Research Article

EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF ETHANOLIC EXTRACT OF *CASSIA FISTULA* (GOLDEN SHOWER TREE) FLOWER AGAINST CCl_4 INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

In this study, we have evaluated the hepatoprotective potential of ethanolic extract of *Cassia fistula* (Golden shower tree) flower on the CCl_4 induced hepatotoxicity. Hepatotoxicity in rats was achieved by intraperitoneal administration of CCl_4 (1ml/kg). The Swiss albino rats (180-200g) were divided into five groups (n=6). Silymarin (100mg/kg) was given as reference standard. The degree of protection against liver toxicity was determined by measuring the serum biochemical parameters namely SGPT (serum alkaline phosphatase), SGOT (serum glutamine oxaloacetate transaminase), ALP (alkaline phosphatase), TB (total bilirubin) and the hepatic antioxidant parameters such as SOD (superoxide dismutase), CAT (catalase) and GSH (glutathione). The result shows that CCl_4 has enhanced the levels of SGPT, SGOT, ALP, total bilirubin. Pretreatment with *C.fistula* (200mg/kg B. wt. and 400 mg/kg B. wt.) brought back the altered levels of biochemical markers to the near normal levels and also it was observed that animals treated with CCl_4 developed a hepatic damage, increase in LPO and decrease in GSH, CAT & SOD whereas extract treated animals restore the antioxidant parameters near normal levels . Histopathological studies also confirmed the hepatoprotective activity of extracts. It can be concluded from the result that the ethanolic extract of *Cassia fistula* flower possesses hepatoprotective activity against CCl_4 induced hepatotoxicity in rats.

Keywords: *Cassia fistula,C.fistula,*hepatoprotective, CCl_4

INTRODUCTION

The entire world population is turning towards natural drugs because of the widespread belief that “green medicines” are healthier and safer than synthetic ones.[1] It is also gaining greater acceptance from the public and the medical profession due to greater advances in understanding the mechanism of action by which herbs can positively influence health and quality of life.[2] Herbal treatments are the most popular form of traditional medicine. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain parts of plants or other plant materials as active ingredients.[3].

The World Health Organization (WHO) has laid emphasis on promoting the use of traditional medicine for health care. Hence, we see a focus on research on traditional and herbal medicine, especially in developing countries, with individual as well as collaborative efforts by national research organizations.[4]

Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced from the voluminous work on their hepatoprotective potentials. Plants that were once considered of no value are now being investigated, evaluated and developed into drugs with no side effects. One such potential plant is *Cassia fistula.*[5]

Cassia fistula is a medium sized deciduous tree, with an oval to rounded shape, 5 to 15 meter in height and 5 to 10 meter wide. *Cassia fistula* tree is well known for its impressive yellow flowers that cover the entire canopy. Has widely spaced petals, about 2 inches wide with 10 stamens. The flowers are produced in pendulous racemes 20–40 cm (7.9–15.7 in) long, each flower 4–7 cm (1.6–2.8 in) diameter with five yellow petals of equal size and shape. The bark of the young tree is a grey, smooth to slightly ridged and slender, and changes to a darker grey-brown when mature.Stems or young twigs sparsely to densely hairy. The leaves are smooth, ovate shape, hairy below, alternate, pinnate, and deciduous, with 3-8 pairs of leaflets. The leaf can range from 15 – 60 cm long, with each leaflet ranging from 7 –

15 cm long, and 2-7 cm broad. Fruit is legume, pendulous, cylindrical, and brown in color, 20 to 60 cm long, 1 to 2.5 cm broad, with a pungent odor and containing several seeds. Seeds lenticular, light brown, lustrous.[6]

It was found that this plant contains flavonoids, alkaloids, cardiac glycosides, tannins.[7] There are reports showed that *Cassia fistula* possess antiinflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity and wound healing activity.[8] The roots are reported to have anti-fertility and antiulcer activities.[9] However there are no scientific bases or reports in the modern literature regarding its usefulness as hepatoprotective agent. Thus the present study was conducted to evaluate the hepatoprotective activity of the ethanolic extract of the cassia fistula flowers by using CCl_4-induced hepatic injury in rats

MATERIALS AND METHODS

Plant material collection

The *Cassia fistula* flower was collected locally and authenticated by a taxonomist Mrs Aparna Upadyaya, Govt.High School Hodavda Madikeri.

