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CASSIA TORA SEEDS EXTRACT REGULATES LIPID METABOLISM IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT: The hypolipidemic activity of *Cassia tora* (Chakvat, Chakunda) (Family: Caesalpiniaceae) seeds extract have been studied in alloxan-induced diabetic rats. In this model oral administration of seed extract (500 mg/kg bw.P.o.) for 15 days in diabetic-dyslipidemic rats exerted significant lipid lowering effect as assessed by the reversal of serum levels of total cholesterol, phospholipids, triglyceride, free fatty acids, protein and lipid peroxide. The decrease of lipids and apoprotein levels of Very Low Density Lipoprotein and Low Density Lipoprotein were followed by stimulation of plasma post-heparin lipolytic activity as well as lecithin cholesterol acyltransferase, hepatic superoxide dismutase, catalase, triglyceride lipase and lipoprotein lipase activities with increase in reduced glutathione. Lipid and apoprotein level of High Density Lipoprotein were also recovered partially on treatment with seeds extract. The results of the present study demonstrated antidyslipidemic and antioxidant activities in seed extract of *C. tora* which could be used in prevention of diabetic dyslipidemia and related complications. The hypolipidemic activity of *Cassia tora* seeds was compared with a standard drug glibenclamide (600 µg/ kg body wt/ day p.o.).

INTRODUCTION: *Cassia tora* Linn (Family: Caesalpiniaceae) commonly known Chakvat, Chakunda and Charota in Hindi, *Foetid Cassia* in English is an herbaceous foetid annual weed, almost on under shrub, up to 90 cm in height.

It grows in tropical and Asian countries especially on way sides and waste places and on hills of low elevations up to 1,800 m as well as in plains. Different parts of the plant (Leaves, seed, and root) are claimed to be effective against a variety of ailments in indigenous medicine ¹.

The leaves and seeds are acrid, thermogenic, laxative depurative, antiperiodic, liver tonic, antihelmintic, cardio tonic and are useful in ringworm, pruritis, leprosy, skin disease, jaundice, helminthiasis, flatulence, dyspepsia, intermittent fevers, constipation, ophthalmopathy, cough,

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bronchitis, cardiac disorders and haemorrhoids^{2,3}. The leaves of *C. tora* are reported to have antirheumatic activity in folklore practice. Decoction of the leaves is used as laxative. The seeds of *C. tora* have been used in Chinese medicine as vision-improving, cardiogenic, hypolipidemic, aperients, antiasthmatic and diuretic agent. Several polyherbal formulations are available in Chinese market for preventing the formation of atherosclerosis plaque⁴.

Cardiovascular diseases are leading cause of death in both industrialized and developing nations. Disorders of lipid metabolism following oxidative stress are the prime risk factors for initiation and progression of heart diseases. The current therapies used for controlling hyperlipidemia; fibrates, statins and bile acid sequestrants are almost inefficient to regulate lipid metabolism. Furthermore, these drugs also cause a number of serious adverse effects in patients. Currently available treatment for hyperlipidemia in modern medicine, fibrates, statins or bile acids sequestrants and their combinations do not regulate lipid metabolism up to an appreciable mark, also have several adverse effects in patients. Therefore, there is a need to develop safe and effective treatment modalities for hyperlipidemia.

Furthermore medicinal plants play an important role in the treatment of lipid disorders, especially due to their lesser toxicity, side effects and cost effectiveness. Therefore, the research and development of hypoglycemic and lipid lowering drugs from natural products are the best option and also are in great demand. In view of the above considerations, the present study was designed to investigate antidyslipoproteinemic activity of *Cassia tora* seeds in hyperglycemic rats⁵.

MATERIAL AND METHODS:

Preparation of seed extract:

Cassia tora seeds were collected from local area of Lucknow and identified taxonomically by Department of Pharmacology, Era's Lucknow Medical College Lucknow. A voucher specimen (CT-005/10) was also submitted. Seeds were crushed and dried under shade. The powder (500g) was extracted with 95 % ethanol in a Soxhlet extractor for 72 h, the extract was concentrated to

dryness under reduced pressure and controlled temperature (50-60 °C), yielding 23g of reddish brown solid (crude extract). This was stored in refrigerator and used to investigate hypolipidemic activity in rats⁵.

