



Fasting lipid profile of experimental rats gavaged ethanol leaves extract of *Gmelina arborea* Roxb

Daberechi Diala Ibe ^{1,*}, Rose Onyekachi Ugboaja ² and Kelechi Charity Lele ¹

¹ Department of Biochemistry Imo state University Owerri.

² Department of Histopathology/Cytology, Faculty of Medical Laboratory Science University of Calabar.

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Abstract

Objective: The present study was designed to evaluate the fasting lipid profile of ethanol leaves extract of *Gmelina arborea*.

Materials and Methods: Crude extract of *Gmelina arborea* was prepared from the dried plant leaves and using ethanol as the extracting solvent. The extract was orally administered to albino rats at dose range of 250, 500 and 1000 mg/kg body weight for a period of 28 days. The effects on body weight and lipid profile were studied.

Results: The administration of ethanol leaves extract of *G. arborea* at 250, 500, and 1000 mg/kg body weight showed a significant decrease in total cholesterol, triglyceride, very low density lipoprotein-cholesterol, low density lipoprotein-cholesterol, and a significant increase in high density lipoprotein-cholesterol in all the treatments compared with the control. There was also no significant weight gain or organ weight gain in all the treatments compared with the control.

Conclusion: Ethanol leaves extract of *G. arborea* could be used in the management of hyperlipidemia and in weight management in experimental animals.

Keywords: *Gmelina arborea*; Total cholesterol; Triglyceride; Very low density lipoprotein-cholesterol; Low density lipoprotein-cholesterol; High density lipoprotein-cholesterol

1. Introduction

Medicinal plants have been commonly used in folklore medicine for the treatment of diverse diseases. Justification for the use of these medicinal plants has rested largely on the long-term ancient clinical practice with little or no scientific data on their efficacy and safety. Conversely, in the recent past, pharmacological and toxicological effects of these plants have begun to receive attention from scientists for the verification of their claimed pharmacological and therapeutic properties [1,2]. *Gmelina arborea* is one of such plants. It is a fast growing deciduous tree [3] that belongs to family verbenaceae. It occurs naturally throughout India, Vietnam, Nigeria, Sierra Leone, Malaysia, China, Laos and Thailand. Its root, bark, fruit and leaf are used for both their medicinal and nutritional purpose [2 -8]. Whereas fasting lipid profile is an assay which measures serum total cholesterol (TC), High density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C) and triglycerides (TAG). Their elevation points towards hyperlipidemia, which is a term for abnormal high levels of lipids in the blood. The build-up of these lipids can lead to plaque in the arteries and increase the risk of heart attack and stroke [9].

*Corresponding author: Ibe D D¹

2. Materials and methods

2.1. Chemicals used

All chemical were of analytical grade.

2.2. Collection of *Gmelina arborea* plant leaves

Fresh mature leaves of *Gmelina arborea* were collected from a public school in Afaraukwu Umuahia in Abia State, Nigeria. The leaves were transferred to the Department of Plant Science and Biotechnology, University of Calabar for identification. A voucher specimen number UNICAL/PSB/2017/020 was assigned to the sample specimen that was deposited at the herbarium of the Department of Plant Science and Biotechnology, University of Calabar

2.3. Preparation of *Gmelina arborea* leaves extract

The collected leaves of *Gmelina arborea* were separated from the stalk wash with clean water and air dried at room temperature on a laboratory bench within 14 days. It was pulverized into a fine powder using a manual blender.

2.4. Plant material extraction

The method used by [10] was adopted. Briefly, 100g of the pulverized leaves was introduced into the extraction chamber of a soxhlet extractor and extraction was carried out with 96% ethanol as solvent. Extraction temperature was maintained at 60°C for 48 hours. At the end of the period, the extract in solution was concentrated in a hot air oven at 40°C to obtain a pasty dark green extract. The process was repeated until enough amount of extract for the study was generated. Extract so obtained was preserved in a refrigerator until use.

