Effect of *Dillenia Indica* leaves against carbon tetrachloride induced hepatotoxicity

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ABSTRACT

In the present investigations, the ethanolic extract of *Dillenia indica* Linn. leaves were subjected to screening for the hepatoprotective activity in Carbon tetrachloride induced hepatotoxicity in albino rats. The study with ethanolic extract showed significant reduction ($p<0.001$) in various biochemical parameters (SGOT, SGPT, ALKP, T.Bil. and lipid peroxidation). Results of histopathological studies supported the findings. In conclusion it is stated that the extract is found to possess pronounced hepatoprotective activity.

KEY WORDS: *Dillenia indica*, Hepatoprotective, Carbon tetrachloride, Silymarin.

Introduction

Dillenia indica Linn. (Dilleniaceae) is a moderate sized evergreen tree, with a dense crown and irregular trunk [1], distributed in the moist evergreen forests of sub- Himalayan tract from Garhwal, Assam to West Bengal, Central and Southern India [2]. The fruits are said to possess tonic and laxative properties, and are used for abdominal pains [3]. The fruits of *Dillenia indica* have been reported to exhibit significant antioxidant activity [4]. Information from Ayurvedic literature [5] and ethno-botanical survey revealed about the use of the leaves in the treatment of jaundice by the tribes in some parts of South Orissa. In the present investigations, the ethanolic extract of *Dillenia indica* leaves was subjected to screening for the hepatoprotective activity in carbon tetrachloride-induced hepatotoxicity in albino rats, to substantiate the related reports.

Materials And Methods

Plant material and Preparation of Extract

The leaves of *Dillenia indica* were collected from near by areas of Naharkanta, Bhubaneswar and are authenticated by Prof. S. K. Dash, Head, Department of Biosciences, College of Pharmaceutical Sciences, Berhampur. A voucher specimen (No. L-Di 32/ 114) is preserved in the museum of Sri Jaydev College of Pharmaceutical Sciences, Bhubaneswar. The leaves were properly cleaned, dried under shade, powdered to 60 mesh size, extracted with ethanol using a soxhlet extractor and concentrated under reduced pressure. Yield of the extract was found to be 14.22%.

Results of preliminary phytochemical screening [7, 8] and TLC analysis [9, 10] indicated the presence of anthraquinone glycosides, flavonoids, tannins, fixed oils, sterols, vitamin-C, proteins and amino acids.

Animals

Wistar strain albino rats (120-180 g) of either sex were selected and maintained under standard laboratory conditions (temperature: 23 ± 2^0 C, relative humidity $55 \pm 10\%$ and 12 hour light and dark cycle). The animals were fed with standard diet pellets and given tap water. The animal experimental protocol was approved by Institutional Animal Ethics Committee and the experiment was carried out by following the guidelines laid by Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

Acute toxicity studies

Rats were divided into isolated groups of six in each lot. After an over night fast, the suspension of extract (5 % w/v) with *Acacia* mucilage were administered orally to the isolated groups in graded doses of 0.2-4 g/ kg body weight and were under continuous observation for the first 2 hours for observing the toxic symptoms and later up to 24 hours to study the mortality rate. The number of dead/ survived animal after 24 hours was recorded and accordingly the LD_{50} was calculated [11].

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Hepatoprotective activity [12]

The rats were divided into 4 groups of 6 each. A control group (group-I) was maintained with the vehicle (5% w/v acacia mucilage at a dose of 1 ml / kg body weight) by oral route. A single oral dose of 1:1 mixture of carbon tetrachloride in olive oil (2.5 ml/kg body weight) is administered to the rats of toxicant group (group-II) to induce hepatotoxicity. The effective dose of the extract was fixed at 300 mg/ Kg body weight after conducting the acute toxicity studies. Animals of test group (group-III) and standard group (group-IV) were given orally with three doses of ethanolic extract and standard drug (silymarin at a dose 25 mg / kg body weight) [13] respectively, at 12 hours of interval. First dose of test extract and silymarin was administered 30 minutes before the administration of single oral dose of toxicant. Blood was collected from all animals after 12 hours of administration of last dose, by puncturing the retro-orbital plexus and allowed to coagulate at 37^0C for 30 minutes. The serum separated by centrifugation at 2500 rpm and various biochemical parameters such as serum glutamic oxaloacetate transaminase (SGOT) [14], serum glutamic pyruvate transaminase (SGPT) [14], serum alkaline phosphatase (SALP) [15] and total bilirubin (T.Bil.) [16] were analyzed. The rats were sacrificed and liver homogenate was prepared to determine the extent of lipid peroxidation by thiobarbituric acid reactive substances assay (TBARS) [17]. The liver from each animal was removed after dissection; liver lobes were fixed for 48 hours in 10% formalin and were embedded in paraffin. Subsequently, 5µ sections were cut on a microtome and stained with haematoxylene and eosin. These sections were observed under light microscope for histopathological changes and compared with normal system.

