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# Hepatoprotective Activity and Antioxidant role of *Hymenodictyon excelsum* Bark Against Paracetamol-Induced Hepatotoxicity in Rats

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**Abstract**

*Hymenodictyon excelsum* Wall. belonging to the family Rubiaceae, is a medium deciduous tree grown in Himalayas and used traditionally in Sikkim, North-East India for different medicinal purposes. The present work investigated the hepatoprotective activity of methanol extract of *Hymenodictyon excelsum* stem bark (MEHE) against paracetamol-evoked hepatic lesions in rats. Liver toxicity was elicited in Wistar albino rats by single oral administration of paracetamol at the dose of 640 mg/kg body weight. Then MEHE was given orally to rats at the doses of 200 and 400 mg/kg body weight for 16 consecutive days. Silymarin (25 mg/kg body weight) similarly was employed as reference drug. Hepatoprotective activity was assessed by the determination of hepatic functions viz. SGOT, SGPT, ALP, billirubin and total protein; hepatic tissue antioxidant parameters namely lipid peroxidation, reduced glutathione, superoxide dismutase, catalase and histopathological examination of liver. In MEHE treated rats, the foregoing liver function and tissue antioxidant parameters were significantly restored, when compared to paracetamol control. Histopathological study of the liver tissue demonstrated maintenance of normal hepatocellular architecture in MEHE treated rats as compared with paraetamol control, thus affirming the protective role of MEHE in paracetamol-induced hepatic toxicity in rats. From the present study in can be concluded that, *Hymenodictyon excelsum* bark possesses marked hepatoprotective potential in paracetamol-invoked hepatic damage in rats by virtue of its antioxidant role *in vivo*.

**Keywords:** *Hymenodictyon excelsum,* Paracetamol, Hepatoprotetive, Silymarin, Antioxidant.

## Introduction

The higher plants play a prime role in the treatment of several hepatic diseases with other metabolic system-related disorders. Liver is the major organ of metabolism of nutrients and toxicants. Liver damage elicited by toxic drugs and chemicals has been established as very common complication resulting in grave aftermath encompassing major metabolic dysfuntions leading to morbidity. Still now, there is no proven synthetic drug to protect liver (1). Medicinal plants and plant derived natural products (phytochemicals) have traditionally being used and gained considerable recognition in recent years owing to their vast range of health beneficial actions including antioxidant and hepatoprotective effects (2, 3).

Paracetamol (N-acetyl-para-aminophenol or acetaminophen) is a commonly used febrifuge and non-steroidal analgesic, the overdose

of which precipitates acute liver toxicity in mammals. Paracetamol is chiefly metabolized in liver to its glucuronide and sulphate conjugates (4). The liver toxicity of paracetamol is due to the generation of hepatotoxic metabolites in liver while paracetamol is converted to highly active metabolite namely N-acetyl-P-benzoquinone imine (NAPQI) via hepatic cytochrome P-450 enzymes (5, 6). NAPQI is normally disposed by conjugation of reduced glutathione (GSH) via mercapturic acid pathway (7). Nevertheless, on toxic overdose, when NAPQI production exceeds the corresponding detoxification by GSH in liver, it induces oxidative stress leading to oxidative degradation tissue macromolecules like lipids or -SH groups of proteins/enzymes and perturbs the calcium and GSH homeostasis.

*Hymenodictyon excelsum* Wall. (family: Rubiaceae), is a medium-size deciduous tree grown in the Himalayan regions of India (8). The leaf of this plant has been reported to contain triglycerides and acetylenic fatty acids together with 11 previously known constituents like 3-oxo-11a,12a-epoxyurs-13ß, 28-olide. The stem bark of the plant possesses hymexelsin, the apioglucoside of scopoletin; along with coumarin apioglucosides viz. diospyroside, adicardin and decuroside (9, 10). This plant has several traditional medicinal usage. The stem bark from *H. excelsum* has been used as an antipyretic, astringent and in treatment of tumors; whereas its leaves are utilized to cure ulcer, sore throat, sialadenitis, tonsillitis and inflammation (8, 10). Previously, the present authors have reported the *in vitro* anti-inflammatory and antioxidant activity of its stem bark (11). Nonetheless, this plant has not been scientifically investigated for its hepatoprotective activities. Therefore, an effort has been made in the present study to investigate hepatoprotective and corresponding *in vivo* antioxidant property of the methanol extract from the stem bark of *H. excelsum*.

