

Original Research Article

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Effects of Ethanol Extract of *Cola lepidota* Seed on Lipid Profile and Haematological Parameters of Albino Wistar Rats

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ABSTRACT

This study was conducted to evaluate the effects of ethanol seed extract of *Cola lepidota* (Monkey kola) on lipid profile and haematological parameters of albino wistar rats. Twenty eight (28) adult albino wistar rats weighing between 170g-200g were randomly divided into four groups of 7 rats each. The control group (I) was fed with normal rat chow ad libitum. Group II received daily oral dose of 100mg/kg of the extract, Group III received daily oral dose of 200mg/kg of extract while Group IV received daily oral dose of 300mg/kg. After 21 days of treatment, the animals were sacrificed and plasma obtained for haematological assay and lipid profile determination. Results indicated that there was a significant decrease ($p < 0.05$) in total cholesterol (mmol/L), triglyceride (mmol/L) and low density lipoprotein (mmol/L) in test groups compared to control. The result showed a significant increase ($p < 0.05$) in packed cell volume (%), haemoglobin (g/dl), mean corpuscular volume (fl), mean corpuscular haemoglobin (pg), WBC count (μ L), platelet count (μ L). There was however a significant decrease ($p < 0.05$) in mean corpuscular haemoglobin concentration (g/ml) compared to control. It was therefore concluded that the extract is capable of stimulating haematopoeisis and is a potent antihyperlipidaemic agent and should therefore be used in the management of cardiovascular disease risk factors.

Keywords

Cola lepidota,
Antihyperlipidaemic,
Haematopoeisis,
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Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value used in drug synthesis and development (Bailey *et al.*, 2009, Sofowara, 1993). Tropical African sub-regions are home to many of these plant species whose potentials have not been fully realized (Ogbu *et al.*, 2014). A good number

of these fruit species are yet to be domesticated. However, tangible economic produce are being harvested from their wild and protected stands in home gardens, farmlands and forest reserves (Okigbo, 1997; Burkill, 2000; Meregini, 2005).

Monkey kola is a common name given to a number of minor relatives of the cola specie that produce edible tasty fruits. They belong to

the same botanical family *Malvaceae* and sub-family *Sterculioideae* with the popular West African plantation kola nuts (*Cola nitida*), grown for their masticatory and stimulating nuts (Bosch *et al.*, 2000; Angiosperm Phylogeny Group, 2011). Among the species commonly referred to by this name (Monkey cola) are *Cola parchycarpa*, *C. lepidota* and *C. laterita* (Meregini, 2005; Brink *et al.*, 2007). *Cola lepidota* has a yellow pulp while the other two being *C. parchycarpa* and *C. laterita* have white and red pulp respectively (Ogbu *et al.*, 2007, Singh *et al.*, 2010). The red variety is rare. The pod of the yellow variety is roundish while the white variety is cylindrical in shape.

Cola lepidota tree grows upto 18m high with a twisted trunk and carliferous lump. *Cola lepidota* also known as monkey cola in West Cameroon, Duala Mbwid, Oji Ochicha (Cockroach cola) or achicha (Iwu, 1993) among the Igbo speaking people of Nigeria grows wild and especially distributed in Lower Guinea, Gabon, Western Cameroon and Eastern Nigeria (Oghenerebo *et al.*, 2013). According to Anya (Anya, 1982), Southeast Nigeria holds rich species diversity of the cola group and had been regarded as the primary centre of early domestication for the monkey kola species.

Cola lepidota fruit is edible, crunchy and tasty. Native people of Southern Nigeria and Cameron relish the fruits as well as primates like baboons, monkeys etc (Essien *et al.*, 2017). *Cola lepidota* is employed in alternative medicine in Nigeria in treatment of pulmonary troubles, fabrigues, cancer (because of its antioxidant property) (Engel *et al.*, 2011; Oghenerebo *et al.*, 2013). The cola species are also used in traditional medicine to prevent dysentery, headache and to suppress sleep (Morton, 1992; Seitz *et al.*, 1992; Odion *et al.*, 2013). Phytochemical studies reveal that monkey kola contains glycosides, saponins,

steroids, flavonoids, β -carotene (Adegboye *et al.*, 2008; Ene-Obong *et al.*, 2016). Monkey kola also has substantial amounts of iron, zinc, copper, selenium, Calcium, sodium, potassium, vitamins A, B and C (Okudu *et al.*, 2015; Ene-obong, 2016).

