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HEPATOPROTECTIVE EFFECT OF *OLDENLANDIA UMBELLATA* LINN. LEAVES

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Abstract

Ethanolic extract of *Oldenlandia umbellata* Linn. (Rubiaceae) leaves were screened for its effect on carbon tetrachloride induced hepatotoxicity in rats. The result showed significant reduction ($p<0.01$) in various biochemical parameters (SGOT, SGPT, ALKP, TBIL and lipid peroxidation) and supported with histopathological findings. Phytochemical analysis showed the presence of anthraquinone glycosides, flavonoids, phytosterols, saponins, tannins and phenolic compounds. The hepatoprotective activity may be exhibited due to the presence of flavonoids in the leaves.

Keywords: *Oldenlandia umbellata* leaves, Hepatoprotective activity.

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Introduction

Oldenlandia umbellata linn., commonly known as Indian madder and Chay root is a small, stiff, highly branched, biennial or perennial herb up to 40 cm long with four angled, scaberulous stem and tuberous roots. Leaves are opposite but often crowded and appearing verticillate, sessile; linear to narrowly lanceolate, 10–20 × 1–3 mm, adaxially punctate and glabrescent, abaxially scaberulous along midrib, apex and base acute, margin flat to revolute and secondary veins indistinct. The plant is distributed in India from Assam to Travancore, Ceylon, Burma, Pakistan in dry sandy grassy places.[1] This plant is well known in Siddha system of medicine for its styptic property. Decoction of the entire plant is administered in the treatment of bronchial asthma. The leaves are reported to act as febrifuge, expectorant and also used in consumptive, asthmatic affections.[2]

Information of ethno-botanical survey has revealed that leaves of this plant are used for the treatment of fever, rheumatism and jaundice by the local communities in some parts of southern Odisha, India. Since there is a lack of scientific validation for hepato-protective activity of the leaves, this project has been undertaken to explore this property by treating carbon tetrachloride induced hepatotoxicity in albino rats.

Materials and methods

Plant material

Fresh plants were collected from the Ganjam District of Odisha, India in second week of July and authenticated after conducting morphological and microscopical analysis. The leaves were washed thoroughly with tap water, dried in shade and powdered to 40 mesh size.

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Preparation of the extract
Powdered material was soxhleted with 90% v/v ethanol for 72 hours and concentrated under vacuum.

Preliminary phytochemical analysis
The extract is subjected to qualitative chemical tests to detect the phytoconstituents.[3]

Animals used
Wistar strain adult albino rats of either sex weighing 150-180 gm were procured and acclimatized to laboratory conditions (temperature: 23 ± 2^0 and 12 hour light and dark cycle). They were fed standard diet pellets and given tap water.

Acute toxicity studies
Albino rats of either sex weighing 150-180 gm were divided into four isolated groups of six each. After an overnight fast, 5% w/v suspension of the extract with acacia mucilage were administered to the isolated groups in the graded doses of 0.2 to 04 g/ kg b.w.p.o. under the continuous observation for the first two hours to observe any toxic symptoms and later up to 24 hours to record mortality.[4]

Hepatoprotective activity
The rats were divided into four groups of six each. The first group (control group) was administered with the vehicle (5% w/v acacia mucilage at a dose of 1 ml/ kg body weight) orally. Single oral dose of hepato-toxin (CCl_4 as a 1:1 solution in olive oil at a dose of 2.5 ml / kg body weight) was given to the second group animals. The effective dose of the extract was determined by conducting acute toxicity studies and fixed at the body weight of 400 mg/Kg. The test group animals (group-III) received the first dose of test suspension 30 minutes before the administration of a single oral dose of hepato-toxin. Two subsequent doses of test suspension were administered at the interval of 12 hours. Three doses (100 mg/kg) of standard drug silymarin (Microlabs Ltd., Bangalore) were administered to the animals of group-IV orally at 12 hours interval. First dose of silymarin was administered 30 minutes before the administration of CCl_4. After 12 hours of administration of last dose, blood samples were collected from all animals by puncturing the retro-orbital plexus and allowed to coagulate at 37^0C for 30 minutes.[5] The serum separated by centrifugation at 2500 rpm was analyzed for serum glutamic oxaloacetate transaminase (SGOT),[6] serum glutamic pyruvate transaminase (SGPT),[7] serum alkaline phosphatase (ALKP)[8] and total bilirubin.[9] After sacrificing rats, the liver homogenate was prepared to determine the extent of lipid peroxidation.[10] For histopathological studies, liver lobes were fixed for 48 hours in 10% formalin and were embedded in paraffin. Subsequently, 5µ sections were cut using microtome and stained with haematoxylin and eosin. These sections were observed under light microscope for histopathological changes and compared with normal liver histology. The animal experiments were conducted following the guidelines of animal experimentation and ethics.

Statistical Analysis
Estimation reports of biochemical and functional parameters were reported with mean value $\pm$ standard deviation (S.D.). Percentage of reduction in biochemical parameters was calculated in consideration with the differences in biochemical parameters between hepatotoxin treated group and control group as 100% level of reduction. The variation in a set of data was estimated by performing one way analysis of variance (ANOVA) and individual comparisons of group mean values were done using Dunnet's t-test. Statistical analysis was carried out using SigmaStat® 3.5 software.

Result and discussion

Phytochemical analysis
Reports of preliminary phytochemical analysis indicated the presence of anthraquinone glycosides, flavonoids, phytosterols, saponins, tannins and phenolic compounds.

Acute toxicity studies
Ethanolic extract of *Oldenlandia umbellata* leaves was found to be non-toxic since no toxic symptoms and mortality were observed even at the dose of 4g/ kg b.w.p.o. in rats. A dose of 400 mg/ kg, b.w.p.o. was fixed for all the screening experiment.