Animals:

In vivo experiments were conducted as per guidelines provided by Animal Ethics Committee of Central Drug Research Institute, Lucknow, India. Male adult rats of Charles Foster strain (200-225g) bred in animal house of the Institute were used. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26 °C, relative humidity 50-60% and 12/12 h light/dark cycle (light from 8:00 am to 8:00 pm) and provided with standard rat pellet diet and water *ad libitum*⁵.

Alloxan induced hyperglycemia:

The rats were divided into four groups having six animals in each as follows: control, hyperglycemic, hyperglycemic treated with *Cassia tora* seeds and hyperglycemic treated with glibenclamide. Diabetes was induced in rats by a single intraperitoneal injection of alloxan monohydrate 150 mg/kg b.w. in 18 animals. After two weeks of diabetes induction, rats with serum glucose level 280-367 mg/dl were taken for the study.

C. tora seeds extract and glibenclamide were macerated with aqueous gum acacia (1% w/v) suspension and fed orally at the doses of 500 mg/kg b.w. and 600µg/kg, b.w., respectively. Control animals received same amount of vehicle.

After 15 days of feeding rats were fasted overnight and blood was withdrawn from the retro-orbital plexus. A group of normal rats without treatment with alloxan was also included to serve as control. Animals were kept in controlled conditions. Temperature 25-26°C, relative humidity 60-70% and 12/12 h light/dark cycle (light from 08:00 AM to 08:00PM), provided with standard pellet diet (Lipton India Ltd.), and water *ad libitum*⁶.

Biochemical analysis of plasma:

Serum from above rats was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein

(HDL) by polyanionic precipitation method ⁷. Serums as well as lipoproteins were analyzed for their total cholesterol: TC ⁸, triglyceride: TG ⁹, phospholipids: PL ¹⁰ and apoprotein ¹¹ by standard procedures reported earlier, serum lipid peroxide (LPO) ¹², free fatty acid (FFA) ¹³, plasma protein ¹⁴, Plasma lecithin cholesterol acyl transferase (LCAT) activity ¹⁵ and post heparin lipolytic activity (PHLA) ¹⁶ were also estimated.

Biochemical analysis of liver:

Liver homogenized (10% w/v) in cold 1 M phosphate buffer (pH7.2) was used for the assay of lipoprotein lipase (LPL) ¹⁶, and triglyceride lipase (TGL) ¹⁷ activities. Liver homogenate 10% w/v in 0.15 M KCl was also used for the estimation of superoxide dismutase (SOD) ¹⁸, Catalase (CAT) ¹⁹ and reduced glutathione (GSH) ²⁰. The lipid extract of each homogenate was used for the estimation of TC, PL and TG by above- mentioned methods.

Biochemical analysis of feces:

Feces spilling of Rat's from all groups over 15 days and processed for the cholic and deoxycholic acid ²¹.

Statistical analysis:

One-way-analysis of variance (ANOVA-Newman's student test) was performed by comparison of values for alloxan-treated group with control, alloxan and drug-treated with alloxan only. All hypothesis testing were two-tailed. P<0.05 was considered statistically significant and the results were expressed as mean \pm SD. The Graph pad INSTAT 3.0 software was used to carried out the statistical analysis ²².

RESULTS:

Effect of *C. tora* seed extract on serum lipid, FFA, LPO and plasma protein in alloxan induced diabetic rats:

The data in **Table 1** show that, acute administration of alloxan markedly increased in the plasma; TC, TG and PL levels 97%, 130% and 45% , FFA 59%, LPO, 250% and decreased plasma protein 31%. However, treatment with *C. tora* seed extract caused reversal in the levels; TC 34%, TG 36%, PL 38%, FFA 15%, LPO 33% and protein by 29%. Glibenclamide reversed the levels; TC 20.0%, TG 25.0%, PL 7.0%, FFA 20%, LPO 39% and Protein 15.0%.

