

Lipid Lowering Activity of Guggulsterone from *Commiphora mukul* in Hyperlipaemic Rats

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The lipid lowering action of guggulsterone, the active constituent of guggulipid, has been studied in triton and cholesterol fed hyperlipaemic rats. Serum lipids were found to be lowered by guggulsterone (50 mg/kg, b.w.) in triton WR-1339 induced hyperlipaemia. Chronic feeding of this drug (5 mg/kg, b.w.) in animals simultaneously fed with cholesterol (25 mg/kg, b.w.) for 30 days, caused lowering in the lipid and apoprotein levels of very low density and low density lipoproteins in experimental animals. Guggulsterone activates lipolytic enzymes in plasma and liver as well as stimulated receptor mediated catabolism of low density lipoprotein. The hypolipidaemic activity of this drug is mediated through inhibition of hepatic cholesterol biosynthesis, increased faecal bile acid excretion and enhanced plasma lecithin:cholesterol acyltransferase activity.

Keywords: *Commiphora mukul*; guggulip; triton model; hyperlipaemia; lipid lowering agents; guggulsterone.

INTRODUCTION

Guggul gum, a resin obtained from an ethyl acetate extract of *Commiphora mukul*, possesses marked hypolipidaemic activity (Nityanand and Kapoor, 1973). A standardized fraction from this resin containing a mixture of lipid steroids, and named as guggulipid, has been marketed as a new hypolipidaemic lipid lowering agent (Nityanand and Kapoor, 1984). The active constituents of this preparation are two isomers (Z and E) of guggulsterone mixed with some other steroids, diterpenes, esters and higher alcohols (Patil *et al.*, 1972; Satyavati, 1991). Pharmacological studies in experimental animals showed guggulsterone caused an alteration in the levels of biogenic amines and dopamine β -hydroxylase activity which may be one of the possible mechanisms of antilipaeamic action of this compound (Srivastava and Kapoor, 1986). Administration of guggulsterone in normal rats caused a decrease in serum and membrane lipids followed by enhanced catabolism of low density lipoprotein (LDL) through hepatic receptors (Singh *et al.*, 1990). With a view to understanding the mode of action of lipid lowering drugs, the effect of guggulsterone on lipid and lipoprotein metabolism was investigated in triton and cholesterol induced hyperlipaemic rats. The lipid lowering potential of guggulsterone was compared with that of gemfibrozil.

MATERIALS AND METHODS

Animals. Adult male Charles Foster rats (150–200 g) bred in the animal house of the Institute were housed in uniform hygienic conditions and kept on a standard pellet diet (Lipton India Ltd.) and water *ad libitum*.

Triton and cholesterol induced hyperlipaemia. The rats were divided into control, triton and triton plus drug treated

groups of six in each. In the acute experiment triton WR-1339 (Sigma Chemical Company, St. Louis, MO, USA) was administered (400 mg/kg, b.w.) by intraperitoneal injection for 18 h. Guggulsterone and gemfibrozil (CIPLA Ltd, Bombay, India) were macerated with 0.2% aqueous gum acacia suspension and fed orally (50 mg/kg, b.w.) simultaneously with triton. In the chronic experiment hyperlipaemia was produced by feeding with cholesterol (25 mg/kg, b.w.) suspended in refined ground nut oil (0.5% w/w) once a day for 30 days. Drugs were administered (5 mg/kg, b.w.) orally simultaneously with cholesterol in drug treated groups. Control animals received the same amount of normal saline or ground nut oil. At the end of the experiments, rats were fasted overnight and blood was withdrawn. The animals were killed and the liver was excised immediately.

Biochemical analysis of plasma/serum. Plasma lecithin:cholesterol acyltransferase (LCAT) activity (Nagasaki and Akanuma, 1977) and post heparin lipolytic activity (PHLA) were assayed (Wing and Robinson, 1968). Serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanionic precipitation methods (Burstein and Legmann, 1982). Serum as well as lipoproteins were analysed for their total cholesterol (TC), phospholipids (PL), triglyceride (TG) and protein by standard procedures reported earlier (Chander *et al.*, 1988; Khanna *et al.*, 1993).

