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Research Article

Evaluation of Hepatoprotective Activity of Indigofera barberi in Rats against Paracetamol Induced Hepatic Injury

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Abstract

Various fractions obtained from chloroform extract of Indigofera barberi (whole plant) was scrutinized in albino rats for hepatoprotective activity on paracetamol instigated hepatic injury. Into 11 groups rats were divided. 5 animals in each group. By giving paracetamol orally at a dose of 2 gm/kg hepatic injury was acquired. With fraction D, hepatoprotective action is achieved by depletion in various serum marker enzymes like AST (aspartate transaminase), ALT (alanine transaminase). Also diminished the high amount of serum bilirubin and ALP (alkaline phosphotase). The hepatoprotective activity of fraction D was additionally confined by histopathological investigations on paracetamol treated animals. With silymarin (100 mg/kg, orally), as a standard drug the effects acquired were collated. Valuable flavonoids in Fraction D had shown hepatoprotective activity via stability, suppressing oxidative stress and restrictive effect on cellular permeability, through their antioxidant characteristic.

Key words: I. barberi; Paracetamol; Hepatoprotective.

Introduction

Major body organ is the Liver. It executes a vital part in the metabolism of lipids, fats, proteins and carbohydrates. It maintains metabolic equilibrium. It plays vital role in biotransformation, detoxification and elimination of multiple environmental, endogenous and pharmaceutical wastes, biochemical's mandatory for digestion (bile pigments), hormones (angiotensinogen), productivity of many coagulation factors, vitamin A, D, B12 and growth factors. It also safeguards the physique from possible dangerous materials specifically endotoxins that are assimilated by the intestinal tract and virulent

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by-products of metabolism [1]. In the previous few years hepatic damage and palsy principally occurred due to disclosure to virulent chemicals, various drugs such as thio-acetamide, chemotherapeutic agents, xenobiotics, carbon tetrachloride, microbes, viruses, environmental pollutants, chronic alcohol consumption and auto-immune diseases [2].

Paracetamol is a commonly used analgesic and antipyretic agent. The main adverse effects of paracetamol overdose are hepatotoxicity and nephrotoxicity, which could be fatal. NAPQI, which is normally detoxified by glutathione is responsible for hepatotoxicity. Excess of NAPQI is formed in paracetamol overdose, which binds to proteins and other macromolecules resulting in hepatic necrosis. The standard therapy for paracetamol overdose toxicity is N-acetylcysteine. It restores hepatic glutathione, which detoxifies NAQPI [3], nevertheless some cases do not respond [4]. In spite of tremendous advances in modem medicine, there are hardly any reliable drugs that protect the liver from damage and/or help in regeneration of hepatocytes. To treat a wide variety of clinical diseases including liver disease many active plant extracts are frequently utilized. Therefore, searching for effective and safe drugs for liver disorders are continues to be an area of interest [5].

I. barberi belongs to Fabaceae family and it is greatly valued endemic herb found in Tirumala Hills. Vernacularly it is Adavineelimanadu mokka. It grows upto 1 m tall as an under shrub. Its branch lets are angled faintly. Leaves are 3 and foliolate, leaflets are pubescent, ovate-oblong, mucronate and obtuse. Flowers are pink and are arranged in racemes of axillary congested form. Pods were deflexed, sub-terete and appressed. They are sharply pointed and white-tomentose in color. Seeds are in the number of 2 to 4. Fruiting and flowering season was extended from Sep. to Dec [6].

For controlling diabetes, I. barberi leaves powder (5 g) is taken along with butter milk orally. Leaves (50 g), pepper (1 g), garlic (1 g) are made to paste and prepared pills (peanut size), to cure jaundice once a day 5 pills are taken for 5 days as suggested by Nakkala and tribal physicians. It is used as a coloring agent (dye). Once a day for 10 days, whole plant powder (5 g) is taken with rice washed H_2O to cure skin diseases, peptic ulcers and to remove intestinal worms [7]. As an antiseptic, leaves juice is used to cure boils, burns, cuts and wounds. The current study was undertaken by keeping these facts in view, to generate a systematic base for the utilization of chloroform extract fractions of I. barberi as an hepatoprotective agent.

