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Effect of Petroleum Ether Extract of *Kigelia africana* Leaf on Serum Lipid Profile in Phenyl hydrazine Induced Anaemic Female Wistar Rats

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Abstract

This research was designed to evaluate the effect of petroleum ether extract of *Kigelia africana* leaf on lipid profile following phenylhydrazine induced anaemia in male Wistar rats. A total of 35 male rats were distributed into five (5) groups of seven (7) rats each per group and experimental Group A served as Normal control (non-anaemic control), group B: Anaemic rats (induced with phenyl hydrazine) without treatment (anaemic control), group C; anaemic rats treated with feroton (Standard control), group D; Anaemic rats with 100mg/kg petroleum ether leaf extract *Kigelia africana* extract (PETLETKG1), and group E; Anaemic rats treated with 200mg/kg between petroleum ether leaf extract *Kigelia africana* extract (PETLETKG2). All administrations were done orally using oropharyngeal cannula once per day for 14days (2 week). Blood was collected by cardiac puncture using disposable syringe and needle to draw blood into plane sterile tubes. Thereafter, the samples were analysed using standard methods. The result depicts the significant ($p<0.05$) increase in serum

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triglyceride, high density lipoprotein and total cholesterol when compared with both normal and standard control following phenyl hydrazine induced anaemia. More so, the petroleum ether extract of *K. africana* significantly ($p<0.05$) decrease on serum low density lipoprotein when compared with both the standard and normal control. The result suggests that the extract might contain some bioactive ingredients or phytonutrients which may reduce or inhibits predisposition to complication(s) associated with cardiovascular related disorders under prolonged anaemic condition.

Keywords: Anaemia, Cardiovascular, Erythropoiesis, Lipid Profile, Phenyl Hydrazine

INTRODUCTION

Serum lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test. The test includes four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. It is usually done in fasting blood specimens. Cholesterol is an essential component of every cell structure in the body. It is necessary for formation and repairs of new and existing cells (Jefcoat *et al*., 2012). Cholesterol is also utilized during the synthesis of cortisol and testosterone by the adrenal gland and testicles respectively (Dasofunjo ,2024). Increased levels of cholesterol or triglycerides are most often indication of genetic or inherited disorders of lipid metabolism. These lipids may also be increased by some common medical conditions such as hypothyroidism, diabetes mellitus (DM), kidney and liver disease (Beckman *et al.,* 2012). Fat – rich diets also affect serum cholesterol and TG concentration negatively (Simon *et al*., 2015). The cholesterol especially the low-density lipoproteins (LDL) have been implicated in cardiovascular diseases.

Anaemia is a disease characterized by the reduction in the concentration of Haemoglobin, circulating RBC and its indices (MCV, MCH and MCHC) and PCV per unit of the peripheral blood below the normal.

Anaemia has significant consequences for human health, as well as for social and economic development. In 2010, anaemia accounted for 68.4 million years of life lived with disability, or 9% of the total global disability burden from all conditions (Kassebaum *et al*., 2014). Anaemia has been associated with negative health and development outcomes, including neonatal and perinatal mortality, low birth weight (Figueiredo *et al*., 2018), premature birth

(Haider *et al*., 2013: Rahman *et al*., 2016) and delayed child development (McCann and Ames, 2007).

The negative effects on health and development outcomes from anaemia arise from the impacts of decreased oxygen delivery to tissues (in which multiple organ systems may be affected), as well as effects related to the underlying causes of anaemia, which are difficult to disentangle. For example, in iron deficiency anaemia (IDA), decreased iron availability has well-established negative effects on brain development and functioning even prior to anaemia development.