Biochemical analysis of liver. Liver was homogenized (10% w/v) in cold 100 mM phosphate buffer pH 7.2 and used for the assay of total lipolytic activity (Wing and Robinson, 1968). The lipid extract of each homogenate was used for estimation of TC, PL, TG and protein. The hepatic rate of cholesterol biosynthesis was investigated using [1- C^{14}]-sodium acetate (Nityanand and Kapoor, 1973). Human serum LDL was prepared, radio-labelled with I^{125} and the binding of this I^{125} LDL with liver plasma membrane preparation (Kovanen *et al.*, 1979) was assayed as described by Singh *et al.* (1990).

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Faecal bile acids. The rat faeces were collected from all groups throughout 30 days and processed for estimation of cholic and deoxycholic acid (Mosbach *et al.*, 1954).

Statistical analysis. Data were analysed using Student's 't'-test. Hyperlipaemic groups were compared with control, hyperlipaemic and drug treated with hyperlipaemic. $p < 0.05$ was considered as significant.

RESULTS

Effect of guggulsterone in triton induced hyperlipaemia

The acute administration of triton WR-1339 caused a marked increase in serum levels of TC (4.27 fold), PL (82%), TG (43%) and protein (59%). Treatment with

guggulsterone caused reversal in these levels of TC (42%) together with a decrease in PL, TG and protein by 19–26% (Table 1). The lipid lowering activity of guggulsterone in the hyperlipaemic rats was comparable to that of gemfibrozil.

Effect of guggulsterone on lipid composition in serum lipoproteins and liver

The data in Table 2 show that administration of cholesterol in rats increased their serum levels of TC, PL and TG by 131%, 91% and 89% respectively. Feeding with guggulsterone and gemfibrozil reversed the levels of these serum lipids (33–40%) in cholesterol and drug treated animals. The analysis of hyperlipaemic serum showed a marked increase in the level of lipids and apoproteins constituting

Table 1. Effect of guggulsterone and gemfibrozil on serum lipids in triton-induced hyperlipaemia

Parameter	Control	Triton treated	Triton and guggulsterone treated	Triton and gemfibrozil treated
Total cholesterol ^a	86.74 ± 5.91	370.39 ± 16.23	214.07 ± 10.32	203.01 ± 4.53
Phospholipid ^a	67.40 ± 3.63	122.78 ± 7.0	90.76 ± 5.12	89.71 ± 4.69
Triglyceride ^a	83.55 ± 3.92	119.55 ± 4.42	90.66 ± 4.81	85.68 ± 4.55
Protein ^b	5.64 ± 0.23	8.97 ± 0.31	7.23 ± 0.44 ^c	6.84 ± 0.37 ^c

^a mg/dL serum, ^b g/dL serum.

Values are mean ± SD from 6 animals $p < 0.001$, except^c ($p < 0.01$), triton group compared with control, triton and drug treated with triton.

Table 2. Effect of guggulsterone and gemfibrozil on blood lipids and lipolytic enzymes in hyperlipaemic rats