Preparation of extract

The extraction of the *Cassia fistuala* flowers was carried out using known standard procedures. The plant material was shade dried and powdered then used for extraction. The powder of *Cassia fistula* flower is initially defatted with petroleum benzene (60-80°C) followed by 1000 ml of ethanol by using a Soxhlet extractor for 72 hrs at a temp not exceeding the boiling point of the solvent. The extract is filtered using Whattman filter paper (No. 1) and then concentrated in vacuum and dried at 45°C for ethanol removal, and the extracts was kept in sterile bottles under refrigerated conditions until use.[10]

Preliminary phytochemical screening[11,12]

Preliminary phytochemical screening of ethanol extracts of *Cassia fistula* flower was carried out as per the methods and tests to confirm the presence and absence of various phytoconstituents. These tests revealed the presence of Alkaloids, Tannins, Flavanoids, Terpenoids, Glycosides, Carbohydrates, Saponins.

Table 1: Preliminary phytochemical screening

S. No.	Test	Result
1	Alkaloids	+
2	Carbohydrates	+
3	Flavanoids	+
4	Glycosides	+
5	Saponins	+
6	Tannins	+
7	Anthraquinone	-
8	Terpenoids	+

Drugs and Chemicals

All chemicals used were of analytical grade and obtained from Himedia Laboratories. The kits for the estimation of SGPT, SGOT, ALP and Bilirubin were purchased from Agape Diagnostics LTD, Kochi. The standard drug Silymarin was purchased from Serum international Ltd, India.

Experimental animals

Healthy Wistar albino rats of either sex weighing 150-200 g were used. Animals used in the study were procured from our college. The animal care and handling was carried out according to CPCSEA guidelines. Animals were acclimatized to the animal quarantine for one week prior to the experiment under controlled conditions of temperature (27 ± 2°C) and were housed in sterile polypropylene cages containing paddy husk as bedding material with maximum of six animals in each cage. The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee, Srinivas College of Pharmacy, Mangalore, Karnataka (Approval No.: SCP/IAEC/F150/F150/P99/2016).

Acute toxicity studies[12,13]

Acute toxicity study of the ethanolic extract of *Cassia fistula* flower was performed as per the OECD guidelines 425 at a limit dose of 2000 mg/kg. The doses were administered by oral route in rats as per scheduled in OECD guidelines 425. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for total 14 days for signs of discomfort, general behavior and mortality. The LD_{50} was calculated by using OECD guidelines 425. In the present study, 200 mg/kg and 400 mg/kg doses were selected.

CCl_4 induced hepatotoxicity studies

Wistar albino rats were randomly assigned into five groups of six animals each. All the animals except Group I was intoxicated by the

administration of CCl_4 (1:1 of CCl_4 in olive oil) on 2nd, 4th, & 6th day of treatment. All the treatment was given orally once daily for seven days.[14]

Group I and II were treated with vehicle. Group III animals were treated with Silymarin 100 mg/kg and group IV and V were treated with *C.fistula* 200mg/kg and *C.fistula* 400mg/kg respectively for seven days. All the animals except Group I was intoxicated by the administration of CCl_4 (1:1 of CCl_4 in olive oil) on 2nd, 4th and 6th day of treatment. On seventh day blood was collected through retro orbital puncture and analyzed for various biochemical parameters. Blood was allowed to clot at room temperature for 30 min, subjected to centrifugation (2000 rpm for 15 min.) and subjected for the estimation of biochemical parameters. Liver was dissected out and placed in 10% formalin solution for histopathological study.[15]

Statistical analysis

All data were expressed as mean ±SEM. The statistical significance between groups was compared using one way ANOVA, followed by Dunnett's (multiple comparisons) test.[16]

OBSERVATION AND RESULTS

I. Evaluation of Biochemical parameters

The results of ethanolic extracts of *Cassia fistula* flower on liver-injury induced by CCl_4 are summarized in Table-2.Administration of CCl_4 to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pre-treatment of rats with *C.fistula* flower extract exhibited marked protection against CCl_4 induced hepatotoxicity. The effects produced by *C.fistula* flower extract were comparable with that produced by the standard Silymarin.

In the CCl_4 intoxicated group (II) ALP, SGOT, SGPT and total bilirubin were increased to 320.8±4.549, 283.2.7±2.344, 246.7±3.073[a] and 2.833 ± 0.307 respectively, whereas these values were showed 140.7±0.9189, 100.03±2.974, 83.83±0.833, 0.7167±0.04 in control group (I), respectively.

There was a significant ($p<0.05$) reduction in the ALP, SGOT, SGPT, and Total Bilirubin levels 184.3±2.929, 143.2±2.104, 91.83±0.9458 and 1.5±0.2236 respectively, after the treatment with Silymarin (100mg/kg).