Methodology

Plant material collection

The I. barberi (whole plant) was clustered from the deciduous plantation of Tirumala Hills in A.P. State, India. Samples were validated and certified by Dr. K. Madhava Chetty, Plant Taxonomist, Department of Botany, S.V. University, Tirupati, A.P., India. The collected whole plant of I. barberi was sorted, thoroughly cleaned and air-dried at room temperature for more than one week. By using the laboratory hammer mill the whole plant was ground to powder. Powdered samples are collected carefully and stored in air, water and insect proof containers protected from direct sunlight, heat and other substances until required for extraction.

Preparation of extracts

The whole plant powdered material (about 30 gm) of Indigofera barberi was extracted successively in soxhlet apparatus with each solvent of increasing polarity i.e., with petroleum ether, chloroform ($CHCl_3$), ethanol and distilled water for 18-20 hours. The yield of Indigofera barberi were 9.7 gm, 6.8 gm, 3.43 gm & 7.79 gm with petroleum ether, chloroform, ethanol and distilled water respectively. The extracts were then thoroughly evaporated to dryness in rota evaporator till free from the different solvents [8].

Isolation of fractions

Thin-layer chromatography (TLC) method was carried out using silica gel aluminum plate 60F-254, 0.5 mm (TLC plates, Merck).

The spots were apprehended, visualized and conceptualized in UV light and 10% of H_2SO_4 in methanol. For further thorough purification, the chloroform extract was carefully subjected to column chromatography (silica gel ≠ 60-100). The column was equilibrated for almost one hour with petroleum ether at effusion rate 5 ml/min. The sample (1 gm dissolve in methanol) was loaded on to the column, 11 fractions were collected using petroleum ether (100%), petroleum ether:ethyl acetate (4:1), petroleum ether:ethyl acetate (2:3), petroleum ether:ethyl acetate (3:2), ethyl acetate (100%), chloroform:methanol (2:3) and chloroform:methanol (3:2). Above yielded product were pooled into four fractions based on TLC.

Phytochemical analysis

Phytochemical analysis of various extracts was carried out for the presence of alkaloids, fixed oils, saponins, carbohydrates, tannins, steroids & sterols, glycosides, proteins, amino acids, terpenoids, flavonoids by different methods [9].

Experimental design

Wistar albino rats weighing 150-200 gm are obtained from Mahaveera Enterprises, Hyderabad (Reg. No.: 1656/PO/Bt/S/12/CPCSEA), are kept in polypropylene cages, 3 in each cage, at an ambient temperature of 25±2oC and relative humidity of 55-65%. A 12 hour light and dark schedule was carefully maintained in the air conditioned animal house. All the rats are fed with common diet for 1 week after arrival and then divided into groups with free access to food and water.

Acute toxicity studies

According to most essential OECD (Organization for Economic Co-operation and development) guidelines 423 and recommendations we have conducted the acute toxicity studies. Experiments were performed using healthy young adult female rats; animals are nulliparous and non-pregnant weighing between 150-200 gm. Female rats were chosen because of their greater sensitivity to treatment. For about 4-5 hours prior to experiments, all the animals are fasted with free access to only distilled water. The suitable extract of I. barberi are administered to 5 different groups of rats in doses of 5, 50, 300, 2000 and 5000 mg/kg orally and observed for mortality and considerable physical and behavioral changes for over 14 days.

Study design

Rats were divided into 11 groups of 5 animals in each [10].