2.5. Animals used

Twenty (20) adult male *Sprague dawley* rats (10-11 weeks old) and weighing between 64g to 82g were used. The rats were purchased from the laboratory animal production unit of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture Umudike. The animals were housed in aluminum cages in an animal house in the Department of Biochemistry University of Calabar where the study was carried out. Unrestricted access to food and water and an acclimatization period of two weeks under exposure to 12hrs light/dark cycle in a tropical climate was allowed for all animals, however animals were starved for 12 hours before commencement of experiments. All investigations involving experimental animals were conducted in accordance with the accepted guideline for laboratory animal use and care as approved by the ethical committee of the Faculty of Medical Sciences, College of Medicine, University of Calabar with approval no: 262MLS1123.

2.6. Experimental design

Twenty of the acclimatized rats were assigned to four groups of five rats each and treated according to the order below:

- Group 1: Control.
- Group 2: Extract (250 mg/body weight).
- Group 3: Extract (500 mg/body weight).
- Group 4: Extract (1000 mg/body weight).

Treatments were oral and for 28 days.

2.7. Animal sacrifice and tissue preparations

At the end of the 28 days, the rats were fasted overnight and sacrificed using chloroform inhalation and blood collected through cardiac puncture. Blood sample was collected into well labelled plain plastic sample container for biomedical analysis. Sera was separated within one hour of blood clotting by centrifugation at 3000 x g for 5 minutes after which they were transferred into a clean and dried plain sample containers for analysis.

2.8. Lipid profile assay

2.8.1. Serum Total Cholesterol (TC)

Colorimetric method: Serum cholesterol was estimated by the cholesterol esterase, oxidase and peroxidase method of Allain *et al.* (1974), Roeschlau *et al.* (1974), Arntz *et al.* (1979). The reagent kit was manufactured by Agape Diagnostics, Switzerland

$$\text{Total cholesterol conc. (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc of standard}$$

2.8.2. Serum triglyceride (TAG)

Colorimetric method: Serum triglyceride was estimated by the glycerol -1- phosphate oxidase- DHBS method coupled with Trinder reaction of Fossati and Prencipe, (1982), McGowan *et al.* (1983). The reagent kit was made by Teco Diagnostics, U.S.A.

$$\text{Triglyceride conc. (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

2.8.3. Serum High Density Lipoprotein cholesterol (HDL-C)

Serum HDL was estimated by the precipitation method of Assmann, (1979). The reagent kit was made by Agape Diagnostics, Switzerland

$$\text{HDL cholesterol conc. (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times N \times 2$$

Where 2 = dilution factor of the sample and N= concentration of standard (50mg/dl)

2.8.4. Low density lipoprotein cholesterol (LDL-C)

This was evaluated from the Friedewald equation described by Friedwald *et al.* (1972).

$$\text{LDL-C} = [\text{Total cholesterol}] - [\text{HDL} - \text{C}] - [\text{VLDL} - \text{C}]$$

$$\text{Where VLDL-C} = \frac{[\text{Triglyceride}]}{5}$$

2.8.5. Very low density Lipoprotein cholesterol (VLDL-C)

This was evaluated from the Friedewald equation described by Friedwald *et al.* (1972).

$$\text{VLDL} = \frac{[\text{Triglyceride}]}{5}$$

2.9. Data analysis

Data from this study were presented as mean and standard deviation in tables. Microsoft ware SPSS version 22 was used to analyze the raw data. ANOVA was used to determined statistical significance. $P \leq 0.05$ was considered significant.

3. Results

Table 1, shows a non significant body weight gain, liver weight gain and kidney weight gain among the groups treated with different doses of the extract compared with the control and also their relative organ weight gains, these values compares well with [2]. In table 2, the serum fasting lipid profile of the experimental animals shows that TC, TAG, LDL-C, and VLDL-C were all significantly reduced in the treatments compared with the normal control whereas HDL-C showed a significant increase in the treatments compared with the normal control.