TABLE 1: EFFECT OF *C. TORA SEED* EXTRACT ON SERUM LIPID, PROTEIN, LIPID PEROXIDE AND FFA IN ALLOXAN-INDUCED DIABETIC RATS

Experimental schedule	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Protein (g/dl)	Serum lipid peroxide (n mol MDA/ml plasma)	Serum free fatty acid (μ mol/L)
Control	74.23 \pm 10.66	77.72 \pm 7.54	86.76 \pm 11.30	6.99 \pm 0.45	2.53 \pm 0.49	1.48 \pm 0.17
Alloxan-treated	146.15 \pm 26.80 ** (+ 97)	178.81 \pm 13.60 ** (+130)	124.88 \pm 14.68 ** (+45)	4.84 \pm 0.36 ** (- 31)	8.88 \pm 1.40** (+250)	2.36 \pm 0.30** (+59)
Alloxan + <i>C. tora seed</i> extract (500 mg /kg b.w.)	96.19 \pm 12.36 *** (-34)	115.17 \pm 6.07*** (-36)	77.25 \pm 7.22*** (-38)	6.23 \pm 0.39** (+29)	6.18 \pm 0.46*** (-33)	2.00 \pm 0.24* (-15)
Alloxan + Glibenclamide (600 μ g/kg b.w.)	117.18 \pm 18.86** (-20)	133.78 \pm 11.24** (-25)	116.45 \pm 11.67 ^{NS} (-7)	5.57 \pm 0.69 * (+15)	5.45 \pm 0.99*** (-39)	1.88 \pm 0.29** (-20)

Values are expressed as mean \pm SD of 6 animals. Values in the paranthesis indicate percent change. Alloxan-treated group was compared with control, alloxan and drug-treated groups with alloxan. ***p<0.001, **p<0.01, *p<0.05, NS= Non significant.

Effect of *C. tora* seed extract on serum lipoprotein profile in alloxan-induced diabetic rats: Analysis of hyperglycemic serum (**Table 2**) showed marked increase in the levels of lipids and apoprotein constituting β -lipoproteins (VLDL and LDL) and these effects were pronounced for

VLDL-TC, PL and TG 53%, 21% and 96% respectively and apoprotein 13%. There was increase in LDL-TC, PL, TG 325%, 43%, 134% respectively and apoprotein 24%. The decrease in HDL-TC, PL, TG and Apoprotein 27%, 23%, 5% and 28% respectively.

The data in **Table 2** also show that treatment with *Cassia tora* seed extract for 15 days significantly reversed the level VLDL-TC 30% and PL, 13 %,TG 27% and Apoprotein 9% and decrease in LDL-TC 41%, PL, TG 24%, 39 % and apoprotein 13%. At the same time *Cassia tora* seed extract increased in the levels of HDL-TC, PL, TG 10%,

4%, 7% respectively, and apoprotein 15%. Glibenclamide decreased VLDL-TC, Apoprotein 26%, 9% respectively PL 8 % and TG 32 % and LDL-TC, TG 37%, 38% respectively, PL 7.0 % and apoprotein 13% respectively with simultaneously increase in HDL-TC, PL, TG, Apoprotein 4 %, 4 %, 5 %, 11% respectively.