Group I : Received only normal saline

Group II : Treated with paracetamol 2 g/kg in a single dose

Group III : Silymarin (100 mg/kg)

Group IV : Fraction A of IB (50 mg/kg)

Group V : Fraction A of IB (100 mg/kg)

Group VI : Fraction B of IB (50 mg/kg)

Group VII : Fraction B of IB (100 mg/kg)

Group VIII : Fraction C of IB (50 mg/kg)

Group IX : Fraction C of IB (100 mg/kg)

Group X : Fraction D of IB (50 mg/kg)

Group XI : Fraction D of IB (100 mg/kg)

The following treatments were given orally for 7 days. On the 7th day, paracetamol suspension was administered at a dose of 2 g/kg body wt. to rats of groups III, IV, V, VI, VII, VIII, IX, X and XI.

Biochemical estimation

After 24 h of the last treatment, blood was collected from retro-orbital plexus; serum was separated by centrifugation at 10000 rpm for 15 min. The serum was then collected and analyzed for various biochemical parameters like aspartate transaminase (AST), alanine transaminase [11] (ALT), alkaline phosphatase[12] (ALP) and bilirubin [13].

Histopathological studies

The liver tissue was collected, fixed in 10% formalin and stained with hematoxylin and eosin for photomicroscopic observation [14].

Statistical analysis

The data was represented as Mean±SEM. Results were analyzed by One-Way ANOVA. The minimum level of significance was set at $p<0.01$, $p<0.05$.

Results

Preliminary phytochemical screening

Phytochemical screening revealed the presence of alkaloids and carbohydrates in fraction A, saponins in fraction B, glycosides in fraction C and flavonoids in fraction D (Table 1).

Biochemical parameters

Acute paracetamol administration increased appreciably the extent and altitude of liver injury marker enzymes like AST, ALP, ALT and bilirubin (Table 2). The liver marker enzymes were 259.3±8.5 U/L, 194.3±6.7 U/L, 639.6±11.5 U/L and 2.34±0.05 mg/dl for the AST, ALP, ALT and bilirubin respectively for paracetamol treated group. Pretreatment for 7 days continuously reduced raising serum enzymes in hepatotoxic rats with fraction D (50 mg/kg and 100 mg/kg). The liver marker enzymes were 161.3±6.9, 132.4±5.5, 428.5±9.5 and 1.96±0.05 for 50 mg/kg and 142.4±7.8, 119.3±4.9, 396.4±8.9 and 1.45±0.08 for 100 mg/kg for the biochemical parameters of AST, ALP, ALT and bilirubin. Pretreatment with fraction A, B and C (50 mg/kg and 100 mg/kg) reported no notable alterations changes in serum AST, ALT, ALP and bilirubin levels compare and correlate to paracetamol induced hepatotoxic rats. These values are compared and equated with the

S. No.	Phytoconstituents	Fraction A	Fraction B	Fraction C	Fraction D
1	Alkaloids	+	---	---	---
2	Tanins	---	---	---	---
3	Saponins	---	+	---	---
4	Glycosides	---	---	+	---
5	Terpinoids	---	---	---	---
6	Carbohydrates	+	---	---	---
7	Flavonoids	---	---	---	+
8	Proteins	---	---	---	---
9	Aminoacids	---	---	---	---
10	Fixed oils	---	---	---	---
11	Steroids & Sterols	---	---	---	---

Table 1: Preliminar y phytochemical screening of Chloroform extract of I. barberi (CEIB)

standard silymarin 100 mg/kg (124.2±7.3, 96.7±8.7, 341.7±12.1 and 1.21±0.09 AST, ALP, ALT and bilirubin respectively).