Statistical analysis

Results of estimation of biochemical parameters have been calculated as mean value ± standard error of mean (S.E.M.). The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test [18]. Percentages of reduction in biochemical parameters was calculated by considering the differences in biochemical parameters between the hepatotoxin treated and control group as 100% level of reduction.

Results and Discussion

In the present studies, rats treated with carbon tetrachloride developed significant ($p<0.001$) liver damage as observed from the elevated serum levels of hepato-specific enzymes as well as severe alteration in other biochemical parameters. A pronounced elevation in the concentration of bilirubin was observed in the carbon tetrachloride intoxicated rats. The level of lipid peroxidation was also increased markedly in the intoxicated rats (Table-1).

Carbon tetrachloride induced increase in SGOT, SGPT, SALP and total bilirubin in blood, is observed to be decreased in the test extract treated animals (Table-1), indicating protection against toxin.

Observations of histopathological studies in the T.S. of liver sections showed normal parenchymal architecture with cords of hepatocytes, portal tracts and central veins in the control group (Fig.2A). Centrilobular necrosis accompanied by fatty changes and ballooning degeneration were seen in the remaining hepatocytes in the livers of rats treated with carbon tetrachloride (Fig.2B). The toxin-mediated changes in livers of test group were of much less intensity than those observed in the livers of the animals of group-II, showing regeneration of hepatocytes in the ethanolic extract treated group of animals (Fig.2C), which is comparable with that of livers of silymarin treated group (Fig.2D). These findings from the histopathological studies too, provided supportive evidence for the biochemical analysis.

Table-1:

Effect of Ethanolic Extract of *Dillenia indica* against Carbon tetrachloride Induced Hepatototoxicity in Albino Rats

Animal Groups	Biochemical parameters, mean ± S. E. M.				
	SGOT (Units/ ml)	SGPT (Units/ ml)	SALP (Units/ l)	T.Bil (mg/dl)	TBARS (mili moles/g)
Group-I	87.94± 3.76	54.54± 2.90	79.87± 4.17	1.17± 0.02	58.78± 2.81
Group-II	309± 11.19*	328 ± 12.06*	331.4± 11.82*	3.32± 0.13*	132.79± 6.89*
Group-III	151.7± 6.07**	113.5± 5.03**	152.1± 6.58**	1.51± 0.03**	72.32± 2.90**
Group-IV	95.5± 3.89**	65.7± 3.21**	117.6± 4.22**	1.36± 0.01**	61.09± 2.13**

Significant as compared with toxicant group (*P<0.001) and significant as compared with control group (**P<0.001), n=6.

