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Kigelia africana extract attenuates hypertension-induced haematological disturbances in Wistar rats

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ABSTRACT

Hypertension is a major health problem worldwide. It can lead to cardiovascular disease and functional disturbances including haematological disturbances. The abnormal changes of haematological parameters may enhance end-organ damage. Therefore, this study assessed the effect of extract of *Kigeliaafricana* (KA) leaf on haematological indices of Wistar rats following L-NAME induced hypertension. Thirty-five (35) male Wistar rats were grouped into seven groups (n=5). Treatment was conducted as follows: group I (normal control) received only normal saline orally, group II (hypertensive control) received L-NAME (40 mg/kg b. wt/day) intraperitoneally; group III (standard control) received L-NAME (40 mg/kg b.wt/day) + 20mg/kg b.wt/day of amlodipine, while groups IV and V got extracts of *Kigeliaafricana* (200 mg/kg b.wt/day and 400 mg/kg b.wt/day respectively). Group VI received (200mg/kg bwt KA + 40 mg/kg b.wt L-NAME), group VII received (400mg/kg b.wt KA + 40 mg/kg b.wt L-NAME). The result showed that the extract of KA administered to hypertensive rats, produced a significant ($p<0.05$) increase in Hb compared with both normotensive and hypertensive controls. There was a significant increase in the RBC and HCT concentrations of all groups compared with the normotensive control. The TWBC, MCV and MCH levels of all groups showed no significant ($P>0.05$) difference compared with the normotensive control. The MCHC levels of test groups showed no significant difference compared with both the normotensive and hypertensive controls, except groups VI and VII that were significantly ($P<0.05$) increased compared with all groups. The platelet count of the hypertensive group was significantly ($P<0.05$) decreased compared with both the normotensive control and the rest of the groups. Treatment with the extract reversed the effect of hypertension with a significant ($P<0.05$) increase in platelet count. The study suggests that platelet count could be useful in the early diagnosis of hypertension and can serve as a simple diagnostic prognosticator. It also suggests that the extract of *Kigeliaafricana*serves to relieve hypertensive disturbances.

Keywords:*Kigelia africana,* hypertension, endothelial cell, blood pressure, haematology

INTRODUCTION

Hypertension is a primary risk factor for cardiovascular disease, including stroke, heart attack, heart failure, and sometimes death [1]. Keeping blood pressure under control is vital for preserving health and reducing the risk of these dangerous conditions. The force exerted by a person's blood on the walls of their blood vessels is known as blood pressure [2]. This pressure depends on the resistance of the blood vessels and how hard the heart must work. The globalisation of unhealthy lifestyles has advanced non-contagious diseases, such as the position of the world's leading cause of mortality and outranking infectious diseases [3]. Globally, cardiovascular diseases account for approximately 17 million deaths annually. It has been reported that complications caused by hypertension account for 9.4million deaths worldwide every year [4]. Early, diagnosis of hypertension may minimise the risks and complications associated with hypertension [5]. Over the years, the use of herbal medicines has been considered 'safe' because they are 'natural', the intake of such herbal preparations maybe with some visible side effects, particularly in older patients treated with polypharmacy [6]. Haematological profiles in both humans and animals are an important index for the physiological state of an individual [7]. Haematology

normally encompasses the evaluation of full blood count (FBC) and the organ in producing blood. Additionally, plants may reduce levels of packed cell volume (PCV), erythrocytes (RBC) and haemoglobin, significantly altering white blood cell value. In some cases, however, medicinal plants do not have harmful effects on haematological and serum biochemical parameters [8].

Kigelia is based on an African name and *africana* means from Africa. The genus *Kigelia* has one species and occurs only in African. *Kigelia* is a genus of flowering plants in the family *Bignoniaceae* [9]. The genus consists of only one species, *Kigelia africana*, which occur throughout tropical Africa [10]. The so-called Sausage tree grows a fruit that is up to 60cm (2feet) long, weighs about 7kg (15 pounds) and resembles a sausage in a casing [11]. The tree is easily propagated from fresh seed sown in river sand in September or from truncheons. The sausage tree *(Kigeliaafricana)* is said to be a popular shade and street tree in tropical Africa and Australia [12]. *Kigeliaafricana*is used to manage infectious diseases, including leprosy, impetigo and worm infestations in blood. Dermal complaints and infections, such as whitlow, cysts, acne and boils, are treated with traditional medicines containing the fruit and used frequently, the bark [13]. Therefore, since there is a dearth of information on the relationship between hypertension and haematological indices hence, this research was designed to determine the effect of *Kigeliaafricana*on haematological profile following L-NAME induced hypertension in Wistar rats.