Lipid profile or lipid panel and differential haematological parameters are a panel of blood tests that serve as an initial broad medical screening and diagnostic tool for disease conditions like heart failure, hypertension, stroke, leukaemia, thrombocytopaenia (Merck Manual, 2012; Togun *et al.*, 2007; Jonas-Mosby, 2005). Lipids such as cholesterol, triglycerides, high and low density lipoproteins and haematological parameters like packed cell volume (PCV), haemoglobin (Hb), white blood cell count and differentials are effective tools in the diagnosis of dyslipidaemia, atherosclerosis, anaemia, compromised immune system (Ugwu *et al.*, 2013; Ezekwesili *et al.*, 2008; Togun *et al.*, 2007; Onyeyili *et al.*, 1992). The results of these tests can identify certain genetic diseases and can determine approximate risks for cardiovascular diseases, acute pancreatitis, cerebrovascular diseases (Enechi *et al.*, 2014, Miller *et al.*, 2011).

Hyperlipidaemia, anaemia and low resistance to infections and their attendant health challenges viz., pancreatitis, cardiovascular disorders, coronary artery diseases, arthrosclerosis, septicaemia still pose a great health challenge especially to people in third world countries which abound in Africa where alternative medical practice holds sway because of high level of poverty, poor education and lack of access to quality healthcare (Leeders *et al.*, 2004; WHO, 2007). It is in view of the above that this research was designed to ascertain the effects of the readily available *Cola lepidota* seed on lipid profile and haemotological parameters so as to give a

scientific backbone to the use of this plant in traditional medicine in the management of obesity, cardiovascular diseases and infective processes.

Materials and Methods

Preparation of experimental materials

Cola lepidota fruits were purchased at the Relief Market, Owerri, Imo State, Nigeria. The fruits were washed and the seeds obtained by peeling the bark and cutting open the fruit pulps. The seeds were chopped into pieces, air dried at room temperature for two weeks and ground into fine particles using an electronic grinding machine. The extract was prepared by mixing 100g of the powder with 100mls of 100% ethanol. After 48 hours, the ethanol was evaporated using a rotary evaporator, leaving the extract which was then dissolved in normal saline after further concentration using electronic incubator at 40°C.

Experimental animals

Twenty eight (28) healthy albino wistar rats weighing between 170-200g were used for the study. These rats were sourced from the stalk of Animal and Environmental Biology Department, Imo State University. The rats were confined in wooden cages made of iron nets and allowed one week for acclimatization. During this time, they were fed with standard rat pellets (Livestocks Feeds Nig. Ltd; Ikeja, Lagos) and water ad libitum. The experiment proceeded for 21 days.

Experimental design

The animals used were randomly divided into four groups, each group consisting of seven rats and labeled as groups I, II, III, IV. Group I served as control and received normal rat feed and water for 21 days. Group II rats, received orally 100mg/kg body weight of *Cola lepidota*

seed extract for 21 days. Group III rats received orally 200mg/kg body weight of *Cola lepidota* seed extract for 21 days. Group IV rats received 300mg/kg body weight of *Cola lepidota* seed extract for 21 days.

All the animals in groups I to IV were allowed access to water and rat diet ad libitum throughout the duration of the experiment.

Blood collection and analysis

At the end of the experimental period, the animals were fasted for 8 hours and then sacrificed under chloroform anaesthesia. Blood samples were collected via cardiac puncture and transferred into:

Labeled tubes containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant for determination of haemoglobin concentration, percentage packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell and platelet count based on methods described by Lewis *et al.*, (2001).

Labeled tubes without anticoagulant and allowed to clot. Serum was obtained by centrifuging at 400rpm for 10 minutes in a wisperfuge centrifuge (Model 1384). The serum obtained was used for determination of total cholesterol concentration, triglyceride concentration, high density lipoprotein concentration (using assay kit from Randox Laboratories, Plasma), plasma level of low density lipoprotein concentration was determined using the Friedewald equation (Friedewald *et al.*, 1972).

Statistical analysis

Statistical analysis of results obtained was done using the SPSS version 15.0. The means and standard deviations were calculated for all

parameters under investigation. Statistical differences between the experimental and control groups were determined using one-way analysis of variance followed by students t-test. Values were considered significant at $p < 0.05$. Results are presented as mean \pm S.D.

