable to that of a standard hepatoprotective agent (Silymarin).

INTRODUCTION

Liver is the important organ concerned with the biochemical activities in the human body. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agent is of grave consequences. There is an ever increasing need of an agent which could prevent it from such damage[1]. In view of severe undesirable side effect of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity[2].

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[3], anthelminitic and antimalarial [4],Antitumour activity and antioxidant[4],adaptogenic activity[6], antidiabetic activity [7].

This plant contain various chemical constituents such as Alkaloid , Glycoside (Bonducin) , tannin , furanoditerpenes – α caesalpin, β caesalpin, γ caesalpin ,δ caesalpin ,ε caesalpin and caesalpin –F . Fatty acids – palmitic ,stearic oleic and linoleic acid . amino acid – aspartic acid, arginine and citrulline. Carbohydrates ,starch β- cartene, gum and resins.

MATERIALS AND METHOD

Plant

The plant was collected from Karwand near Shirpur in the month of July. The plant was authenticated by Dr. D.A. Patil, Department of Botany, S.S.V.P.S College of Science, Dhule, Maharashtra, India.

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Extraction methodology

The seed coat was broken and the kernels and seed coat were separated and size reduced to a coarse powder. The Kernels Powder was extracted with Pet ether. (60-80). The marc was dried and further extracted with ethanol.

Animals

Healthy adult swiss albino mice of either sex weighing between 25 to 30 gm were used for acute toxicity study. Healthy Albino wistar rats of either sex were used for hepatoprotective activity.

Toxicity study 8:

Acute oral toxicity (AOT) was conducted for extract on albino mice. None of the animal showed any toxic effect or mortality in an observation period of 14 days up to dose 5000 mg/kg.

Effective dose (ED50) of extract was selected based on LD50 obtained from acute toxicity studies.

Experimental procedure

Carbon tetrachloride (CCl4) induced liver damage 9:

Procedure:

Albino wistar rats (150-250g) were used. All the animals were divided into the seven groups each group consisting of 6 rats and they received the treatment as follows.

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

Group II: Toxicant (CCl4 on 2nd & 3rd day 1ml/kg s.c.)

Group III: Standard drug (Silymarin 50mg/kg p.o. + (CCl4 on 2nd & 3rd day 1ml/kg s.c)
Group IV: Vehicle treated (1%CMC)

The vehicle (Distilled water) or extract were administered orally for 7 days. Paracetamol suspension (1% CMC) was administered in a dose of 3g/kg p.o on 5th day. 48 hrs after paracetamol administration, blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical investigations viz. ALT, AST, ALP, LDH, TB, and DB.

Group V: Extract treated (Ethanolic extract 125 mg/kg p.o. + CCl4 on 2nd & 3rd day 1ml/kg s.c)
Group VI: Extract treated (Ethanolic extract 250 mg/kg p.o. + CCl4 on 2nd & 3rd day 1ml/kg s.c)
Group VII: Extract treated (Ethanolic extract 500mg/kg p.o. + CCl4 on 2nd & 3rd day 1ml/kg s.c)

The test drugs or vehicles (1% CMC in distilled water) were administered orally for 5 days. Hepatotoxicity was induced in all groups by an injection of CCl4 (1 ml/kg, 1:1 with Olive oil s.c.) up to 5th day 48 hours after CCl4 administration, blood sample from each animal from all Groups was obtained by puncturing retro-orbital plexus. The blood samples were allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical investigations viz. ALT, AST, ALP, LDH, TB, and DB.
The livers of all animals were removed and processed for histological investigations.
Paracetamol induced liver damage 10:
Procedure:

Albino wistar rats (150-250g) were used. All the animals were randomly divided into the six groups each group consists of 6 animals and they received the treatment as follows
Group I: Normal (Distilled water p.o.)
Group II: Toxicant (On 5th day Paracetamol 3g/kg, 1% CMC p.o.)
Group III: Standard (Silymarin 50mg/kg p.o. + on 5th day Paracetamol 3g/kg, p.o.).
Group IV: Extract treated (Methanolic extract 125 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).
Group V: Extract treated (Methanolic extract 250 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.).
Group VI: Extract treated (Ethanolic extract 500 mg/kg p.o. + On 5th day Paracetamol 3g/kg, p.o.)

The livers of all animals were removed and processed for histological investigations.

Ethanol induced liver damage [11]:

Procedure:

Albino wistar rats of either sex (150-250g) were used. All the animals were divided into the six groups each group consists of 6 animals and they received the treatment as follows.

