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Evaluation of hepatoprotective activity of *Haldinia cordifolia* against paracetamol induced liver damage in rats

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ABSTRACT

Hepatoprotective activity of ethanol extract of *Haldinia cordifolia* against paracetamol induced hepatic damage in albino rats was observed. In the present study the effect of ethanol extract of *Haldinia cordifolia* on serum markers such as SGPT, SGOT, ALP and Serum total bilirubin, anti oxidant studies such as SOD, CAT and LPO and Histopathological studies have been studied to find out the possible mechanism of hepatoprotection. It was observed that extracts of *Haldinia cordifolia* has reversal effects on the levels of above mentioned parameters in paracetamol hepatotoxicity. The extract of *Haldinia cordifolia* functions as a hepatoprotective agent and this hepatoprotective activity of *Haldinia cordifolia* may be due normalization of impaired membrane function activity.

Keywords: Paracetamol, hepatoprotective activity, Silymarin, *Haldinia cordifolia*, Serum markers.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction.^[1] The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins, and abused by poor drug habits, and alcohol and prescribed & over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease.^{[2][3]} They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders.

It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity^[4]. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Plant extracts of many crude drugs are also used for the treatment of liver disorders. Extracts of different plants of about 25 plants have been reported to cure liver disorders^[5].

In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell^[6]. There are however, members of drugs employed in traditional system of medicine for liver affections^[7]. Many formulations containing herbal extracts are sold in the Indian market for liver disorders. But management of liver disorders by a simple and precise herbal drug is still an intriguing problem. Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to possess strong antioxidant activity^[8-10].

Haldinia cordifolia (Roxb.) belongs to family Rubiaceae, is found throughout the India in deciduous and semievergreen forests in the lowland and lower hills. The plant fairly large and grows upto 30-40 meters tall. It has been reported to possess astringent, antipyretic and wound healing properties^[11]. Phytochemical evaluation of the bark extract showed the presence of alkaloids, tannins, flavanoids, and steroids etc., till now *Haldinia cordifolia* has not been the subject of any pharmacological research. Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute liver damage if overdoses are consumed. The present study was undertaken to study the possible hepatoprotective role of ethanol extract of stem bark of *Haldinia cordifolia* against paracetamol induced liver damage.

MATERIALS AND METHODS

Plant material:

The stem bark of *Haldinia cordifolia* was collected from Sri Venkateswara University, Chittoor Dist, Andhra Pradesh and identified by Prof. Sri Madhavachetty, voucher specimen (No.561) has been deposited at the Herbarium of the Department of Pharmacology, Bharathi College of Pharmacy, Karnataka.

Preparation of extracts

The stem bark of *Haldinia cordifolia* was washed, shade dried, powdered, passed through a #60 mesh sieve and were extracted with alcohol (95% v/v) in a soxhlet apparatus by continuous heat extraction. The extract was concentrated in a rotary flash evaporator at a temperature not exceeding 50°C. The alcohol extract was prepared in distilled water containing 0.2% w/v CMC (as a suspending agent) for experimental purpose.

Experimental animals

Albino rats of either sex (150-200 gm) were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions, at 25 ± 1 °C, humidity ($60 \pm 10\%$) with 12 hour day and night cycle, with food and water *ad libitum*. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC- BCP/IAEC/PCL/03) of Bharathi College of pharmacy, Bharathinagara, Mandya. Studies were performed in accordance with the CPCSEA guidelines.

Hepatoprotective activity

The LD₅₀ is >2g/kg. No toxic effects or mortality were observed with doses ranging from 50mg/kg to 2g/kg for four weeks. Acute toxicity studies did not detect any changes in vital organ function tests.¹² Hence hepatoprotective activity of alcohol extracts of *Haldinia cordifolia* bark was studied by following methods.