Researchers over the years have shown that medicinal plants can be useful in the management of anaemia, cardiovascular and degenerative diseases. A prototype of these plants includes *Kigelia africana*. *Kigelia africana* (Lam.) Benth. syn. *Kigelia pinnata* belongs to the family *Bignoniaceae* and is the only species in the genus *Kigelia* (Nsubuga, 2018). The generic name *Kigelia* comes from the Mozambican name for sausage tree, "kigeli-keia". *Kigelia africana* is native to Africa, thus the derivation of the species name *"africana"*. The tree is deciduous, with a rounded crown, thick trunk, dark-grey to light-brown, scaly slash creamy-white with a green edge, low-branching, branches and branchlets spreading and lenticellate (Dhungana *et al*., 2017). The tree reaches maturity within four to six years, with a height of up to 24 meters (Dhungana *et al*., 2017). The leaves are alternate, pinnate and stipules absent; rachis up to 50 cm long; leaflets three to six opposite pairs, usually with a terminal leaflet, elliptic to elongated lanceolate, 7–20 cm long, 4–12 cm wide, apex abruptly to gradually shortly acuminate, base slightly asymmetrical, rounded to cuneate, margin sentire or sometimes lightly toothed, coriaceous or papyraceous, shiny green and usually scab rid above, dull green and glabrous to tomentose below; midrib impressed above, major lateral veins 7–12 pairs and prominent below (Dhungana *et at*., 2017). The flowers of *K. africana* are hermaphrodite, zygomorphic and five-merous. The calyx is campanulate approximately 1–4-cm-long, 1–2-cm-wide, fleshy, irregularly five-lobed, the lower lobes generally longer at maturity and the calyx mouth thus oblique. The corolla is greenish-yellow to purplish-red or bright claret, 5–12-cm-long, the throat rather abruptly expanded, limb 9–18 across with the two upper lobes smaller than the three lower and velvety inside; stamens *four* fertile and one staminode about half the fertile stamens. The ovary is conical, tapering into a slender style subequalling the stamens (Adam and Alhameed, 2013; Hussain *et al*., 2016). They possess a very unpleasant scent, which is most notable at night, indicating their reliance on pollination by bats, which visit them for pollen and nectar (Hussain *et al*., 2016). The fruits

are indehiscent, woody, greyish-brown, sausage-shaped and pendulous, up to 50-cm-long and 15 cm in diameter, with elongated pedicels. The seeds are numerous, unwinged, obovate and 1.25-cm-long (Adam and Alhameed, 2013). The fruits usually weigh 10 kg (Priya *et al*., 2013). The mature fruits can be found on trees throughout the year (Singh *et al.,* 2018), (Hussain *et al*., 2016). Although not eaten by humans, they find wide applications in traditional medicine (Dhungana *et al*., 2017). Due to the unusual fruits and large attractive flowers, *K. africana* is considered as tricking ornamental plant, and the fruits are used as florists' materials. The tree is sometimes planted as a boundary marker but usually at roadside sand for shade.The baked fruits of *Kigelia african*a are used for fermentation of beer. It also has internal application including treatment of dysentery, ringworm, tapeworm, malaria, diabetes, pneumonia, haemorrhage, and tooth care (Gills, 2012,Dasofunjo, 2024). The fruits and bark ground and boiled water are taken orally or used in treating children's stomach ailment usually tapeworm (Navab *et al*., 2006). This present research is designed to determine the effect of *Kigelia africana* extract on lipid profile following phenylhydrazine induced anaemia in Wistar rats.

MATERIALS AND METHODS

Materials

Plant Materials

Fresh leaves of *K. africana* were collected from the CRUTECH environment, Okuku, Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 205 has been deposited for future reference at the department's herbarium.

Chemicals and Reagents

All chemicals and reagents (Phenyl hydrazine, ethanol, sulphuric acid, ferric chloride, chloroform, hydrochloric acid, Mayer's reagent and Wagner's reagent) used were of analytical grade. Fresh distilled water was used throughout the experimental period. Assay kits used in the analysis in this study were products of Randox Laboratories (England).

Experimental animals

Twenty-five (35) male Wistar rats was obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals

were allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively with 12 hours natural light-dark cycle. They were allowed food and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages.

Methods

Preparation of extract of *K. africana* leaf

The leaves of *K. africana* were collected around CRUTECH and air dried at room temperature for a period of 21days until constant weight was obtained. The dried leaves were then pulverized to powdered form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*K. africana*) was dissolved in 1200ml of 70% petroleum ether for 72 hours. This was followed with vacuum filtration and extracts was concentrated using an evaporator water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

Induction of haemolytic anaemia

Haemolytic anaemia was induced by intraperitoneal (I.P.) injection of phenyl hydrazine (PHZ) at 10 mg/kg for 7 days. Anaemia was considered to be induced by comparing the PCV of the PHZ-induced animals with that of the normal control (non-induced) animals after 24hours of the last induction. The PCV was carried out by the capillary tube method whose procedures are as follows; blood was collected from the tail into the capillary tube and one end of the tube was sealed with plastacin. The capillary tube was place inside a haematocrite centrifuge and spun for five minutes at 2000 rpm.