The rats treated with CCI_4, developed significant ($p<0.01$) liver damage as observed from the elevated serum levels of hepato-specific enzymes as well as severe alteration in other biochemical parameters. Pronounced elevation in the concentration of bilirubin was observed in the CCI_4 intoxicated rats. The level of lipid peroxidation was

also increased markedly in the intoxicated rats (table 01).

Treatment with the ethanolic extract of *Oldenlandia umbellata* leaves and sylimarin decreased CCl_4 induced SGOT, SGPT, ALP and total bilirubin in blood. The level of lipid peroxidation was also found to be decreased in the groups of extract and sylimarin treated rats (table 01).

Histological analysis showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins in the control group (Fig. No. 01A). Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were observed in the liver of the rats treated with carbon tetrachloride (Fig. No. 01B). The toxin mediated changes in liver of test group (group-III) animals were of much less intensity than those observed in the livers of the group-II animals indicating regeneration of hepatocytes (Fig. No. 01C) which was comparable with that of the livers of silymarin treated group (Fig. No. 01D). These findings from the histopathological studies provided supportive evidence for the biochemical analysis.

Conclusion

This study showed hepatoprotective effect of ethanolic extract of *Oldenlandia umbellata* leaves against CCl_4 induced liver damage in albino rats which was comparable with the standard drug silymarin.

Table No. 01: Effect of silymarin and ethanolic extract of *Oldenlandia umbellata* leaves on rat liver biochemical parameters in CCl_4 induced hepato toxicity.

Groups	Biochemical parameters, mean ± S. D.				
	SGOT (Units / ml)	SGPT (Units / ml)	ALKP (Units / l)	Total Bilirubin (Mg/dl)	Lipid Peroxidation Values (Millimoles/ gms)
Group- I	86.4 ± 6.87	58.1 ± 5.76	76 ± 8.33	0.2 ± 0.03	55.18 ± 6.47
Group- II	307 ± 14.27	327 ± 19.71	331.9 ± 12.64	1.64 ± 0.16	133.05 ± 11.45
Group- III	153.4 ± 15.33* (69.56)	113.29 ± 12.01* (78.75)	158.1 ± 7.99* (67.69)	0.55± 0.05* (75.00)	68.39 ± 7.03* (81.53)
Group- IV	94.1 ± 8.27* (95.67)	69.1 ± 6.18* (96.26)	117.9 ± 9.7* (83.00)	0.28 ± 0.02* (93.05)	59.01 ± 4.31* (97.86)

Results are expressed as mean ± S.D., n = 6, one way ANOVA followed by Dunnet's t-test.
*P< 0.01 compared with CCl_4 treated group. Value in parenthesis indicates percentage of reduction

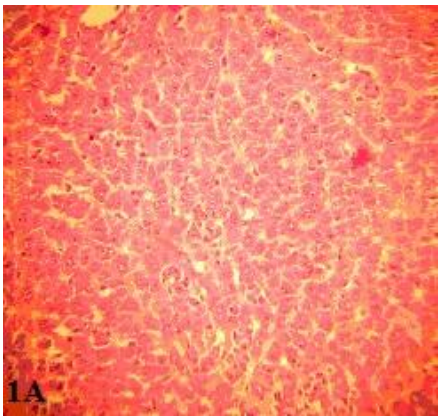

Fig. No. 01A: Photomicrographs of liver T.S. of control group; 100X magnification.

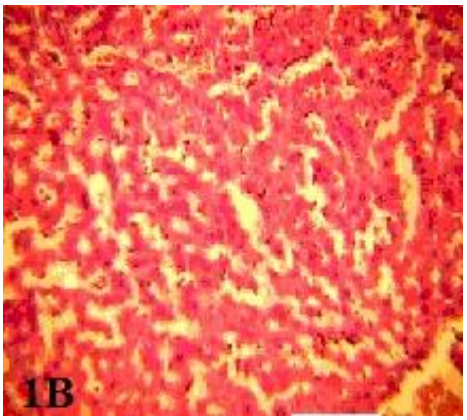

Fig. No. 01B: Photomicrographs of liver T.S. of CCl_4 treated rats; 100X magnification.

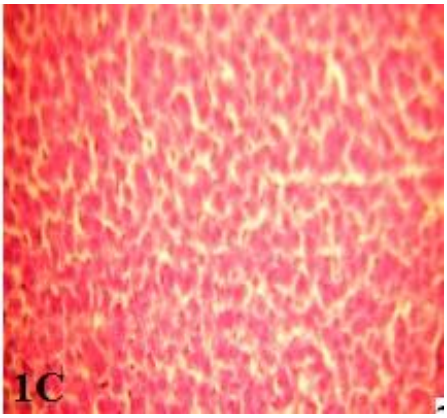

Fig. No. 01C: Photomicrographs of liver T.S. of ethanolic extract of *Oldenlandia umbellata* leaves and CCl_4 treated rats; 100X magnification.

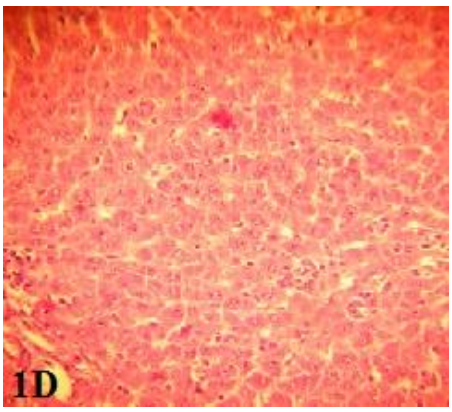

Fig. No. 01D: Photomicrographs of liver T.S. of silymarin and CCl_4 treated rats; 100X magnification.

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