1. Paracetamol induced-hepatotoxicity:¹³

- Group A- Normal control
- Group B- Toxicant (paracetamol 750mg/kg, *p.o.*)
- Group C- Served as Standard (Silymarin 100 mg/kg, *p.o.*)
- Group D- Ethanol extract of *Haldinia cordifolia* bark (200mg/kg, *p.o.*)
- Group E- Ethanol extract of *Haldinia cordifolia* bark (400mg/kg, *p.o.*)

Experimental procedure

Wistar rats of either sex weighing between 150-200 g were divided into five groups of six rats each. Group A was maintained as normal control, which was given distilled water only. Group B received paracetamol 750 mg/kg body wt by *p.o.* at every 72 hours for 10 Days. Group C animals were treated with Silymarin (100 mg/kg *p.o.*) which served as standard. Groups D and E animals were treated with two different doses of alcohol extract of *Haldinia cordifolia* (medium, high) respectively. Group C, D and E were in toxicated with paracetamol (750 mg/kg) 1 hr

before the administration of Silymarin or extract for 10 days. The animals were then anesthetized using anesthetic ether, and blood collected by retro orbital puncture and biochemical parameters were estimated.

Measurement of Biochemical Parameters¹⁴

Blood samples were collected from retro-orbital plexus under ether anaesthesia and the serum was used for the assay of marker enzymes namely SGPT, SGOT, ALP and bilirubin. The enzyme levels were assayed using standard kits obtained from Coral clinical systems, Verna Goa, India. The liver homogenate was prepared and the clear supernatant was used for the estimation of lipid peroxidation (MDA), total protein and antioxidant enzymes viz. Catalase (CAT) and superoxide dismutase (SOD) level.

Histopathological Examination¹⁵

A portion of liver tissue from each group was preserved in a 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed at a magnification of 3100.

Statistical analysis

The values were expressed as mean \pm SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Student 't' test. P values < 0.05 were considered as significant.

RESULTS

Phytochemical screening:

Preliminary phytochemical evaluation revealed the presence of alkaloids, flavonoids, tannins, sterols and saponins in the ethanolic extract of the *Haldinia cordifolia*.

Biochemical Parameters

The animals treated with Paracetamol exhibited a significant (P,0.01) rise in SGOT, SGPT, ALP and bilirubin levels when compared to the control group. This was significantly (P, 0.01) reduced after treatment with EEHC-first, which was almost similar to that of Silymarin [Table1].

Lipid Peroxidation

The liver MDA, which is an index of tissue lipid peroxidation, was found to be significantly (P, 0.01) higher in the Paracetamol-treated group than measured in the control group. Treatment with EEHC first decreased the elevated MDA levels. The MDA level for Silymarin was also found to be significantly decreased.

Total Protein

Total protein level was significantly (P, 0.01) reduced in the Paracetamol-treated group when compared to the control and was significantly elevated in the EEHC-first-treated groups. This was comparable to that of Silymarin-treated group [Table 1].

Antioxidant Enzymes and Glutathione Levels

The levels of antioxidant enzymes such as CAT and SOD and LPO were decreased significantly (P, 0.05) after Paracetamol treatment and was significantly (P, 0.01) elevated in EEHC -first-treated group. This was comparable with that of Silymarin-treated group [Table 1].

Table 1 : Effect of ethanolic extract of *Haldinia cordifolia* on biochemical parameters in paracetamol induced hepatic toxicity