Treatment of animals

The experimental rats were randomly divided into five (5) groups, with five animals per group and treated for a period of fourteen (14) days.

Group A: Normal control (non-anaemic control)

Group C:Anaemic rats treated with feroton (Standard control)

Group B: Anaemic rats(induced with phenyhdrazine) without treatment (anaemic control)

Group D: Anaemic rats treated with 100mg/kg bwt petroleum ether leaf extract *Kigelia africana* extract (PETLETKG1)

Group E: Anaemic rats treated with 200mg/kg bwt petroleum ether leaf extract *Kigelia africana* extract (PETLETKG2)

All administrations were done orally using oropharyngeal cannula once per day for 14days (2 week).

Blood sample collection

Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle draw blood into plane sterile tubes. The specimens were labelled with the identification alphabets/ number. The samples were kept at room temperature until processing, which occurred within 30 minutes of collection.

RESULTS

The result below reveals the effect of petroleum ether extract *Kigelia africana* leaf on serum lipid profile in phenyhdrazine induced anaemia in Wistar rats. Following the administration of the extract of *Kigelia africana* , the extract *significantly*($P<0.05$) increased the serum HDL, TG and TC when compared to the normal ,standard control (table1).

More so, the extract of *Kigelia africana produced a significant* ($P<0.05$). *decrease in serum* LDL when compared to the normal and standard control in phenyhdrazine induced anaemicWistar rats (table1).

Table 1: Effect of petroleum ether extract *Kigelia africana* leaf on serum lipid profile in phenyhdrazine induced in Wistar rats

Group	TC (mg/dL)	TGL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
NC	103.33 ± 3.33^{a}	79.33 ± 3.18^{a}	49.67 ± 2.40^{a}	159.67 ± 2.91^{a}
Anaemia+ Feroton	161.33 ± 4.10^{b}	353.00 ± 4.04^{b}	104.33 ± 2.33^{b}	228.67 ± 4.37^{b}
AnaCpPHZ	230.67 ± 10.04^{c}	454.67 ± 8.45^{c}	34.67 ± 1.45^{c}	364.33 ± 7.45^{c}
Anaemia +PETLEKA$_1$	170.67 ± 1.76^{b}	232.33 ± 0.50^{d}	115.00 ± 1.16^{d}	138.33 ± 4.41^{d}
Anaemia +PETLEKA$_2$	158.33 ± 3.33^{b}	222.67 ± 2.91^{d}	108.67 ± 2.33^{b}	132.00 ± 3.06^{d}

Values are expressed as mean$\pm$SEM. (n = 5). Values with different superscript along the columns are statistically significant ($P<0.05$). NC: Normal Control, Standard: Anaemic rats+ Feroton (10mg/Kgbwt), AnaCpPHZ; Anaemic rats control, induced with phenylhydrazine, Anaemia + PETLEKA$_1$: Anaemic rats + Petroleum ether leaf extract of

Kigelia africana (100mg/Kgbwt) , Anaemic rats + PETLEKA$_2$: Anaemic rats + Petroleum ether leaf extract of *Kigelia africana* (200mg/Kgbwt).

DISCUSSION

The recent shift from orthodox medicines to ethno botanicals and pharmacognosy or plants with medicinal potentials is a major contributory factor to a decline in the prevalence of degenerative diseases or management of the disposable risk factor in most developing nations specifically in Africa continent, Asia and beyond (Dasofunjo *et al.*, 2013). Assessment of the alteration on the lipid profile of major lipids like Total Cholesterol (T.c), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL) and Triglycerides (T.G) might reveal a clinical basis to understanding the metabolism of lipids and it role in predisposing humans to atherosclerosis, coronary heart diseases and other cardiovascular related disorder (Dasofunjo *et al.,* 2018). Elevation of serum lipid levels increases the risk of atherosclerosis and coronary heart disease (Yang *et al.*, 2015). Its effect on health increases with age, thus great attention is paid to abnormal levels of lipids and its associated factors (Ashar *et al.*, 2015; Sotuneh *et al.*, 2014).

