ORIGINAL ARTICLE

A preliminary study on hypolipidemic effect of aqueous leaf extract of *Clerodendron glandulosum*.Coleb

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Aqueous extract of *Clerodendron glandulosum*. Coleb (CG) (400 mg/kg/day) was orally administered to rats rendered hyperlipidemic chronically (by feeding high-fat diet; HL) to assess its possible lipid-lowering potential. The hyperlipidemic rats were administered CG extract by oral gavage from 30-90 days along with high fat diet. Plasma lipid profile was monitored on 30th, 60th and 90th days to assess the effect of CG extract. Observations revealed a decrement in body weight (9.6%), plasma TC (15.63%), TG (42.99%), PL (13.91%), LDL-C (81.36%) and VLDL-C (43%) along with an increase in HDL-C (52.84%) at 90 days (after 60 days of CG extract feeding) compared to high levels at 30 days. Fecal lipid analysis revealed high content of TC, TG and PL in HL + CG group. Lipid-lowering property of the CG extract in chronic hyperlipidemic rats validates its use traditionally as a part of folklore medicine in North-eastern India, though there is no scientific evaluation to date.

Key words: Cholesterol, *Clerodendron glandulosum.* Coleb, hyperlipidemia, lipoproteins

INTRODUCTION

Hyperlipidemia is a condition associated with increased levels of lipid and cholesterol in plasma leading to various physiological disorders including coronary artery disease (CAD).[1] CAD has been reported as the most common cause of death in developed as well as developing nations.[2-4] Since synthetic drugs have been shown to have side effects, clinical importance of the herbal drugs has received considerable attention in recent years[5] as medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesterolemic properties.[6,7] North-eastern states of India (biodiversity hotspots) with geographical and climatic diversity, house a treasure trove of plants with novel medicinal properties.[8,9] These plants have found a prime place in the indigenous system of medicine and are in focus for evaluation of their active ingredients.[10] Various species of *Clerodendron* (Verbenaceae) have been reported to have medicinal properties like anti-inflammatory effect (*C. serratum*), regulation of blood pressure and sedative action (*C. trichotomum*), hepatoprotective action (*C. inerme*) and hypolipidemic effect *(C. colebrookianum*).[11-14] Dried powdered leaves of *C. glandulosum.*Coleb (CG) are widely used traditionally in North East India by a cross-section of people as a home remedy against obesity, hypertension and diabetes. Urban people grow CG in kitchen gardens, and the leaves are sold in towns of different states of North East India. Use of CG in folklore medicine makes an interesting case to scrutinize its possible lipid-lowering effect at the organism level in detail, as there is no scientific report to date on its hypolipidemic or hypocholesteraemic property. Hence, this inventory is an effort to investigate the possible lipid-lowering property of an aqueous extract of CG on plasma lipid metabolite load in a chronic hyperlipidemic animal model.

MATERIALS AND METHODS

Plant Material

The CG plant was collected locally and authenticated by Dr. Hemchand Singh, Department of Botany, D.M college of Science, Manipur University, Imphal (voucher specimen no. 405). Fresh leaves of CG were collected from local market of Imphal, Manipur, India, from time to time and stored after shade drying.

Preparation of the Aqueous Extract of Plant

Fresh extract was prepared every third day and stored at 4°C. Shade-dried leaves were grounded and boiled in distilled water for 60 minutes. Residues of leaves were removed by filtering with an autoclaved muslin cloth and the filtrate was concentrated by heating. A semi-solid paste was obtained by this process that was further freeze dried at 0°C. The net

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yield of extract was 28% w/w.

Animals

Female Albino rats of *Charles Foster* strain (180-190 g body weight) were maintained as per CPCSEA guidelines and were fed with normal diet or high fat diet and water *ad libitum*.

Experimental Design

Hyperlipidemia was induced by feeding rats with high fat diet.[15]

Group I: Control group (NC); fed with standard laboratory diet for 90 days.

Group II: Hyperlipidemic group (HL); fed with hyperlipidemic diet for 90 days.

Group III: Hyperlipidemic + CG extract fed group (HL + CG); hyperlipidemic diet from day 1 to day 90 and CG extract (400 mg/ kg BW in 0.5% carboxyl methyl cellulose (CMC)) via gastric intubation from day 31 to day 90 of study. The NC and HL groups received vehicle (0.5% CMC) from day 31 to day 90.

Blood and Feaces Collection

Blood was collected by retro-orbital puncture on 0, 30th, 60th and 90th day of experimentation in EDTA-coated tubes and centrifuged at 3000 rpm at 4°C. Plasma was separated and stored at −80°C. Fecal samples of each group were collected every third day from day 75 to day 90 (totally 5 samples).

