HEPATOPROTECTIVE ACTIVITY OF *RUBIA CORDIFOLIA*

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Summary

R. cordifolia Linn. (Rubiaceae) is commonly known as Indian Madder and Manjistha. It is used in many polyherbal formulations for various ailments and cosmetic preparations. Traditionally the drug is utilized for its inflammatory, antiseptic and galactopurifier activity. Various extracts of roots of *R. cordifolia* were screened for its hepatoprotative activity using Thioacetamide induced hepatotoxicity in rats. The activity was assessed through estimation of biochemical parameters viz. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT), further the results were supplemented with histopathological studies on Liver samples of the treated animals.

The methanolic extract could protect the liver of the animals against thioacetamide induced hepatotoxicity. Histology of the Liver sections of animals treated with methanolic extract showed the normal hepatic architecture with absence of necrosis, which further evidence the hepatoprotective activity.

Keywords: Hepatoprotective; Manjisth; *Rubia cordifolia*

R.cordifolia belonging family *Rubiacae,* commonly known as Manjishth grows throughout India mainly in Himalayas, Nilgiris and other hilly districts.[1] It has been claimed in traditional literature to be valuable against wide variety of diseases. Literature describes the uses of roots in the treatment of number of ailments including anti-inflammatory, rheumatism, skin diseases, urinary diseases and as blood purifier. The root extract have been studied for its antioxidant, anti-inflammatory, analgesic and anti-cancer activity. The roots are also known for their dyeing properties.

The roots are well known source of anthraquinones. The predominant anthraquinones characterized include alizarin (1,2-dihydroanthraquinones), purpurin (trihydroxy anthraquinones), and munjistin (xanthopurpurin-2-carboxyllic acid).[2, 3] The other constituents like sterol, terpenes and saponins are reported in literature.

Methods

Dried roots of drug were collected from Local Market and authenticated at Agharkar Research Institute, Pune. Thioacetamide was obtained from Alpha Laboratory Chemical, India.

Preparation of plant extracts: The coarse powder of dried roots of *R. cordifolia* was exhaustively extracted by various solvents of increasing polarity viz. pet tether (60 – 80^0C), chloroform, acetone, methanol and water, in a Soxhlet Extractor. The extracts were concentrated under reduced pressure to dryness.

Animals: Wistar albino rats of either sex weighing 150 –200 gm were housed at controlled temperature, humidity and light. These were fed with the standard rodent diet and water *ad libitum*.

Toxicity studies: The animals were fasted over night prior to the study. The animals were grouped randomly, each group having six animals and were administered grading does till 1 gm./ kg. b.w. i.p. The treated animals were then observed continuously for a period of 2 hrs. and finally after 24 hrs. for any apparent signs of toxicity and mortality.

Evaluation of Hepatoprotective activity: The protocols of the experiments were approved by the Institutional Ethics Committee. The experimental protocols for evaluation of hepatoprotective activity in thioacetamide induced hepatotoxicity are presented in **Table I** and **Table II.** Thioacetamide was administered in a dose of 100 mg/kg b.w. s.c., 20-30 minutes later administration of test solution and vehicle.

Table I Protocols for evaluation of hepatoprotective activity of acetone, methanol and aqueous extracts of *R. cordifolia* in thioacetamide induced hepatotoxicity

Groups	Day 1	Day 2	Day 3	Day 4
Control	Vehicle	Vehicle	Vehicle	Blood withdrawal
Toxicant	Vehicle	Vehicle	Vehicle + Thioacetamide	Blood withdrawal
Test	Test solution	Test solution	Test solution + Thioacetamide	Blood withdrawal

Toxicant- thioacetamide (100 mg/kg b.w. s.c.)
Vehicle- 5 % w/v aqueous solution of Acacia
Test solutions: acetone, methanol and aqueous extracts of *R. cordifolia* suspended in vehicle and were administered in a dose of 100 mg/kg b.w.i.p.

Table II : Protocols for evaluation of hepatoprotective activity of pet ether and chloroform extracts of *R. cordifolia* in thioacetamide induced hepatotoxicity

Sample	Day 1	Day 2	Day 3	Day 4
Control	Vehicle	Vehicle	Vehicle	Blood withdrawal
Toxicant	Vehicle	Vehicle	Vehicle + Thioacetamide	Blood withdrawal
Test Solution	Test solution	Test solution	Test solution + Thioacetamide	Blood withdrawal

Toxicant- thioacetamide (100 mg/kg b.w. s.c.)
Vehicle- Olive oil
Test solution: pet ether and chloroform extracts of *R. cordifolia* suspended in olive oil (vehicle) and were injected in a dose of 100 mg/kg b.w.i.p.

