

***Clerodendron glandulosum*.Coleb METHANOLIC EXTRACT AMELIORATES
HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN MALE RATS**

Patel D.K., Bhatt R.K., Desai S.N., Thounaojam M.C., Jadeja R.N., Ansarullah,
Salunke S.P., Padate G.S., Devkar R.V.* and Ramachandran A.V.

Division of Phytotherapeutics and Metabolic Endocrinology, Department of Zoology,
Faculty of Science, The M.S. University of Baroda, Vadodara-390002, Gujarat, INDIA.

Phyto_met@yahoo.com

*corresponding author

Summary

In recent times, herbal drugs with lipid lowering potential have received considerable attention because these drugs have relatively less side effect unlike synthetic drugs. This study assesses the lipid lowering property of *Clerodendron glandulosum*.Coleb (CG) methanolic extract in experimentally induced hyperlipidemia by feeding rats with hyperlipidemic diet (HL). It was observed that CG extract restored the plasma lipid profile of HL rats to levels comparable to control, with increase in high density lipoproteins being the most significant finding. CG restored the high fat diet induced alterations in liver and renal functions as was evident from the activity levels of relevant enzymes. Elevated levels of lipids and triglycerides eliminated through faeces further substantiate the role of CG as a potent lipid lowering agent. Results envisaged herein, indicates the ability of CG extract as a potent lipid lowering agent in experimentally induced hyperlipidemia.

Key words: *Clerodendron glandulosum*.Coleb, hyperlipidemia, hypercholesterolemia

Introduction

Metabolic syndrome, a cluster of clinical disorders which includes abdominal obesity, insulin resistance, dyslipidemia (Hypertriglyceridemia, hypercholesterolemia and low HDL level) and high blood pressure is associated with a high incidence of cardiovascular diseases (CVD) [1, 2]. Hyperlipidemia is now regarded as independent risk factor of CVD [3] and is also responsible for liver damage [4]. CVD have been reported as the most common cause of death in developed and developing nations [5, 6, 7]. Majority of the synthetic hypolipidemic drugs reduce plasma cholesterol level [8, 9] but are not efficient in lowering plasma triglyceride levels and elevating HDL levels. In recent times, herbal drugs with lipid lowering potential have received considerable attention because these drugs have no side effect like synthetic drugs and improve safety profile [10, 11, 12, 13].

North eastern states of India (biodiversity hotspots) house a treasure-trove of plants with novel medicinal properties [14]. These plants have found a prime place in the

indigenous system of medicine and are in focus for evaluation of their beneficial effects [15]. *Clerodendron glandulosum*. Coleb (CG) is a herb found in north eastern parts of India and used by rural and urban people against diabetes, obesity and hypertension. Apatani tribe uses leaves of CG for controlling hypertension and fever [16], while the tender shoots are used by people of Debru biosphere reserve as an antipyretic agent [17].

In our laboratory acute oral toxicity of CG was performed on Swiss albino mice. There was no mortality and behavioral changes in plant treated group as compared to normal untreated group. Activity levels of plasma enzymes analyzed to assess liver and kidney functions were similar and comparable to the control groups (Unpublished observation). The aim of this study was to assess the influence of methanolic extract of CG on altered lipid metabolism in hyperlipidemic rats.

Materials and Methods

Plant material:

Clerodendron glandulosum. Coleb plant is found in North eastern part of India. CG leaves were collected from Imphal district, India in the month of June and shade dried. The plant was identified and authenticated 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 extract:

The leaves of the plant were washed and rinsed with tap water and shade-dried. The dried leaves were subjected to extraction using methanol in soxhlet apparatus and resultant filtrate was concentrated under reduced pressure by rotary evaporator (Buchi, Germany). A semisolid paste obtained by this process was stored at 0°C. The extractive value of the CG methanolic extract was 18.39% w/w, which further dissolved in 0.5% CMC (Carboxy methyl cellulose).

