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Hepatoprotective Effect of *Cassia Fistula* Linn.

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ABSTRACT

A number of herbal preparations are used throughout the world for the management of the hepatic disorders. However, many of them have not been investigated for their described effects. *Cassia fistula* Linn. is one such drug used in the treatment of hepatitis in folk medicine. Therefore, an attempt was made to investigate the hepatoprotective effect of leaves and bark of *Cassia fistula* against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. Sixty albino Wistar rats were divided into six equal groups of 10. Four groups received extracts leaves/bark of *Cassia fistula* and intraperitoneal (i.p.) CCl_4 (0.2 ml/100 g) either before or after administration of extracts. Two groups were controls, one treated with CCl_4 and one with normal saline. Liver damage was assessed by plasma concentration of bilirubin and enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Treatment with aqueous extract of leaves and bark significantly reduced CCl_4 -induced elevation in plasma enzyme and bilirubin concentration in rats. This study demonstrated that CCl_4 -induced liver damage in rats can be ameliorated by treatment of extracts from leaves and bark.

KEYWORDS: *Cassia fistula*, hepatoprotective effect, aminotransferases, carbon tetrachloride.

INTRODUCTION

Liver is the key organ for detoxification and disposition of endogenous substances. It is continuously and widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions[1]. *Cassia fistula* (Leguminoceae) is known as amaltas (Hindi), bahava (Marathi). It is a deciduous, medium sized tree upto 24 m in height with straight bole upto 15 m, found both wild and cultivated almost throughout India. The leaves reported to contain flavonoids like quercetin, kaempferol along with anthraquinone glycosides while bark is rich in flavonoids like lupeol, epicatechin and tannins[2]. Literature survey revealed that no scientific study is reported regarding its hepatoprotective activity of *Cassia fistula*. Therefore, the present study was conducted to investigate its hepatoprotective activity.

MATERIAL & METHODS

Plant Material

Leaves and bark of *Cassia fistula* were obtained from Amaravati District, Maharashtra. The plant samples were identified and authenticated in the Herbarium by botanist Dr. Prabha Y. Bhogaonkar, VMV, Botany Department, Amaravati, Maharashtra, India. The leaves and bark were macerated by using water as a menstrum. The obtained extract was dried and concentrated under freezed drier and kept tied closed into container.

Animals

The Institutional Animal Ethics Committee approved the animal studies. It includes Albino Wistar rats of either sex, weighing 180-200 g. All animals were given standard diet and water ad libitum. They were maintained at a relative humidity of 65 to 86%, a temperature of 23 to 25°C, and in a schedule of 12 h of light and 12 h of dark. Rats were weighed at the beginning and end of the study.

Chemicals

CCl_4 was obtained from Sigma-Aldrich. All other chemicals used were of analytical grade.

Assessment of Hepatoprotective Activity

Sixty animals were randomly divided equally into six groups of 10 each.

Group 1 (controls): received normal saline orally (0.2 ml/100 g) for 16 consecutive days.

Group 2 (pretreatment experiment-leaves extract): allowed free access to aqueous extract of leaves ad libitum for 28 consecutive days and treated with i.p. CCl_4 on Days 14, 15, and 16 of the treatment period.

Group 3 (post-treatment experiment-leaves extract): given aqueous extract of leaves ad libitum for 14 consecutive days and treated with i.p. CCl_4 on Days 1, 2, and 3 of the treatment period.

Group 4 (pretreatment experiment-bark extract): allowed aqueous extract of bark ad libitum for 28 consecutive days and treated with i.p. CCl_4 on Days 14, 15, and 16 of the treatment period.

Group 5 (post-treatment experiment-bark extract): given aqueous extract of bark ad libitum for 14 consecutive days and treated with i.p. CCl_4 on Days 1, 2, and 3 of the treatment period.

Group 6 (CCl_4 -treated control): injected i.p. with a fresh mixture of equal volumes of CCl_4 and olive oil for three consecutive days at the dose of 0.2 ml/100 g of body weight/day.

Twenty-four hours after the last treatment, the rats were euthanized by injecting i.p. sodium pentobarbitone (100 g/kg). Hepatoprotective activity was calculated[3].

Hepatoprotective activity (%) = 1 - (PC - S)/(C - S) x 100 where PC, C, and S are the measurable variables in rats treated with *Cassia fistula* leaves/bark extract with CCl_4, CCl_4, and saline-treated animals, respectively.

