

Experimental Studies on the Hypolipidemic and Haematological Properties of Aqueous Leaf Extract of *Cleistopholis Patens* Benth. & Diels. (Annonaceae) in Hypercholesterolemic Rats

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Abstract

Background: *Cleistopholis patens* (Cp) (Annonaceae) is a popular medicinal herb used in the treatment of cardiovascular disorders in Nigerian ethnomedicine.

Objectives: The present study investigated the effects of the aqueous leaf extract of Cp on lipid and haematological profile of hypercholesterolemic Albino rats.

Methods: The animals were divided into 5 groups (A – E) of 5 animals per group. All the animals in groups A – D, along with their normal diet, also received 400 mg/kg of cholesterol suspended in 2 % Tween 80 (p.o) daily for 60 days. Six hours after each cholesterol feeding, the animals were treated respectively with a hypolipidemic drug, simvastatin; 10 mg/kg, C. patens extract; 400 mg/kg, 600 mg/kg and 2 % Tween 80 (negative control). The last group (positive control) received cholesterol free diet and 2 % Tween 80. All the treatments were administered orally for a period of 60 days. Thereafter, the lipid and haematological profile of serum and blood samples collected were determined respectively.

Results: Treatment with simvastatin (10 mg/kg), the extract (400 and 600 mg/kg) significantly ($P = 0.033$) lowered the total cholesterol, triglycerides, low density lipoproteins (LDL) levels and significantly ($P = 0.05$) increased the level of high density lipoproteins (HDL) in comparison with the negative control group. There was no significant difference between the cholesterol lowering effect of the extract and that of simvastatin ($P = 0.991$). The haemoglobin concentration, packed cell volume, red and white blood cell counts of treated animals did not produce any significant change ($P = 0.705$). The LD_{50} showed that the extract has a wide margin of safety.

Conclusion: The extract showed marked hypolipidemic activity.

Keywords: *Cleistopholis patens*; hypolipidemic activity; haematological parameters.

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Introduction

The elevation of serum total cholesterol, triglycerides and low density lipoprotein (LDL) along with decreased HDL levels are known to cause hyperlipidemia which is responsible for

initiation and progression of atherosclerosis impasse.^{1, 2} This is the hallmark of early cardiovascular disease. Cardiovascular diseases have been implicated as leading causes of death in developed and the third world countries. In Africa and in most other third world countries, where the use of folk medicine is prevalent, the search for herbal cure is a common practice. There is an increasing interest in the use of natural products as protective agents against cardiovascular diseases.

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In China, green tea is increasingly being used as a protective agent against cardiovascular diseases.^{3,4} The effect of drinking green tea on plasma lipoproteins appears to be characterised by decreasing LDL cholesterol and increasing HDL cholesterol levels.^{4,5}

Cleistopholis patens (Benth.) Engl. & Diels. (Family: Annonaceae) is a tree of about 30 m high, occurring from Sierra Leone eastwards into Uganda and Zaire. In Nigeria, the Igbo tribe of Southeastern region refers to it as 'ojo' while the Yorubas call it 'apako' in their native dialect.⁶ In Liberia, the tree trunks are used to float heavy timber while in Ghana the trunks are used as floats, to make drums, canoes and roof-beams.⁶ In Nigeria, it is used to make canoes and has been called 'canoe wood'.⁶ In Ghana, leaf-infusion, with lemon grass, papaya or other plants, has been used for infective hepatitis. The leaves have been used as a vermifuge in many countries.⁶ In Nigeria, (South East), leaf extracts from this plant are used in the treatment of cardiovascular diseases by traditional healers [oral information]. The leaf oil contains beta-cimene as the main constituent, while the fruit oil contains linalool oxides. The main components of the stem-bark oil are myrcene, P-cymene, and germacrene D. The root bark of *Cleistopholis patens* collected in Ghana yielded two sesquiterpenes and five alkaloids.⁶ This work was therefore, designed to evaluate the effects of the aqueous leaf extract of *C. patens* on lipid and haematological profile in hypercholesterolemic rats.

Materials and Methods

The experimental protocol used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka, and conforms with the guide to the care and use of animals in

research and teaching of University of Nigeria (Nsukka, Enugu State, Nigeria).

Animals

The rats (males and females) used in this study (150-232g) of about 10 weeks old were obtained from the animal house of the Department of Veterinary Obstetrics and Gynaecology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The males were separated from the females to avoid mating. The animals were acclimated for 2 weeks and were housed in aluminium cages. They were fed *ad libitum* on Vital® pelleted grower feed mash and water while the study lasted.

Plant Collection and Identification

The leaves of *C. patens* were obtained from Nsukka locality in Enugu state, Nigeria, in April, 2009 and were identified in the Department of Botany, University of Nigeria, Nsukka, by a plant taxonomist. A voucher specimen has been deposited in the Department's herbarium for reference purposes.