Experimental Animals:

Male *Charles foster* albino rats (200-250 gm) were housed and maintained in clean polypropylene cages under controlled room temperature (24±2°C) and were fed with commercially available rat chow (SLD; M/s Pranav Agro Ltd., Vadodara, India) or high fat diet (HFD)[18]; 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:

Group-I Normal control (NC): Animals were fed with SLD and received 0.5% CMC via gastric intubation for 42 days.

Group-II Hyperlipidemic (HL): Animals were fed with HFD and received 0.5% CMC via gastric intubation for 42 days.

Group-III (HL+CG400): Animals were fed with HFD and received methanolic extract of CG (400mg/kg BW) orally via gastric intubation for 42 days.

Lipid profile:

Plasma Total cholesterol (TC), Triglyceride (TG) and High density lipoprotein (HDL) were analyzed using commercially available kits (Eve's diagnostics, Vadodara, India). Very low density lipoprotein (VLDL) and Low density lipoprotein (LDL) were calculated by Friedewald's formula [19]. Hepatic and fecal lipids were extracted using chloroform: methanol (2:1) mixture and total lipids were estimated gravimetrically [20]. Dried lipid extract was dissolve in 1% triton X 100 [21] and TC and TG were analyzed using above mentioned kits. Atherogenic index was calculated as a ratio of plasma LDL/HDL.

Fecal Cholic (CA) and Deoxycholic acid (DCA):

Fecal samples from each experimental group were collected on every 3rd day between days 31 to 42 of study. Dried fecal samples were eluted with absolute alcohols, filtered and CA and DCA were estimated [22].

Liver and Kidney function tests:

Enzymes associated hepatic (SGOT, EC 2.6.1.1 and SGPT, EC 2.6.1.2) and renal functions (ACP, EC 3.1.3.2 and ALP, EC 3.1.3.1) were assayed in plasma of control and experimental rats using kits (Eve's diagnostics, Vadodara, India).

Statistical analysis:

Data are expressed as the mean \pm SEM using Graph pad prism version 3.0 for Windows, Graph Pad Software, San Diego California, USA. Statistical significance was evaluated using analysis of variance (ANOVA) with the Bonferroni test.

Tables

Table.1. Effect of CG methanolic extract on food intake, water intake, weight gain, feed efficiency ratio, liver and epididymal fat pad weight

Parameters	Groups		
	NC	HL	HL+CG 400
Food intake ^{\$}	11.20 \pm 0.37	11.61 \pm 0.66 ^{ns}	11.27 \pm 0.46 ^{ns}
Water intake [@]	24.40 \pm 0.50	24.28 \pm 1.13 ^{ns}	28.16 \pm 1.18 [*]
Weight gain [¥]	25.09 \pm 11.50	51.75 \pm 4.70 ^{##}	11.50 \pm 1.84 ^{***}
Feed efficiency ratio	1.75 \pm 0.17	5.26 \pm 0.27	1.00 \pm 0.17
Liver weight [†]	9.02 \pm 0.39	12.28 \pm 0.43 ^{##}	10.03 \pm 0.59 [*]
Epididymal fat pad weight [†]	6.97 \pm 0.41	11.18 \pm 0.45 ^{###}	7.82 \pm 1.15 [*]

Where, \$ = g/day, @ = ml/day and ¥ = g and † = gm/100gm BW

When # = comparison of NC v/s HL and * = comparison of HL v/s HL+CG400

Table.2. Effect of CG methanolic extract on plasma lipid profiles

Groups	Parameters		
	TC†	TG†	FFA†
NC	45.78±3.45	53.09±5.78	38.17±1.85
HL	89.09±5.87 ^{###}	120.90±5.42 ^{###}	61.27±1.02 ^{###}
HL+CG 40	56.74±5.25 ^{**}	76.78±4.98 ^{***}	48.03±1.46 ^{***}

Where, † = mg/ dl

When # = comparison of NC v/s HL and * = comparison of HL v/s HL+CG 400

Table.3. Effect of CG methanolic extract on plasma lipoprotein profiles and Atherogenic Index (AI)