Group	Treatment	AST (U/L)		ALP (U/L)		ALT (U/L)		Bilirubin (mg/dl)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
I	Control	143.3	9.6	79.4	5.4	251.3	10.1	0.75	0.02
II	Paracetamol (2 gm/kg)	259.3	8.5	194.3	6.7	639.6	11.5	2.34	0.05
III	Silymarin (100 mg/kg)	124.2	7.3	96.7	8.7	341.7	12.1	1.21	0.09
IV	Fraction A (50 mg/kg)	230.1	9.2	180.2	4.5	501.2	10.9	2.11	0.06
V	Fraction A (100 mg/kg)	211.3	8.4	171.6	8.3	409.3^{+}	11.2	1.96	0.07
VI	Fraction B (50 mg/kg)	249.6	6.2	176.3	7.2	508.5	12.3	2.07	0.06
VII	Fraction B (100 mg/kg)	201.3	8.1	164.5	6.9	479.3	11.8	1.85*	0.05
VIII	Fraction C (50 mg/kg)	223.5	7.9	169.5	7.8	511.4	10.9	2.23	0.06
IX	Fraction C (100 mg/kg)	194.6	10.2	153.4	4.9	464.3	10.8	1.99	0.08
X	Fraction D (50 mg/kg)	161.3*	6.9	132.4	5.5	428.5	9.5	1.96*	0.05
XI	Fraction D (100 mg/kg)	142.4^{+}	7.8	119.3	4.9	396.4	8.9	1.45^{+}	0.08

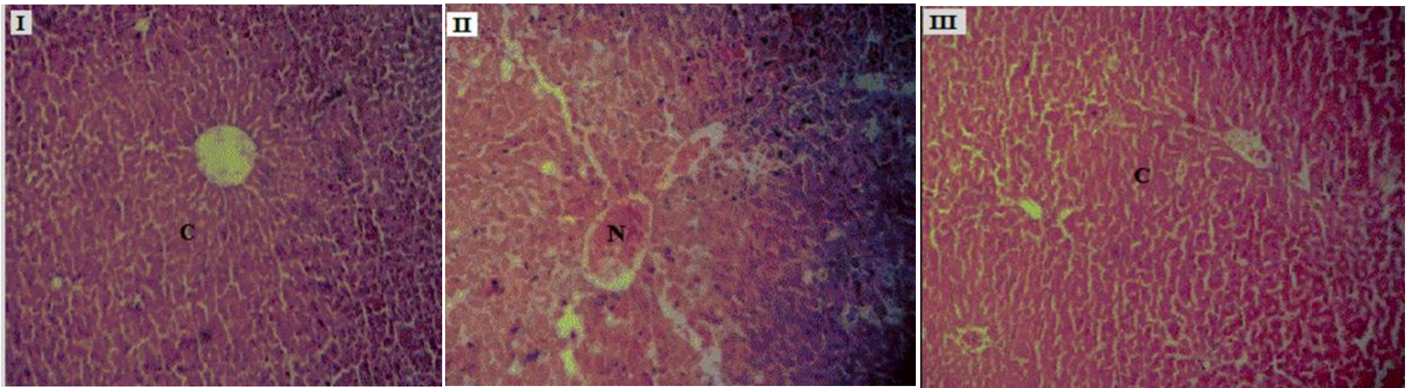
Table 2: Effect of CEIB fractions on the serum AST, ALP, ALT and bilirubin levels on albino rats

The results are indicated as Mean±SEM for each group. The data was calculated by One-Way ANOVA. P<0.01, P<0.05 was considered statistically significant.

