
Hepatoprotective Effect of *Cassia angustifolia* Vahl

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The plant *Cassia angustifolia* vahl has been used in various diseases. The alcoholic extract of the plant was investigated for the hepatoprotective properties against carbon tetrachloride-induced liver damage. Various biochemical parameters were studied to evaluate the hepatoprotective activity of crude extract. In serum, total bilirubin, total protein, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were determined. In addition to these glutathione (GSH) and lipid peroxides (LPO) were determined in liver tissue and is supported by histopathological studies of liver before and after the treatment. Results of this study revealed that leaves of *Cassia angustifolia* could afford a significant protective action in CCl₄-induced hepatocellular injury.

The plant *Cassia angustifolia* Vahl (Leguminosae) is commonly called in Sanskrit as bhumiari or pitapushpi and in Tamil as nattunelavarai¹. The leaves being an excellent laxative and febrifuge, have been used in the treatment of anaemia, typhoid, cholera, jaundice², tumours and probably leprosy. In spite of its reported use in jaundice, no systematic studies of its liver protective activity have been reported. Hence an effort has been made to establish the hepatoprotective effect of the alcoholic extract of *Cassia angustifolia* leaves against CCl₄-induced liver damage in rats. Hepatocellular damage can be induced by CCl₄^{3,4}.

MATERIALS AND METHODS

The plant material used in this study was collected in the month of November from the Herbal garden at Adhiparasakthi College of Pharmacy, Melmaruvathur, Tamilnadu. This plant material was authenticated by the Department of Botany, Captain Srinivasamoorthy Drug Research Institute, Arumbakkam, Chennai-600 106. Powdered leaves were extracted with 90% alcohol in a Soxhlet apparatus, solvent was removed by distillation

under reduced pressure and dried. The extracts were dissolved in 1% carboxy methyl cellulose (CMC) and used for the experiment. Male Wistar rats were procured from Tamilnadu veterinary and animal sciences university, Chennai. They were fed on commercial diet (Hindustan lever, Bangalore) and tap water *ad libitum* during the experiment.

Experimental design:

The animals were divided into three groups (n=6). The rats weighing in the range of 150-200 g were selected. The animals in control group were served as normal control for 7 d. The toxic control group animals were received a single dose of CCl₄ (50% v/v of CCl₄ in olive oil 4 ml/kg, p.o.) on day 6. Treated group (*cassia angustifolia*) for 7 d (500 mg/kg, p.o., suspended in 1% CMC) and single dose of CCl₄ (50% v/v of CCl₄ in olive oil 4 ml/kg, p.o.) given on day 6.

All animals were sacrificed on day 8 by decapitation. Four to five millilitres of blood was collected by carotid bleeding and serum was used for estimation of serum bilirubin⁵, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase⁶ (GPT) and total

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protein⁷. The liver was dissected out, washed in ice-cold saline and homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant used for the estimation of marker enzymes such as reduced glutathione⁸ (GSH) and lipid peroxidation⁹ (LPO).

Histopathology:

Livers were excised quickly and fixed in 10% buffered neutral formalin and then fixed in bovine solution, they were processed for paraffin embedding following the standard microtechnic¹⁰. Sections of the liver stained with alum-haematoxylin and eosin, were observed microscopically for histopathological changes.

Student 't' test was used for statistical analysis of data. The level of significance of $p < 0.001$ was chosen for determining significant differences.

RESULTS

Cassia angustifolia protects the liver injury in rats with reference to biochemical changes in serum and liver were shown in Table 1. There was a significant increase

($p < 0.001$) in the levels of total bilirubin, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) in group II (CCl_4 -treated) as compared to group I (normal control). Pretreatment with *Cassia angustifolia* (group III) resulted in significant ($p < 0.001$) protection against the increase of total bilirubin, GOT, GPT in group III rats compared to group II (CCl_4 -treated), on marked decrease in the level of total protein in group II (CCl_4 -treated) when compared to group I (normal control). The total protein level was significantly increased ($p < 0.001$) in group III, when compared to group II.

The changes in liver activity of the lipid peroxidase (LPO) of group II (CCl_4 -treated) rats showed highly significant ($p < 0.001$) increase when compared to group I, (normal control) and group III (*Cassia angustifolia* + CCl_4 -treated). There was a marked decrease in the level of reduced glutathione (GSH) in group II (CCl_4 -treated) when compared to group I (normal control). The GSH level was significantly increased ($p < 0.001$) in group III, when compared to the group II.