## Materials and Methods

### *Plant material*

The mature stem bark of *H. excelsum* was obtained in September 2011 from the plants grown in the mountainous regions of East Sikkim, India. The species was authenticated by Dr. M. S. Mondal, taxonomist of the Central National Herbarium (CNH), Botanical Survey of India (BSI), Howrah, West Bengal, India. The relevant specimen (HPI-SOA/01) was preserved at our laboratory for further records. The collected stem bark was air-dried under shade at ambient temperature (24–26°C) and ground mechanically to a coarse dust.

### *Extraction*

The ground plant substance (200 g) was macerated at ambient temperature (24–26°C) with methanol (450 ml) for 4 days with frequent shaking, then by re-maceration with methanol (350 ml) for 3 more days. After 7 days, the extract was collected by filtration. The extract was then evaporated to dryness at 40°C *in vacuo* by rotary vacuum evaporator. The dried extract thus obtained (MEHE, yield: 9.18%) was placed within a freeze for use in the experiments. Preliminary phytochemical screening was performed on MEHE (12).

### *Drugs and chemicals*

Paracetamol was procured from M/s Cipla Ltd., Baddi, H.P., India. Silymarin was supplied by M/s Panacea Biotech Ltd, New Delhi, India. The rest reagents and chemicals and used in the study were of analytical grade obtained commercially. All the test kits were purchased from Arkray Health Care Private Limited (Autospan), Gujarat, India.

### *Animals*

Male Wistar albino rats between 170-200 g body weight were employed for the investigation. The rats were kept within polypropylene cages and housed at 25 ± 2ºC under 12 h dark/light cycle and were given dry pellet diet and had access to water *ad libitum.* The experimental protocol for usage of animals in the present study was approved by the Institutional Animal Ethics Committee, School of Pharmaceutical

Science, Siksha O Anusandhan Deemed to be University, Bhubaneswar, Odisha 751003, India (Reg. No. 1171/PO/Re/S/08/CPCSEA).

### *Acute toxicity*

The acute oral toxicity of MEHE was performed as per the OECD guideline 425 in male Swiss albino mice (13).

### *Experimental design*

The experimental rats were divided in five groups ($n$ = 6). Group I was normal control (received 0.9% w/v NaCl, *p.o.*). Groups II to group V received a single dose of paracetamol (640 mg/kg in 1% methyl cellulose; *p.o.*) and the group II stood as toxin (paracetamol) control. After 45 minutes of paracetamol administration, groups III and IV got MEHE at the doses of 200 and 400 mg/kg body weight, *p.o.* respectively on every day for 16 consecutive days. Group V received the reference agent silymarin (25 mg/kg body weight; *p.o.*) similarly for 16 consecutive days (14). All rats were sacrificed by cervical dislocation after 24 h of last treatment and 18 h of starving. Blood and livers were collected for the biochemical estimations, antioxidant assays and histopathological studies.

### *Biochemical estimation*

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP), serum total protein and bilirubin contents were determined from the collected blood, by using commercially available kits mentioned under drugs and chemicals subheading.

### *Antioxidant estimation*

The livers of sacrificed rats were immediately dissected and cleaned with ice-cold isotonic saline, blotted to dryness and weighed. Tissue homogenate of 25% (w/v) was prepared by using 1.15% KCl solution and then centrifuged at 3000 *g* for 1 h. The supernatant liquid was subjected to the estimation of antioxidative parameters viz. lipid peroxidation (LPO) expressed as malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) by employing the commercially available kits mentioned under drugs and chemicals subheading.

### *Histopathological observation*

The livers were dissected from the rats of each group and cleaned with isotonic saline. The tissues were then fixed using 10% buffered neutral formalin followed by bovine serum albumin solutions. Then these were subjected to paraffin embedding procedure for microtome sectioning. The sections were obtained at the thickness of 50 µ, duly processed by alcohol-xylene solutions and were double stained by alum-haematoxylin and eosin (2). The sections were then mounted and observed microscopically to find out the histopathological alterations.

### Statistical analysis

The values were given as mean ± standard error of mean (SEM). The data were statistically assessed as per one way analysis of variance (ANOVA) with Dunnett's *post hoc* test by SPSS software. $P < 0.001$ were regarded as statistically significant.