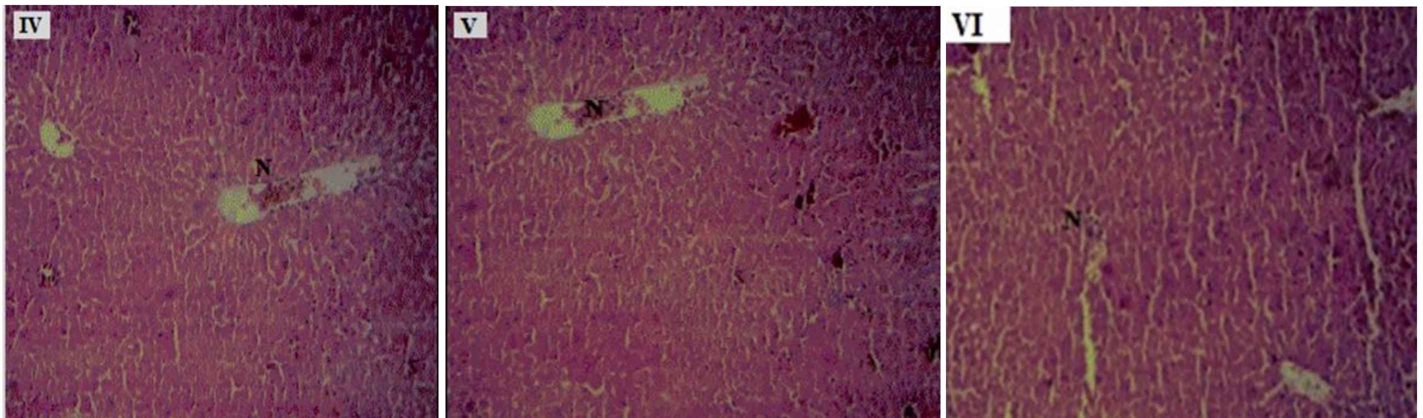
Histopathological examination

Photographs demonstrating histopathology of liver sections of rats (5-10 μ), 40X, Haematoxylin-eosin stain are shown in Figure. 1. Control group (normal saline) displayed normal histology. Group treated with paracetamol (2 gm/kg) manifested cellular degeneration, hydropic changes, fatty changes with wide spread hepatocellular necrosis. Silymarin (100 mg/kg) treated group showed almost normal histology. Group treated with fraction A (50 mg/kg) displayed hepatocellular damage, fatty changes and loss of liver architecture. Fraction A (100 mg/kg) treated group manifested hepatocellular damage and loss of liver architecture. Group treated with fraction B extract (50 mg/kg) showed hepatic necrosis, cellular degeneration, hydropic alterations and fatty changes. Fraction B extract (100 mg/kg) treated group showed cellular degeneration and fatty change. Group treated with fraction C extract (50 mg/kg) manifested hepatocellular damage and fatty changes. Fraction C extract (100 mg/kg) treated group showed cellular degeneration and fatty change. Group treated

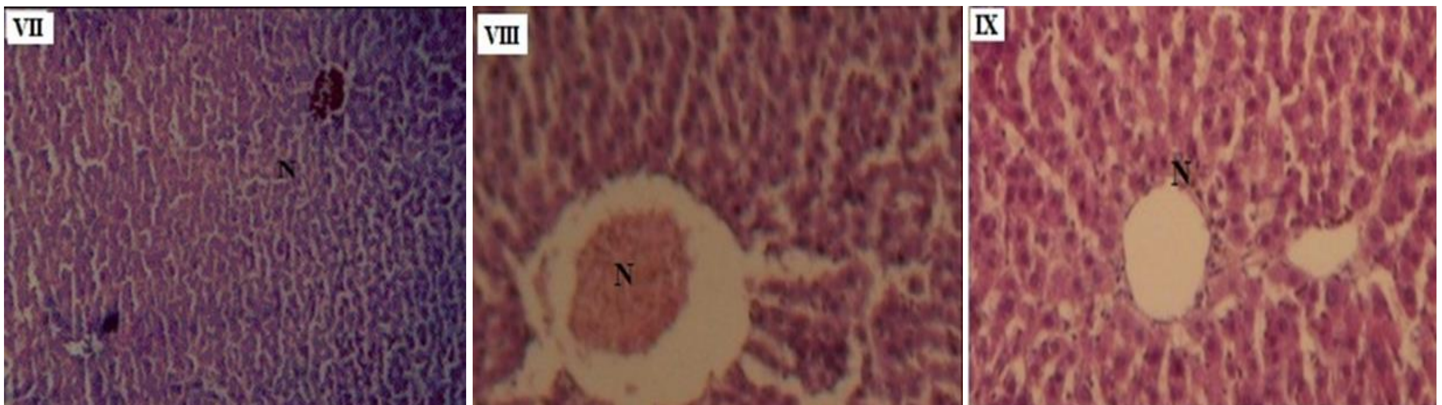
with fraction D (50 mg/kg) manifested no hepatocellular damage excepting areas of focal degeneration and sinusoidal dilation. Fraction D (100 mg/kg) treated group showed almost normal histology.



