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Artigo

Clerodendron glandulosum Coleb., Verbenaceae, ameliorates high fat diet-induced alteration in lipid and cholesterol metabolism in rats

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RESUMO: "*Clerodendron glandulosum* Coleb., Verbenaceae, melhora a dieta rica em gordura induzida por alteração no metabolismo de lipídios e colesterol em ratos." Este estudo foi realizado para avaliar a eficácia do extrato liofilizado das folhas de *Clerodendron glandulosum* Coleb., Verbenaceae (FECG), em alterar o metabolismo de lipídios e colesterol em ratos hiperlipidêmicos alimentados em uma dieta rica em gordura. Plasma e perfil lipídico hepático, lipídeos e enzimas que metabolizam o colesterol em tecidos-alvo e o conteúdo de lipídeos fecais totais e ácidos biliares foram avaliados em ratos normolipidêmicos e hiperlipidêmicos tratados com FECG. Os resultados foram comparados com a droga sintética hipolipemiante Lovastatina (LVS). Os resultados indicam que FECG foi capaz de regular positivamente a hiperlipidemia induzida experimentalmente por alteração significativa no perfil lipídico do plasma e tecidos. Estes resultados podem ser atribuídos à absorção reduzida, a eliminação efetiva e catabolismo aumentado de lipídeos e colesterol, possivelmente devido ao alto teor de saponina e fitoesteróis em *C. glandulosum* Coleb. Uso de extrato de *C. glandulosum* como um possível agente terapêutico contra a hipercolesterolemia e hipertrigliceridemia é indicado.

Unitermos: *Clerodendron glandulosum,* Verbenaceae, hiperlipidemia, hipercolesterolemia.

ABSTRACT: The present study was undertaken to evaluate the efficacy of freeze dried extract of *Clerodendron glandulosum* Coleb., Verbenaceae, leaves (FECG) on alteration in lipid and cholesterol metabolism in high fat diet fed hyperlipidemic rats. Plasma and hepatic lipid profiles, lipid and cholesterol metabolizing enzymes in target tissues and fecal total lipids and bile acid contents were evaluated in FECG treated normolipidemic and hyperlipidemic rats. These results were compared with synthetic hypolipidemic drug Lovastatin (LVS). Results indicate that FECG was able to positively regulate induced experimental hyperlipidemia by significant alteration in plasma and tissue lipid profiles. These results can be attributed to reduced absorption, effective elimination and augmented catabolism of lipids and cholesterol possibly due to high content of saponin and phytosterols in *C. glandulosum*. Use of *C. glandulosum* extract as a potential therapeutic agent against hypercholesterolemia and hypertriglyceridemia is indicated.

Keywords: *Clerodendron glandulosum,* Verbenaceae, hyperlipidemia, hypercholesterolemia.

INTRODUCTION

Diets containing high amount of fats or cholesterol lead to both hypercholesterolemia and hypertriglycedemia which are major prognosis for cardiovascular diseases (Reiner & Tedeschi-Reiner, 2006); and leading causes of death in developing and developed countries (Yokozawa et al., 2003) The World Health Organization (WHO) estimates that sixty per cent of the world's cardiac patients will be Indians by 2010 (Radhakrisnan, 2008). In view of the adverse effects of synthetic lipid-lowering drugs, the search for natural products with lipid-lowering potential and with minimal or no side effect is recommended (Adaramoye et al., 2008). In recent times research interest has focused on various herbs that possess hypolipidemic property that may be useful in reducing the risk of cardiovascular disease (Craig, 1999). Because of their perceived effectiveness, minimal side effects and, relative low cost, herbal drugs are prescribed widely even when their biologically active compounds are unknown (Valiathian, 1998). North eastern states of India (biodiversity hotspots) house a treasure-trove of plants with novel medicinal properties (Albert & Kuldip, 2006). These plants have found a prime place in the indigenous system of medicine and are in focus for evaluation of their beneficial effects (Sudhir, 2002). *Clerodendron glandulosum* Coleb., Verbenaceae, is a herb

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found in many part of north east India and used by rural and urban people against diabetes obesity and hypertension. Apatani Tribe uses the leaves of *C. glandulosum* for controlling hypertension and fever (Kala, 2005), while the tender shoots are used by people of Debaru biosphere region against abdominal pain (Puryakashtha et al., 2005). Previous study from our laboratory had documented antioxidant property of *C. glandulosum* extract (Jadeja et al., 2009).This inventory is an effort to evaluate efficacy of *C. glandulosum* extract on alterations in lipid and cholesterol metabolism in hyperlipidemic rats.