Preparation of the Extract

The fresh leaves of *C. patens* were dried at room temperature and then reduced to coarse powder. The powder (150 g) was soaked in 600 ml of petroleum spirit to defat the substance. The dried marc was later soaked in distilled water kept in a beaker. They were shaken at regular intervals of 2 hours. After 24 hours, the extract was filtered with number 1 Whatman filter paper. The concentration of the aqueous extract and the yield were determined. It was then stored in the refrigerator at 4°C before the study commenced.

Chemicals, Solutions/Reagents

Enzymatic kits for serum triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL)-

cholesterol and low density lipoprotein (LDH)-cholesterol were purchased from Sigma Chemical, USA. The cholesterol used came from BHD, USA; simvastatin (Ranbaxy, India).

Acute Toxicity

A total of 25 Albino Wistar (males and females kept separate) rats weighing between 150 and 232 g were used to determine the acute toxicity of the aqueous extract of *Cleistopholis patens* (leaves). They were assigned to 5 (A, B, C, D, and E) groups of 5 rats per group. The animals were deprived of water for 16 h before the administration of the extract. The animals were administered orally with the extract at doses of 200, 400, 600, 800 and 1000 mg/kg respectively. The rats were given food and water *ad libitum* and were observed over a period of 24 h for acute toxicity signs such as dullness, ruffled hair, depression, clumping together and death.

Determination of Serum Cholesterol and Triglycerides

Five groups of rats (n=5) were selected for the study. The animals were grouped according to their sexes and treated as follows: The first group received cholesterol (400 mg/kg) and simvastatin (10 mg/kg). The second and the third groups received cholesterol orally as in the first group and were treated with 400 and 600 mg of *C. patens* respectively. The fourth group served as the negative control. This group received only cholesterol (400 mg/kg) and 2 % Tween 80 while the fifth group served as the untreated control that received only 2 % Tween 80. Cholesterol was suspended in Tween 80. The extract and the drug were given 6 h after administration of cholesterol. The treatment lasted for 60 days.

Lipid Profile

The lipid profile such as Total cholesterol (TC), Triglycerides (TG), high density lipoproteins (HDL) and low density lipoproteins were estimated by standard methods.⁷

Haematological Analyses

At day 60, rats in all the groups were bled through the retroorbital plexus.⁸ Blood samples for the haematological analyses were collected into a bottle containing EDTA anticoagulant while the samples for biochemical analyses were collected into bottles without anticoagulant and allowed to clot. Serum was harvested from the clotted blood after centrifugation at 1500 rpm. Red blood cell count (RBC) and total leukocyte count (TLC) were done using haemocytometer⁹, haemoglobin concentration (HbC) determination was carried out using Cyanomethaemoglobin method,¹⁰ and packed cell volume (PCV) was determined by microhaematocrit method.⁸

Statistical Analysis

The data collected were subjected to statistical analysis using the procedure outline for a completely randomised design¹¹. Values were recorded as mean and standard deviation using SPSS 17.0. Test for significance was performed using the One way analysis of variances (ANOVA) and the appropriate post hoc test.

Results

Extraction

The aqueous extract of *Cleistopholis patens* (leaves) was brown in colour and the concentration was 37 mg/ml. The yield was 0.55 % w/w.

Acute Toxicity

No death was recorded even at the highest dose of 1000 mg/kg body weight.

Lipid Profile

The lipid profile showed significant decreases in the concentration of total serum cholesterol, triglycerides and the LDL ($P = 0.033$; $P = 0.01$ and $P = 0.009$ respectively), along with significant ($P = 0.01$) increases in the level of

HDL in the groups treated with the extract and simvastatin when compared with the group treated with cholesterol only. (Table 1).

Haematological Analyses

The haematological parameters monitored include, PCV, haemoglobin concentration, RBC and WBC counts. These parameters did not change significantly; $P = 0.705$. (Table 2).

Table 1: The Effects of Aqueous Extract of *C. patens* on Rats Fed Cholesterol for 60 Days

Lipid Profile	Mean \pm SEM				
	A Simvastatin (10mg) + Cholesterol	B Extract (400mg/kg) + Cholesterol	C Extract (600mg/kg) + Cholesterol	D Cholesterol Only	E Control
Total Serum Cholesterol (mg/dl)	38.00 \pm 8.87**+	42.00 \pm 5.29*+	31.00 \pm 2.52**	73.00 \pm 4.34 ⁺	30.00 \pm 7.75**
Triglycerides (mg/dl)	53.13 \pm 16.44*	46.88 \pm 12.88*	37.50 \pm 7.22*	62.50 \pm 7.22	46.75 \pm 10.71*
HDL (mg/dl)	28.10 \pm 3.27*	27.50 \pm 5.66*	27.86 \pm 3.83*	22.00 \pm 5.66	30.20 \pm 2.52*
LDL (mg/dl)	15.96 \pm 2.90**	16.71 \pm 2.30**	13.18 \pm 5.42**	35.63 \pm 1.06	16.04 \pm 8.37**

* $P = 0.033$, ** $P = 0.01$ in comparison with the negative control (cholesterol group only)

Table 2: The Mean Haematological Changes in Rats Fed Cholesterol for 60 Days and Treated with Aqueous Extract of *C. patens*