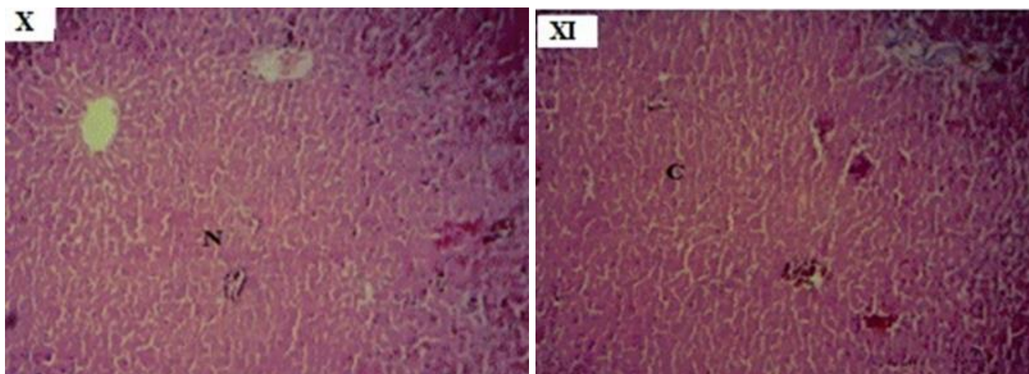
I) Liver tissue of control rats; II) Liver tissue treated with 2 gm/kg of paracetamol; III) Liver tissue treated with silymarin 100 mg/kg



IV) Liver tissue treated with 50 mg/kg of fraction A; V) Liver tissue treated with 100 mg/kg of fraction A VI) Liver tissue treated with mg/kg of fraction B



VII) Liver tissue treated with 100 mg/kg of fraction B; VIII) Liver tissue treated with 50 mg/kg of fraction C; IX) Liver tissue treated with 100 mg/kg of fraction C



X) Liver tissue treated with 50 mg/kg fraction D; XI) Liver tissue treated with 100 mg/kg of fraction D

Figure 1: Hepatoprotective activity of CEIB fractions on paracetamol induced hepatotoxicity.

Discussion

Paracetamol is routine analgesic and antipyretic drug. Many investigations had evidenced the initiation of liver cells necrosis by paracetamol elevated doses in test animals and anthropoids[15]. For safeguarding of hepatoprotective activity, paracetamol-initiated hepatotoxicity had been employed as a authenticated procedure. It is metabolized predominantly in liver and abolished by cojoin with sulfate and glucuronide, then eliminated by the kidneys. Furthermore, its hepatotoxicity has been ascribed to the generation of unsafe metabolites, when a component of paracetamol is triggered by liver cytochrome P-450 to a extremely reactive metabolite[16] Nacetyl-p-benzoquinoneimine (NAPQI). NAPQI could oxidize and alkylate intracellular GSH, which inturn ends in liver glutathione (GSH) exhaustion eventually resulting to elevated lipid peroxidation by withdrawing H2 from a poly-unsaturated fatty acid and furthest, liver destruction due to elevated quantity of paracetamol[17-18]. Active metabolites could employ inceptive cell stress by a broad span of procedure counting exhaustion of GSH or binding to lipids, nucleic acids, enzymes and other cells [19].

Acute paracetamol administration significantly increased the level of liver injury marker enzymes like AST, ALP, ALT and bilirubin. A similar experimental procedure also used by other researchers to report the hepatoprotective effect of natural products [20].

The present study reported the degree of protection, measured by using biochemical parameters like AST, ALP, ALT and bilirubin in fractions treated rats. Studies showed that the pretreatment with I. barberi fraction D (100 mg/kg) for seven days offers considerable protection ($P<0.05$) to liver as evidenced from the levels of biochemical parameters.

Conclusion

Valuable flavonoids in Fraction D had shown hepatoprotective activity via stability, suppressing oxidative stress and restrictive effect on cellular permeability, through their antioxidant characteristic.

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