MATERIAL AND METHODS

Plant material

Clerodendron glandulosum Coleb., Verbenaceae, leaves were collected from Imphal district India in the month of June and shade dried. The plant was identified by Dr. Hemchand Singh, at the Department of Botany, D.M. College of Science Manipur, Imphal and a sample (voucher specimen No.405) was deposited at the herbarium of the Department of Botany.

Preparation of freeze dried extract

Leaves of *C. glandulosum* were shade dried and fine powdered. Powdered leaves (100 g) was boiled in distilled water at 100 ºC for 3 h. Resulting filtrate was concentrated by heating to obtain a semisolid paste that was later freeze dried resulting in a total yield of 28% w/w. Different doses of freeze dried extract (FECG) were prepared by dissolving a known weight of dried paste in 0.5% carboxy methylcellulose (CMC).

Phytochemical analysis

The quantitative phytochemical analysis of *C. glandulosum* leaves for saponins (Xi et al., 2007), phytosterols (Goad & Akihisa, 1997), ascorbic acid (Barakat et al., 1973), polyphenols (Yen & Hsieh, 1998) and flavanoids (Chang et al., 2002) was carried out.

Experimental animals

Female *Charles foster* albino rats (180-220 g) were housed and maintained in clean polypropylene cages under controlled room temperature (24±2 ºC) and were fed with commercially available rat chow (SLD; M/s Pranav Agro Ltd., Baroda) or hyperlipidemic diet (HFD; Rathi et al., 1984) and provided with water *ad libitum*. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethical Committee (Approval No.827/ac/04/CPCSEA).

Experimental design

Fifty four animals were divided into nine groups (n = 6 per group).Groups I and V were fed with SLD and HFD respectively. Groups II, III, and IV were maintained on SLD and orally administered with 200, 400 or 800 mg/kg FECG extract daily. Groups VI, VII and VIII were maintained on HFD and administered with FECG extract as mentioned above. Group IX was fed with HFD and received synthetic hypolipidemic agent, lovastatin (LVS; 5 mg/kg BW). Animals were maintained for a total of 42 days (six weeks) during which they were orally administered with vehicle, extract or LVS by gastric intubation.

Lipid profile

Plasma Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) were analyzed using commercially available kits (Merck diagnostics Ltd, Mumbai, India). Very Low Density Lipoproteins (VLDL) and Low Density Lipoprotein (LDL) were calculated by Friedwald's formula (Friedewald et al., 1972). Hepatic and fecal lipids were extracted in chloroform:methanol (2:1) mixture and dried. Total Lipids (TL) were estimated by gravimetric analysis (Folch et al., 1957). Dried lipid extract was dissolved in 1% triton X 100 (Thounaojam et al., 2009) and TC and TG were analyzed using above mentioned kits.

Fecal cholic acid (CA) and deoxycholic acid (DCA)

Fecal samples from each experiment group were collected on every third day between days thirty one and forty two of study. Fecal samples were dried, eluted with absolute alcohol, filtered and processed for estimation of CA and DCA (Mosback et al., 1954).

Estimation of lipid metabolizing enzymes

Freshly excised tissues (liver and adipose tissues) were rinsed with 0.5 M KCl and kept in -80 ºC for further use. Activity levels of Lipoprotein lipase (LPL; EC 3.1.1.34) were estimated in fresh tissue homogenates (Mays & Felts, 1968; Itaya & Ui, 1965). Heparin (50 units/rats) was injected intravenously to facilitate release of endothelium bound LPL, two min before blood collection. Plasma was separated and post-heparin lypolytic activity (PHLA) was assayed (Qi et al., 2006).