Results and Discussion

The effect of *Cola lepidota* seed extract on lipid profile markers in wistar albino rats are shown in Table 1. Feeding the rats with *Cola lepidota* seed for 21 days at 200mg/kg (Group III) and 300mg/kg (Group IV) was found to produce a significant decrease ($p < 0.05$) in the concentration of total cholesterol, triglyceride and low density lipoprotein when compared with control. Conversely there was a significant increase ($p < 0.05$) in the concentration of high density lipoprotein. There was however no statistically significant ($p > 0.05$) difference in the concentration of total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein following 100mg/kg *Cola lepidota* seed feeding for 21 days.

Table 2 shows the effects of *Cola lepidota* seed on some haematological parameters, namely: packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count and platelet count of albino wistar rats.

In the animals fed with *C. lepidota* seed extract for 21 days at 100mg/kg (Group II), 200mg/kg (Group III) and 300mg/kg (Group IV), the PCV, Hb, MCV, MCH and WBC count were found to be significantly increased ($p < 0.05$) when compared with the control. Also there was a statistically significant decrease ($p < 0.05$) in MCHC and platelet count in the animals fed with *C. lepidota* seed for 21 days at 100mg/kg (Group II) 200mg/kg

(Group III) and 300mg/kg (Group IV) when compared with the control.

One of the efficient ways of managing the ever increasing cases of hyperlipidaemia and its complications such as atherosclerosis and hypertension is diet therapy (Enechi *et al.*, 2014). This helps to achieve the control of major risk factors such as blood cholesterol and triacylglycerol that predispose to these disorders (Ghasi *et al.*, 2000). Hyperlipidaemia is characterized by elevated levels of cholesterol, triglycerides and phospholipids and changes in lipoprotein (Badage *et al.*, 1991). High density lipoprotein is the smallest of the lipoprotein species containing approximately 20% cholesterol ester and very little triglyceride. It is strongly and independently related to coronary heart disease. Unlike LDL, the relationship is inverse, a high LDL being an important risk factor for coronary heart disease (CHD) and high HDL level protecting against CHD (Udenze *et al.*, 2012).

The results of this study clearly indicate that the administration of extract of *Cola lepidota* seeds produced hypolipidaemic effect in experimental animals. Phytochemical analysis of *Cola lepidota* shows that it contains flavonoids, cardiac glycosides, steroids and saponins (Adegboye *et al.*, 2008; Ene-obong *et al.*, 2016). Previous studies showed that these phytochemicals acting wholly or partly may be responsible for the lipid lowering action of some plant extracts (Gaamoussi *et al.*, 2010). The underlying mechanism of lipid lowering effect of *Cola lepidota* could be by inhibition of lipid absorption due to the presence of saponin in *Cola lepidota* (Ram *et al.*, 1997; Ahmed *et al.*, 2010) or inhibition of cholesterol esterase, activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate (Sharmila *et al.*, 2007).

Table.1 Effects of *Cola lepidota* seeds on some lipid profile markers of albino wistar rats

Rat Group	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
I	2.71 ± 0.07	1.91 ± 2.01	0.95 ± 0.09	1.90 ± 0.04
II	2.59 ± 0.09	1.68 ± 1.10	1.10 ± 0.02	0.68 ± 0.02
III	2.51 ± 0.13*	1.57 ± 0.08*	1.22 ± 0.05*	0.47 ± 0.01*
IV	1.41 ± 0.05*	1.43 ± 0.03*	2.45 ± 0.01*	0.24 ± 0.05*

Values are presented as mean ± S.D of three determinants n = 7

TC = Total Cholesterol

TG = Triglyceride

HDL = High Density Lipoprotein

LDL = Low Density Lipoprotein

Values found in the same column and bearing asterisk (*) are statistically significant (p<0.05) from control (Group I).