Assessment of Hepatoprotective Activity: On forth day, 24 hrs after the administration of thioacetamide, the blood samples from each animal was collected separately by puncturing retro- orbital plexus. Serum was separated by centrifugation (at 2500 rpm for 10 min.) and subjected to biochemical investigation of Serum Glutamyl Pyruvate Transaminases(SGPT) and Serum Glutamate Oxalacetate Transaminases (SGOT) using Reitman and Frankel Method[4]. Liver was excised and fixed in 10% neutral formalin. The Liver specimen of different groups was then subjected to histopathological studies.

Statistical Analysis [5] : Result expressed as mean $\pm$ S.D. One way variance (ANOVA) Followed by Dunnet 't' test was applied .

Results

The administration of thioacetamide to the animals resulted in marked increase in SGPT and SGOT enzymes activities. The toxic effect of thioacetamide was controlled in the animals treated with methanolic extract (100mg / kg b.w.i.p.) by the restoration of the levels of SGPT and SGOT .

In the histopathological examination of liver section of control group, indicated the central vein was prominent with normal hepatocytes. The appearance of centrilobular necrosis with mild to moderate leucocytic infiltration and granular degeneration is probably the most accurate reflection of liver toxicity in the intoxicated group in both the experimental sets.

In the histopathological profile of group treated with the methanolic extract, regenerative changes such as normal hepatocytes without centrilobular necrosis were observed indicating almost complete protection against thiocetamide induced hepatotoxicity. The other groups treated with extracts viz. pet ether, chloroform, acetone and aqueous, histopathological studies showed mild to moderate centrilobular necrosis with mild to moderate leucocytic infiltration, indicating nil protection offered to restore normal architecture.

Table III: **Effect of administration of Pet ether and Chloroform Extract of Roots of *R. cordifolia* on Biochemical Parameters of rats intoxicated with Thioacetamide:**

Groups	**Biochemical parameters mean ± S.D.**	
	SGPT (IU/L)	**SGOT (IU/L)**
Control	56.00 ± 6.55	175.33 ± 8.51
Toxicant (Thiacetamide)	125.10 ± 8.54	444.66 ± 16.29
Pet ether extract	247.66 ± 12.50	402.33 ± 9.29
Chloroform extract	67.66* ± 7.63	389.16 ± 10.48

Values as mean ± S. D., n = 6, For SGPT: * $p<0.01$ (as compared with toxicant)

F_{cal} ($p<0.0001$) =426.17 : F_{tab} ($p<0.0001$) = 6.55 For SGOT: F_{cal} ($p<0.0001$) =331.01 : F_{tab} ($p<0.0001$) = 6.55

Table IV: **Effect of administration of Acetone, Methanol and Water Extract of Roots of *R. cordifolia* on Biochemical Parameters of rats intoxicated with Thioacetamide:**

Group	Biochemical parameters Mean ± S.D.	
	SGPT (IU/L)	**SGOT (IU/L)**
Control	59.25 ± 4.20	184 ± 9.60
Toxicant(Thioacetamide)	245 ± 9.35	468 ± 13.50
Acetone extract	238.76 ± 8.16	460.25 ± 12.20
Methanol extract	56.5 ± 3.00*	191.26 ± 5.20*
Aqueous extract	234.68 ± 7.21	451.75 ± 10.7

Values as mean ± S. D., n = 6, For SGPT: * $p<0.01$ (as compared with toxicant), F_{cal} ($p<0.0001$) =1064.8 : F_{tab} ($p<0.0001$) = 4.43, For SGOT:
* $p<0.01$ (as compared with toxicant), F_{cal} ($p<0.0001$) =986.47 : F_{tab} ($p<0.0001$) = 4.43

Discussion

In the present study an attempt has been made to study the effect of the extracts in protecting liver from thioacetamide induced liver toxicity. The continuous administration of drug is reported to produce sever liver toxicity. Literature reports that the extracts have potential to counteract the toxicity induced due to CCl_4.[6] The results obtained in the present study indicate that there is significant reduction potential antihepatotoxic activity against thioacetamide induced hepatoxicity . Thioacetamide is metabolized to its nitrite metabolic form due to simple hydrolysis and oxidation by oxidizing enzymes in the liver and induces liver damage by altering semi permeable character of cell wall, which leads to imbalance of ions, rise in calcium and inhibition of mitochondrial activity thus causing death of liver cells.[7]

The methanolic extract of roots significantly reduced the elevated enzyme levels viz. SGPT and SGOT due to administration of Thioacetamide. It can therefore be concluded that the methanolic extract of root possess the hepatoprotective activity.

