HYPOLIPIDEMIC ACTIVITY OF CASSIA TORA SEEDS IN HYPERLIPIDEMIC RATS

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ABSTRACT

The hypolipidemic activity of Cassia tora (Chakvat, Chakunda) (Family: Caesalpiniaceae) seeds extract has been studied in two hyperlipidemic models of rat. These are triton injected and cholesterol rich HFD fed model of hyperlipidemia. In triton WR-1339 induced hyperlipidemia, feeding with root extract (500 mg/ kg body wt/ day p.o.) exerted lipid lowering effect as assessed by reversal of plasma levels of total cholesterol (TC), phospholipids (PL), triglyceride (TG) and reactivation of Post Heparin Lipolytic Activity (PHLA) of plasma. The other model was fed with cholesterol rich HFD and seeds extract of Cassia tora (500 mg/ kg body wt/ day p.o.) simultaneously for 30 days. This also caused lowering of lipid levels in plasma and liver homogenate and reactivation of plasma post heparin lipolytic activity, hepatic total lipoprotein lipase activity. The hypolipidemic activity of Cassia tora seeds was compared with a standard drug guggulipid (200 mg/ kg body wt/ day p.o.), a known lipid lowering drug in both models.

Key Words: Cassia tora seeds, Triton model of hyperlipidemia, Cholesterol rich HFD, Hypolipidemic agent, PHLA, Hepatic LPL activity, Hepatic steatosis.

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INTRODUCTION

Cassia tora (Chakvat, Chakunda) Cassia tora is a plant of family Caesalpiniaceae. Chakvat grows throughout India especially on way sides and waste places, on hills of low elevations up to 1,800 m as well as in plains. Plant of Cassia tora is a herbaceous foetid annual weed, almost an under shrub, up to 90 cm in height; leaves pinnately compound, rachis grooved with a conical gland between each of the two lowest pairs of leaflets, leaflets three pairs, obviate-oblong, membranous, base somewhat oblique, main nerves 8-10 pairs; flowers yellow, sub sessile pairs, in the axils of the leaves, the upper ones crowded, stamen serven, perfect and three staminodes; fruits subtetragenous obliquely septate pods, 15-23 cm long, the sutures very broad, rhombohedral, 23-30 per pod. The leaves and seeds of these plants are used for medicinal purpose. The leaves and seeds are acrid, thermogenic, laxative depurative, antiperiodic, liver tonic, antihelmintic, cardiotonic and are useful in ringworm, pruritis, leprosy, skin disease, hepatopathy, helminthiasis, flatulence, dyspepsia, intermittent fevers, constipation, ophthalmopathy, cough, bronchitis, cardiac disorders, haemorrhoids, antifungal, hypolipidemic, hepatoprotective, and hypotensive activities (1- 6).

The plant is claimed to be effective against a variety of ailments in indigenous medicine such as in treatment of jaundice. In Chinese medicine, it is highly valued for the treatment of hyperlipidemia. Several polyherbal, formulations are available in Chinese market for preventing the formation of atherosclerosis plaque (7). The aim of present work is to further evaluate the hypolipidemic activity of cassia tora which grows as a common weed in India.

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 these diseases (8). The known lipid lowering drugs such as fibrates, statins, bile acid sequestraints have many side effects in patients (9). Therefore, the research and development of 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 hypolipidemic activity of Cassia tora seeds in hyperlipidemic rats.

MATERIALS AND METHODS

Preparation of root 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 (10) 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 rats. Guggulipid, a potent lipid lowering agent from Commephora mukul (Guggulipid) developed in Central Drug Research Institute, Lucknow, was used as a standard drug.

Preparation of Cholesterol rich high fat diet

Deoxycholic acid (5g) was mixed thoroughly with 700g of powdered rat Chow diet supplied by Ashirvad Industries, Chandigarh, India. Ingredient and nutrient composition of the normal rat diet was: casein 210; corn starch 440; sucrose 100; maltose dextrin 100; cellulose 50; soya bean oil 50; vitamin mix 10 and minerals 35g/kg. Other Ingredients included choline bitrate (2g/kg) and t-butyl hydroquinone (0.008 g/kg). Proximate analysis of diet showed it contained crude protein 21; crude fat 5; crude fiber 4; and ash 8%. Simultaneously Cholesterol (5g) was dissolved in 300g warm coconut oil. This oil solution of Cholesterol was added slowly into powdered mixture to obtain homogeneous soft cake. This Cholesterol rich (HFD) was molded in shape of pellet of about 3g each (10).

