

## ORIGINAL RESEARCH ARTICLE

**Phytochemical Analysis and Hepatoprotective Effect of Stem Bark of *Oroxylum indicum* (L) Vent. on Carbon Tetrachloride Induced Hepatotoxicity in Rat****Bichitra NandaTripathy\*, S.K.Panda, S. Sahoo, S.K.Mishra, L.Nayak**

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**ABSTRACT**

The present study was carried out to screen and evaluate the hepatoprotective activity of bark extracts of *Oroxylum indicum* (L.) Hepatoprotective activities of petroleum ether, chloroform, Methanolic and aqueous extracts of *O. indicum* were examined against carbon tetrachloride induced liver damage in mice using silymarin as control. The extracts were screened for alkaloids, steroids, proteins, flavanoids, saponins, mucilage, carbohydrates, tannins, glycoside. Enzyme activities of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) were analyzed. Results indicate that alcohol bark extracts of *O. indicum* had activity over carbon tetrachloride treatment as compared to control. Results of the present investigation confirm the traditional uses of this plant as a potential hepatoprotective agent. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnet's t-test. *P*-values <0.05 were considered as significant.

**Key words:** *Oroxylum indicum* (L.); Hepatoprotective activity; Serum Glutamate Oxaloacetate Transaminase (SGOT); Serum Glutamate Pyruvate Transaminase (SGPT); Alkaline Phosphatase (ALP).

**INTRODUCTION**

*Oroxylum indicum* (L.), belongs to the family Bignoniaceae and is popularly known as Indiantumpet flower (Eng). It is a tree which can reach a height of 12 m (40 ft). The large leafstalk wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. The tree is a night-bloomer and flowers are adapted to natural pollination by bats. They form enormous seed pods that hang down from bare branches. Those long fruits curve downward and resemble the wings of a large bird or dangling sickles or swords in the night. It is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern China, in Indo-China and the Malesia ecozone.<sup>[1]</sup> It is also found in some hill area of Odisha. The leaf contains chrysin and baicalein. Tetra-O-glucoside of baicalein, is reported in the seed. Other flavonoids, Oroxindin has also been isolated from *Oroxylum indicum* whereas oroxylin A is reported in the root bark.<sup>[2,3]</sup> The literature survey reveals that in traditional systems of medicine, different parts

(leaves, bark, root, fruit, seed) have been recommended for many more presumed beneficial uses and for the treatment of expectorant, digestive, Carminative, Febrifuge, Diuretic, Antimicrobial, Antifungal, Anti-inflammatory and Tonic. Stem bark paste is applied for the cure of scabies, and to treat arthritis. Leaf Decoction is given in treating stomachache, ulcers, rheumatism pain and enlarged spleen. Mature fruits are useful in treating Cough, Bronchitis, Piles, Jaundice, dyspepsia, Smallpox, colic, leucoderma, pharyngodymia, cardiac disorders, gastropathy, hemorrhoids and cholera. Seeds are used as purgative.<sup>[4,5,6,7]</sup> In the present study we have evaluated the hepatoprotective potential of *Oroxylum indicum*.

**MATERIALS AND METHODS****Collection of Plant Material**

The stem barks were collected from the tribal belts of the Kapilash forest of Dhenkanal district. The plant was identified, confirmed and authenticated by the taxonomist Dr. N.K. Dhal, Institute of Minerals and Materials Technology Bhubaneswar, Orissa, India, Vide Voucher

specimen no. (V.N. no -12,500). After authentication stem barks were collected in bulk and washed under running tap water to remove adhering dirt. Then the stem barks were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder.

**PREPARATION OF EXTRACTS:**

The coarse powder was taken in Soxhlet apparatus and extracted successively with petroleum ether, chloroform, methanol and water. The extraction was done for 72 hours. The marc of each extract was dried and used for extraction with successive solvent. The liquid extracts were concentrated separately under vacuum and resulting extracts were kept in desiccator until further<sup>[8,9]</sup>.

**Phytochemical investigation**

Chemical tests were carried out on all the extracts for the qualitative determination of phytochemical constitute<sup>[8,9]</sup>.

**Experimental animals**

Inbred wistar albino male rats (100 – 120gm) were used for the evaluation of 2<sup>0</sup>c, relative±pharmacological activities. They were kept in colony cages at 25<sup>0</sup> c. All the animals were acclimatized for a week before use. They were fed with standard animal feed and water adlibitum. The test compounds and the standard drugs were administered in the form of a suspension using 5% Gum acacia as vehicle; to each group consisted of six animals. All the pharmacological experimental protocols were performed according to the recommendation of the institutional animals ethics committee (No- 787/03-SCPCSEA/Regd).