TABLE 1: EFFECTS OF *CASSIA ANGUSTIFOLIA* ON CCl_4 -TREATED HEPATOTOXICITY IN RATS

Biochemical Parameters	Treatment (Group)		
	Normal Control (I)	CCl_4 (II)	<i>Cassia angustifolia</i> + CCl_4 (III)
SERUM			
Total Bilirubin (mg/dl)	0.24±0.020	0.70±0.036*	0.35±0.020**
GOT U/ml	22.6±1.37	70.48±1.40*	29.66±1.40**
GPT U/ml	21.13±0.54	92.25±0.52*	23.97±0.63**
Total Protein (mg/dl)	5.09±0.06	3.21±0.03*	4.93±0.04**
LIVER			
GOT (µmol of pyruvate liberated/mg protein/min)	34.19±1.77	77.81±1.47*	43.26±2.14**
GPT (µmol of pyruvate liberated/mg protein /min)	31.94±0.38	60.77±0.54*	41.45±0.52**
Total Protein (mg/g tissue)	0.50±0.006	0.31±0.002*	0.43±0.009**
GSH (µmol/mg protein)	5.87±0.03	2.65±0.04*	5.56±0.03**
LOP (nmol of MDA/mg protein)	1.86±0.05	7.14±0.29*	4.06±0.11**

All values are mean ± SEM of 6 animals in each group. Group II compared with Group I (* $p < 0.001$) and Group III compared with Group II (** $p < 0.001$).

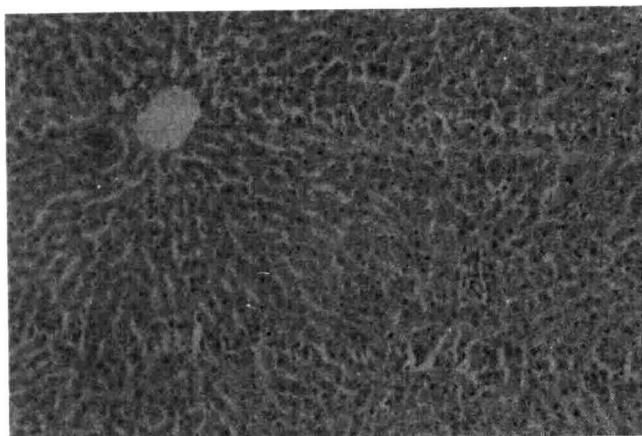


Fig. 1: Alum-Haematoxylin & Eosin 10X10x=100x

Histopathology:

Histology of liver from normal control group (fig. 1) shows normal arrangement of hepatic cells with central vein. Microscopical examination of CCl_4 -treated liver section (fig. 2) shows various degree of pathological changes starting from cloudy swelling and necrosis of hepatic cells and central lobular fatty degeneration. Liver section from *Cassia angustifolia*-treated group (fig. 3) shows moderate protection in CCl_4 -induced liver damage.

DISCUSSION

Our results indicated that leaves of *Cassia angustifolia* provides protective effect against CCl_4 -induced hepatotoxicity in rats. CCl_4 widely used experimentally as hepatotoxin is biotransformed by the cytochrome p-450 system to produce the trichloromethyl free radical which in turn covalently binds to cell membranes and organells to elicit lipid peroxidation, disturbs calcium haemostasis and finally results in death¹¹. The elevation of enzymes in group II reflects that the liver injury indicated by CCl_4 .

Alcoholic extract decreases the CCl_4 -induced elevated enzyme levels suggests the protection of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells by the extract. Decrease in serum bilirubin level of the treatment with alcoholic extract indicates the effectiveness of the normal functional status of liver.

Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in levels of lipid peroxides in liver of CCl_4 -treated animal shows free radical induce liver damage¹², while the animals



Fig. 2: Alum-Haematoxylin & Eosin 10X10x=100x

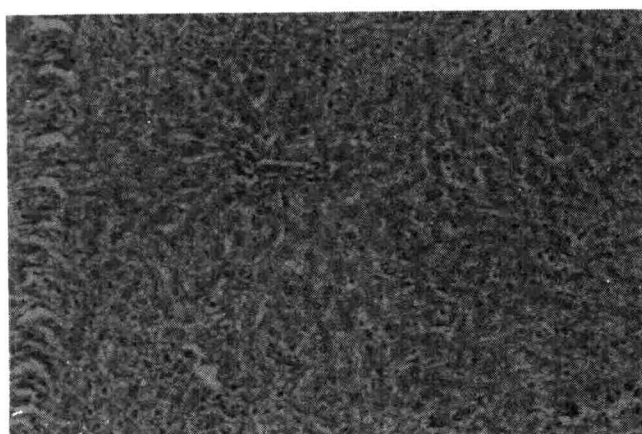


Fig. 3: Alum-Haematoxylin & Eosin 10X10x=100x

pretreated with alcoholic extract of *Cassia angustifolia* showed moderately reduce the level of lipid peroxide indicating the protective action of this plant on cellular membrane.

Reduced glutathione, the cellular antioxidant was lowered in animals treated with CCl_4 . While the animals treated with *Cassia angustifolia* and CCl_4 , GSH level was increased suggesting protective effect of *Cassia angustifolia* cellular defences against free radicals are many and varied. The significant elevation of GSH contents by *Cassia angustifolia* suggest GSH-dependent detoxification of free radicals. It will interesting to note the role of flavinoids in *Cassia angustifolia*¹³ in protecting the liver against free radicals.

The results of biochemical observations are supplemented by histopathological examination of rat's liver sections. The histological changes induced by CCl_4 -treatment are reversed by administration of *Cassia angustifolia*.

Our results suggest that *Cassia angustifolia* prevents fatty liver, and exhibits membrane stabilising and antioxidant activity, to protect liver from sever damage caused by CCl₄.

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