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 polypropioline cages and kept in uniform hygienic conditions, temperature 25-26 °C, relative humidity 50-70% and 12/12 h light/dark cycle (light from 8:00 a.m.to 8:00 p.m.) and provided with standard rat pellet diet and water ad libitum (10).

Triton and Cholesterol rich HFD induced hyperlipidemia

The rats were divided into four groups: control, hyperlipidemic, hyperlipidemic treated with Cassia tora seeds or guggulipid (standard drug). Containing six animals in each group. In the acute experiment to induce hyperlipidemia, triton WR-1339 (Sigma Chemical Company, St.Louis, MO, USA) was administered (400 mg/ kg body wt/ day p.o.) by intraperitonial injection. Cassia tora seeds extract and guggulipid were macerated with aqueous gum accacia (0.2 % w/v) suspension and fed orally at the doses of 500 and 200 mg/ kg body wt/ day p.o.), respectively, simultaneously with triton and blood was collected after 18 hrs (10). In the chronic experiment, hyperlipidemia was produced by feeding with cholesterol rich HFD for 30 days. Drugs were administered orally at the same doses as above simultaneously with cholesterol rich HFD in the drug treated groups. Control animals received the same amount of vehicle. At the end of experiment, rats were fasted overnight, anaesthetized with thiopentone solution (50mg/ kg body wt/day i.p.), prepared in normal saline. Blood was withdrawn from retro-orbital plexus using glass capillary in EDTA coated tubes (3mg/ ml blood). There after animals were sacrificed liver was excised immediately washed with cold 0.15 m KCl and kept it – 40 °C till analyses. Blood was centrifuged and plasma was taken (10).

Biochemical analysis of plasma and Liver

Plasma Post heparin lipolytic activity (PHLA) was assayed in plasma spectrophotometrically using Intralipid as artificial substrate (11) plasma was diluted with normal saline in a ratio of 1:3 and used for the analysis of total cholesterol TC (12), phospholipids PL (13) and triglyceride TG (14) using standard enzymatic kits supplied by Merck India Ltd. Mumbai India. Liver was homogenized (10%w/v) in cold 100mM phosphate buffer pH 7.2 and used for the assay of total Lipolytic; the lipoprotein lipase (LPL) activity (11). The lipid extract of each homogenate prepared in a mixture of CHCl3: CH3OH (2:1, v/v) was used for estimation of TC (12), PL (21) and TG (14). Plasma and tissue were also estimated for protein content (15).

STATISTICAL ANALYSIS

One way analysis of variance (ANOVA-New man's student t- test) was performed by comparison of values for hyperlipidemic groups with control, hyperlipidemic and drug treated groups with hyperlipidemic All hypothesis testing were two-tailed. $P<0.05$ was considered statistically significant and results were expressed as mean ± SD of six rats. The graph pad INSTAT 3.0 software carried out the statistically analysis. (16).

RESULTS

Effect of Cassia tora seeds extract in triton induced hyperlipidemia

The data in Table 1 shows that in acute administration of triton WR-1339 in rats caused marked increase in their plasma levels of TC (2.86 fold), PL (2.76 fold) and TG (2.68 fold) following inhibition of PHLA by 26%.Treatment with Cassia tora seeds extract exerted a decrease in these levels of TC (25%), PL (26%), TG (28.0 %) simultaneously with reactivation of PHLA by (22%). The hyperlipidemic action of guggulipid (31-35 %) was comparatively higher to that of Cassia tora seeds extract.

Effect of Cassia tora seeds extract in cholesterol rich HFD induced hyperlipidemia

In this model of hyperlipidemia (table-2), feeding with cholesterol rich HFD in rats caused marked increase in their

plasma levels of TC (2.34 fold), PL (1.71 fold) and TG (2.18 fold) following inhibition of PHLA by (32%). Treatment with Cassia tora seeds extract for 30 days, reversed these plasma levels of TC (25%), Pl (21%) and TG (31%) simultaneously with reactivation of PHLA by (21.0%). Feeding with cholesterol rich HFD in rats also caused marked accumulation of TC (1.53 fold), PL (1.72 fold) and TG (1.57 fold) following diminution of LPL activity (37%) in their liver (Table-3). However, treatment with Cassia tora seeds extract decrease in these levels of TC.

Effect of Cassia tora seeds extract in cholesterol rich HFD diet induced steosis in Live

(26%), PL (22%) and TG (29%) was observed following reactivation of LPL activity (26%) in hyperlipidemic animals. Guggulipid was more effective hypolipidemic to that of Cassia tora seeds.