TABLE 2- EFFECT OF CASSIA TORA SEED EXTRACT ON LIPOPROTEIN PROFILE IN ALLOXAN-INDUCED DIABETIC RATS

Experimental schedule	VLDL				LDL				HDL			
	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	Apo-protein (mg/dl)	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	Apo-protein (mg/dl)	TC (mg/dl)	PL (mg/dl)	TG (mg/dl)	Apo-protein (mg/dl)
Control	7.98 ±0.63	17.11 ±1.98	38.17 ±3.95	7.15 ±1.04	16.17 ±1.78	12.28 ±1.11	20.17 ±1.11	17.33 ±1.61	52.83 ±3.80	40.97 ±3.35	13.77 ±1.43	178.13 ±11.34
Alloxan treated	12.19*** ±1.20 (+53)	20.72*** ±2.48 (+21)	74.76*** ±5.70 (+96)	8.11* ±0.57 (+13)	68.68*** ±8.65 (+32)	17.59*** ±1.43 (+43)	47.21*** ±6.29 (+134)	21.55* ±1.46 (+24)	38.71*** ±3.75 (-27)	31.52*** ±2.27 (-23)	13.00* ±1.28 (-5)	127.42*** ±12.33 (-28)
Alloxan + <i>Cassia tora</i> seed extract	8.52*** ±0.99 (-30)	17.98* ±1.51 (-13)	54.29*** ±4.85 (-27)	7.36NS ±6.80 (-9)	40.51*** ±3.46 (-41)	13.41* ±0.90 (-24)	28.66** ±4.28 (-39)	18.80NS ±1.44 (-13)	42.52NS ±4.41 (+10)	32.82NS ±2.90 (+4)	13.25NS ±1.25 (+7)	146.80* ±13.27 (+15)
Alloxan+ glibenclamide	9.04 ±0.96* (-26)	19.30 ^{NS} ±2.39 (-8)	51.02*** ±5.62 (-32)	7.30 ^{NS} ±0.86 (-9)	43.33*** ±3.80 (-37)	17.44 ^{NS} ±1.40 (-7)	29.24** ±4.36 (-38)	18.70 ^{NS} ±1.52 (-13)	40.12 ^{NS} ±4.22 (+4)	32.12 ^{NS} ±1.55 (+4)	13.59 ^{NS} ±1.28 (+5)	140.96 ^{NS} ±12.41 (+11)

Values are expressed as mean ± SD of 6 animals. Values in the parenthesis indicate percent change. Alloxan-treated group was compared with control, alloxan and drug-treated groups with alloxan. ***p<0.001, **p<0.01, *p<0.05, NS= Non significant

Effect of *C. tora* seed extract on hepatic SOD, CAT, TGL, LPL and reduced GSH in alloxan-induced diabetic rats:

The data in **Table 3** shows that administration of alloxan in rats decreases the levels of SOD 33%, CAT 25%, TGL 21% and LPL 31% and depletion

of GSH 45% respectively. Treatment with *C. tora* seed extract for 15 days reactivates SOD 40% , CAT 24 % , TGL 17% LPL 29% and recovered GSH 35%. Glibenclamide increases the levels of SOD 26% , CAT 16%, TGL 17 % , LPL 23% and recovered GSH 41 % respectively) (**Table 3**).

TABLE 3: EFFECT OF CASSIA TORA SEED EXTRACT ON HEPATIC SOD, CAT, TGL, LPL AND GSH IN ALLOXAN-INDUCED DIABETIC RATS

Experimental schedule	SOD (Unit /min /mg protein)	CAT (Unit /min /mg protein)	TGL (n mol FFA released/hr/mg protein)	LPL (n mol FFA released/hr/mg protein)	Reduced glutathione μmoleGSH/g
Control	3.21±0.20	3855±248.17	75.11±5.78	95.69±7.71	5.23±0.58
Alloxan treated	2.14±0.16*** (-33)	2873±402.08*** (-25)	59.63±4.62** (-21)	66.36±5.46** (-31)	2.75±0.32*** (-47)
Alloxan + <i>C. tora</i> seed extract	3.00±0.13*** (+40)	3550±504.59*** (24+)	69.71±4.88* (+17)	85.34±9.79*** (+29)	3.71±0.48*** (+35)
Alloxan + Glibenclamide	2.70±0.13*** (+26)	3342±482.02* (+16)	69.85±7.08* (+17)	81.89±8.68** (+23)	3.87±0.50*** (+41)

Values are expressed as mean ± SD of 6 animals. Values in the parenthesis indicate percent change. Alloxan-treated group was compared with control, alloxan and drug-treated groups with alloxan. ***p<0.001, **p<0.01, *p<0.05, NS= Non significant.

Effect of *C. tora* seed extract on faecal bile acids, plasma LCAT, PHLA in alloxan induced diabetic rats: Administration of alloxan in rats markedly decreased in the levels of cholic acid 28% and deoxycholic acid 35% in feces, as well as

LCAT 33% and PHLA 28%, in plasma. The treatment with *C. tora* seed extract for 15 days increased the levels of cholic acid 25% and deoxycholic acid 62% in feces and LCAT 51.0 % and PHLA 39 % in plasma of alloxan induced

diabetic rats. Glibenclamide decreased the levels of cholic acid 28% and deoxycholic acid 26% in feces and also LCAT 28% and PHLA 18% in plasma (Table 4).