Groups	Simvastatin (10mg) +Cholesterol	Extract (400mg/kg) +Cholesterol	Extract (600mg/kg) + Cholesterol	Cholesterol only	Control
PCV (%)	42.40 \pm 0.97	41.20 \pm 1.02	41.60 \pm 1.36	39.60 \pm 0.68	41.60 \pm 1.21
HB (g/dl)	13.30 \pm 0.43	11.52 \pm 0.73	11.92 \pm 0.43	10.94 \pm 0.53	12.84 \pm 0.51
RBC($\times 10^6/\mu\text{L}$)	6.84 \pm 0.17	6.31 \pm 0.24	6.44 \pm 0.17	6.03 \pm 0.15	6.72 \pm 0.21
WBC($\times 10^3/\mu\text{L}$)	10.06 \pm 1.22	10.30 \pm 2.01	9.86 \pm 1.31	9.58 \pm 7.93	9.88 \pm 6.94

Discussion

This work investigated the hypolipidemic effect of *C. patens* in hypercholesterolemic rats. Feeding the animals with 400 mg/kg of cholesterol for 60 days resulted in hyperlipidemia as evidenced in the negative control group. (Table 1). Hyperlipidemia has

been implicated in the development of atherosclerosis¹². In this study, all the doses of the extract (400 and 600 mg/kg) and simvastatin (10 mg/kg) showed significant decreases in the blood lipid levels when compared with the negative control. The total cholesterol, triglycerides and low density lipoproteins levels were significantly

decreased along with significant increases in the level of HDL. Several classes of hypolipidemic drugs or agents are known. They may differ in both their impact on the cholesterol profile and adverse effects. Some may lower the "bad cholesterol" low density lipoprotein (LDL) more than others, while others may preferentially increase high density lipoprotein (HDL) "the good cholesterol".¹³ The statins or β -hydroxymethylglutaryl coenzyme A (HMG CoA reductase inhibitors) are a class of drugs used to lower plasma cholesterol level. Statin drugs lower LDL and total cholesterol. They have a moderate effect on boosting artery-cleansing HDL.¹⁴ They lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Inhibition of this enzyme in the liver results in decreases in cholesterol synthesis as well as increases in the synthesis of LDL receptors resulting in an increased clearance of low-density lipoprotein (LDL) from the bloodstream.¹⁵ Dietary plant sterols have been shown to inhibit this rate limiting enzyme, HMG-CoA reductase.¹⁶ Monacolin-K, a compound found in standardised red yeast rice extract, is a naturally occurring statin. In clinical studies, red yeast rice along with healthy lifestyle practices lowers cholesterol as effectively as the prescription drug, simvastatin. A substantial body of supportive research further indicates that monacolin-K also reduces triglycerides and boosts HDL, thus offering a safer and broader spectrum alternative to taking synthetic prescription statins.¹⁷⁻²³

Studies in the past showed that the extract contains saponins, flavonoids, alkaloids, steroids, glycosides²⁴ and both volatile and

fixed oils such as beta ocimene, linlool, Z-linalool oxides, myrcene, P-cymene and germacrene D²⁵ as chemical constituents. The lipid lowering effect of *C. patens* may be ascribed to these chemical constituents. When there are plenty of plant sterols and dietary fibre in the intestine, especially soluble fibre from fruits, vegetables, oats, peas and beans, cholesterol absorption decreases.^{26, 27} Plant metabolites such as flavonoids and saponins are also known to reduce cholesterol levels.² Saponins and bile acids bind with cholesterol in the intestine, making cholesterol unavailable for absorption and then excreted with the feces.² Oral administration of saponins from some medicinal plants, significantly reduced triglycerides and cholesterol levels in rat. The usage of diet with high saponin content is also suggested to reduce heart diseases.²⁸ Saponins have also been shown to increase the lipoprotein lipase activity (LPL) and this enhances faster removal of free fatty acids from circulation thereby, causing a decrease in total cholesterol.^{29, 30} HDL functions in the transport of cholesterol away from the peripheral tissues to the liver, thus preventing the genesis of atherosclerosis. The observed significant increase in the level of HDL further points to the cardiac protective activity of the extract.

Investigation into the haematological changes in the animals used in this study did not show any significant change (s) among the extract, simvastatin and the control groups. This is an indication that the extract may not have adverse effect on the haematological parameters investigated. The results and the parameters obtained in the study are limited to the duration the study lasted. Increase in the duration of the study may have given a different result. More large scale studies should be carried out in this perspective.

In conclusion therefore, the aqueous extract of *C. patens* showed very strong hypolipidemic activity in this study. This study has therefore, validated the use of this extract as a hypolipidemic and hypocholesterolemic agents in folkloric medicine by the natives of some part of Eastern Nigeria. This plant extract did not show any adverse effects on the haematological profile of the animals used in the study. The mechanism (s) of the action was not investigated. We therefore, recommend that detailed studies on the mechanism (s) of action will be carried out in our subsequent work on this plant material.

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