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Research Article

Investigating the Activity of Ethanol Extract of *Gongronema Latifolium* Leaf on High Fat Diet-Induced Hyperlipidemia in Wistar Rats

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Abstract

Hyperlipidemia, a disorder of lipid metabolism, is rising at alarming rate in the global population and has been noted to pose various cardiovascular health challenges. Synthetic drugs used in the treatment of this disorder are associated with remarkable adverse effects and consequently, poor patient compliance. Plant remedies are not only advancing in the treatment of human ailments, but also have advantages of local availability, better patient tolerance and environmental friendliness. *Gongronema latifolium* is a flora of Tropical Africa and commonly used in tradomedicinal practice in South Eastern Nigeria in the treatment of various human ailments, including heart-related diseases. This work was designed to investigate the activity of ethanol extract of *G. latifolium* leaf on high fat diet-induced hyperlipidemia in wistar rats. This was done by extracting the plant material, randomizing thirty adult wistar rats into six groups (n=5), subjecting the animals to various treatment per oral, and estimating the lipid profile of each group. The result shows that oral treatment with the plant extract produced significant ($p<0.05$) dose-dependent decrease in serum total cholesterol, triglyceride, low density lipoprotein, very low-density lipoprotein and simultaneous increase in high density lipoprotein in hyperlipidemic rats. The finding of this study led to conclusion that *G. latifolium* leaf possesses antihyperlipidemic activity, hence, authenticating its tradomedicinal claim.

Keywords: Hyperlipidemia; Plant extract; *G. latifolium*

Introduction

Hyperlipidemia is a metabolic disorder that has posed global health challenge and contributed significantly to prevalence and severity atherosclerosis that predisposes to cerebrovascular and ischemic heart diseases [1,2]. It is an aberration in lipid metabolism, or in serum lipid transport, or in degradation of lipoproteins [3]. It is characterized by an increase in serum level of any or all of the bad lipids such as Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL), and by a decrease in serum level of High-Density Lipoprotein (HDL): the good cholesterol [4]. Although the etiology of the disease is linked to genetic factor, habit consumption of high cholesterol-containing diets contributes greatly to development of hyperlipidemia [3-6]. Cholesterol is a soft waxy substance found in the bloodstream and useful to the body as a major component in the formation of cell membranes and hormones [7]. High level of serum cholesterol can result from either increased absorption through the gut or by enhanced endogenous synthesis, therefore, there are two possible ways to reduce hyperlipidemia: to diminish absorption or to block endogenous synthesis [8]. Currently available synthetic antihyperlipidemic drugs can act by any of the two ways mentioned, but are associated with a number of adverse effect and high cost. Plants, apart from being a rich source of medicines used for centuries in treatment of disease, products from plants are locally available, cheap, safe, environmentally friendly, and have made significant contributions to advancement of modern health care [9].

G. latifolium belonging to the family of Asclepiadaceae, is an edible plant commonly found in South Eastern Nigeria and other Tropical African countries [10,11]. *G. latifolium* is claimed by Trado-medicine as a remedy for heart-related diseases. Reported activities of *G. latifolium* include: anti-inflammatory activity [12], antidiabetic activity [13], antioxidant activity [14,15], immunomodulatory activity [16], antihyperlipidemic activity [17]. Based on this background, this study would agree or disagree with tradomedicinal claim on *G. latifolium* as a remedy for heart-related diseases. This study therefore, was designed investigate activity of ethanol extract of *G. latifolium* leaf on high fat diet-induced hyperlipidemia in wistar rats.

Materials and Methods

Collection and confirmation of plant material

Matured fresh leaves of *G. latifolium* were collected from Okigwe, identified and confirmed by a taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt Rivers State, Nigeria. For reference purpose, voucher specimen with herbarium number, UPH/P/1471, was deposited in the Department.

Animal ethics approval

Animal ethics approval with reference number, MAU/SERC/A/22, was granted by Senate Ethics and Research Committee of Madonna

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Experimental animal

Adult Wistar rats that weighed 200 g - 220 g, and about 16 to 18 weeks old, were used in this work. The rats were bred at Animal Facility Center, Madonna University, Nigeria in polyethylene cages adequately floored with wood shavings. The rats had free access to food and clean drinking water, and were handled in accordance with international guidelines for animal studies [18].

Plant material extraction

About 250 g of finely powdered plant material wrapped in Whatmann number 1 filter paper was extracted by placing in the holding chamber of reflux extractor and reflux extraction was performed at 40°C for 48 hours, using 500 ml of 80% ethanol as extraction solvent. The process was repeated three times to obtain sufficient amount of extract. The extract was concentrated using rotary evaporator to expel the ethanol. The concentrated extract was stored in air-tight container for subsequent use.