Materials and Methods

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 205 has been deposited for future reference at the department's herbarium.

Experimental animals

Thirty-five (35) male Wistar rats were obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals were allowed to acclimatize for 7 days, in a well-ventilated room at room temperature and relative humidity of 29±2°C and 70%, respectively, with 12 hours natural light-dark cycle. They were allowed food and water *ad libitum*. Daily cleaning and removal of faeces and spills from the animal cages were done to maintain good hygiene. The animals were handled in line with ethical guidelines and approved by the Faculty of Basic Medical Sciences Ethical Committee, CRUTECH.

METHODS

Preparation of extract of *K. africana* leaves

The leaves of *K. africana* were collected around CRUTECH and air-dried at room temperature for a period of 21days until a constant weight was obtained. The dried leaves were then pulverised to powdered form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*K. africana*) was dissolved in 1200 mL of 70% petroleum ether for 72 hours. Then 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.

Experimental design

Majithiya*et al.* (2005) method were used to induce hypertension by intraperitoneal administration of L-NAME (40 mg/kg b.wt/day) in distilled water. Treatment was conducted as follows: group I (normal control) received only the vehicle (normal saline) orally, group II (hypertensive control) received L-NAME (40 mg/kg b.wt/day) intraperitoneally, group III (standard control) got L-NAME (40 mg/kg b.wt/day) +20mg/kg b.wt /day of amlodipine, while groups IV and V (reference groups) received only extract of *Kigelia africana* (200mg/kg b.wt/day and

400 mg/kg b.wt/day respectively). Group VI received 200mg/kg b.wt KA +40L-NAME, while group VII received 400mg/kg b.wt KA + 40L-NAME.

Blood sample collection

The collection of blood from the test rats and control was through cardiac puncture using disposable syringes and needles. The blood collected was put into Ethylene Diamine Tetra-acetic Acid (EDTA) tubes. The specimens were labelled with the identification letters/ number. The EDTA samples were maintained at room temperature until processing, which occurred within 30 minutes of collection.

Determination of haematological parameters

Blood samples collected in EDTA bottles were analysed for haematological parameters using a haematologyanalyser (Sysmex, Kobe, Japan) following the manufacturer's instructions. The parameters analysed include white blood cell count (WBC) and the differentials, platelets, red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Procedures

Each blood sample was mixed well and then approximately 20 μL was aspirated by allowing the analyser's sampling probe into the blood serum sample and depressing the start button. Results of the analysis were displayed after about 30 seconds, after which the analyser generated a paper copy of the results on thermal printing paper.

Statistical analysis

The data obtained were analysed using a one-way analysis of variance (ANOVA). The SPSS software version 20.0 was used to establish statistical significance at $P<0.05$.

RESULTS

The result below shows the effect of extract of *Kigeliaafricana* leaves extract on haematological indices following L-NAME induced hypertension in Wistar rats. Following L-NAME induced hypertension in Wistar rats. RBC and HCT of all groups significantly ($p<0.05$) increased compared with the negative control. While the Hb of the test groups increased significantly ($P<0.05$) compared with both the normal and negative controls, Neu % was not clear-cut, but there was a general decrease in all groups, which were significant in groups III, IV and VII compared with the normal control (Table1). The effect of administration of *K. africana* extract on serum total white blood cells (TWBC) showed no significant ($P>0.05$) difference compared with both normotensive and hypertensive controls. There was an indication of decreased TWBC when the extract alone (group IV) was administered, however, it was not significant ($P>0.05$) compared with the normotensive group (Table 2). More so, there was a significant ($P<0.05$) increase in LYMP of all groups compared with the normotensive control, except group V, which compared well with the normal control (Table 2). There was also a significant ($P<0.05$) increase in the MID of all groups compared with the normotensive control, however, groups III and IV were significantly ($P<0.05$) increased compared with both the normotensive and hypertensive controls. The platelets (PLT) were significantly ($P<0.05$) decreased in the hypertensive control compared with the rest of the groups. However, the PLT of the test groups were significantly increased compared to both the normotensive and hypertensive controls (Table 2). There was a general decrease in MCV and MCH of all groups compared with the normal control but it was not significant ($P>0.05$). The MCHC of the negative control was not altered compared with the normal control. However, groups VI and VII were significantly increased compared with both normal and negative controls (Table 3)

