JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

How to cite this article:
DAS S, SARMA G,BARMAN S.HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF FRUIT PULP OF CASSIA FISTULA (AFCF) AGAINST CARBON TETRACHLORIDE (CCL_4) INDUCED LIVER DAMAGE IN ALBINO RATS.Journal of Clinical and Diagnostic Research [serial online] 2008 October [cited: 2008 October 6]; 2:1133-1139.
Available from
http://www.jcdr.net/back_issues.asp?issn=0973-709x&year=2008&month=October&volume=2&issue=5&page=1133-1139&id=290

ORIGINAL ARTICLE

Hepatoprotective Activity Of Aqueous Extract Of Fruit Pulp Of Cassia Fistula (AFCF) Against Carbon Tetrachloride (CCL_4) Induced Liver Damage In Albino Rats.

DAS S*, SARMA G, BARMAN S*****

ABSTRACT

Objective: To evaluate Hepatoprotective activity of aqueous extract of fruit pulp of Cassia fistula (AFCF) against Carbon tetrachloride (CCL_4) induced liver damage in albino rats and compared to standard drug silymarin.

Materials And Methods: Healthy albino rats of either sex weighing 150-200gm were divided into four groups of six animals each.

Group A (Normal control) – 3% gum acacia (2ml/kg/day) orally and olive oil s.c.

Group B (Exp.Control) – 3% gum acacia orally and CCL_4 and olive oil (1:1 v/v) s.c.

Group C (Test) – AFCF (200mg/kg/day) orally and CCL_4 s.c.

Group D (Standard) - Silymarin (100mg/kg/day) and CCL_4 s.c.

Hepatic injury was induced to animals belonging to group B, C and D by giving CCL_4 & olive oil mixture s.c on 2nd and 3rd day of experiment. Standard and test drugs were administered for 5 days. Blood samples were collected on 6th day for determination of enzyme markers viz, aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and total protein (TP). Histopathological examinations of liver tissues were also performed.

Results: One way ANOVA followed by Dunnett's multiple comparison test were used for statistical analysis. Values of $p < 0.01$ were considered significant. There was significant ($p<0.01$) increase in all serum marker enzymes and total bilirubin and significant ($p<0.01$) decrease in total protein in group B. The AFCF and Silymarin resulted in significant ($p<0.01$) reduction in serum levels of AST, ALT, ALP, TB and increase in TP as compared to group B. Fatty changes, necrosis and fibrosis were observed in group B on histopathology, while in group C and D it was near normal.

Conclusion: As revealed by the study aqueous extract of fruit pulp of Cassia fistula possesses significant hepatoprotective activity.

Keywords: silymarin, carbon tetrachloride, hepatotoxicity, Cassia fistula.

Key Message: Cassia fistula has been used for a variety of diseases in traditional medicine. Very few studies have been done, so this study was done to evaluate its hepatoprotective activity.

* MD (pharmacology)
** (P.G student, pharmacology)
*** (P.G student, pharmacology)
Department of Pharmacology,
Assam Medical College & Hospital
Corresponding Author:
DasS, , MD (pharmacology)
Department of Pharmacology,
Assam Medical College & Hospital
Dibrugarh, Assam, Pin: 786002,
phone: 9435030220
E-mail: sdasbarua@yahoo.co.in

Introduction

Native to India, Amazon and Sri Lanka, Cassia fistula Linn. (Leguminosae-Caesalpinoideae) a semi-wild Indian Labernum also known as Golden Shower, has become extensively diffused in various