Estimation of cholesterol metabolizing enzymes

Activity levels of Lecithin-Cholesterol Acyltransferase (LCAT, EC 2.3.1.43) was assayed in plasma by the method of Legraud et al. (1979) with modifications by Hitz et al. (1983) HMG CoA reductase (HMG-CoA reductase, EC 1.1.1.34) activity was assayed as per the method of Rao & Ramakrishnan (1975)

and expressed as inversely proportional to the mevalonate content. Cholesterol ester synthase (CES, EC 2.3.1.26) was assayed by the method of Kothari et al. (1973) in liver and intestine. Protein content in liver, adipose tissue and intestine was analyzed by the method of Lowry et al. (1951).

Acute oral toxicity in mice

Balb/c mice of either sex were divided into groups of six mice each (three male & three female). They were orally administered with a single dose of FECG at 1.6 g, 3.2 g, 6.4 g and 8.0 g/kg bodyweight. They were observed for 48 h post administration.

Statistical analysis

Statistical evaluation of the data was done by one way ANOVA followed by Bonferroni's multiple comparison test .The results are expressed as mean±S.E.M using Graph Pad Prism version 3.0 for Windows, Graph Pad Software, San Diego California USA.

RESULTS

Phytochemical analysis and acute oral toxicity

Quantitative phytochemical analysis of *C. glandulosum* leaf extract recorded 4.36% flavanoids, 6.39% sterols, 5.23% saponins, 3.22% polyphenols and 5.96% vitamin C. There was no mortality and gross behavioral changes in acute oral toxicity test. Plasma analysis for liver and kidney functions did not record any adverse effect in FECG administered rats (Data not shown).

Plasma and hepatic lipid profile

FECG did not record any significant effect in SLD fed rats but, the extract had a dose dependent effect in reversing the altered serum and hepatic lipid profile induced by HFD (Table 1 and 2). A comparison of FECG treated groups with LVS treated rats revealed a better response in the former with respect to plasma triglyceride and HDL levels (Table 1).

Fecal lipid profile and bile acid content

HFD rats did not show any significant changes in fecal TL, TC, TG, cholic acid and deoxycholic acid contents (Table 3). The extract fed rats recorded a highly significant increment in fecal TL, TC and TG contents with all the three doses compared to HFD rats ($p<0.001$). HFD+FECG recorded a dose dependent increment in fecal CA and DCA compared to HFD groups ($p < 0.001$). HFD+LVS rats recorded highly significant increment in fecal TL and TC ($p<0.001$) and a non significant increment in fecal TG, CA and DCA.

Lipid metabolizing enzymes

HFD rats recorded significant decrement in activity levels of PHLA, and LPL in adipose tissue and liver ($p<0.001$). HFD+FECG 200 mg/kg did not record any significant effect in PHLA and LPL activities. HFD+FESR (400 and 800 mg/kg) significant resistance towards decrease in PHLA and LPL activities compared to HFD rats ($p<0.001$). HFD+LVS rats registered non significant effect in PHLA and LPL activities compared to HFD group (Table 4). The extract had no significant effect in control rats fed with SLD (Table 4).

Cholesterol metabolizing enzymes

HFD rats recorded a significant decrement in plasma LCAT activity ($p<0.001$) and increment in hepatic and intestinal CES ($p<0.001$). Hepatic HMG Co A reductase activity registered a non significant alteration in HFD rats. Higher doses of FESR (400 and 800 mg/kg) depicted dose dependent decrement in the activity level of HMG Co A reductase ($p<0.05$ and $p<0.01$) and prevented decrease in plasma LCAT activity ($p<0.001$). HFD+FECG rats recorded a dose dependent protective effect against decrement in hepatic and intestinal CES activity (Table 6). HFD+LVS rats recorded significant decrease in HMG Co A reductase activity ($p<0.001$) along with protective effect against the decrease in intestinal and hepatic CES activities ($p<0.01$) and a non significant change in plasma LCAT activity compared to HFD rats. The extract had no significant effect in hepatic HMG Co A reductase, plasma LCAT and, CES activities in liver and intestine in SLD fed rats (Table 5).