Animal treated with low dose (200mg/kg) of ethanolic extract of *Cassia fistula* flower showed a reduction in the ALP, SGOT, SGPT, and Total billirubin levels 239.5±3.304, 182.5±6.551, 163.7±1.978 and 1.86±0.3108 respectively, and animal treated with high dose(400mg/kg) of ethanolic extract of *Cassia fistula* flower showed a significant ($p<0.05$) reduction in the ALP, SGOT, SGPT, and Total billirubin levels 199.8±4.693, 152.8±6.013, 94.5±2.553 and 1.667±0.2108 respectively.

Table 2: Effect of Silymarin and ethanolic extract of *Cassia fistula* flowers on SGOT, SGPT, ALP and Total Bilirubin in CCl_4 induced liver toxicity.

Groups	Treatment	ALP (U/l)	SGOT (U/l)	SGPT (U/l)	TB (mg/dl)
Vehicle control	Olive oil 1ml/ Kg	140.7±0.9189	100.03±2.974	83.83±0.833	0.7167±0.04
Toxic control	CCl_4 1ml/Kg p.o	320.8±4.549[a]	283.27±2.344[a]	246.7±3.073[a]	2.833±0.3073[a]
Standard	Silymarin 100mg/Kg, p.o	184.3±2.929***	143.2±2.104***	91.83±0.9458***	1.5±0.2236***
Low dose	*C.fistula* flower extract 200mg/Kg, p.o	239.5±3.304*	182.5±6.551*	163.7±1.978*	1.86±0.3108*
High dose	*C.fistula* flower extract 400mg/Kg, p.o	199.8±4.693**	152.8±6.013**	94.5±2.553**	1.667±0.2108**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test. [a]p< 0.001 when compared with vehicle treated control group.*p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

II. Evaluation of liver endogenous antioxidant enzymes

Table-3 shows the effects of extracts of *C.fistula* on LPO, SOD, GSH and CAT concentrations in rat liver after challenging with CCl_4.

It was observed that animals treated with CCl_4 developed a hepatic damage, increase in LPO and decrease in GSH, CAT & SOD when compared to normal control. Animals treated with standard (Silymarin) showed extremely significant (P<0.001) increase in GSH, CAT & SOD and decrease in LPO.

Ethanolic extract of *Cassia fistula* flowers (200mg/kg) treated animals showed significant (P<0.05) decrease in LPO and significant (P<0.05) increase in GSH, CAT & SOD.

Ethanolic extract of *Cassia fistula* flowers (400mg/kg) treated animals showed moderately significant (P<0.01) decrease in LPO and moderately significant (P<0.01) increase in GSH, SOD & CAT.

Table 3: Effect of Silymarin and ethanolic extract of *Cassia fistula* flowers on LPO, SOD, GSH, and CAT in CCl_4 induced liver toxicity

Groups	Treatment	LPO (Abs at 535 nm)	SOD (Abs at 560 nm)	GSH (Abs at 412nm)	CAT (Abs at 620 nm)
Normal Control	Olive oil 1ml/Kg	3.333± 0.2108	20.33± 0.6667	31.33± 1.406	40.33± 01.498
Toxic control	CCl_4 1ml/Kg p.o.	16.5± 0.4282[a]	8.25± 1.897[a]	15.1± 0.9847[a]	21± 1.183[a]
Standard	Silymarin 100mg/Kg p.o	7.8± 0.5182***	13.77± 1.035***	22.33 ± 2.394***	28.17± 2.028***
Low dose	*C.fistula* flower extract 200mg/Kg p.o	10.533± 0.5817*	9.5± 0.4687*	20.89± 1.6745*	22.75± 0.8269*
High dose	*C.fistula* flower extract 400mg/Kg p.o	8.778± 0.6827**	12.6± 0.8221**	21.33± 1.2553**	23.33± 1.626**

All the values are Mean±SEM, n=6. One way ANOVA followed by Dunnett's t test, [a]p<0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.

Histopathological Profile

The histopathological evaluation of CCl_4 toxicity in all the groups was examined and shown in following figure.

Liver section of normal group shows liver parenchyma with intact architecture. Most hepatocytes appear normal. In toxic control group shows inflammation, centrilobular degeneration and necrosis. Treatment with ethanolic extract of *Cassia fistula* flowers (200mg/kg & 400mg/kg) found to reduce inflammation, centrilobular and bridging necrosis. Liver section of this group shows normal hepatocytes with significant reduction in areas of necrosis when compared to toxic group. These changes show protective effect of the drug against hepatic damage induced by CCl_4.