countries including Mauritius, India, South Africa, Mexico, China, West Indies, East Africa and Brazil as an `ornamental tree for its beautiful bunches of yellow flowers [1] . It is found throughout India in all deciduous forests and hilly tracts[2]. In Sanskrit it is known as Savarnangah[2]. It is commonly known as Sonaru in Assamese [3]. In the Indian literature, this plant has been reported useful against skin diseases, liver troubles, tuberculous glands and in treatment of haematemesis, pruritus, leucoderma and diabetes[4].Its antifungal, antibacterial, laxative and antitussive properties have been established[5]. Butanol extract of residue of Cassia fistula from 70% alcohol fraction has been shown to have antiviral effect, while hot water extracts have proved useful in treatment of uterine, menstrual disorders and fever [6]. Other uses of this plant include antidiarrhoel and anti dysentery [7] effects.Its antitumor[8], antifertility [9], and antioxidant [10] properties have been reported. Hepatoprotective activity has been evaluated recently [11] and very few studies have been done on this property. In view of this, the present study was aimed at evaluating the hepatoprotective activity of aqueous extract of fruit pulp of Cassia fistula (AFCF) against carbon tetrachloride (CCL4) induced hepatotoxicity in albino rats.

The compounds isolated from the pods are :- - rhein; 1, 8-dihydroxy-3-anthraquinone carboxylic acid[12]; free rhein complexed with sennidin like compounds[13]; fistulic acid; anthraquinone acid[14]; 3-formyl-1-hydroxy-8-methoxy anthraquinones[15]; flavan-3-ols and proanthracyanidins like catechin, epicatechin, procatechin, procyanidin B-2 and epiafzelechin[16]; diterpene, 3β-hydroy-17-norpimar-8(9)-en-15-one[17]; sugars; volatile oils[5], 5-nonatetracontanone, 2-hentriacontanone, tricontane,16-hentriacontanol and β-sitosterol[2].

Materials And Methods

Experimental animals: Healthy Wister albino rats of either sex weighing 150-200 g were used for experiments, which were taken from the Central Animal House, Assam Medical College & Hospital, Dibrugarh, Assam.. The animals were acclimatized to laboratory conditions for 5 days prior to experiments and standard animal diet was maintained with bengal gram, wheat, maize and carrot in sufficient quantities daily. Before commencing the study, permission from the Institutional Animal Ethical Committee (Regd. No. 634/02/a/CPCSEA) was obtained.

Plant Materials: Ripe fruits of Cassia fistula were collected in month of May-June,2007 from Assam Medical college campus, Dibrugarh and authenticated by Department of Botany, Dibrugarh University. Fruits were peeled off and seeds were separated from fruits. About 250 gm of pulp material was boiled in distilled water for 30 min, kept for 3 days with intermittent shaking, filtered and concentrated using rotary flash evaporator to obtain the aqueous extract. Extract was dried in a desiccator and yield was 30% w/w[18].

Experimental Procedure:

Acute oral toxicity test :Healthy Wister albino rats of either sex weighing 150-200 g maintained under standard laboratory conditions were used for acute toxicity test according to OECD guidelines 425 (OECD guideline, 2000).A total of five animals were used which received a single oral-dose (2000mg/kg, body weight) of AFCF. Animals were kept overnight fasting prior to drug administration of AFCF. After administration of AFCF, food was withheld for further 3-4 h. Animals were observed individually at least once during first 30 min after dosing, periodically during first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Observations were done daily for changes in skin and fur, eyes and mucus membrane (nasal), respiratory rate, circulatory signs(heart rate and blood pressure), autonomic effects(salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central

nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes[19]. None of the mentioned toxic signs and symptoms or mortality were observed in the animals at above mentioned dose. So one tenth of this dose i.e., 200 mg/kg, p.o. of AFCF was selected for evaluation of antihepatotoxic activity.

Hepatoprotective Study

The study of hepatoprotective activity was carried out as described by Jalalpure SS et al[20]. Healthy Wister albino rats of either sex weighing 150-200 g were divided into four groups with six animals in each group (n=6 in each group). Group A (normal control) and group B (CCL_4- treated control) were given 5% gum acacia (2ml/kg, b.w) for 5 days. Group C and D were pretreated with AFCF (200 mg/kg, p.o.) and silymarin (100 mg/kg, p.o.) respectively for 5 days. Liver damage was induced in all groups (except group A) with 1:1 (v/v) mixture of CCL_4 and olive oil (1 ml/kg, s.c) injected on days 2 and 3 while olive oil (0.5ml/kg,s.c) was injected to group A. The animals were killed under light ether anaesthesia after 48 h of CCL_4 treatment, that is, on sixth day. Blood was withdrawn from the carotid artery, allowed to coagulate at 37 degree C for 30 min, serum separated by centrifugation at 2500 rpm for 10 min and biochemical analysis were carried out to asses liver function viz., serum transaminases [aspartate transaminase (AST) , alanine aminotransferase (ALT) [21], alkaline phosphatase (ALP) [22], total bilirubin[23] and total protein (TP)[24].