DISCUSSION

The present study has investigated the effects of FECG on lipid and cholesterol metabolisms in normolipidemic and hyperlipidemic rats. The cholesterol lowering property of FECG could be due to increased excretion of cholesterol and bile acids (Cholic acid & Deoxycholic acid) in feaces. These observed effects can be attributed to presence of phytosterol in FECG as; phytosterols possesses greater affinity for micelles than cholesterol and reduce incorporation of cholesterol in micelles (Ikeda & Sugano, 1998). Liver serves as the primary site for elimination of cholesterol from the body. In the liver, free cholesterol is either secreted directly into bile or it can be converted to oxysterols (Zhang et al., 2001). Oxysterols stimulate expression of CYP7A1, thereby increasing conversion of cholesterol to bile acids (Lehmann et al., 1997). High content of fecal bile acids in HFD+FECG rats (CA & DCA) indicates that FECG stimulates removal of excess cholesterol via increased bile

acid excretion in feaces.

Observed decrease in HMG Co A reductase activity in HFD+FECG rats, indicates diminished *de novo* cholesterol synthesis. Decreased hepatic cholesterol levels are known to induce LDL receptor expression to promote LDL clearance from circulation in order to maintain hepatic cholesterol homeostasis (Ma et al., 1986). Thus it can be speculated that FECG decreases circulating LDL via increased LDL-R expression. A decreased plasma LDL level recorded in HFD+FECG groups corroborates this hypothesis.

Cholesterol ester synthase (CES) in known to be involved in esterification and incorporation of free cholesterol in chylomicron and VLDL (Klein & Rudel, 1983; Rudel & Shelness , 2000). Recorded low activity of CES in intestine and liver of HFD+FECG rats indicates decreased esterification of cholesterol resulting in impaired incorporation of Cholestryl ester into chylomicrons and VLDL leading to, low levels of circulating cholesterol (Table 2) along with high fecal cholesterol content (Table 4). These observed effects are in accordance with other published reports on the effect of plant products on CES activity (Song et al., 2002).

A low level of HDL is directly correlated with an increased risk of cardiovascular disease (Wilson et al., 1988). FECG recorded a dose dependent increment in plasma HDL levels in HFD fed rats (Table 2) that can be correlated with an increased plasma LCAT activity (Table 6). Flavanoids and polyphenols are known to increase HDL levels (Daniel et al., 2003). Presence of flavanoids in FECG (4.36%) could thus be responsible for the observed higher HDL levels.

Phytocompounds like saponins are known to inhibit pancreatic lipase in HFD fed animals, leading to greater fat excretion due to reduced intestinal absorption of dietary fats (Han et al., 2002). Hence, increased elimination of TG through feaces of HFD+FECG rats seen in the present study can be related with the high saponin content (4.5%) in FECG. LPL hydrolyses triacylglycerols (TG) from chylomicrons thereby generating nonesterified fatty acids (NEFA) for subsequent tissue uptake and metabolism (Sattler et al., 1996) HFD rats in the present study recorded suppressed post heparin lypolytic activity levels (PHLA) while HFD+FECG rats recorded significantly elevated levels. These observations are in accordance with the previous studies in hyperlipidemic rats fed with plant products (Khanna et al., 1996) .Similarly, lipoprotein lipase (LPL) is related to hydrolysis of TG molecules and lypolytic activity in tissues. Elevated LPL activity in hepatic and adipose tissues of HFD+FESR rats could be held responsible for reducing tissue lipid load.

In recent times, therapeutic approach for treatment of hyperlipidemia has been shifted towards a combination therapy with synthetic drugs (i.e. niacin extended lovastatin release tablet) as, cholesterol lowering drugs are having a moderate effect on triacylglyceride levels (Richard, 2008). Another drawback of synthetic drugs is their inability to increase HDL levels (Wilson, 1990). Present study clearly indicates that treatment with *Clerodendron glandulosum* Coleb. (Verbenaceae) is able to significantly lower plasma TG levels and elevates plasma HDL levels, and can therefore be a candidate for alternative therapy for treatment of hypercholesterolemia and hypertriglyceridemia.

Table 1. Effect of FECG and LVS on plasma lipid profile.