	Normal control	Toxicant control	Standard	EEHC 200mg/kg	EEHC 400mg/kg
SGPT	83.50 \pm 2.92	140.55 \pm 9.2 ⁺⁺⁺	95.86 \pm 1.39 ^{***}	113.62 \pm 2.63 ^{**}	105.08 \pm 1.12 ^{**}
SGOT	73.93 \pm 2.96	113.41 \pm 8.47 ⁺⁺⁺	96.16 \pm 2.31 ^{**}	109.66 \pm 3.16 [*]	102.03 \pm 3.08 ^{**}
ALP	114.72 \pm 5.26	181.48 \pm 1.9 ⁺⁺⁺	123.0 \pm 1.65 ^{***}	136.33 \pm 1.01 ^{**}	127.66 \pm 3.66 ^{***}
TOTEL BILIRUBIN	0.82 \pm 0.05	3.4 \pm 0.06 ⁺⁺⁺	1.2 \pm 0.12 ^{**}	2.7 \pm 0.14	1.35 \pm 0.07 ^{**}
TOTAL PROTEIN	8.55 \pm 0.18	7.15 \pm 0.11 ⁺⁺⁺	8.42 \pm 0.22 ^{**}	7.80 \pm 0.14 [*]	8.12 \pm 0.11 ^{**}
CAT	78.66 \pm 7.671	28 \pm 5.17 ⁺⁺⁺	59 \pm 0.63 ^{***}	36.83 \pm 2.01 ^{**}	48.66 \pm 2.64 ⁺⁺⁺
SOD	34.09 \pm 4.590	14.32 \pm 1.84 ⁺⁺⁺	27.37 \pm 0.75 ^{***}	18.98 \pm 0.74 ^{**}	23.73 \pm 1.81 ^{**}
LPO	8.650 \pm 1.994	3.58 \pm 1.224 ⁺⁺⁺	3.58 \pm 1.224 ⁺⁺⁺	4.80 \pm 1.55 ⁺	6.25 \pm 0.67 ^{**}

Values are expressed in mean \pm SEM (n=6) one way ANOVA followed by student 't' test. Where, * represents significant at $p < 0.05$, ** represents highly significant at $p < 0.01$, *** represents very significant at $p < 0.001$

Histopathology

The histopathological examination showed that treatment with Paracetamol caused typical centribular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis, especially in the periportal hepatocytes and portal triaditis as compared with control liver. Liver tissues exposed to EEHC -first and Silymarin were almost similar to the control in histology, size and staining properties and showed only mild congestion. In the formulation-treated group, there was reduction in inflammation and it significantly prevented the degeneration of hepatocytes. Thus, histological examination clearly demonstrated the protection of liver against Paracetamol cytotoxicity.