Groups	Parameters			
	HDL†	LDL†	VLDL†	AI
NC	21.89±1.98	34.50±1.99	10.61±1.35	1.57
HL	13.67±1.89 [#]	99.6±2.01 ^{###}	24.18±1.78 ^{###}	7.28
HL+CG 400	23.42±2.00 ^{***}	68.59±2.13 ^{***}	15.23±1.12 ^{**}	2.92

Where, † = mg/dl

When # = comparison of NC v/s HL and * = comparison of HL v/s HL+CG 400

Table.4. Effect of CG methanolic extract on tissue and fecal lipid profiles

	Parameters	Groups		
		NC	HL	HL+CG 400
Liver	TL ^{\$}	52.93±0.59	91.61±0.85 ^{###}	72.18±1.766 ^{***}
	TC ^{\$}	10.98±0.44	22.09±0.99 ^{###}	14.51±0.51 ^{***}
	TG ^{\$}	21.89±0.29	49.53±0.35 ^{###}	35.95±0.56 ^{***}
Faeces	TL [@]	42.18±0.85	45.79±0.60 [#]	63.02±1.69 ^{***}
	TC [@]	12.34±0.63	14.91±0.60 [#]	21.47±1.20 ^{**}
	TG [@]	9.84±0.24	10.88±0.25 [#]	21.80±1.29 ^{***}
	CA [‡]	29.87±0.88	40.07±3.35 [#]	56.37±4.29 [*]
	DCA [‡]	35.40±1.05	35.43±1.80 ^{ns}	57.10±1.15 ^{**}

Where \$ = mg/g tissue, @ = mg/g, and ‡ = µg/gm faeces

When # = comparison of NC v/s HL and * = comparison of HL v/s HL+CG 400

Table.5. Effect of CG methanolic extract on enzymes of hepatic and renal function

Groups	Parameters			
	SGOT†	SGPT†	ACP©	ALP©
NC	82.68±4.90	35.98±3.09	20.03±2.30	1.00±0.30
HL	123.09±5.13###	53.27±2.90##	56.89±2.76###	4.24±0.29###
HL+CG 400	76.21±4.78***	35.11±2.87**	34.90±2.22***	1.38±0.28***

Where, † = Unit/ml and © = KA units

When # = comparison of NC v/s HL and * = comparison of HL v/s HL+CG 400

Results

1. Body weight and food intake

HL group recorded 51.51% increase in body weight gained as compared to NC group. The feed efficiency ratio was higher (66.73%) as compared to NC group, though there was no significant change in food intake in any of the experimental group. However, HL+CG group recorded 77% decrement in body weight gained coupled with 80.94% decrement in feed efficiency ratio. Although, water intake was significantly more 13.77% in HL+CG group, it was unchanged in all other groups (Table 1).

2. Tissue weight

The whole weight of epididymal fat pad increased by 76.38% in HL control group as compared to NC group whereas, it decreased by 61.45% in HL+CG group as compared to HL group. The weight of liver increased by 14.76% in HL control group as compared to NC group whereas; decreased by 7.45% in HL+CG group as compared to HL control group (Table 1).

3. Plasma lipid profile

HL group recorded significantly elevated TC (48.61%), TG (56.08%), FFA (37.70%), LDL (65.35%) and VLDL (56.12%) as compared to NC group whereas; the HDL levels were significantly decreased (37.55%). HL+CG group recorded significant decrease in TC (36.31%), TG (36.49%), FFA (21.60%), LDL (31.13%) and VLDL (37.01%) as compared to HL group, whereas HDL level were elevated (41.63%) (Tables 2 and 3). Atherogenic index was highest (7.28) in HL group followed by HL+CG group (2.92) and was least in NC group (1.97; Table3).

4. Hepatic TL, TC and TG levels

Hepatic total lipids (TL), total cholesterol (TC) and triglycerides (TG) contents were higher (42.22%, 50.29%, and 55.80%) in HL group as compared to NC group whereas, they were lower (21.20%, 34.31% and 37.77%) in comparing with HL+CG group (Table 4).

5. Fecal lipids

TL, TG, TC and CA eliminated through faeces of HL group were 7.88%, 9.55%, 17.23%, 25.45% higher respectively as compared to NC group. There was no significant change in fecal DCA level in HL group as compared to NC. The fecal TL (27.34%), TG (50.09%), TC (30.55%), CA (28.91%), DCA (37.95%) levels eliminated from the faeces were higher in HL+CG group as compared to HL group (Table 4).