Phytochemical screening

Standard procedure proposed by [19] was employed to test for the presence or absence of phytochemicals in ethanol extract of *G. latifolium* leaf.

Acute toxicity test

Using guideline specified by [20], acute oral toxicity of ethanol extract of *G. latifolium* leaf was conducted in 16-18 weeks old adult Wistar rats that weighed between 200 g - 220 g. In the test, five rats were allowed free access to water throughout the experiment but fasted of food for four hours before and two hours after per oral administration of the plant extract. Initial sighting study was conducted by per oral administration of the plant extract at 4000 mg/kg to a rat and then monitored for 24 hours for signs of toxicity. From the obtained result, similar dose was administered per oral to the rest of the rats and were observed for signs and symptoms of toxicity for 24 hours at interval of 30 minutes, and then for 14 days consecutively at interval of 24 hours.

High fat diet induction of hyperlpipidemia

Adult Wistar rats that weighed between 200 g-220 g were made hyperlipidemic by feeding with High Fat Diet (HFD) which consisted of normal rat feed (47%), sucrose (40%), coconut oil (10%) cholesterol (2%) and cholic acid (1%).

Experimental Protocol

Thirty adult Wistar rats were randomized into six groups (n=5). The animal groups were treated as follows for first 15 days:

1. Group 1 (normal control): received non-HFD (i.e. normal feed) and water, per oral
2. Group 2 (negative control): received HFD and 5 ml/kg 3% v/v Tween 80, per oral.
3. Group 3 (positive control): received HFD and 100 mg/kg Artovastatin, per oral.
4. Group 4: received HFD and 100 mg/kg Plant Extract, per oral.
5. Group 5: received HFD and 200 mg/kg Plant Extract, per oral.
6. Group 6: received HFD and 400 mg/kg Plant Extract, per oral.

After the first 15 days (i.e., from 16th day), HFD was withdrawn from groups 2 to 6, and replaced with non-HFD (i.e., normal feed) for second 15 days.

Standardization and calculation of dose

Weight of beaker+extract=51.37 g

Weight of empty beaker alone=49.37 g

Weight of the extract=2.0 g

20 ml (3% v/v Tween 80)+2 g extract=2 g/20 ml=100 mg/ml.

Therefore, the standardized dose of the extract is 100 mg/ml.

Doses of extract for different groups were calculated as follows:

Group 4: received extract at 100 mg/kg b.w

Dose per gram of body weight=100 mg/1000 g= 0.1 mg/g

Converting 0.1 mg/g dosage into its equivalent in milliliter per gram (ml/g) of the standard gave:

$$1.0 \text{ ml}/100 \text{ mg} \times 0.1 \text{ mg/g} = 0.001 \text{ml/g} = 1.0 \text{ ul/g}$$

Therefore, rat of body weight "Y" gram in Group 4 received:

$1.0 \text{ ul/g} \times Y \text{ g} = Y \times 1.0$ ul of the standardized extract

Where Y=body weight (g) of rat: ul=microliter

For example, a 200 g b.w rat received 200×1.0 ul=200 ul of standardized extract

Because of two-fold increase in doses for the groups:

Group 5 rats, based individual b.w of rat "Y" (g), received $Y \times 2.0$ ul of standardized extract

Group 6 rats, based on individual b.w of rat "Y" (g), received $Y \times 4.0$ ul of standardized extract

Collection of blood sample and estimation of serum lipid

On 16th and 31st day of treatment, under mild anesthesia, blood samples were collected from the rats by retro-orbital sinus puncture. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum from clotted cells [21]. Standard procedures were used to estimate TC [22,23], TG [24], HDL [25], LDL [26] and VLDL [26] levels in the serum.

Statistical data analysis

Data were presented in the tables as Standard Error of Mean (SEM). One Way Analysis of Variance (ANOVA) was used to statistically analyze the data, followed by Duncan's multiple comparison. Probability values less than 0.05 ($p<0.05$) were considered statistically significant.

Results

All the tested phytochemicals in ethanol extract of *G. latifolium* leaf were found to be present except anthraquinones as shown in Table 1.

The result of oral acute toxicity did not indicate any signs and symptoms of toxicity nor death within 24 hours and 14 days of observation in rats, at the limit test dose of 4000 mg/kg.

As shown in Tables 2 and 3, effect of the plant extract at 400 mg/kg on lipid profile is comparable to that of the standard drug, artovastatin.

Discussion

Phytochemicals, also called bioactive compounds, are produced in plants as secondary metabolites, and have beneficial effects on

Table 1: Phytochemistry of Ethanol Extract of Gongronema Latifolium Leaf.