Groups	Treatment	Total cholesterol$	Triglycerides$	HDL$	LDL$	VLDL$
I	SLD	32.80±3.57	40.86±5.31	20.70±1.71	20.27±3.00	8.17±0.86
II	SLD+200mgCG	33.91±8.34	39.63±2.45	19.24±1.51	22.59±2.49	7.92±0.49
III	SLD+400mgCG	28.57±2.87	44.26±2.01	19.48±2.31	18.43±2.03	8.85±0.40
IV	SLD+800mgCG	30.99±5.53	44.48±3.00	17.67±1.51	22.22±6.01	8.89±0.59
V	HFD	93.36±5.36^{C}	170.29±10.99^{C}	9.20±1.21C	118.21±5.06^{C}	34.05±1.02^{C}
VI	HFD+200mgCG	54.84±5.96^{b}	80.69±6.69^{b}	16.49±1.00^{a}	47.50±3.07^{c}	16.13±0.34^{b}
VII	HFD+400mgCG	42.36±3.69^{c}	56.17±9.67^{c}	22.31±1.21^{b}	31.28±2.36^{c}	11.23±0.56^{c}
VIII	HFD+800mgCG	32.03±3.79^{c}	48.05±5.69^{c}	32.51±3.95^{c}	20.15±2.67^{c}	9.61±0.93^{c}
IX	HFD+5mgLVS	36.43±4.60^{c}	72.05±5.65^{b}	18.68±1.68^{a}	28.16±3.96^{c}	14.41±1.13^{b}

Where $ = mg/dL; CG = *C. glandulosum*; a = p<0.05, b = p <0.01, c = p<0.001 and ns = non significant when, Group V vs. Group VI, VII, VIII and IX. A = p <0.05, B = p<0.01, C = p<0.001and NS = non significant when, Group I vs. Group V.

Table 2. Effect of FECG and LVS on liver lipid profile.

Groups	Treatment	Total lipids#	Cholesterol#	Triglycerides#
I	SLD	61.30±1.30	9.04±0.49	19.50±1.11
II	SLD+200mgCG	61.70±1.41	9.90±0.76	19.70±1.19
III	SLD+400mgCG	63.10±1.39	9.96±1.08	20.60±1.72
IV	SLD+800mgCG	62.00±1.94	11.80±0.59	18.90±0.545
V	HFD	127.20±5.21^{C}	22.80±1.49^{C}	51.50±1.67C
VI	HFD+200mgCG	96.70±5.78^{c}	14.80±0.96^{b}	38.80±0.94^{c}
VII	HFD+400mgCG	89.30±2.81^{c}	13.70±1.67^{c}	32.80±2.60^{c}
VIII	HFD+800mgCG	69.30±1.54^{c}	10.90±1.63^{c}	23.70±1.45^{c}
IX	HFD+5mgLVS	74.80±2.53^{c}	12.60±0.20^{c}	41.70±1.07^{b}

= mg/g; CG = *C. glandulosum*; a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ and ns = non significant when, Group V vs. Group VI, VII, VIII and IX. A = $p < 0.05$, B = $p < 0.01$, C = $p < 0.001$and NS = non significant when, Group I vs. Group V.

Table 3. Effect of FECG and LVS on fecal lipids and bile acid content.

Groups	Treatment	Total lipids$	Cholesterol$	Triglycerides$	Cholic acid#	Deoxycholic acid#
I	SLD	28.30±2.07	8.43±0.45	8.82±0.49	29.90±1.23	20.60±1.67
II	SLD+200mgCG	29.30±2.16	6.42±0.27	8.52±0.31	31.00±1.43	22.00±1.55
III	SLD+400mgCG	32.00±1.55	5.00±0.54	9.13±0.15	31.70±1.80	23.40±2.46
IV	SLD+800mgCG	31.20±2.20	4.04±0.28	10.30±0.34	32.00±2.31	21.80±1.71
V	HFD	30.00±1.42^{NS}	6.93±1.39^{NS}	11.30±0.57^{NS}	35.50±3.07^{NS}	24.40±2.30^{NS}
VI	HFD+200mgCG	50.20±1.78^{c}	19.08±1.52^{c}	21.90±1.23^{c}	58.90±4.17^{b}	36.10±2.29^{a}
VII	HFD+400mgCG	66.70±2.15^{c}	24.15± 0.03^{c}	25.40±1.35^{c}	25.40±1.35^{e}	69.00±2.52^{e}
VIII	HFD+800mgCG	77.60±1.48^{c}	27.66±0.66^{c}	29.00±0.87c	77.60±7.50^{c}	53.90±3.81^{c}
IX	HFD+5mgLVS	41.10±3.43^{a}	14.25±1.90^{a}	15.80±2.29^{ns}	15.80±2.29^{ns}	33.80±1.74^{ns}