Determination of Plasma and Fecal Lipid Profile

Total cholesterol (TC), triglyceride (TG), phospholipids (PL), high-density lipoprotein cholesterol (HDL-C) were assayed using kits (Reckon Diagnostics Ltd, Baroda) and very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were calculated by Friedewald's formula: VLDL=TG÷5, and LDL=Total cholesterol- HDL+VLDL.[16] The ratios of TC: HDL-C (atherogenic index) and LDL-C: HDL-C were also calculated for effective interpretation of data. Total fecal lipids were extracted using 2:1 chloroform: methanol mixture.[17] Dried lipid samples were dissolved in 1% Triton ×100,[18] and TC, TG and PL were estimated using above-mentioned kits.

Statistical Analysis

All the values are expressed as mean ± S.E.M using graph pad prism version 3.0 for windows, Graph pad software, San Diego, California, USA and data were analyzed statistically by Student's *t*-test. $P < 0.05$ were considered significant. Significance was calculated for comparison between NC and HL at 30th, 60th and 90th day and between HL and HL + CG at 60th and 90th days of experimentation.

RESULTS

The NC rats showed a gradual increase in body weight during the 90-day study period. Rats fed with HL diet showed a significant increment in body weight (24%) during the same period. However, HL rats given CG extract showed body weight change that was more comparable with control rats [Figure 1].

HL rats depicted significant increase in plasma TC level (65.85%). However, CG treatment to HL rats from 31st to 90th day significantly lower TC level (15.63%) [Figures 2 and 3].

Plasma TG increased significantly (64.79%) from 0 to 90 days in HL rats, while treatment with CG extract significantly decreased the same to levels comparable to the NC group [Figure 3]. Plasma PL content also showed a significant increase in HL rats (55.40%), while treatment not only retard the increment initially but also brought down the level towards control level (23.59%) [Figure 4].

Plasma HDL-C showed no significant change either in NC or HL groups, while in the HL + CG group there was significant increase (56.56%) [Figure 5].

Plasma LDL-C and VLDL-C showed significantly increasing level between 30-60 and 60-90 days. However, circulating

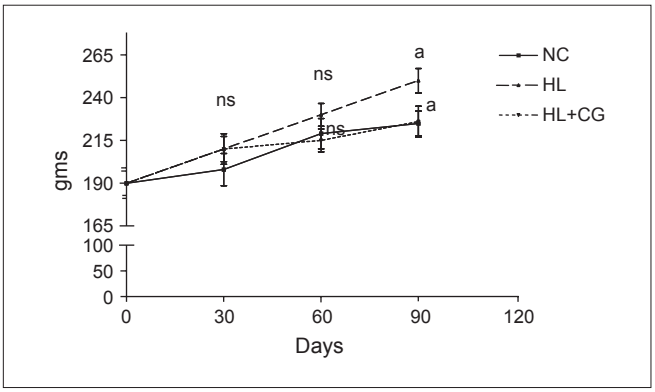

Figure 1: Effect of *C. glandulosum.*Coleb extract on body weight. Values = mean ± SEM (n = 5). Where NS = Non-significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

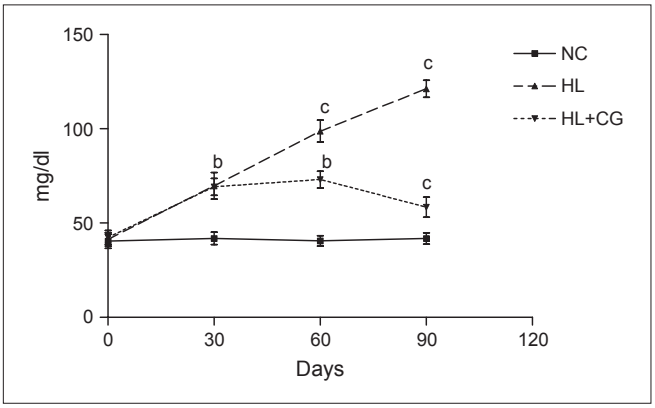

Figure 2: Effect of *C. glandulosum.*Coleb extract on plasma total cholesterol. Values are expressed as mean ± SEM (n = 5). Where NS = Non-significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

levels of LDL-C and VLDL-C plummeted by 90.16% and 59.66%, respectively in the HL + CG group and were very much comparable to the NC group [Figures 6 and 7].

Total lipid content in faeces showed an increase in the HL + CG rats compared to the HL group during day 75 to day 90 of study. HL rats recorded an increase in fecal TC (34.49%), whereas TG and PL did not show a significant change. However, the HL + CG group recorded significant increment in fecal TG , TC and PL content amounting to an overall increment of 44.23%, 48.86% and 58.65%, respectively [Figure 8].