Table 1: Effect of extract of *K. Africana* on RBC, HB, HCT and NEU parameters following L-NAME-induced hypertensive Wistar rats

Group	RBC (X10^{12}/l)	HB (g/dl)	HCT (%)	NEU (%)
I (Normal control)	5.30±0.14^{a}	12.20±0.20^{a}	33.00±1.53^{a}	58.17±3.88^{a}
II (Negative control)	7.95±0.10^{b}	12.50±0.21^{a}	43.33±0.88^{b}	51.00±3.22^{abc}
III (Positive control)	7.89±0.10^{b}	13.83±0.23	41.00±1.00^{b}	47.83±0.73^{bc}
IV (200mg/kg b.wt KA)	8.18±0.08^{b}	14.47±0.50^{bd}	44.00±1.73^{b}	46.53±1.27^{c}
V (400mg/kg b.wt KA)	7.92±0.07^{b}	14.07±0.03^{b}	42.00±0.00^{b}	56.70±3.31^{a}
VI (200KA +40L-NAME)	8.17±0.46^{b}	15.03±0.29^{cd}	43.33±2.03^{b}	52.07±2.04^{a}
VII (400KA+40L-NAME)	8.20±0.83^{b}	15.57±0.0.7^{c}	43.67±0.67^{b}	48.97±0.38^{b}

Values are expressed as mean ±SEM. (n = 5). Values with different superscripts along the columns are statistically significant (P<0.05). Legend: Normal control = group that received normal saline, Negative control = group treated with 40mg/kg b.wt L-NAME, Positive control = group that received 40 mg/kg b.wt L-NAME + Amlodipine, L-NAME = N (gamma)-nitro-L-arginine methyl ester, KA= *Kigeliaafricana*

Table 2: Effect of extract of *K. africana*on TWBC, LYMP, PLT and MID parameters following L-NAME-induced hypertensive Wistar rats

Group	TWBC (X10^{9}/l)	LYMP (%)	PLT (X10^{9}/l)	MID (%)
I (Normal control)	8.50±1.90^{a}	33.17±1.16^{a}	317.67±2.33^{a}	5.50±0.76^{a}
II (Negative control)	7.97±0.76^{a}	41.13±0.94^{b}	306.00±2.08^{b}	7.60±1.08^{c}
III (Positive control)	9.10±2.32^{a}	41.00±1.53^{bd}	403.67±9.70^{c}	11.17±1.88^{b}
IV (200mg/kg b.wt KA)	6.83±1.80^{a}	43.73±1.16^{d}	384.00±10.04^{c}	10.33±0.33^{b}
V (400mg/kg b.wt KA)	9.87±2.03^{a}	35.57±2.19^{ac}	339.67±11.49^{d}	7.73±1.27^{c}
VI (200 KA +40L-name)	9.83±0.71^{a}	39.23±2.45^{bc}	360.67±7.67^{d}	9.03±0.48^{bc}
VII (400 KA+40L-name)	8.37±0.93^{a}	42.53±0.30^{d}	431.33±4.06^{e}	8.50±0.27^{c}

Values are expressed as mean ±SEM. (n = 5). Values with different superscripts along the columns are statistically significant (P<0.05). Legend: Normal control = group that received normal saline, Negative control = group treated with 40mg/kg b.wt L-NAME, Positive control = group that received 40 mg/kg b.wt L-NAME + Amlodipine, L-NAME = N (gamma)-nitro-L-arginine methyl ester, KA= *Kigeliaafricana*