Where $ = mg/g feaces, # = µg/g feaces; CG = *C. glandulosum*; a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ and ns = non significant when, Group V vs. Group VI, VII, VIII and IX. A = $p < 0.05$, B = $p < 0.01$, C = $p < 0.001$and NS = non significant when, Group I vs. Group V

Table 4. Effect of FECG and LVS on enzymes of lipid metabolism.

Groups	Treatment	Post heparin lipolytic activity$	Lipoprotein lipase$ (Liver)	Lipoprotein lipase$ (Adipose)
I	SLD	14.41±0.42	130.20±2.18	68.93±1.46
II	SLD+200mgCG	14.00±0.40	131.70±2.49	73.73±2.17
III	SLD+400mgCG	14.00±0.44	133.10±2.19	77.01±1.76
IV	SLD+800mgCG	13.20±0.44	132.00±2.29	76.77±3.21
V	HFD	8.36±0.34^{C}	82.74±2.23^{C}	40.23±3.02^{C}
VI	HFD+200mgCG	9.28±0.36^{NS}	86.76±3.49^{ns}	50.20±2.48^{ns}
VII	HFD+400mgCG	12.60±0.59^{c}	106. 30±2.06^{c}	64.50±2.91^{c}
VIII	HFD+800mgCG	14.50±0.54^{c}	110.50±2.54^{c}	71.27±1.15^{c}

$ = µmol of FFA formed /hr/dl plasma; CG = *C. glandulosum*; a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ and ns = non significant when, Group V vs. Group VI, VII, VIII and IX. A = $p < 0.05$, B = $p < 0.01$, C = $p < 0.001$and NS = non significant when, Group I vs. Group V.

Table 5. Effect of FECG and LVS on enzymes of cholesterol metabolism.

Groups	Treatment	Hepatic HMG CoA reductase activity[$]	Plasma lecithin cholesterol acyl transferase[#]	Hepatic cholesterol ester synthase[@]	Intestine cholesterol ester synthase[@]
I	SLD	2.22±0.11	60.80±1.14	11.20±.41	12.80±0.84
II	SLD+200mgCG	2.49±0.19	60.80±2.08	9.86±0.79	14.70±1.72
III	SLD+400mgCG	2.16±0.23	59.00±1.62	10.40±0.56	14.00±0.70
IV	SLD+800mgCG	2.42±0.15	60.50±1.30	9.50±0.70	12.90±0.45
V	HFD	2.61±0.35^{NS}	32.30±1.09^{C}	29.60±2.05^{C}	35.00±2.08^{C}
VI	HFD+200mgCG	3.90±0.48^{ns}	38.50±1.26^{ns}	15.40±1.19^{c}	24.10±1.91^{b}
VII	HFD+400mgCG	4.47±0.17^{a}	43.50±1.77^{c}	12.20±0.56^{c}	24.00±0.75^{b}
VIII	HFD+800mgCG	4.92±0.15^{b}	49.40±1.89^{c}	10.20±1.37^{c}	21.00±1.40^{c}

$ = HMG CoA /mevalonate ratio. CG = *C. glandulosum*; # = nmol of cholesterol esterified /hr/l plasma. @ = µmol of cholesterol esterified/hr/mg protein.a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ and ns = non significant when, Group V vs. Group VI, VII, VIII and IX. A = $p < 0.05$, B = $p < 0.01$, C = $p < 0.001$and NS = non significant when, Group I vs. Group V.

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