Parameter	Control	Cholesterol treated	Cholesterol and guggulsterone treated	Cholesterol and gemfibrozil treated
A. Serum				
Total cholesterol ^a	86.66 ± 5.10	200.58 ± 15.12	124.37 ± 7.81	124.21 ± 6.00
Phospholipid ^a	88.00 ± 7.70	168.80 ± 12.30	113.33 ± 4.51	113.37 ± 8.12
Triglyceride ^a	106.88 ± 9.00	201.93 ± 14.44	128.21 ± 5.59	120.77 ± 7.16
Protein ^a	6.05 ± 0.17	10.88 ± 0.33	6.23 ± 0.17	6.21 ± 0.19
B. VLDL				
Total cholesterol ^a	8.32 ± 0.41	32.43 ± 2.12	23.09 ± 1.62	21.53 ± 1.33
Phospholipid ^a	14.87 ± 0.31	30.18 ± 1.24	20.12 ± 1.80	20.00 ± 0.31
Triglyceride ^a	38.69 ± 1.27	86.77 ± 5.12	55.01 ± 2.82	53.94 ± 3.00
Apoprotein ^a	6.30 ± 0.50	12.12 ± 1.90	9.15 ± 0.64	7.75 ± 0.52
C. LDL				
Total cholesterol ^a	13.23 ± 0.88	64.16 ± 5.72	40.29 ± 3.67	41.30 ± 2.88
Phospholipid ^a	12.14 ± 0.47	43.36 ± 3.36	30.41 ± 2.73	29.73 ± 1.64
Triglyceride ^a	15.12 ± 0.17	36.62 ± 2.68	27.12 ± 2.12	25.00 ± 2.00
Apoprotein ^a	17.56 ± 1.00	28.62 ± 1.88	19.50 ± 1.33	16.30 ± 1.08
D. HDL				
Total cholesterol ^a	45.38 ± 3.71	38.14 ± 2.80 [°]	44.28 ± 4.00 ^{NS}	44.19 ± 3.64 ^{NS}
Phospholipid ^a	37.41 ± 2.61	28.81 ± 2.14	32.83 ± 2.66 ^{NS}	33.00 ± 2.21 ^{NS}
Triglyceride ^a	15.14 ± 1.10	12.13 ± 0.94	15.09 ± 1.14	15.00 ± 0.82
Apoprotein ^a	168.20 ± 13.5	120.35 ± 14.4	140.80 ± 7.50 [°]	148.24 ± 10.0
E. Plasma				
LCAT activity ^c	67.59 ± 3.94	37.77 ± 2.66	48.39 ± 2.42	50.26 ± 3.32
PHLA ^d	17.66 ± 1.06	10.38 ± 0.70	13.72 ± 0.64	14.00 ± 0.66

^a mg/dL serum, ^b g/dL serum, ^c nmol cholesterol released/h/L plasma, ^d nmol free fatty acid formed/h/mL plasma.

Values are mean ± SD from 6 animals; $p < 0.001$, except[°] ($p < 0.05$), NS, not significant, cholesterol compared with control, cholesterol and drug treated with cholesterol.

Table 3. Effect of guggulsterone and gemfibrozil on hepatic biochemical parameters and faecal bile acids excretion in hyperlipaemic rats

Parameter	Control	Cholesterol treated	Cholesterol and guggulsterone treated	Cholesterol and gemfibrozil treated
A. Liver				
LPL activity ^a	130.37 ± 8.84	71.23 ± 3.42	81.81 ± 6.12*	88.95 ± 5.02
Total cholesterol ^b	6.62 ± 0.14	10.04 ± 0.32	8.42 ± 0.10*	8.00 ± 0.31
Phospholipid ^b	23.33 ± 2.00	36.12 ± 1.87	19.92 ± 1.70	19.08 ± 1.00
Triglyceride ^b	10.34 ± 0.70	15.72 ± 0.88	12.22 ± 1.1	11.87 ± 0.89
Protein ^b	150.3 ± 12.5	217.5 ± 15.0	180.0 ± 10.3*	174.2 ± 12.6*
Specific binding of ¹²⁵ I-LDL ^c	40437 ± 300	19668 ± 138	27684 ± 3.77	24948 ± 369
Lipid biosynthesis				
Total sterol ^e	2488 ± 161	1533 ± 77	1212 ± 61*	1181 ± 57*
Cholesterol digitonoid ^e	1228 ± 53	666 ± 33	530 ± 27*	500 ± 40
Free fatty acid ^e	2008 ± 90	1558 ± 50*	1203 ± 37*	1166 ± 35*
B. Faecal bile acids				
Cholic acid ^d	81.47 ± 4.87	47.62 ± 3.14	58.12 ± 3.90*	54.42 ± 4.00
Deoxycholic acid ^d	53.66 ± 3.00	23.31 ± 1.66	37.28 ± 2.41	34.40 ± 2.33

^anmol free fatty acid formed/h/mg protein; ^bmg/g; ^ccount/min/mg protein; ^dμg/g. Values are mean ± SD from 6 animals; *p* < 0.001, except* (*p* < 0.01), cholesterol compared with control, cholesterol and drug treated with cholesterol.