DISCUSSION

The present study assesses the lipid-lowering potential of CG extract in a hyperlipidemic rat model. The therapeutic value of the extract is also well reflected in the pattern

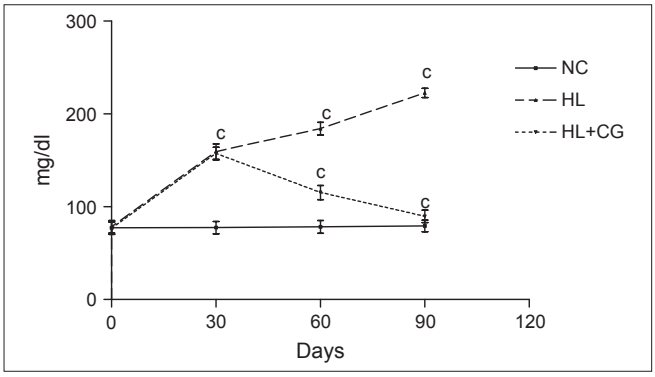

Figure 3: Effect of *C. glandulosum.*Coleb extract on plasma triglycerides.Values are expressed as mean ± SEM (n = 5). Where NS = Non-significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

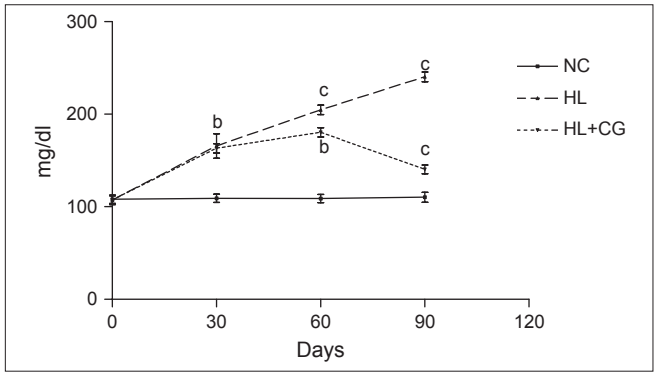

Figure 4: Effect of *C. glandulosum.*Coleb extract on plasma phospholipids. Values are expressed as mean ± SEM (n = 5). Where NS = Non-significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

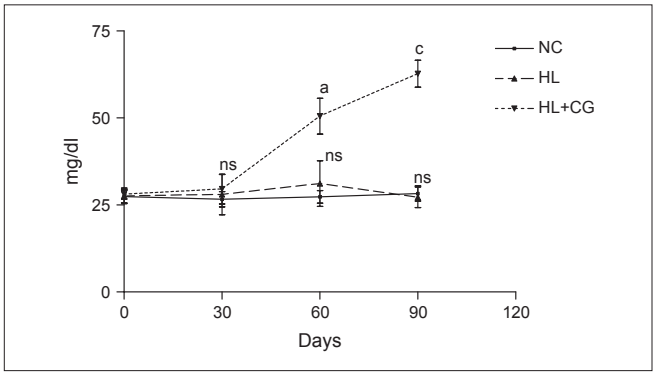

Figure 5: Effect of *C. glandulosum.*Coleb extract on plasma high-density lipoprotein cholesterol. Values are expressed as mean ± SEM (n = 5). Where NS = Non significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

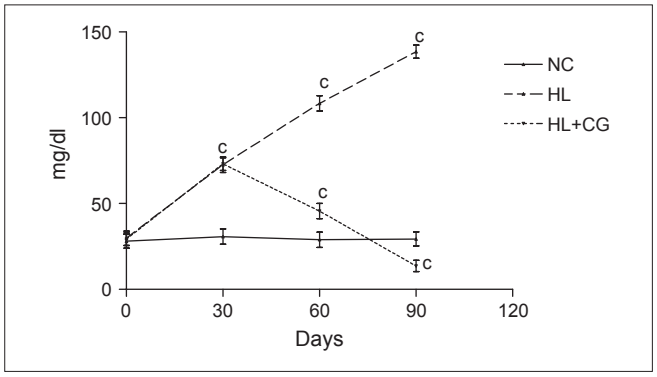

Figure 6: Effect of *C. glandulosum.*Coleb extract on plasma low-density lipoprotein cholesterol. Values are expressed as mean ± SEM (n = 5). Where NS = Non-significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