Blood Sampling

Blood was collected in heparinized tubes from the inner canthus on the 29th day or the 16th day in the pre or post-treated groups, respectively. Plasma was separated by centrifugation at 900 rpm for 10 min at 4°C and used for

determining the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin concentration using the Hitachi autoanalyzer-902.

Statistical Analysis

Values reported are means ± SE (n = 10). Experimental results were statistically analyzed using the Student's t -test followed by ANOVA. P value less than 0.01 considered significant.

Results

The CCl_4 -treated animals exhibited a significant increase ($P < 0.01$) in plasma enzyme activity and bilirubin concentration compared with saline-treated control rats (Table 1).

Table 1: Effect of pre- and post- treatment with aqueous *Cassia fistula* leaves and bark on CCl_4 induced liver damaged in albino Wistar rats.

Treatment	Aspartate aminotransferase (U/L)	Alanine aminotransferase (U/L)	Alkaline phosphatase (U/L)	Bilirubin (µmole/ L)
Control	108.6±3.29	35.43±1.33	156.2±2.64	0.17±.03
CF Leaves pretreatment	134.6±3.12	46.80±1.78*	181.5±1.98*	0.19±.01
CF Leaves post treatment	124.0±4.27*	44.31±0.76*	193.2±4.82*	0.20±.01
CF Bark pretreatment	163.1±4.63*	59.60±1.56*	180.90±3.03*	0.21±.01
CF Bark post treatment	158.7±6.45*	56.90±1.23*	171.8±10.13	0.20±0.00
Carbon tetrachloride	283.4±2.99*	85.6±1.99*	253.6±5.45*	3.30±0.07*

CF: *Cassia fistula*; Data are expressed as ± SE (n=10), * Significantly different than control at P<0.01.

A significant reduction was found in elevated AST, ALT, and ALP values in rats subjected to both pre- and post-treatments with the aqueous extracts of both leaves and bark. Liver enzyme values were higher in the four experimental groups than in the saline-treated controls, but the liver enzyme values were decreased to about half of those found in CCl_4 -treated control animals for all liver function tests except bilirubin. Expressed in percentage of protection provided, both *Cassia fistula* leaves and bark given pre- or post-treatment were hepatoprotective (Table 2).

Table 2: Hepatoprotective activity of leaves and bark of *Cassia fistula* in CCl_4 induced hepatotoxicity in albino Wistar rats.

Clinical chemistry liver function indicator	Aspartate aminotransferase	Alanine aminotransferase	Alkaline phosphatase	Bilirubin
CF Leaves pretreatment (% protection)	83.06	76.60	75.16	99.23
CF Leaves post treatment (% protection)	89.89	82.32	61.97	99.07
CF Bark pretreatment (% protection)	68.66	51.11	71.37	96.03
CF Bark post treatment (% protection)	71.23	54.99	82.54	99.03

CF: *Cassia fistula*; % Protection = 1-(PC-S)/ (C-S) × 100, where PC, C and S are the measurable variables in rats treated with CF leaves or bark extract with CCl_4, CCl_4 and saline treated animals respectively

Discussion

Liver cirrhosis induced by CCl_4 is perhaps the best-studied model of liver cirrhosis[4] .Several mechanisms underlying this toxicity have been suggested[5]. The reduction of CCl_4 -induced elevated plasma activities of AST, ALT, ALP, and bilirubin level in animals pre- and post-treated with the aqueous extracts of *Cassia fistula* leaves and bark showed their ability to restore the normal functional status of the poisoned liver and also to protect against subsequent CCl_4 hepatotoxicity. The mechanism by which the fruits pulp and seeds induces its hepatoprotective activity is not certain. The inactive metabolite (CCl_4), is transformed to a free radical through the microsomal cytochrome P-450-dependent enzyme, resulting in activation of CCl_4 toxicity. Hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration. On that basis, it is suggested that flavonoids in *Cassia fistula* could be a factor for exhibiting the hepatoprotective activity.

Conclusion

This study clearly demonstrates that aqueous extracts of *Cassia fistula* leaves and bark are effective in the treatment and prevention of CCl_4 -induced hepatic cytotoxicity. The data suggest that the daily oral consumption of an aqueous extract of the *Cassia fistula* leaves and bark 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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