β -lipoproteins and these effects were pronounced for VLDL-TG (130%) and LDL-TC (384%). Treatment with guggulsterone and gemfibrozil significantly reduced these levels of VLDL lipids (22–38% and 34–39%) as well as LDL-TC (37% and 36%), PL (30% and 31%), TG (26% and 32%) and apo-LDL (32% and 43%) respectively in hyperlipaemic rats. At the same time the decreased levels of HDL-lipids and apo-HDL in these animals were partially recovered (Table 2). The increased levels of TC, PL, TG and protein in liver (52%, 55%, 52% and 45%) of cholesterol fed rats were observed to be lowered by their treatment with drugs (Table 3).

Effect of lipolytic enzymes

Cholesterol feeding caused the inhibition of plasma LCAT (44%), and PHLA (41%) respectively (Table 2). and total lipolytic activity (45%) in liver (Table 3). Treatment with guggulsterone and gemfibrozil partially reactivated these lipolytic activities in plasma and liver of hyperlipaemic rats.

Hepatic cholesterol biosynthesis and ¹²⁵I-LDL catabolism

Administration of cholesterol suppressed (51%) the specific binding of ¹²⁵I-LDL in the liver membrane. Guggulsterone and gemfibrozil partially reversed the receptor mediated catabolism of LDL (40% and 27% respectively) in treated animals (Table 3). Administration of guggulsterone in hyperlipaemic rats inhibited the overall hepatic lipid biosynthesis as observed by the decreased incorporation of [¹⁴C] sodium acetate in total sterol (51%), cholesterol digitonoid (57%) and FFA (40%) fractions of liver lipids compared with the control group and these effects were comparable to gemfibrozil (Table 3).

Effect on faecal excretion of bile acids

Feeding with cholesterol caused a significant decrease in the faecal excretion of cholic acid (42%) and deoxycholic acid (56%) and these levels were shown to be recovered by the treatment with guggulsterone (22%–59%) and gemfibrozil (14%–48%) in cholesterol and drug fed animals.

DISCUSSION

Guggulsterone and gemfibrozil both cause a significant decrease in the serum level of lipids in triton induced hyperlipaemic rats and this model has been successfully used for the evaluation of lipid lowering activity of natural products in rats (Nityanand and Kapoor, 1973; Khanna *et al.*, 1992, 1993). The present investigation with cholesterol fed hyperlipaemic animals shows that guggulsterone could increase the level of HDL by increasing the activity of LCAT, which plays a key role in lipoprotein metabolism. The increase of the receptor mediated catabolism of LDL as well as the lipolytic activity in liver and the level of blood HDL-TC followed by the decrease of β -lipoprotein-lipids and the suppression of hepatic cholesterol biosynthesis by guggulsterone are of great utility for regressing atherosclerosis. The stimulation of LDL catabolism by guggulsterone in hyperlipaemic animals may be due to a significant decrease in the levels of serum and tissue lipids. This drug may also enhance the synthesis of LDL apoprotein (Apo B) as well as receptor protein to accelerate the turnover of cholesterol. Increased synthesis of receptor protein decreased the rate of hepatic cholesterol biosynthesis and inhibition of oxidative modifications in LDL may regulate the cholesterol level in the body (Windler *et al.*, 1980; Henriksen *et al.*, 1983) Guggulsterone has been reported to decrease the levels of lipid peroxidation products in the liver membranes of treated animals (Singh *et al.*, 1990; Kaul and Kapoor, 1989). Recently we have found that guggulsterone inhibits the oxidative changes in LDL-lipids and protein induced by Cu⁺² and Fe⁺² *in vitro*. It

has been reported that the hypolipidaemic activity of natural products such as *Achyranthus aspera*, *Terminalia chebula* and picroliv (Khanna *et al.*, 1992, 1993) may be linked with increased faecal bile acid excretion and with the inhibition of cholesterol biosynthesis.

In conclusion, the lipid lowering action of guggulsterone may be due to activation of LCAT and tissue lipolytic enzymes, enhanced catabolism of LDL, increased faecal bile acid excretion as well as inhibition of hepatic cholesterol biosynthesis, and some of these effects were

comparable to that of gemfibrozil. The study reveals that guggulsterone is a better drug as a natural product, in its hypolipidaemic and antioxidant activities as well as in the rapid catabolism of low density lipoprotein.

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