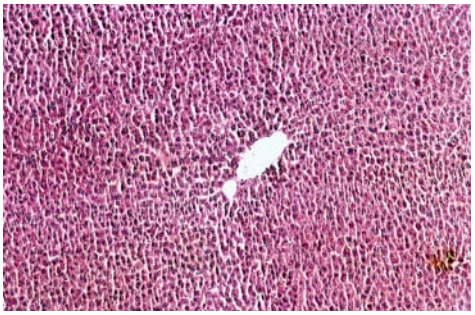

Fig 1 Histopathological Examination of Liver of control group

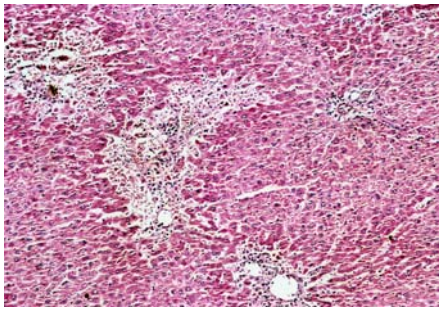

Fig 2 Histopathological Examination of Liver Sections of Toxicant

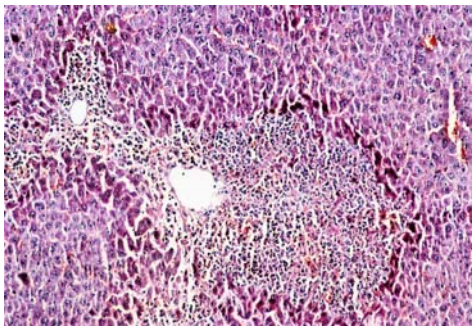

Fig 3 Histopathological Examination liver of Acetone extract Group

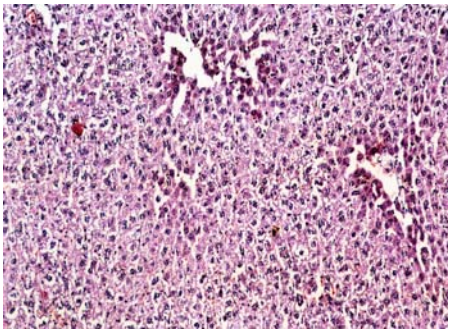

Fig 4 Histopathological Examination of Liver of Methanol extract Group

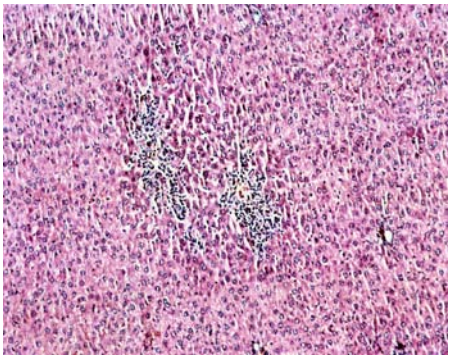

Fig 5 Histopathological Examination of Liver of Chloroform extract Group

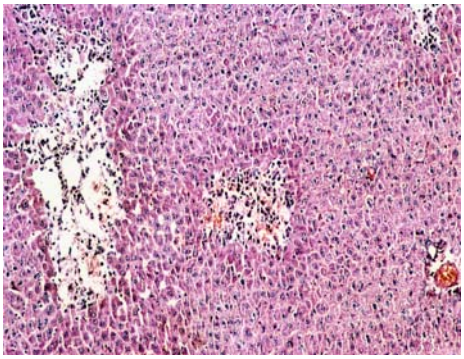

Fig 6 Histopathological Examination of Liver of Olive Toxicant Group

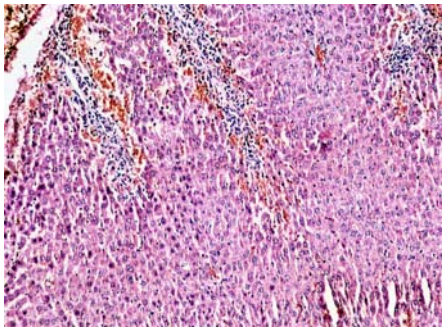

Fig 7 Histopathological Examination of Liver of Pet ether extract Group

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