Histopathological Study

A portion of liver tissue of all animal groups were excised and then washed with normal saline. They were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h and then processed for paraffin embedding. By using a microtome, sections of 5 mm thickness were taken, processed in alcohol-xylene series and were stained with alum-haematoxylin and eosin [25] and subjected to histopathological examination.

Results

Biochemical Assessment

The CCL_4 treated group showed significant ($p<0.01$) increase in serum hepatic enzyme levels viz., AST, ALT, ALP and TB; and a significant ($p<0.01$) decrease in TP levels compared to normal control group indicating liver injury. Whereas in animals pretreated with AFCF and silymarin there was a significant (<0.01) decrease in serum hepatic enzymes and total bilirubin and an increase in total protein as compared to experimental control showing that AFCF has hepatoprotective activity [Table/Fig 1].

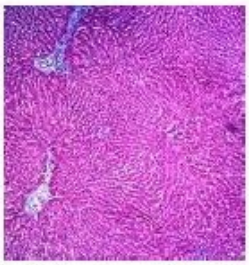
[Table/Fig 1] THE HEPATOPROTECTIVE EFFECTS OF FRUIT PULP OF CASSIA FISTULA AGAINST CCL, INDUCED HEPATOTOXICITY ON ALBINO RATS

Groups	Dose	AST (U/L)	ALT (U/L)	ALP (KA units)	TB (mg %)	TP (gm %)
Group–A (Normal control)	5% gum acacia (2ml/kg/day p.o) and olive oil (0.5ml/kg,s.c)	46 ± 2.0	24± 1.238	13 ± 1.155	0.5 ± 0.1155	4.8 ± 0.1414
Group–B (experimental control	5% gum acacia (2ml/kg/day p.o) and CCL_4 and olive oil (1:1 v/v, s.c)	240 ± 1.770*	132±1.414*	30 ± 1.390*	3.2±0.1155*	2.7 ± 0.1155*
Group–C (AFCF)	200 mg/kg/day, p.o	120± 1.713**	36±1.183**	22 ± 1.183	2.1±0.1155**	3.8±0.1461**
Group– D (Silymarin)	100mg/kg/day p.o.	110±1.183**	28±1.862**	16±1.414**	1.2±0.1155**	4.517±0.1621**
ANOVA	F	2285	1267	33.75	102.3	43.30
	P	<0.01	<0.01	<0.01	<0.01	<0.01

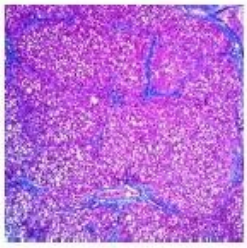
Values expressed as Mean ± SEM; n = 6 in each group; *p < 0.01 when compared to normal control; ** p <0.01when compared to experimental control; ANOVA followed by Dunnet's Multiple Comparision Test

Histopathological Examination

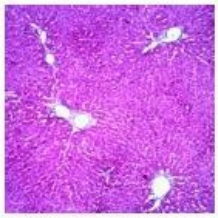
Histopathological examination of normal control group showed normal hepatocytes [Table/Fig 2]. CCL_4 treated rat liver revealed fatty degeneration, necrosis, and fibrosis [Table/Fig 3]. Administration of AFCF preserved the histological structure of liver to near normal though there was congestion and regeneration of liver tissue [Table/Fig 4]. Sections of liver taken from Silymarin treated group showed hepatic architecture similar to that of normal control group [Table/Fig 5]



[Table/Fig 2] Section of the liver tissue of control showing normal histology.(60 X)