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

Group II: Toxicant (28.50 % Ethanol 3ml/100g/day p.o.) Group III: Standard (Silymarin 50mg/kg p.o. + 28.50 % Ethanol 3ml/100g/day p.o) Group IV: Extract treated (Ethanolic extract 125 mg/kg p.o + 28.50 % Ethanol 3ml/100g/day p.o) Group V: Extract treated (Ethanolic extract 250 mg/kg p.o. + 28.50 % Ethanol 3ml/100g/day p.o) GroupVI: Extract treated (Ethanolic extract 500mg/kg p.o. + 28.50% Ethanol 3ml/100g/day p.o) The vehicle (distilled water) or extracts was administered orally for 30 days. Alcohol (28.50 %) solution in distilled water was administered in a dose of 3ml/100g/day p.o. for 30 days in three divided doses. Twenty-four hours after last dose of alcohol, blood was withdrawn from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical investigations viz. ALT, AST, ALP, LDH, TB, DB, and Cholesterol.

The livers of all animals were removed and processed for histological investigations.

Statistical analysis

The data represent mean S.E.M. Result were statistically by one-way ANOVA followed by dunnet's test. The minimum level of significance was set at $p<0.005$.

RESULTS

Administration of CCl4, Paracetamol, and Ethanol of rats caused significant increase in liver weight (Table1,3,5) and serum enzyme like AST, ALT, LDH, ALP, cholesterol and bilirubin compare with treated group. Treatment with Ethanolic extract of Caesalpinia bonduc caused reduction of this value (Table 2,4,6) , dose- dependently, almost comparable to the Silymarin treated group.

CCl4 induced hepatotoxicity

Table no. 1.Physical parameter

Group	Body weight (gm)	Liver weight (gm)	Liver volume (ml)
Normal	245	8	6
CCl4 Treated	259	14	11.5
STD+ CCl4	225	9	8
125 mg/kg	239	11	10
250mg/kg	226	11	9
500mg/kg	268	10	9.5

Table no. 2.Biochemical parameter

Group	SGOT (I.U./L)	SGPT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	73.09 ±0.6**	57.97 ±0.6*	153.2 ±0.8*	251.3 ± 1.3*	0.16± 0.009*	0.077±0.004**
CCl_4 Treated	132.4 ±0.7**	95.84 ±0.3**	249.4± 0.8*	326.8 ±1.6*	0.83± 0.039**	0.47 ±0.02
STD+ CCl_4	81.98 ±0.4	69.73 ±0.4**	192.1 ±5.5*	277.9 ±0.8*	0.46 ±0.59	0.13± 0.008**

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125 mg/kg	93.35 ±0.4**	79.14 ±0.5**	217.7 ±0.5*	279.3 ±0.8*	0.56± 0.018*	0.34± 0.01*
250mg/kg	87.5 ±0.6*	76.55 ±0.2	207.1 ±0.4*	270.9 ±0.8*	0.44± 0.01*	0.29± 0.02*
500mg/kg	83.06 ±0.3	72.24 ±0.3*	195.7 ±0.5*	261.7 ±0.7	0.42± 0.008*	0.17 ±0.019

Paracetamol induce Hepatotoxicity

Table no. 3.Physical parameter

Group	Body weight (gm)	Liver weight (gm)	Liver volume(ml)
Normal	265	9	7.5
Paracetamol treated	229	12	10.5
STD+ Paracetamol	256	9	7
125 mg/kg	232	10.5	10
250mg/kg	252	10.5	9
500mg/kg	268	10	9.5

Table no. 4.Biochemical parameter

Group	SGOT (I.U./L)	SGPT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	64.19± 1.2*	47.09± 0.6*	185.1± 1.7*	146.3 ± 1.3*	0.18± 0.006	0.17± 0.004**
Paracetamol Treated	172.9± 1.3**	125.8 ±3.2	352.5± 3.2*	273.5 ± 1.4*	1.08± 0.1*	0.6± 0.016
STD + Paracetamol	95.91 ±1.1	71.03 ±0.8	210.3± 0.9*	177.3 ± 3.5*	0.35± 0.01*	0.24± 0.004
125 mg/kg	135.5 ±1*	107.1 ±0.6	305.3± 1.2*	230.8 ± 1.6*	0.6± 0.03**	0.50 ± 0.01*
250mg/kg	114.9 ±0.9	96.86 ±0.7**	281.9± 2.4*	215.7 ± 1.8*	0.5 ±0.02	0.42± 0.01*
500mg/kg	103.8 ±1	85.88 ±0.3	240.7 ±2.6*	196.8 ± 0.4*	0.45 ±0.01	0.32± 0.01*

