Hepatoprotective Activity of Cassia fistula Linn. Bark Extracts against Carbon Tetra Chloride Induced Liver Toxicity in Rats

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Citation

Abstract
In the present study cassia fistula (Caesalpinaceae) bark was extracted with water. The extracts were vacuum dried to yield Aqueous Extract (AQET). The extracts were evaluated for hepatoprotective activity against Carbon tetrachloride (CCl₄) induced liver damage at 250 mg/kg and 500 mg/kg dose level. The biochemical parameters observed in serum were total bilirubin, Alkaline phosphatase (ALP), Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) levels and total protein. Aspartate transaminase (AST), Alanine Transaminase (ALT) and total protein levels in liver were also evaluated. Histopathological study on the liver tissue was also performed. The extracts exhibited dose dependant reduction in total bilirubin, ALP, SGOT, SGPT, AST, ALT and increase in total protein (serum and liver) levels. The extract treated groups shows mild hepatatocytic damage compared to the CCl₄ treated group. CONCLUSION: Cassia fistula attenuates the hepatotoxic effect of CCl₄.

INTRODUCTION
Cassia fistula linn (Caesalpinaceae) tree is one of the most widespread in the forests of India, usually occurring in deciduous forests The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice (Anonymous,1992, Kirtikar and Basu 1991). A new bioactive flavone glycoside 5,3',4'-tri-hydroxy -6- methoxy -7-O- alpha -L- rhamnopyranosyl - (1-->2) -O- beta-D-galactopyranoside with antimicrobial activity was reported by (Yadava and Verma, 2003). Four new compounds, 5-(2-hydroxyphenoxymethyl)furfural, (2'S)-7- hydroxyl -5- hydroxymethyl -2- (2'-hydroxypropyl) chromone, benzyl 2-hydroxy-3,6-dimethoxybenzoate, and benzyl 2beta-O-D-glucopyranosyl-3,6-dimethoxybenzoate, together with four known compounds, 5-hydroxymethylfurural, (2'S)-7- hydroxy-2-(2'-hydroxypropyl) -5- methylchromone, and two oynamthraquinones, chrysophanol and chrysohanine, were also isolated from the seeds of Cassia fistula by Kuo et al. (2002). The hepatoprotective activity of leaf extracts (Bhakta et al., 1999; Bhakta et al., 2001) and the hypoglycaemic activity have been reported. However, a detailed pharmacological screening of the Cassia fistula bark extracts have not been reported. The present study reports the Haepatoprotective activity of Cassia fistula bark extracts. In the present study C.fistula bark was extracted with water by soxhlet extraction. The extracts were vacuum dried to yield the aqueous extract (AQET). The extracts were evaluated for hepatoprotective activity against carbon tetrachloride (CCl₄) induced liver damage at the dose level of 600 mg/kg/day.

MATERIALS AND METHOD
PLANT MATERIAL AND EXTRACTION
The barks of Cassia fistula Linn (Caesalpinaceae) were collected from Vedharaniyam, TamilNadu, India during the month of September and identified by Dr.G.Prema Gupendran B.S.M.S. The barks were dried in shade for a week. Then powdered and extracted with water (AQET) by Soxhlet extraction (24 h) to yield the extract. The extracts were vacuum dried in a rotary vacum film evaporator and the extractive yields 26%(w/dry wt. of bark).

ANIMALS
Inbred wistar albino male rats (100 – 120gm) were used for the evaluation of pharmacological activities. They were kept in colony cages at 25±2 °c, relative humidity 45 – 55% under 12 h light and dark cycles. All the animals were acclimatized for a week before use. They were fed with standard animal feed (Hindustan Lever Ltd) and water adlibitum. The test
compounds and the standard drugs were administered in the form of a suspension using 5% 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.

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 (CCl₄ treated), Group-III (CCl₄ + AQET 250 mg/kg treated) and Group-IV (ccl₄ + 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 AQET 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).

On the seventh and eighth day animals of Group II, III, and IV & V were administered orally with a single dose of CCl₄ with 5% acacia mixture (600 mg/kg/day). After thirty minutes of CCl₄ administration Group III and Group IV rats were treated with AQET 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 from jugular veins 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.

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.

STATISTICAL ANALYSIS

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.

RESULTS AND DISCUSSION

Figure 1

Table 1: Hepatoprotective parameters of AQET against CCl₄ induced damage

The extracts did not cause mortality up to 2000 mg/kg and were considered as safe. ALP, SGOT, AST, ALT, and total bilirubin were significantly increased and total protein (serum and liver) was significantly decreased in CCl₄ treated group. The extracts exhibited dose dependent reduction in total bilirubin, ALP, SGOT, SGPT, AST, ALT and increase in total protein (serum and liver) levels (Table 1).

Histology of liver from normal control group (Fig.1a) showed the central vein surrounded by cords of hepatocytes and showed normal arrangement of hepatocytes with clearly brought out nuclei, cytoplasm, central vein and portal triad. Microscopical examination of CCl₄ treated liver (Fig 1b) showed cloudy swelling, sinusoidal dilatation, Individual hepatocytic necrosis of hepatic cells and centrilobular fatty changes with clear space representing fatty materials or lipids. AQET (250 mg/kg) treated animals (Fig. 1c) showed
individual focal hepatocyte damage and necrosis. AQET (500 mg/kg) treated animals (Fig 1d) showed the mild focal hepatocytic damage and necrosis. The silymarin treated group (Fig 1e) showed central vein with cords of hepatocytes with occasional focal hepatocytic damage.

**Figure 2**
Figure 1a: Control group

**Figure 3**
Figure 1b: CCl treated group

**Figure 4**
Figure 1c: CCl and AQET 250 mg/kg treated group

**Figure 5**
Figure 1d: CCl and AQET 500 mg/kg treated group

**Figure 6**
Figure 1e: CCl and Silymarin 25 mg/kg treated group
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DISCUSSION

The reactive metabolites such as trichloromethyl (CCl₃-) and trichloromethyl peroxy (CCl₃OO-) radicals emanated from CCl₄ initiate peroxidation of membrane unsaturated fatty acids. This lipid peroxidation of membrane seriously impairs its function and produces liver injury.

CCl₄-induced damage produces alteration in the antioxidant status of the tissues, 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).

C. fistula restored all these changes. AQET (250 mg/kg) treated animals (Fig. 1c) showed individual focal hepatocyte damage and necrosis. AQET (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 CCl₄ by acting as an in vivo antioxidant and thereby inhibiting the initiation and promotion of lipid peroxidation.

References

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