Test	Observation
Resins	+
Saponins	+
Tannins	+
Anthraquinones	-
Alkaloids	+
Terpenoids	+
Glycosides	+
Phenols	+
Flavonoids	+
Steroids	+
Proteins	+
Carbohydrates	+

+ = present, - = absent

human health when consumed as nutrients [27,28]. Plants have been in use for past decades as remedy for human diseases [29]. Similar to previous reports by [16,30], this study has reestablished the presence of a number of phytochemicals in ethanol extract of *G. latifolium* leaf. These phytochemicals, particularly alkaloids and flavonoids, may be responsible for antihyperlipidemic activity of the plant. This finding is justified by reports that alkaloids [31,32] and flavonoids [33] exhibit antihyperlipidemic activity.

In this study, the rats were fed with High Fat Diet (HFD) and average daily food intake per rat was noted to be 40.17 g ± 6.32 g. This daily consumption of HFD caused significant increase and decrease in bad and good cholesterol respectively, as noted in the in the result obtained from negative control (group 2) vis-à-vis the normal control (group 1). This finding correlates with the report that feeding rodents with HFD increases serum and tissue level of bad cholesterol (hyperlipidemia) [21,34,35]. Elevated level of serum TC, TG, LDL, VLDL, and low-level HDL are associated coronary heart disease [1,36-39], while higher serum level of HDL has been documented to be physiologically beneficial [40]. From the result of this study, the animal groups treated with plant extract and artovastatin showed significant ($p<0.05$) reduction in TC, TG, LDL, VLDL and increased HDL, thus suggesting antihyperlipidemic activity. This finding is similar to the report by [17] on ethanol root extract of the same plant.

Elevation of serum level of cholesterol occurs in two ways: either by increased absorption from the gut which can be blocked by diminishing gastrointestinal tract absorption or by endogenous enzymatic biosynthesis which can be interrupted by blocking the activity of liver and intestinal enzymes (e.g., thiolase) involved in endogenous lipid biosynthesis [8]. In this study, the ability of the plant extract to significantly decrease the HFD-induced hypercholesterolemia may suggest an activity mediated by either or both ways.

Conclusion

The finding of this study has revealed the antihyperlipidemic activity of *G. latifolium* leaf, hence, confirming the tradomedicinal claim on the plant as a remedy for heart-related diseases.

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Table 2: Serum Lipid Profile of the First 15 days Treatment with Plant Extract and Artovastatin in HFD-Induced Hyperlipidemic Rats.

Treatment Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1.	83.62 ± 3.34	60.28 ± 2.13	27.76 ± 3.07	56.15 ± 0.98	41.96 ± 2.77
2.	112.41 ± 3.76	83.51 ± 1.96	28.17 ± 2.75	75.81 ± 1.64	57.22 ± 3.67
3.	86.18 ± 2.31*	63.46 ± 3.04*	39.46 ± 1.88*	61.75 ± 2.32*	42.34 ± 1.95*
4.	97.24 ± 3.11*	80.71 ± 2.64*	32.53 ± 2.63*	72.68 ± 4.16*	54.19 ± 2.18*
5.	89.33 ± 2.59*	72.25 ± 1.62*	34.67 ± 1.12*	69.23 ± 3.55*	50.55 ± 2.05*
6.	88.42 ± 2.20*	67.53 ± 2.72*	35.14 ± 2.48*	64.26 ± 1.82*	47.91 ± 3.43*

Values represent ± SEM of n=5, *significant relative to negative control at $p<0.05$

Table 3: Serum Lipid Profile of the second 15 days Treatment with Plant Extract and Artovastatin without HFD (Normal Feed) in Rats.

Treatment Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1.	84.12 ± 3.69	59.76 ± 3.41	27.13 ± 3.69	56.91 ± 4.04	42.33 ± 0.85
2.	114.64 ± 2.91	82.35 ± 2.14	29.52 ± 1.85	76.11 ± 2.86	56.65 ± 1.46
3.	82.25 ± 3.18*	60.15 ± 1.37*	48.85 ± 2.22*	55.37 ± 2.90*	40.4 ± 1.73*
4.	92.36 ± 1.82*	76.08 ± 3.37*	35.18 ± 3.05*	70.19 ± 2.34*	50.73 ± 4.07*
5.	87.59 ± 3.55*	70.29 ± 2.08*	40.6 ± 1.77*	68.52 ± 3.13*	47.28 ± 2.47*
6.	84.64 ± 1.17*	65.77 ± 4.11*	42.73 ± 2.61*	60.94 ± 1.65*	45.82 ± 3.30*

Values represent ± SEM of n=5, *significant relative to negative control at $p<0.05$

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