Ethanol induced hepatotoxicity

Table no. 5.Physical parameter

Group	Body weight (gm)	Liver weight (gm)	Liver volume(ml)
Normal	260	7.5	6.5
Ethanol treated	232	10	8.5
STD+ Ehanol	260	8	6.5
125mg/kg	238	9	7.5
250mg/kg	252	9	7.2
500mg/kg	255	8.5	6.8

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Table no. 6.Biochemical Parameter

Group	SGOT (I.U./L)	SGPT (I.U/L)	ALP (I.U./L)	LDH (I.U./L)	TB (% mg)	DB (%mg)	Chole-sterol (mg/dl)
Normal	72.87±1.3**	52.38± 1.6**	144.2± 2.5**	256.3± 0.7**	0.16±0.007***	0.12±0.02**	89.19± 0.7**
Ethanol trated	139.2±0.8**	100.2± 1.1**	247.9±3.0**	353.5± 2.3**	1.07±0.008**	0.34±0.01***	143.3± 1.5**
STD + Ethanol	87.95±0.8**	70.53± 0.5**	173.5± 1.0**s	275.1± 0.8**	0.24±0.01**	0.23±0.05***	99.92±0.8**
125mg/kg	127.2±0.7**	90.39±0.5**	228.7±1.3**	333±0.9**	0.76±0.008**	0.30±0.004***	130.5±1.0**
250 mg/kg	119.5±1.9**	84.14± 0.6**	213.4± 0.7**	316.9± 1.7**	0.61±0.02	0.27±0.007***	115.8±.2.0**
500mg/kg	102± 0.9**	76.49± 1**	197.7± 1.0**	304.4± 1.7**	0.44±0.02**	0.24±0.007***	106.3± 0.7**

DISCUSSION

Damage to the structural integrity of liver is reflected by an increase in level of serum transaminases and bilirubin because these are cytoplasmic in location and are release into circulation after cellular damage[12]. The present study has also demonstrated that bersgenin in vivo has a hepatoprotective activity against liver injury induced by CCl_4, Paracetamol, and Ethanol.

CCl induced hepatotoxicity by metabolic activation, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is metabolically activated by the Cytochrome p 450 dependent mixed oxidase in the endoplasmic reticulum to from trichloromethyl free radical (CCl_3) which combined with cellular lipid and protein in presence of oxygen to induced lipid Peroxidation [13].

Paracetamol (N-acetyl p-amono phenol) a widely used analgesic and antipyretic drug is known to cause hepatotoxicity in experimental animals and humans at high doses[14] It is mainly metabolized in liver to excretable glucuronide and sulphate conjugates . However, hepatotoxicity of Paracetamol has been attributed to formation of toxic metabolites when a part of Paracetamol is activated by hepatic Cytochrome p 450 to a highly reactive metabolite N-acetyl-p-benzoquinoneimine, which is normally conjugated with GSH and excreted in the urine as conjugates. Overdose of Paracetamol leads to mitochondrial dysfunction followed by acute hepatic necrosis [15].

The hepatocytes contain three main pathways for ethanol metabolisms to give acetaldehyde; each located in the different subcellular compartment namely alcohol dehydrogenase pathway of cytosol, the microsomal ethanol oxidizing system located in the endoplasmic reticulum, and catalase located in the peroxisomes. Chronic ethanol feeding results in the appearance of a form of Cytochrome P-450 also differing by its catalytic activity from other Cytochrome P-450 species, which leads to glutathione depletion and due to CYP-450 directly or through its active metabolites like acetaldehyde, increases the free radical concentration in body[16]. These free radicals trigger the oxidative damage and further stimulate lipid peroxidation. Ethanol induced hypoxia has also been evoked as a possible cause of hepatotoxicity [17].

The present study revealed a significant in activities of SGOT, SGPT, ALP, Cholesterol and serum bilirubin level on exposure to CCl_4, Paracetamol and Ethanol , indicating considerable Hepatocellular injury . Administration of Ethanolic extract of *Caesalpinia bonduc* at 125mg/kg ,250mg/kg and 500mg/kg dose level attenuated the increased level of of the serum enzymes, produced by CCl4, Paracetamol and Ethanol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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