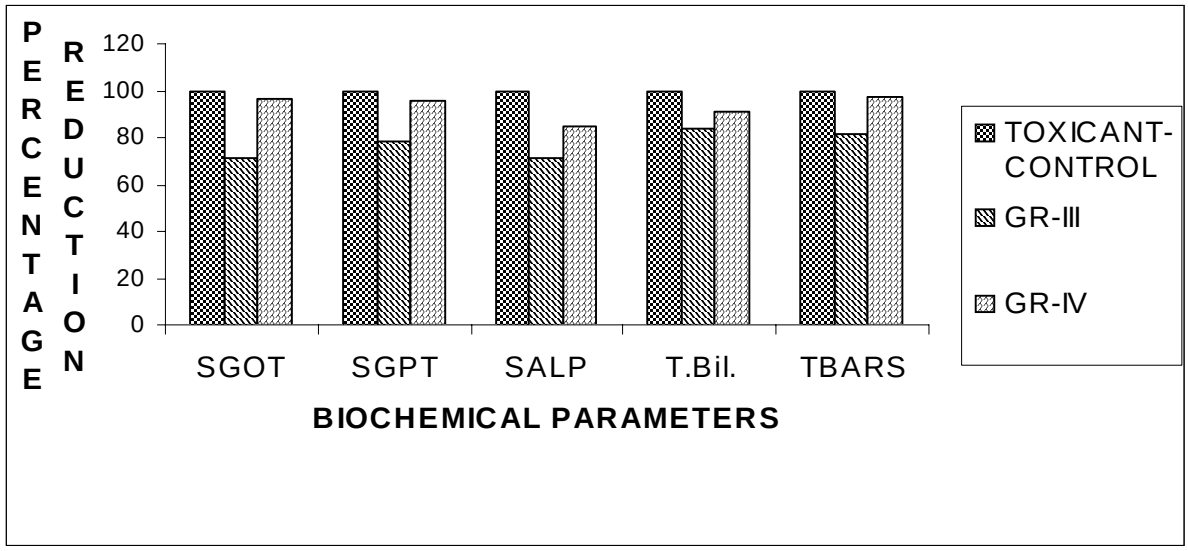

Fig. 1: Percentage of Reduction in the Biochemical Parameters and Lipid Peroxidation.

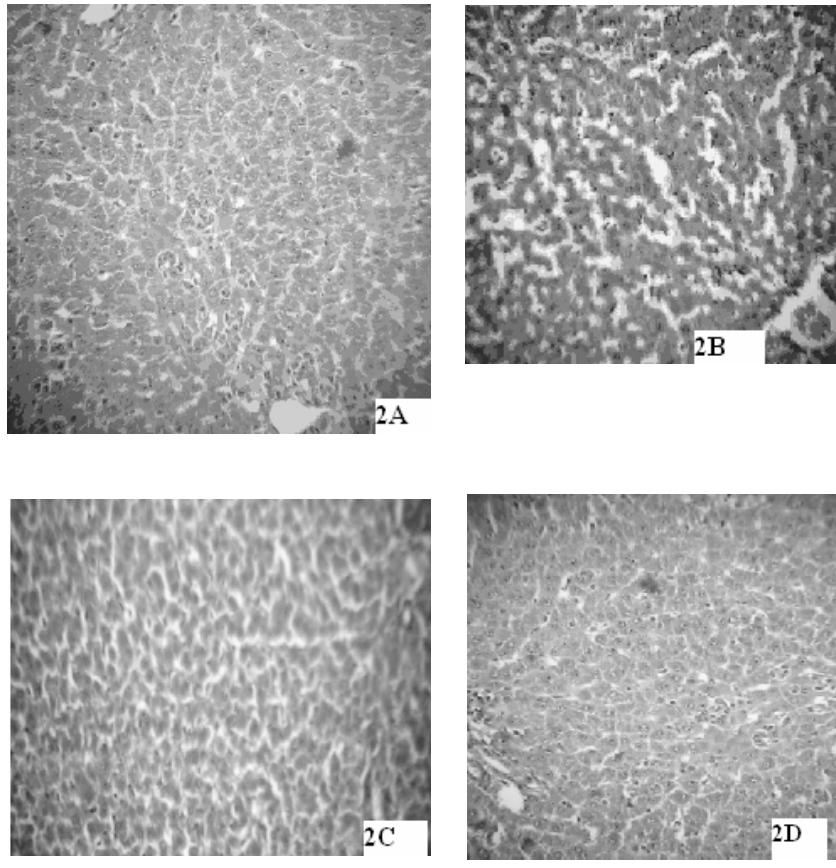

Fig. 2: Photomicrographs of Liver sections (T.S.) at 100X magnification, haematoxylene eosin staining (Fig. 2A: Normal rat of control group; Fig. 2B: Carbon tetrachloride induced rat of toxicant group; Fig. 2C: Test extract and Carbon tetrachloride treated rat; Fig. 2D: Silymarin and carbon tetrachloride treated rat.)

The results of the present study indicated that under the present experimental conditions, ethanolic extracts of leaves of *Dillenia indica* at 300 mg/ Kg body weight showed hepatoprotective effect against carbon tetrachloride induced liver damage in rats and was comparable with the standard drug silymarin.

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