**Acute oral toxicity**

Acute oral toxicity test was performed as per OECD-423 guidelines (acute toxic class method). Wister albino mice (n=3) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for 3-4 hours providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg by intra gastric tube and observed for 3 days. If mortality was observed in 2-3 animals

then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg.

**Hepatoprotective activity**

Wistar albino male rats were selected by random sampling technique. Rats were divided into four groups, each comprising of six rats. The groups were named as Group I (control), Group-II (CCl<sub>4</sub> treated), Group-III (CCl<sub>4</sub> +ET 250 mg/kg treated) and Group-IV (ccl<sub>4</sub> + ET 500 mg/kg treated) and Group-V (CCl<sub>4</sub> + Silymarin 25 mg/kg treated). For the first seven days of study Group-I and II were fed only with normal lab feed and water. Group-III and IV animals were treated orally with ET 250 mg/kg/day and 500 mg/kg/day respectively for seven days and Group-V animals were treated with Silymarin (25 mg/kg/day)<sup>[10,11,12,13]</sup>.

On the seventh and eighth day animals of Group II, III, and IV & V were administered orally with a single dose of CCl<sub>4</sub> with 5% acacia mixture (600 mg/kg/day). After thirty minutes of CCl<sub>4</sub> administration Group III , IV and Group V rats were treated with ET 250 and 500 mg/kg/day and Silymarin (25 mg/kg/day) respectively. All the animals were sacrificed by cervical decapitation under light ether anesthesia on the Ninth day. Blood was collected by heart puncture and centrifuged (300 rpm for 10 mins) to obtain serum. The serum was used for the assay of total bilirubin, alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel. 1957), and total protein<sup>[14,15,16]</sup>.

The Liver was dissected out immediately after sacrifice, washed in ice-cold saline. Small pieces of liver tissue were collected and preserved in 10% formalin solution for histopathological studies.

**Table 1: Phytochemical analysis**

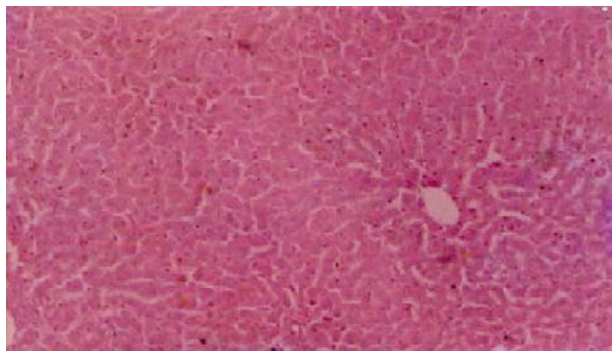
Various extracts of <i>Oroxylum indicum</i>	Alkaloid	Glycoside	Flavonoid	Protein and amino acid	Phenolic com.	Saponin	Steroid	Carbohydrate
Petether extract	+	+	+	-	+	-	-	-
Chloform extract	+	+	+	-	+	-	-	-
Ethnol extract	+	+	+	-	+	-	-	-
Aquous extract	+	+	+	-	+	-	-	-

‘+’ = Present; ‘-’ = Absent

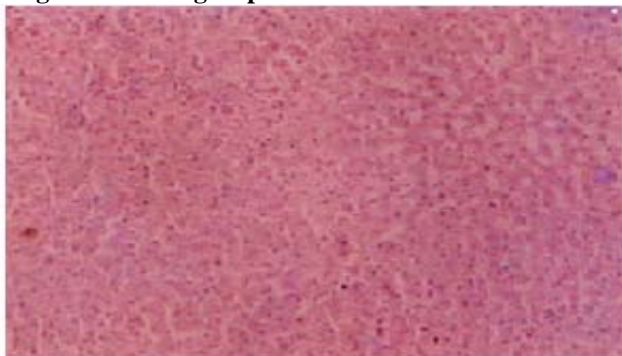
**Table 2: Hepatoprotective parameters of Ethanol Extract of *Oroxylum Indicum* against CCL<sub>4</sub> induced damaged**

Biochemical Parameters	Control group	CCL <sub>4</sub> treated group	Oroxylum indicum Ethanol Extract		Silymarin (25mg/kg)
			250 mg/kg	500 mg/kg	
Total bilirubin (mg/dl)	0.46 ± 0.03	23 ± 0.16	2.13 ± 0.20	0.8 ± 0.05	0.55 ± 0.03
ALP (KA Units)	114.78 ± 3.45	243.65±5.0	236.67±4.92	178.83±4.8	146.6±5.83
SGOT (U/ml)	27.17±1.7	82.83±4.89	70.42±1.22	34.5±2.13	32.83±1.25
SGPT (U/ml)	24.66±3.1	60.48±4.13	52.17±1.34	28.5±0.77	26±0.16
Total Protein (mg/dl)	5.46±0.15	3.77±0.12	4.47±0.23	4.7±0.13	5.17±0.16