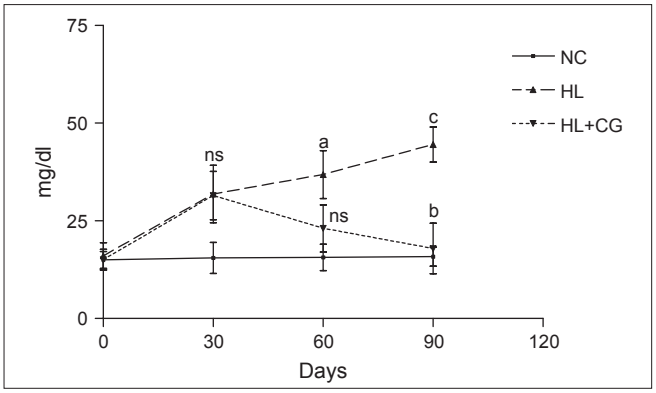

Figure 7: Effect of *C. glandulosum.*Coleb extract on plasma very low-density lipoprotein cholesterol. Values are expressed as mean ± SEM (n = 5). Where NS = Non significant. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$

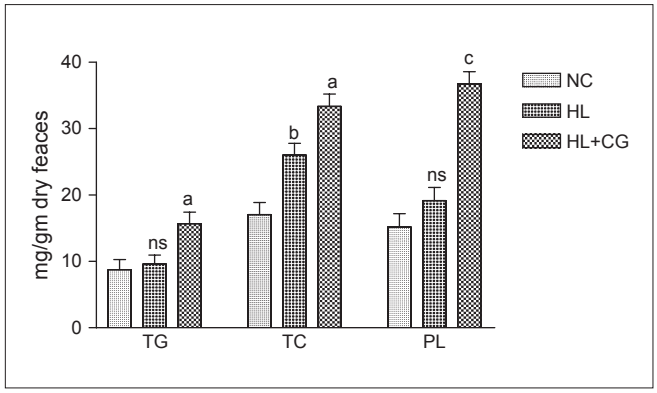

Figure 8: Effect of *C. glandulosum.*Coleb extract on fecal lipids. Values are expressed as mean ± SEM. Where NS = Non-significant, $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$. Significance was calculated by comparison of NC vs. HL and HL vs. HL + CG

of the body weight gain. Rats of the HL group showed 24% increase in body weight during experimental period, whereas the HL + CG group showed only 15.92% increase, that was comparably with 15.55% increase in the NC group. Furthermore, the HL + CG group had the significant hypolipidemic effect marked by a pronounced ability to lower plasma TC and TG levels in HL + CG rats compared to the HL group. The potent hypolipidemic effect is clearly indicated by the recorded 51.80% and 59.73% decrement in plasma TC and TG, respectively, both independently capable of leading to coronary artery disease.[19,20] A generalized lipid-lowering activity is also indicated by the recorded 41.58% decrease in plasma PL. It is presumable that *CG* could be a potent herbal agent against cardiovascular problems, as the HL + CG rats in present experiment have shown a decrease in VLDL-C (59.73%) and LDL-C (90.16%) along with an increase in HDL-C (56.56%). Higher plasma LDL-C level is related with greater deposition of cholesterol in artery and aorta thereby increasing risk for CAD,[21] whereas low HDL-C is the prevalent lipoprotein abnormality reported among the Indian populace.[22,23] Hence, recorded decrement in LDL-C and increment in HDL-C suggests usefulness of CG in the treatment of hypercholesterolemia. Also, synthetic hypercholesterolemia drugs lower both TC and HDL-C, simultaneously;[24] CG formulation could prove to be more effective therapy due to its ability to significantly increase HDL-C while lowering TC.

Highly potent nature of CG to prevent both atherosclerosis and cardiovascular problems is clearly indicated by the significantly lowered atherogenic index (TC: HDL-C) in the HL + CG group (0.93) versus HL (4.44) or NC (1.47) groups. Also, the LDL-C: HDL-C ratios were lowered by CG treatment (0.21) as compared to HL (5.08) and NC (1.03) rats further validating its positive effects on lipid metabolism [Table 1].

There are reports that suggest that lipid-lowering effect of plant extracts is due to reduced gastrointestinal absorption which is reflected in the concurrent increase in fecal lipid load.[25] The present study validates the contention to a certain extent as fecal samples of HL + CG animals show significantly higher TC, TG and PL contents compared to both control and HL animals. However, the significant alterations seen in plasma lipoprotein fractions also suggest the favourable metabolic effect of CG extract in controlling body lipid load, the mechanics of which need to be investigated.

Table 1: Effect of CG extract on TC: HDL and LDL: HDL ratios

	Atherogenic index	LDL-C: HDL-C
NC	1.47	1.03
HL	4.44	5.08
HL + CG	0.93	0.21

CONCLUSION

The present study provides a preliminary scientific basis for hypolipidemic effects of CG, a plant which has been extensively used as a folklore medicine in North-eastern region of India. Further studies are however required to decipher the possible mechanism(s) of action. Pilot studies have identified the presence of flavonoids, steroids, saponins and other related compounds. The potential for using CG for developing hypolipidemic formulations is very much indicated.

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