[Table/Fig 3] Section of the liver tissue of animal treated with CCL4 showing fatty degenration,necrosis and fibrosis. (60X)



[Table/Fig 4] Section of the liver tissue of aqueous extract of fruit pulp of cassia fistula treated animals showing normal arrangment of hepatocytes,absence of necrosis and mild fatty change.(60 X)

[Table/Fig 5] Section of the liver tisue of silymarin treated animals showinsg normal hepatocytes with central hepatic vein.(60X)

Discussion

CCL_4 is one of the most commonly used hepatotoxin in experimental study of liver diseases[26] . CCL_4 is biotransformed by cytochrome p-450 in liver to produce highly reactive trichloromethyl free radical. This, in presence of oxygen generated by metabolic leakage from mitochondria causes lipid peroxidation of membrane lipid. This leads to loss of integrity of cell membranes and damage of hepatic tissue[27] which is evidenced by increased levels of serum marker enzymes, namely AST, ALT and ALP and TB. AFCF significantly reduced these liver enzyme levels. Further, AFCF increased the levels of total protein which indicates hepatoprotective activity comparable with standard drug silymarin. Stimulation of protein synthesis accelerates regeneration process and production of liver cells[28].

Histopathological studies showed that CCL_4 caused fatty degeneration, necrosis and fibrosis of liver tissue. Pre-treatment with AFCF showed protection of liver tissue, which confirmed the results of biochemical studies.

The bioactive actions ascribed to polyphenols are almost certainly mediated partly by their free radical scavenging and antioxidant actions[29], their ability to decrease localised oxygen concentration and to decompose peroxides[30] .Total phenolics and particularly flavin 3-ol derivatives are known to be potential antioxidant prophylactic agents [31] and it has already been mentioned earlier that antioxidant activity is important in protection against CCL_4 induced liver lesion [32]. So, hepatoprotective activity of AFCF may be probably due to presence of these antioxidants though it has to be confirmed yet.

Thus the present study revealed that Cassia fistula possesses significant hepatoprotective effect. However further studies on other models and clinical trials are required to confirm these results and to establish the exact mechanism of action and active principles involved in hepatoprotective effect.