TABLE 4: EFFECT OF *C. TORA SEED* EXTRACT ON FAECAL BILE ACIDS, PLASMA LECITHIN CHOLESTEROL ACYLTRANSFERASE AND PLASMA POST-HEPARIN LIPOLYTIC ACTIVITIES IN ALLOXAN-INDUCED DIABETIC RATS

Experimental schedule	Faecal bile acids		Plasma lecithin cholesterol acyl transferase activity (n mol Cholesterol released/hr/l)	Plasma post-heparin lipolytic activity (n mol FFA formed/h/l)
	Cholic acid (µg/g feces)	Deoxycholic acid (µg/g feces)		
Control	74.31±6.80	54.76±11.30	61.58±4.90	15.29±1.78
Alloxan-treated	53.68 ± 6.49*** (-28)	35.34±7.83*** (-35)	41.04±3.76*** (-33)	10.96 ± 0.98** (-28)
Alloxan + <i>C. tora seed</i> extract (500 mg/kg b.w.)	67.08 ± 1.76** (+25)	57.36 ± 11.36*** (+62)	62.17 ± 4.92** (+51)	15.19 ± 1.19* (+39)
Alloxan + Glibenclamide (600 µg/kg b.w.)	68.67 ± 3.81** (+28)	44.61 ± 2.08** (+26)	53.32 ± 1.23** (+28)	12.95 ± 1.16** (+18)

Values are expressed as mean ± SD of 6 animals. Values in the paranthesis indicate percent change. Alloxan-treated group was compared with control, alloxan and drug-treated groups with alloxan. ***p<0.001, **p<0.01, *p<0.05.

DISCUSSION: In the present study, seed of *C. tora* were tested for their anti-dyslipidemic and antioxidant activities in alloxan induced diabetic rats. Alloxan causes reversible damage to insulin producing β-cells found in the pancreas, and that is why this animal model have been used for primary screening of test drug for anti dyslipoproteineic activities. In the present study we found that, intoxication with alloxan caused increased levels of TC, PL, TG, FFA, LPO as well as decrease in protein. The analysis of the lipid and apoprotein components of β lipoproteins showed that alloxan intoxication in rats also caused significant increase in TC, PL, TG and Apoproteins components of VLDL and LDL. On the other hand levels of TC, PL, TG and Apoproteins components were decreased in HDL. Due to alloxan intoxication levels of SOD, CAT, TGL, LPL and GSH were also decreased.

Fecal bile acids, LCAT, PHLA were also decreases by alloxan. Similar observations were reported by others. Lipases play a significant role in lipoprotein metabolism and decreased lipoprotein lipases activities in diabetes are main cause of atherosclerosis²³. However, treatment with *C. tora* seed extract for 15 days reversed these effects.

beneficial effects may be due to bioactive compounds like typical alkaloids, anthraquinones, chrysophanol, emodin, rhein, euphol, bas-seol

The abnormal high concentration of serum lipid in diabetes is mainly due to the increase in the mobilization of free fatty acid from the peripheral depots, since insulin inhibits the hormone sensitive lipase²⁴. On the other hand, glucagons, catecholamine and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded because of the unregulated actions of lipolytic hormones on the fat depots²⁵. *C. tora* and glibenclamide both caused a significant decrease in the plasma levels of TC, TG, PL and FFA in alloxan-induced hyperglycemia. In alloxan induced diabetic rats, *C. tora* seed extract could increased the level of HDL by increasing the activity of LCAT, which might contribute to the regulation of blood lipids.

LCAT play a key role in lipoprotein metabolism and most of the lipoprotein changes are the outcome of primary abnormality owing to the diseases related with lipid metabolism⁶. *C. tora* seed extract enhanced the excretion of bile acids through feces and this contributed to regress the cholestestosis in liver damage. In conclusion, the lipid lowering activity of *C. tora* might be due to inhibition of hepatic cholesterol biosynthesis, activation of tissue lipases, SOD, CAT and these phenolic glycosides namely: ru- brofusarine triglucoside, nor-rubrofusarin gentiobioside, demethyl flavasperone gentiobioside, torochryson

gentio- bioside, torachryson tetra- glucoside and torachryson apioglucoside²³

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