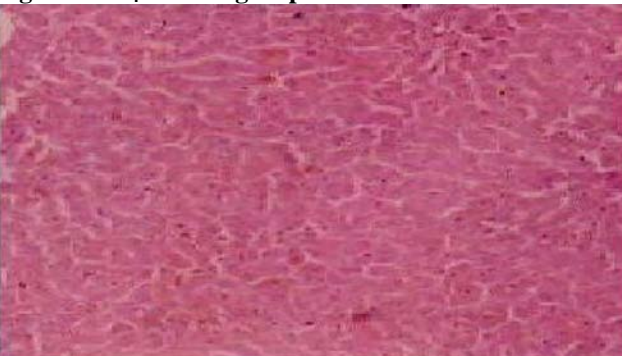
The results are expressed as mean ± SEM, in each group \* p<0.05 when compared with the CCL<sub>4</sub> control group (ANOVA, followed by Dunnett's t-test)



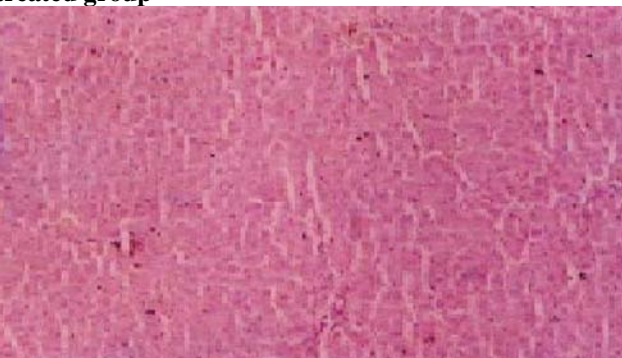
**Fig 1a: Control group**



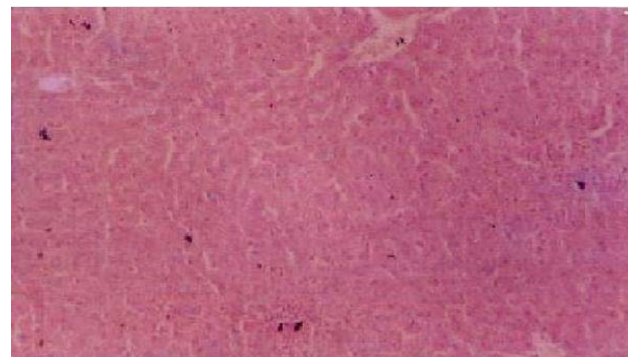
**Fig 1b: CCl<sub>4</sub> treated group**



**Fig 1c: Oroxylum indicum, ethanol extract 250mg/kg treated group**



**Fig 1d: Oroxylum indicum, ethanol extract 500mg/kg treated group**



**Fig 1e: CCl<sub>4</sub> and Silymarin 25 mg/kg treated group**

## RESULTS AND DISCUSSION

Phytochemical studies revealed that alkaloids, flavonoids, glycoside, and phenolic compound in all extracts, while carbohydrate, saponin, sterolids, protein and amino acid were absent in all the extracts. The reactive metabolites such as trichloromethyl (CCl<sub>3</sub>-) and trichloromethyl peroxy (CCl<sub>3</sub>OO-) radicals emanated from CCl<sub>4</sub> initiate peroxidation of membrane unsaturated the phytochemicals studies of *Oroxylum indicum bark extrats*, indicated that the presence of fatty acids. This lipid peroxidation of membrane seriously impairs its function and produces liver injury, which is manifested as an abnormal histopathology like cloudy swelling, sinusoidal dilatation, Individual hepatocytic necrosis of hepatic cells and centrilobular fatty changes in Fig. (1b). CCl<sub>4</sub>-induced damage produces alteration in the antioxidant status of the tissues, *Oroxylum indicum* restored all these changes. ET (250 mg/kg) treated animals (Fig. 1c) showed individual focal hepatocyte damage and necrosis. ET (500 mg/kg) treated animals (Fig 1d) showed the mild focal hepatocytic damage and necrosis. So, it can be concluded that the herb is a potential antioxidant and attenuates the hepatotoxic effect of CCl<sub>4</sub> by acting as an *in vivo* antioxidant and thereby inhibiting the initiation and promotion of lipid peroxidation

## CONCLUSION

Preliminary phytochemical screening indicated that the stem bark of *Oroxylum indicum* was rich in flavonoids. Alkaloids, tannins, and anthraquinones. It is concluded from our study

that alcoholic extract of stem bark of *Oroxylum indicum* showed significant hepatoprotective activity. The hepatoprotective activity of this plant can be correlated with its antioxidant enzyme. Thus, this plant may serve as a useful adjuvant in several clinical conditions associated with liver damage, which is evident from the results of biochemical assays and histopathological study.

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