Table 1 Result of the body and organ weights of experimental animals gavaged ethanol leaves extract of *Gmelina arborea*

Treatments	NC	250 mg	500 mg	1000 mg
Initial body weight (g)	64.02±0.10 ^a	65.74±0.05 ^b	68.28±0.32 ^c	81.50±0.65 ^d
Final body weight (g)	121.28±12.25 ^{a,b}	120.00±13.42 ^{a,b}	115.52±7.19 ^a	132.14±03.62 ^b
% Weight gain	89.43±19.04 ^b	82.52±20.38 ^{a,b}	69.23±11.13 ^{a,b}	62.15±4.94 ^a
Liver weight (g)	6.29±0.51 ^{a,b}	6.17±0.35 ^a	6.06±0.10 ^a	6.70±0.31 ^b
Kidney weight (g)	0.97±0.03 ^a	1.03±0.06 ^b	0.99±0.02 ^{a,b}	1.03±0.02 ^b
Relative kidney weight	5.2±0.53 ^a	5.18±0.46 ^a	5.26±0.29 ^a	5.07±0.16 ^a
Relative liver weight	0.81±0.08 ^a	0.86±0.09 ^a	0.86±0.04 ^a	0.78±0.03 ^a

Values are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly different from any paired mean across the row.

Table 2 Lipid profile level of adult experimental animals gavaged ethanol leave extract of *Gmelina arborea*

Group	Treatments	TC(mg/dl)	HDL-C (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
1	Normal Control	99.20±4.35 ^c	61.98±1.45 ^a	72.10±1.64 ^b	22.80±4.38 ^c	14.42±0.33 ^b
2	250 mg/kg	87.36±1.60 ^b	64.32±0.72 ^b	70.20±1.32 ^b	9.00±1.27 ^b	14.04±0.26 ^b
3	500 mg/kg	86.50±1.29 ^{a,b}	66.06±0.99 ^b	66.84±1.68 ^a	7.07±2.22 ^{a,b}	13.37±0.34 ^a
4	1000 mg/kg	82.88±2.65 ^a	65.42±2.53 ^b	66.74±2.32 ^a	4.11±2.31 ^a	13.35±0.46 ^a

Values are presented as mean ± standard deviation (n = 5); and values with different letter superscripts are significantly different from any paired mean within the column is significantly different at P<0.05. Group 1- Normal control, Group 250mg/kg body weight, Group 3- 500mg/kg body weight, Group 4- 1000mg/kg body weight.

4. Discussion

Lipids are vital for synthesizing cell membranes, energy storage, intracellular messaging, and transporting other essential organic substances such as vitamins [11]. Chylomicrons, low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), high-density lipoproteins (HDL) and Very low-density lipoproteins (VLDL), are the 5 major types of lipoproteins [11]. These lipoproteins transport hydrophobic lipids such as phospholipids, triglycerides, and cholesterol in the plasma and extracellular fluids [11, 12]. Significant decrease in serum VLDL-C, LDL-C, total cholesterol, triglycerides and a significant increase in HDL-C as observed in our study indicates a positive health outcome, mainly a reduced risk of cardiovascular disease as a result of ingestion of the extract by the experimental animals. This is as high levels of these lipoproteins are associated with the development of atherosclerosis [11, 13, 14] and factors such as diet, sex, and race significantly influences their production and secretion [15].

5. Conclusion

In conclusion, the results indicate that ethanol leaves extract of *Gmelin arborea* might be an important source of phytonutrients which could be used in the management of hyperlipidemia and in weight management in experimental animals. This conclusion was based on the findings of the study.

Compliance with ethical standards

Acknowledgments

This work was carried out in collaboration between the authors: Ibe, DD designed, supervised, performed the analysis, interpreted the data and wrote the manuscript of the study. Author Ugboaja, R.O, assisted in provision of essential materials and collection of samples data. Author Lele, K.C, read and approved the final manuscript.

Disclosure of Conflict of Interest

There is no conflict of interest between and among authors in any aspect either financially or otherwise.

Statement of ethical approval

This study involves the use of animal subjects, approval was sought and approval given by the faculty animal research ethics committee (FAREC-FBMS) faculty of Basic Medical Sciences University of Calabar, Nigeiria with the ethical committees reference number reference 262MLS1123. The research was conducted, by following the guidelines set by National Institute of Health, USA as approved and adapted by the faculty animal research ethics committee (FAREC-FBMS) faculty of Basic Medical Sciences University of Calabar, Nigeria.

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