References

[1]. Theeshan Bahorun,Vidushi S Neergheen1, Okezie I Aruoma. Phytochemical constituents of Cassia fistula. African Journal of Biotechnology 2005; Vol. 4 (13): 1530-40.
[2]. Indian Herbal Pharmacopoea. Indian drug manufacturers Association, Mumbai, 2002; 1: 106-113.
[3]. Bimala Patwari. A glossary of medicinal plants of Assam and Meghalaya. 1992;1:27.
[4]. Asolkar LV, Kakkar KK, Chakre OJ). Second supplement to glossary of Indian medicinal plant with active principles. In: Publication and Information Directorate, New Delhi. CSIR1992; I: p. 177.
[5]. William Charles Evans. Phenols and phenolic glycosides. Trease and evans Pharmacognosy. Elsevier, China, 2004; 15: 214-52.
[6]. Kaji N. N., Khorara M. L, Sanghari M.M. One more glycoside from C. fistula pod. Ind. J Pharm 1965; 27 (3) :71 - 72.
[7]. Manandhar N. P. Medicinal Plants used by Chepang Tribes of Makawanpar District, Nepal Fitoterapia 1989;Vol LX (1): 61-68
[8]. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumour activity of methanolic extract of Cassia fistula L. seed against Ehrlich ascites carcinoma. J Ethnopharmacol 2000; 72:151-56.
[9]. Yadav R, Jain GC . Antifertility effect of aqueous extract of seeds of Cassia fistula in female rats. Adv Contraception1999; 15: 293-301.
[10]. Chaminda T, Munasinghe J, Seneviratne CK, Thabrew MI, Abeysekera AM. Antiradical and anti - lipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. Phytother Res 2001; 15: 519-23.
[11]. Bhakta T, Banerjee. S, Subhash C. Heptoprotective activity of Cassia fistula leaf extract Phytomedicine 2001; 8 (3): 220-24.
[12]. Modi FK, Khorana ML. A study of Cassia fistula pulp. Indian J Pharm 1952;4: 61-63.
[13]. Kapadia GJ, Khorana ML. Studies of active constituents of Cassia fistula pulp. I. Colorimetric estimation of free rhein and combined sennidin-like compounds. Lloydia 1966;25: 55-58.
[14]. Agrawal GD, Rizvi SAI, Gupta PC, Tewari JD. Structure of fistulic acid a new colouring matter from the pods of Cassia fistula. Planta Med 1972; 2:150-55.
[15]. Rani M, Kalidhar SB. A new anthraquinone derivative from Cassia fistula Linn. Pods. Indian J Chem 1998;37B: 1314-15.
[16]. Kashiwada Y, Toshika K, Chen R, Nonaka G, Nishioka I. Tannins and related compounds. XCIII. Occurrence of enantiomeric proanthocyanidins in the Leguminosae plants, Cassia fistula L.; Cassia Javanica L. Chem Pharm Bull 1996; 38: 888–93.
[17]. Misra TR, Singh RS, Pandey HS, Singh BK. A new diterpene from Cassia fistula pods. Fitoterapia 1997; LXVIII (58):375.
[18]. Kokate CK, Purohith AP, Gokhale SB. Pharmacognosy. Pune, India : Nirali Prakashan; 1990.
[19]. OECD (2000). Acute oral toxic class method. Guideline 423, adopted 23.03.1996. In: Eleventh Addendum to the OECD guidelines for the Testing of Chemicals. Organisation for Economic Co-Operation and Development, Paris: June 2000.
[20]. Jalalpure ss, Patil MB, Prakash NS, Hemalata K, Manvi FV. Hepatoprotective activity of fruits of Piper longum Linn. Indian J Phrm Sci 2003; 65:363-66.
[21]. Reitman S, Frankel S. A Colorimetric method for the determination of serum glutamic oxoloacetic and glutamic pyruvic transaminase. Am J Clin Pathol 1957;28:56-63.
[22]. Bessey OA, Lowery DH, Brock MJ. A method for the rapid determination of alkaline phosphatase with five cubic meters of serum. J Biol Chem 1964; 164:321-29.
[23]. Mallory HT, Evelyn EA. The determination of bilirubin with photoelectric colorimeter. J Biol Chem 1937; 119:481-85.
[24]. Kingsley SR, Frankel SJ. The determination of serum totol protein albumin and globulin by the biuret reaction. J Biol Chem 1939; 128:131-37.
[25]. Galigher AE, Kayloff EN. Essentials of practical microtechniques. Philadelphia: Lea and Febiger; 1971.
[26]. Johnson DE, Kroening C. Mechanism of early carbontetrachloride toxicity in cultured rat hepatocytes. Pharmacol Toxicol 1998; 83:231-39.
[27]. Recknagel RO, Glende EA, Hruszkewyez AM. Free radicals in biology. In:Proyer EA, editor.Vol.3. Academic press; 1976.p.97.
[28]. M. Uma Maheswari, P.G.M. Rao. Antihepatotoxic effect of grape seed oil in rat. Indian J Pharmacol 2005; 37(3):179-82.
[29]. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Biol Med 1996; 20: 933-56.
[30]. Aruoma OI. Methodological considerations for characterizing potential

antioxidant actions of bioactive components in plant foods. Mut Res 2003; 544: 203-15.

[31]. Neergheen V, Bahorun T. Optimisation of growth andpolyphenolic production in Cassia fistula callus cultures. Asian J Microbiol Biotechnol Environ Sci 2002; 4: 181-85

[32]. Srivastava SP, Chen NO, Holtzman JL. The in vitro NADPH dependent inhibition by CCL_4 of the ATP dependent calcium uptake of the hepatic mcrosomes from male rats. Studies on the mechanism of inactivation of the hepatic microsomal calcium pump by the CCL_3 radical. J Biol Chem 1990;265:8392-99.