## Results and Discussion

Preliminary phytochemical analysis indicated the presence of alkaloids, triterpenes, steroids, flavonoids and polyphenolics in MEHE.

In acute toxicity assessment, MEHE did not demonstrate any mortality or toxic manifestation orally in mice up to the dose of 2 g/kg body weight, accordingly; the doses 200 and 400 mg/kg body weight of MEHE were employed for the present study.

Single high dose paracetamol administration to the rats led to increase in activities of serum transaminases viz. SGPT and SGOT, alkaline phosphatase (ALP) with bilirubin content, whereas serum total protein content was reduced when compared with normal control

group animals. The animals treated with MEHE and silymarin exhibited a significant decline ($p < 0.001$) in the higher serum hepatic markers like SGPT, SGOT, ALP, bilirubin and significant ($p < 0.001$) elevation of the total protein content (Table 1). This demonstrated normalization of the liver functions.

Toxic overdose of paracetamol markedly lowered the activities of endogenous antioxidative enzymes namely SOD, CAT and non-enzymatic antioxidant (GSH) with aggravated lipid peroxidation (LPO) in the hepatic tissue, as observed in paracetamol or toxin control rats. MEHE treatment dose

**Table 1.** Effect of MEHE on serum enzyme activities, total bilirubin and total protein content of normal and paracetamol-intoxicated rats.

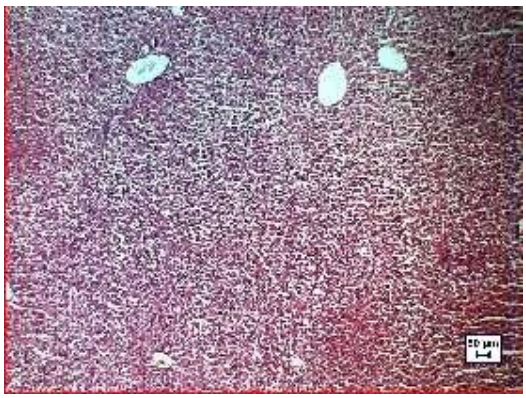
| Treatments | SGOT (IU/L) | SGPT (IU/L) | ALP (IU/L) | Total Bilirubin (mg/100 ml) | Total protein (mg/dL) |
|---|---|---|---|---|---|
| Normal control | 53.81±1.16 | 23.98±1.39 | 9.30±1.18 | 1.13±0.22 | 7.70±0.56 |
| Paracetamol (640 mg/kg) | 139.16±8.10# | 126.13±7.31# | 45.12±3.53# | 3.52±0.17# | 4.01±0.23# |
| MEHE (200 mg/kg) | 106.11±1.37* | 79.64±3.91* | 26.07±3.15* | 2.53±0.44* | 5.47±0.33* |
| MEHE (400 mg/kg) | 79.09±3.33* | 52.84±2.88* | 18.11±2.06* | 1.73±0.14* | 5.98±0.28* |
| Silymarin (25 mg/kg) | 58.56±1.43* | 27.46±1.85* | 11.81±1.33* | 1.14±0.18* | 6.42±0.71* |

The values are expressed as mean ± SEM ($n = 6$), #paracetamol control group *vs*. normal control group ($p < 0.001$), *all treated groups *vs*. paracetamol control group ($p < 0.001$)

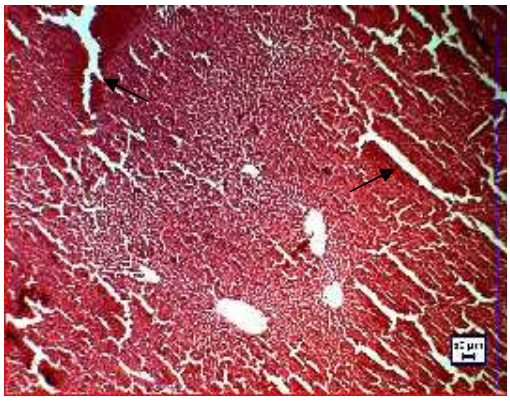
**Table 2.** Effect of MEHE on LPO, GSH and CAT levels of normal and paracetamol-intoxicated rats.