6. Enzymes activity

Activity levels of ACP, ALP, SGPT and SGOT were significantly elevated (64.79%, 76.46%, 32.45%, 32.82% respectively) in HL control group as compared to NC group whereas, significantly decreased (38.61%, 67.45%, 34.09%, 38.08% respectively) in HL+CG group as compared to HL control group (Table.5)

Discussion

The present study accesses lipid lowering potential of CG methanolic extract in hyperlipidemic rat model. The therapeutic value of extract is well reflected in the pattern of body weight gain. Rats of HL group showed 51.51% increase in body weight during the experimental period whereas HL+CG group showed 77.77% decrease in body weight as compared to HL rats (Table 1). Further, HL+CG rats recorded significant hypolipidemic effect that was marked by a pronounced decrement of plasma TC, TG and FFA levels as compared to HL group (Table 2). This finding is important and significant because high TC, TG and FFA in plasma are independently capable of inducing coronary artery disease (CAD) [23, 24].

Hepatic TC and TG content increased in HL group whereas HL+CG rats recorded significant decrease suggesting a positive homeostatic change in lipid metabolism of hyperlipidemic rats. This could have been possibly achieved due to low activity of cholesterol ester synthetase (CES) in liver resulting in poor esterification of free cholesterol and its incorporation into VLDL [25]. Previous studies have reported poor CES activity in liver and decreased VLDL content in plasma in HL rats fed with aqueous extract of *Sida rhomboides* Roxb [26]. It was observed in the present study that significantly increased levels of TC, TG, CA (Cholic acid) and DCA (Deoxy cholic acid) were eliminated in faeces of HL+CG rats suggesting reduced quantum of absorption of lipids in intestine. These results can be attributed to decreased activity levels of CES in intestine suggesting poor esterification of dietary free cholesterol resulting in the reduced absorption [27]. High levels of cholesterol eliminated through faeces have been attributed to dietary phytosterols [28] and high phytosterol content in CG extract has already been reported [26]. Increased elimination of TG through faeces in HL+CG rats can be attributed to increased saponin content in CG because saponins are known to induce greater fat excretion by inhibition of pancreatic lipase activity [29]. Hence, it can be hypothesized from the present study that CG could possibly act as a potent herbal agent against cardio vascular problems because HL+CG rats recorded decrement in LDL and VLDL respectively along with increased in HDL level. LDL and VLDL are known to increase the deposition of cholesterol in arteries and aorta thus increasing the risk of CAD [30] and is a prevalent abnormality reported among Indian population [31]. Hence,

decrement in LDL and increment in HDL suggests usefulness of CG extract in treatment of hyperlipidemia. Significantly lowered atherogenic index (LDL/HDL) in HL+CG group (42.20%) v/s that observed in HL (78.43%) provides ample testimony to this hypothesis. It is well known that hyperlipidemia induces changes in liver weight; plasma SGOT, SGPT, ACP and ALP levels [32]. In present study, increased activity of SGOT (32.82%), SGPT (32.45%), ACP (64.79%) and ALP (76.46%) levels were recorded in the HL group. However, a significant decrease of plasma SGOT (38.08%), SGPT (34.09%), ACP (38.61%) and ALP (67.45%) levels were detected in HL+CG group with decrease in liver weight compared to that of HL group. Results envisaged herein, indicates the ability of CG extract to manifest corrective changes in HL induced alteration in liver and kidney functions. Overall, it can be concluded that CG methanolic extract acts as a potent lipid lowering agent in experimentally induced hyperlipidemia thus, validating its use as an ethno- medicine by general populace of North-East India.

Acknowledgment

Authors acknowledge Post graduate students of S.P University, V.V. Nagar, Gujarat, India and P.M. Patel Science College, Anand, Gujarat, India for the help rendered. Financial assistance in form of JRFSMS provided to the first author by University grant Commission, New Delhi, India is also acknowledged.

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