Table 1: Effect of Cassia tora (seeds) extract and guggulipid on plasma lipids in triton induced hyperlipidemia

Experimental Schedule	Total cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	PHLA (n mol Free Fatty Acid released /h/L)
Control	82.46±3.33	90.66±8.69	87.52±6.11	16.86±0.95
Triton treated	235.89***±12.09	250.57***±24.86	234.60***±12.09	11.56**±1.34
	(+2.86 fold)	(2.76 fold)	(2.68 fold)	(-25.50 %)
Triton + *Cassia tora* (seeds) extract	177.89***±8.69	185.88**±12.80	169.87***±6.14	14.11*±0.70
	(-24.58%)	(-25.81%)	(-27.59%)	(+22.56%)
Triton + Guggulipid	159.14***±7.85	149.14***±8.23	153.13***±8.65	14.36*±0.68
	(-32.53%)	(-32.49%)	(-32.72%)	(+24.22%)

Values are mean ± SD of 6 animals. Values in parenthesis indicate percent change. Triton treated group is compared with control, triton and drug treated with triton *P<0.05; **P<0.01; ***P<0.001.

Table 2: Effect of Cassia tora (seeds) extract and guggulipid on plasma lipids in Cholesterol Rich HFD induced hyperlipidemia.

Experimental Schedule	Total cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	PHLA (n mol Free Fatty Acid released /h/L)
Control	88.59±6.98	80.62±4.29	114.80±11.30	19.31±1.30
Cholesterol rich HFD treated	208.18***±22.10 (2.34 fold)	138.27***±24.86 (1.71 fold)	251.35***±24.59 (2.18 fold)	13.11***±1.18 (-32%)
Cholesterol rich HFD + *Cassia tora* (seeds) extract	155.36**±11.13 (-25.37%)	108.86***±6.78 (-21.26%)	172.88**±6.49 (-31.21%)	15.88*±1.78 (+21.12%)
Cholesterol rich HFD+ Guggulipid	153.39***±8.83 (-26.31%)	100.30***±8.77 (-27.46%)	169.92***±6.09 (-32.72%)	16.33 *±1.64 (-24.56%)

Values are mean ± SD 0f 6 animals. Values in the parentheses indicate percent change. Cholesterol rich HFD treated group is compared with control, cholesterol rich HFD and drug treated groups with cholesterol rich HFD. *P<0.05; **P<0.01; ***P<0.001.

Table 3: Effect of Cassia tora (seeds) extract and guggulipid on Liver lipid in Cholesterol Rich HFD induced hyperlipidemia.

Experimental Schedule	Total cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglyceride (mg/dl)	LPL activity(µ mol Free Fatty Acid released/h/mg protein)
Control	6.75±0.30	22.43±2.71	5.44±0.60	79.85±4.82
Cholesterol rich HFD treated	10.33***±0.62 (1.553 fold)	38.58***±5.33 (1.72 fold)	8.56***±1.02 (1.57 fold)	50.26***±2.72 (-37%)
Cholesterol rich HFD+ Cassia tora (seeds) extract	7.67***±0.62 (-25.75%)	2940***±3.60 (-22.29%)	5.22***±0.74 (-28.62%)	63.09*±3.75 (+25.52%)
Cholesterol rich HFD +Guggulipid	7.36***±0.90 (-28.75%)	28.47***±5.04 (-26.20%)	5.11***±0.62 (-31.19%)	64.99**±4.66 (-29.30%)

Values are mean ± SD of 6 animals Values in the parenthesis indicate percent change, cholesterol rich HFD treated groups were compared with control cholesterol rich HFD and drug treated groups with cholesterol rich HFD. *P<0.05; **P<0.01; ***P<0.001.

DISCUSSION

Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting into increased blood lipid concentration (10, 17). The lipid lowering effect of Cassia tora seeds extract may be due to an early clearance of lipids from circulation in Triton model and it may be due to reactivation of lipolytic enzymes as evidenced by increased PHLA. Investigations with cholesterol rich HFD fed hyperlipidemic animals showed that seed extract stimulates PHLA and hepatic LPL activity, both of which play a key role in lipid catabolism and their utilization in the body (18), leading to decrease in the level of plasma and liver lipids in above models. It is reported that hypolipidemic action of guggulsterone, the active principle of guggulipid, is mediated through activation of PHLA, LPL and LCAT, inhibition of hepatic cholesterol biosyntheses and increased faecal bile acid excretion (19, 20, and 21). The same mechanism may also interplay in the hypolipidemic effect of Cassia tora seeds extract.

Here we have tested crude extract of Cassia tora seeds which however, upon research and development, may produce a more potent lipid lowering natural product or a pure compound like guggulipid/ guggulsterone from commephora mukul (22). Further work on drug metabolism and assessment the biological activity in vivo and in vitro Cassia tora seeds and its fraction is in progress to substantiate the present findings.

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