| Treatments | LPO (MDA, nano m/mg wet tissue) | GSH (mg/mg wet tissue) | CAT (mM of $H_2O_2$ decomposed/min/mg wet tissue) | SOD (U/mg wet tissue) |
|---|---|---|---|---|
| Normal control | 20.56 ± 1.68 | 5.33±0.23 | 1.12±0.08 | 10.8±0.86 |
| Paracetamol (640 mg/kg) | 98.83±4.16# | 2.03±0.11# | 0.43±0.06# | 6.3±0.53# |
| MEHE (200 mg/kg) | 57.39±3.44* | 3.46±0.21* | 0.79±0.03* | 8.3±0.33* |
| MEHE (400 mg/kg) | 48.68±1.29* | 3.93±0.43* | 0.93±0.07* | 9.2±0.70* |
| Silymarin 25 mg/kg | 34.08±3.87* | 5.07±0.35* | 0.97±0.02* | 10.1±0.88* |

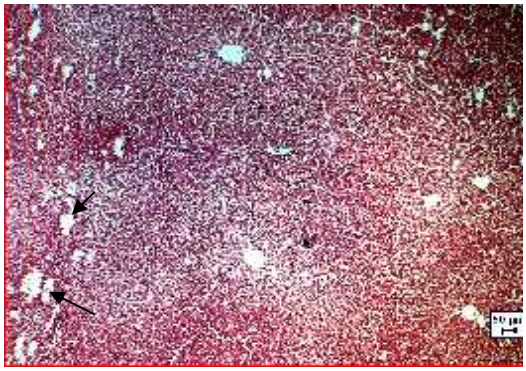
The values are expressed as mean ± SEM ($n = 6$), #paracetamol control group *vs*. normal control group ($p < 0.001$), *all treated groups *vs*. paracetamol control group ($p < 0.001$)



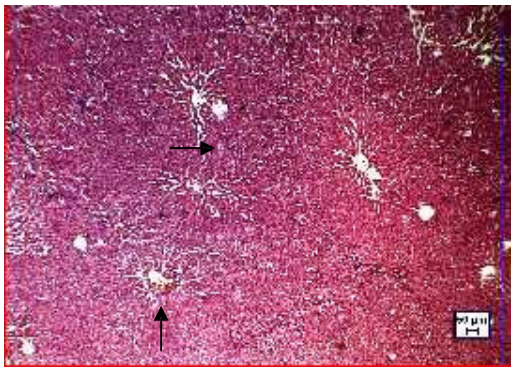
A. Normal control



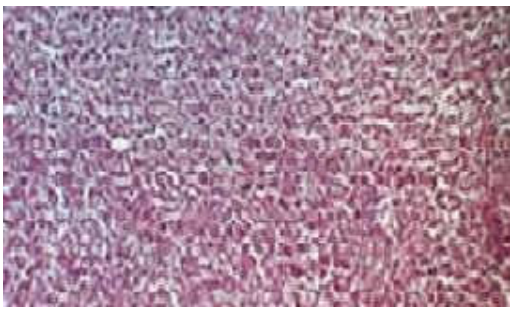
B. Paracetamol control



C. MEHE (200 mg/kg)



D. MEHE (400 mg/kg)



E. Silymarin (25 mg/kg)

Fig. 1. Liver sections A; normal control, B; Liver section of Paracetamol-induced rat showing large necrosis, C; Liver section of MEHE (200 mg/kg) treated rat, showing reduction in necrosis, D; Liver section of MEHE (400 mg/kg) treated rat showing signs of recovery and E; Liver section of reference silymarin (25 mg/kg) treated rats showing also the signs of recovering.

dependently and significantly ($p < 0.001$) restored the enzymatic as well as non-enzymatic antioxidative parameters including LPO as also observed in silymarin treatment (Table 2).

The histopathological study of the liver of normal saline control group rats exhibited normal hepatocellular architecture i.e., definite hepatic cells, central vein and sinusoidal

spaces (Fig. 1A). Non-arrangement of regular hepatocytes with centrilobular and interlobular necrosis, inflammatory and fatty changes with vacuolization of cytoplasm were detected in paracetamol-induced rat livers (Fig. 1B). The livers of the rats treated with both doses of MEHE (Figs. 1.C and D) and silymarin (Fig. 1.E) demonstrated the prevalence of normal hepatocellular architecture, reduction and non-appearance of necrosis and less inflammatory changes near the central vein - which all indicated the signs of recovery from paracetamol toxicity.