Table.2 Effect of *Cola lepidota* seeds on haematological parameters in albino wistar rats

Group	PCV%	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/ml)	WBC Count x 10 ³ /μL	Platelet count x 10 ³ /μL
I	0.32 ± 0.6	9.06 ± 1.49	78.02 ± 15.73	29.18 ± 6.50	39.68 ± 4.49	45.10 ± 2.64	317.56 ± 2.16
II	2.38 ± 0.08*	13.30±1.66*	112.36±24.57*	39.12±4.88*	35.50±4.07*	67.60±4.50*	292.82±3.61*
III	2.36 ± 0.08*	12.92±1.60*	101.08±23.46*	35.88±2.06*	38.72±4.86*	70.25±3.42*	287.31±4.11*
IV	3.38 ± 0.05*	13.36±0.76*	104.64±23.88*	44.52±2.10	35.54±3.56*	86.34±2.51*	243.64±3.42*

Values are presented as mean ± SD of three determinants. n = 7.

Means with asterisk are significantly different (p<0.05) from control (Group I).

PCV = Packed Cell Volume

Hb = Haemoglobin

MCV = Mean Corpuscular Volume

MCH = Mean Corpuscular Haemoglobin

MCHC = Mean Corpuscular Haemoglobin Concentration

WBC = White Blood Cell

Cola lepidota extract may also inhibit cholesterol absorption from the intestine due to the formation of complexes with compounds such as glycosides and saponins (Enechi *et al.*, 2014). Another plausible mechanism of hypolipidaemic effect of *Cola lepidota* could be modulated by the flavonoid content. Flavonoids from plants have been variously implicated in the reduction of lipid level by inhibiting hepatic HMG-CoA (Jung *et al.*, 2006). Flavonoids decreased the total cholesterol and triglycerides of rats (Miyake *et al.*, 1998).

The above findings show that oral administration of *Cola lepidota* facilitates lipid metabolism and hence can be antiatherogenic. This is evidenced in this study as treatment with *Cola lepidota* seed extract reduced atherogenic index (TG/HDL) in a dose dependent manner, achieving the most favourable reduction at the highest dose. Such reduction has been reported in previous works where plants and their extracts containing such phytoconstituents reduced the atherogenic indices in diabetic rats (Gupta *et al.*, 2009). Increase in serum HDL is an improvement of antiatherogenicity. This is an

advantage since HDL is responsible for transportation of cholesterol from peripheral tissues to the liver for metabolism. HDL exerts part of antiatherogenic effect by counteracting LDL oxidation (Adaramoye *et al.*, 2006). It also inhibits oxidation of LDL by transition metal ions and also prevents 12-lipoxygenase mediated formation by lipid hydroperoxide (Nofer *et al.*, 2002).

The white blood cells, red blood cells and related indices play a great role in the pathogenesis of cardiovascular diseases and the body's immune response to organisms. The assessment of hematological parameters is a useful guide to ascertaining the effect of foreign substances including plant extracts on a given organism (Nwankpa *et al.*, 2017). They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs (Magalhaes *et al.*, 2008; Oyedemi *et al.*, 2011). Following the administration of *Cola lepidota* seed extract, Hb, PCV, MCH, MCV increased appreciably. This gives indication that the plant extract may contain some phytochemicals that can stimulate the formation and/or secretion of erythropoietin in the stem cells of the animals. This erythropoietin secretion in the kidneys of the experimental animals increases the synthesis of RBC (Mbaka *et al.*, 2010). The ability of the plant extract to increase RBC and its related indices may be attributed to its ability to lower lipid peroxidation level that causes haemolysis of erythrocytes (Ashafa *et al.*, 2009). Previous studies on this plant revealed presence of flavonoids, zinc, selenium, vitamin C (Adegboye *et al.*, 2008, Ene-obong, 2016). These compounds have been reported to possess strong antioxidant capacity (Akah *et al.*, 2007) and therefore inhibit production of polyunsaturated fatty acids in the cells (Torell *et al.*, 1986; Faure *et al.*, 1991).

However, there was a significant decrease in MCHC upon the administration of the extract and this is in conformity with the findings of (Osuchukwu *et al.*, 2016). There was also a significant increase in white blood cell count in the wistar rats upon the administration of the extract.

The presence of some phytochemicals with ability to stimulate production of red blood cells in the extract could be responsible for the observed result in treated rats (Akinpelu *et al.*, 2008). The decrease in platelet count as seen in the study is at variance with the findings of (Oyedemi *et al.*, 2011) where the administration of a plant extract with similar phytoconstituent led to an increase in platelet count.

In conclusion, it would be appropriate to say that *Cola lepidota* seed administration has a strong antiatherogenic and haematopoietic effect and hence could be cardioprotective and reduce risk of cardiovascular disease as well as boost the body's ability to fight infection.

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