Anaemia is a very common disease and is estimated that one-quarter of the world's population suffer from it. Preschool children and women are more susceptible to develop anaemia. This problem dramatically increases in people who live in Asia and Africa. About two-thirds of preschool-aged children living in Africa are anaemic. There are studies reporting the beneficial effect of anaemia on lipid profile. Type of anaemia does not influence the lowering of lipid levels (Chowta *et al.*, 2017). It has been reported that anaemia may be associated with increased risk of long-term complications of cardiovascular events and death (Winther *et al.*, 2014).

HDL is an anti-atherogenic lipoprotein; it acts as cholesterol scavengers and transports cholesterol from peripheral tissues into the liver and thereby acts as a protective factor against coronary heart disease. Increase in HDL might be due to an increase activity of certain enzyme known as lecithin cholesterol acyl transferase (LCAT) an enzyme responsible for incorporating free cholesterol into HDL (Geetha *et al.*, 2011). High levels of HDL cholesterol can lower the risk of heart disease and stroke. The observed significant increase in serum level of HDL from this research work after administration of *Kigelia*

africana leaf suggest that the extracts might be a panacea to lowering the risk or predisposition to cardiovascular related disorders following the induction of anaemia.

Elevated serum triglyceride concentrations contributes to increased risk of cardiovascular disease, the increased risk of acute pancreatitis is an additional consideration when a patient's triglyceride level is very high (Hodis *et al*., 2010). The two main sources of plasma triglycerides (also known as triacylglycerol) are exogenous (i.e., from dietary fat) and carried in chylomicrons, and and endogenous (from the liver) and carried in very-low-density lipoprotein (VLDL) particles. Chylomicrons are hydrolyzed by lipoprotein lipase into free fatty acids. After a meal, over 90% of the circulating triglycerides originate in the intestine and are secreted in chylomicrons. The observed increase in serum triglyceride may be due to increased production from the liver and intestine (by means of upregulated synthetic and secretory pathways) or through decreased peripheral catabolism (mainly from reduced lipoprotein lipase activity) (George *et al*., 2009). The increase in serum triglyceride following the administration of the extract indicates that the plant extract possesses hypertriglyceridemic activity which is a major risk factor for development of atherosclerosis and other cardiovascular related disorders under anaemic condition.

Cholesterol is a lipid that has multiple functions, is a source of bioactive molecules such as steroid hormones, vitamin D and bile acids, which in turn can regulate cellular metabolism and both intracellular and extracellular communication. It is also important for signal transduction (Liu, 2009). It forms a vital part of the membranes of the spinal cord, nervous system, peripheral nerves and the brain. It is the main constituent of myelin sheath that functions as an insulation layer. Cholesterol is also a forerunner of important hormones such as testosterone, estradiol. The main factors responsible for high cholesterol levels are hereditary factors, stress, smoking, obesity and dietary cholesterol (Gersh *et al.,* 2010). Abnormally high levels of cholesterol can also cause coronary heart disease. From this study, the observed increase in serum cholesterol at 100mg/kg and 200mg/kg body weight indicates that the extract may increase the risk factor for cardiovascular disease such as coronary heart disease and stroke under anaemic condition.

Low-density lipoprotein (LDL) partiles transport cholesterol and its esters in the human bloodstream. Clinically, LDL plays a central role in the development of cardiovascular diseases, in particular atherosclerosis. High serm LDL levels have been shown to increase the risk of Atherosclerosis (Linsel-Nitschenke and Tall, 2005). From this research, the

increase in serum LDL suggest that the extract possess cholesterol lowering or clearing ability or a hypocholesterolemic agent which might be a tool in the management of certain related disorders, atherosclerosis and disorder cardiovascular dysfunction under anaemic condition.

CONCLUSION

It can be inferred from this present study that the extract of *Kigelia africana* might contain some bioactive ingredients or phytonutrients which may reduce predisposition to complication associated with cardiovascular related disorder under anaemic condition.

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