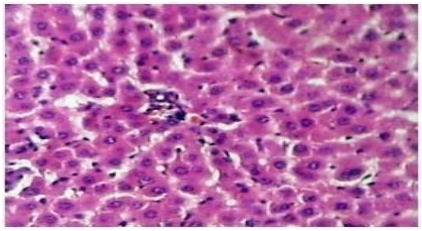

Fig 1: Liver of normal rat

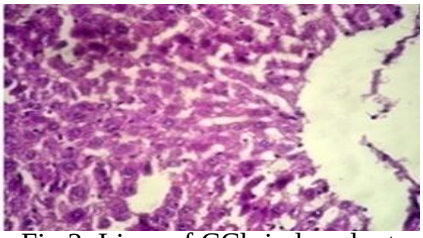

Fig 2 :Liver of CCl_4 induced rat

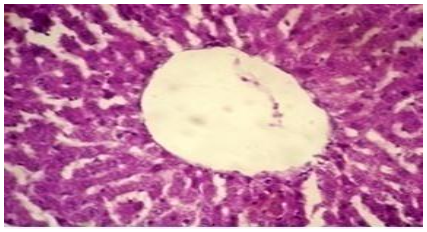

Fig 3 : Liver of Silymarin treated rat

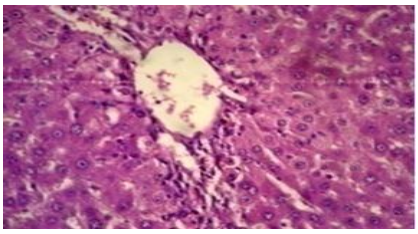

Fig 4 :*C.fistula* 200 mg/kg treated rat

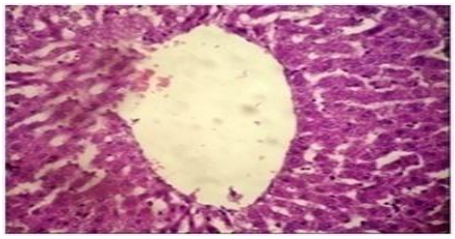

Fig 3 : *C.fistula* 400mg/kg treated rat

DISCUSSION

The liver is one of the vital organs in our body responsible for detoxification of toxic chemicals and drugs. Thus it is the target organ for all toxic chemicals. Numerous studies noted that CCl_4 is widely used to induce liver damage because it is metabolized in hepatocytes by cytochrome P450, generating a highly reactive carbon centered trichloromethy radical, leading to initiating a chain of lipid peroxidation and thereby causing liver fibrosis which in turn disrupts the structure & function of lipid & protein macromolecules in the membrane of the cell organelles.[16] CCl_4 not only initiates lipid peroxidation but also reduces tissue CAT and SOD activities, and this depletion may result from oxidative modification of these proteins. It has been reported by previous findings that CCl_4 causes necrosis steatosis and degeneration of hepatocytes, increase in mitotic activity and cirrhosis in liver. It has also been reported that CCl4 causes apoptosis in liver.

Pretreatment with ethanolic extract of *Cassia fistula* flower significantly improved the structure of hepatic cells and markedly reversed hepatotoxicity caused by the CCl_4. Our results showed that administration of *Cassia fistula* flower extract effectively protected against the loss of antioxidant activities after CCl_4 administration, and it is well known to serve diverse biological functions, including protection of cells from oxidative damage and from free radicals.

The ethanolic extract of *Cassia fistula* flowers -200 & *C.fistula*-400 showed dose dependent decrease in the elevated serum biomarkers (SGPT, SGOT, ALP and bilirubin) and endogenous enzymes(GSH, SOD, CAT), it also observed that increase in total protein levels which were comparable to the standard and significant reduction in the level of lipid peroxidation.

The number of investigators has reported that antioxidants, terpinoids, steroids, saponins and other phenolic compounds are known to possess hepatoprotective activity in animals.[17] It is therefore to speculate that the phytoconstituents present in this plant extracts might responsible for the observed hepatoprotective activity.

CONCLUSION

Conclusion of this study clearly demonstrates that ethanolic extracts of *Cassia fistula* flower was effective in the treatment and prevention of CCl_4 -induced hepatic cytotoxicity. The data

suggest that the daily oral consumption of an ethanolic extract of the Cassia fistula flowers may alleviate CCl_4 toxicity and provide protection to liver. This research work supports its traditional use for the treatment of hepatic disorder.

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