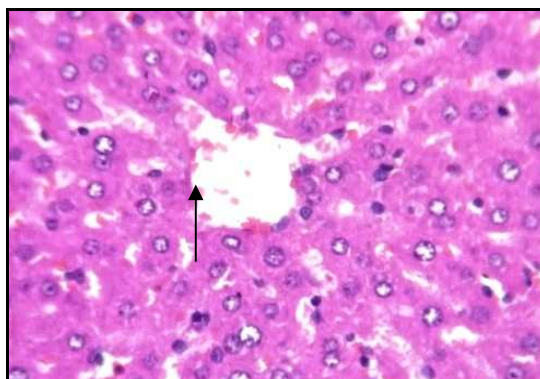


Fig. 1 Liver tissues of control rats

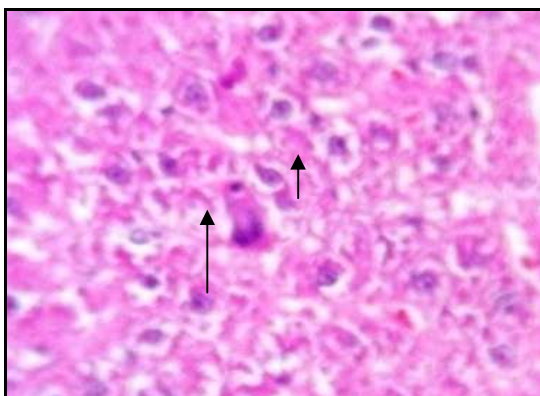


Fig.2 Liver tissue of Paracetamol treated rats

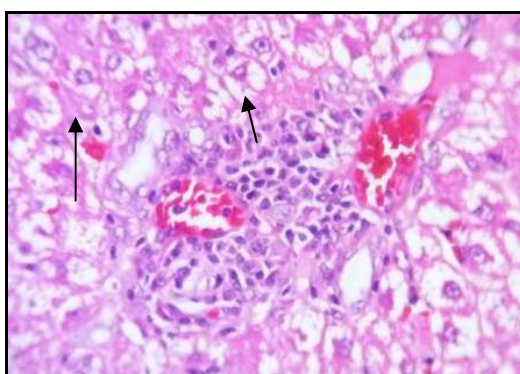


Fig. 3 Liver tissues of Silymarin + Paracetamol treated rats



Fig.4 Liver tissues of EEHC (200 mg/kg) + Paracetamol treated rats

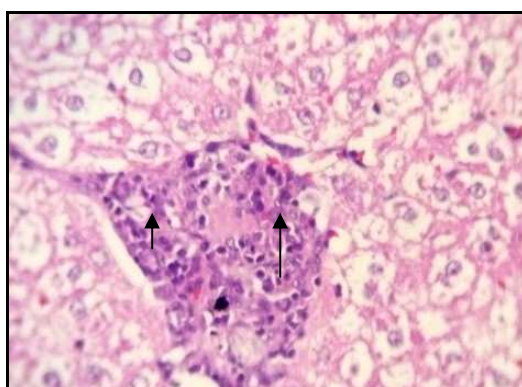


Fig. 5 Liver tissue of EEHC (400 mg/kg) + Paracetamol treated rats

DISCUSSION

Paracetamol is a common antipyretic agent, which is safe in therapeutic doses but can produce fatal hepatic necrosis in man, rats and mice with toxic doses¹⁶. Protection against paracetamol-induced toxicity has been used as a test for potential hepatoprotective activity by several investigations^{17, 18, 19}. The covalent binding of N- acetyl-pbenzoquinoneimine, an oxidation product of paracetamol, to sulfhydryl groups of protein resulting in cell necrosis and lipid peroxidation induced by decrease in glutathione in the liver as the cause of hepatotoxicity have been reported earlier^{20, 21} which is one of the most important natural antioxidants of the hepatocytes, renders the cell remarkably susceptible to oxidative stress²².

In the assessment of liver damage by Paracetamol, the determination of enzyme levels was used. Serum SGPT, SGOT, ALP and bilirubin are the most sensitive markers used in the diagnosis of hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the Paracetamol-induced hepatocellular damage^[23-26]. The reduction of Paracetamol-induced elevated plasma activities of these enzyme levels in animals treated with the formulation showed their ability to restore the normal functional status of the damaged liver.^[25,26] The determination of malondialdehyde (MDA) level is one of the most commonly used methods for monitoring lipid peroxidation.^[24]

The result suggests that there was a dramatic increase in lipid peroxidation after Paracetamol treatment and it was inhibited by the treatment with the extraction revealing that it exhibits potent hepatoprotective activity. Measurement of protein concentration was mainly used to calculate the level of purity of a protein. Maximum doses of Paracetamol cause depletion of total proteins indicating tissue damage which was also evidenced in this study.

Treatment with Paracetamol significantly decreased GSH, CAT and SOD stores indicating that they were used for the detoxification of toxic metabolites of the drug. The extraction restored the antioxidant enzyme levels significantly and reduced the Paracetamol-induced oxidative injury, thus proving its antioxidant potential.^[27]

The histopathological examination of the liver of the control group showed normal hepatocytes with portal triad [Figure 1]. The liver section of Paracetamol-treated rats showed typical centrilobular hepatocytic steatosis (both macrovesicular and microvesicular) and necrosis, limiting plate necrosis, apoptosis especially in the periportal hepatocytes and portal triaditis [Figure 2]. This could be due to the formation of highly reactive free radicals because of oxidative stress caused by Paracetamol. Simultaneous administration of formulation along with Paracetamol prevented these effects [Figures 4 and 5]. Thus, histopathological studies revealed that concurrent administration of Paracetamol with the extraction exhibited protection of liver cells, which further confirmed the above results.

CONCLUSION

In conclusion, the result of this study demonstrated that ethanolic extract of *Haldinia cordifolia* bark (400mg/kg) shows significant ($P < 0.001$) hepatoprotective activity against paracetamol induced liver damage rats. Hence the present study justified the traditional use of *Haldinia cordifolia* bark in the treatment of liver diseases.

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