Liver is the largest organ of metabolism of nutrients and toxicants and it is the target of toxicity owing to its detoxifying role in metabolizing chemicals and xenobiotics. Drug-induced liver toxicity happening occasionally may be fatal and can precipitate other liver diseases. Paracetamol in overdoses, can elicit liver toxicity. The binding of N-acetyl-P benzoquinoneimine, the active metabolite of paracetamol produced in the liver, to tissue sulphydryl groups (proteins, enzymes) resulting in tissue damage (necrosis) and oxidative lipid peroxidation due to depletion of reduced glutathione (GSH) content in the liver to be the cause of hepatotoxicity of paracetamol as already mentioned in introduction section (15).

Determination of the activities of serum biomarker enzymes viz. SGPT, SGOT and ALP is the definite assessment of normal liver functions. As hepatic cell membrane is damaged due to necrosis, the endoenzymes typically present in the cytoplasm, are leaked into blood. Their determination in blood serum serves as very helpful quantitative biomarker regarding the nature and extent of hepatocellular damage occurred (14, 16). The increased activities of the serum biomarker enzymes recorded in paracetamol-intoxicated rats in the present study, is attributable to the hepatic damage caused by the hepatotoxin. Normalization of the activities of these liver function marker enzymes by MEHE and silymarin treatments is the clear indication of hepatoprotective effect of MEHE and reference silymarin. Hike in bilirubin level of toxin control rats indicated the incidence of jaundice, which was nearly alleviated in MEHE-treated rats, further substantiating its hepatoprotective activity. The reduced total protein content in paracetamol-induced rats generally implies hepatopathy. The normalization total protein content in the MEHE treated rats indicates its hepatoprotective potential.

The inhibition of the over generation of reactive oxygen species (ROS) i.e., antioxidant activity of MEHE is the important mode of protection from paracetamol-induced liver lesions, as one of the main events of paracetamol-invoked liver damage is the production of lipid peroxides i.e., lipid peroxidation - induced by the oxidative/nitrosative free radicals (2, 14). Mammalian body normally possesses efficient antioxidative defense mechanisms, consisting of the endogenous antioxidant enzymes (first line of defense) involving superoxide dismutase, catalase and non-enzymatic antioxidants (second line of defense), like reduced glutathione (14, 17). In paracetamol-induced hepatotoxicity, the control within ROS overproduction and the endogenous antioxidative defenses gets perturbed or lost, resulting in oxidative stress leading to lipid peroxidation and eventually hepatic tissue death (6). MEHE treatment demonstrated significant augmentation in these antioxidant defense parameters as compared with the toxin control rats, and it confirms the *in vivo* antioxidant effect of MEHE. The extent of hepatic lipid peroxidation (MDA production) indicates the harm occurred in the structure and functions of cellular membranes and hence is a sign of cell membrane and liver tissue damages incurred (17, 18). Treatment with MEHE dose dependently and significantly restored all these malign changes.

Histopathological assessment of liver tissue unveils that, the typical liver tissue architecture was damaged by the toxicity of paracetamol. The livers from the paracetamol-induced rats treated with MEHE or silymarin, the normal hepatocellular architecture was evidently

preserved as compared with paraetamol control rats, thus affirming the protective role of MEHE against paracetamol-induced hepatic leisions in rats.

It becomes obvious from the present investigation that, MEHE exerted marked hepatoprotective effect in a dose related way in experimental rats. Preliminary phytochemical screening revealed the prevalence of flavonoids and polyphenolics in MEHE. Flavonoids are rigorously known as putative phytochemical antioxidants (19, 20). Previously the authors have reported that, the stem bark of *H. excelsum* contained polyphenolics which had antioxidant activity *in vitro* (11). The antioxidative activity recorded *in vivo* may be owing to the presence of flavonoids and polyphenolics. The current results demonstrate remarkable antihepatotoxic potentiality of *H. excelsum* stem bark plausibly mediated by the amelioration of tissue oxidative impact. To the best of the knowledge of the authors, this is the first record of hepatoprotective effect of *Hymenodictyon excelsum*. Further studies are necessary to elucidate the mechanisms of action and the constituents responsible for the observed effects.

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**Conflict of interest**

The authors declare no conflicts of interest.

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