Table 3: Effect of extract of *K. africana*on MCV, MCH and MCHC parameters following L-NAME-induced hypertensive Wistar rats

Group	MCV (Fl)	MCH (Pg)	MCHC (g/l)
I (Normal control)	40.00±1.00^{a}	21.73±3.89^{a}	451.67±4.81^{a}
II (Negative control)	39.60±1.29^{a}	17.77±0.50^{a}	450.00±1.73^{a}
III (Positive control)	38.43±0.12^{a}	17.50±0.29^{a}	457.33±7.45^{ab}
IV (200mg/kg b.wt KA)	38.60±0.87^{a}	17.63±0.43^{a}	458.00±8.96^{ab}
V (400mg/kg b.wt KA)	39.17±0.03^{a}	17.70±0.10^{a}	453.33±3.336^{a}
VI (200 KA +40L-name)	38.00±0.32^{a}	18.07±0.03^{a}	477.00±3.51^{c}
VII (400 KA+40L-name)	38.07±0.72^{a}	17.77±0.27^{a}	466.67±1.86^{b}

Values are expressed as mean ±SEM. (n = 5). Values with different superscripts along the columns are statistically significant (P<0.05). Legend: Normal control = group that received normal saline, Negative control = group treated with 40mg/kg b.wt L-NAME, Positive control = group that received 40 mg/kg b.wt L-NAME + Amlodipine, L-NAME = N (gamma)-nitro-L-arginine methyl ester, KA= *Kigelia africana*

DISCUSSION

High blood pressure (hypertension) is a major driver of cardiovascular diseases that can lead to life-threatening conditions such as myocardial infarction, coronary heart failure, renal failure, and stroke [14]. The incidence of hypertension has been increasing worldwide, particularly in developing countries. For instance, from 80 million adults in the early 2000s, the number of hypertension cases in sub-Saharan Africa is expected to rise to 150 million by 2025 [15]. The pathogenetic mechanisms of hypertension encompass complex interactions of signalling pathways, genetic and environmental factors. Nonetheless, it is well-established that oxidative stress contributes to the development of hypertension through nitric oxide (NO) deficiency [16]. The vascular endothelial cells produce NO as a potent vasodilator with important roles in the growth and resistance of blood vessels [17]. NO synthase inhibitors induce endothelial dysfunction and oxidative stress by decreasing NO activity. Sub-chronic administration of laboratory rodents with NO synthase inhibitors, such as N-nitro-L-arginine methyl ester (L-NAME), results in chronic hypertension [18], hence the common use of this chemical for developing hypertension in experimental models. Haematological studies are useful in the diagnosis of many diseases and investigations of the extent of damage to blood [19, 20]. Blood that is a vital special circulatory tissue is composed of cells suspended in a fluid intercellular substance (plasma) with the major function of maintaining homeostasis [21]. Haematologicalindexes are valuable in monitoring feed toxicity especially with feed constituents that affect the blood as well as the health status of animals [22]. The prognostic situation of hypertension is closely related to the modification of haematological parameters. The association between hypertension (HTN) and haemoglobin (Hb) level has been linked to arginase enzyme effects on nitric oxide (NO) bioavailability [23]. During HTN, there is a possibility of haemolysis. But, whether haemolysis is a cause or effect of hypertension remains unclear. Most studies suggest that hypertension is a complication of haemolysis and associated with haemolyticanaemia [24]. During haemolysis, haemoglobin and arginase enzyme are released into circulation from erythrocytes. This free Hb is a scavenger of nitric oxide, which is produced in the endothelial cells that line the blood vessels and is important for the relaxation of blood vessels [25].Haemoglobin is an important factor in determining the viscosity of blood and causes a rise in systolic and diastolic pressure as it increases [26]. It can be considered that this outcome is not the case here as Hb is considered not cell-free in this work, but is encapsulated in the RBC and therefore increase in the Hb on the administration of the extracts to hypertensive rats did not diminish endothelial relaxation of blood vessels [27].It has also been reported that haematocrit (HCT) affects blood pressure (BP) to the point where a decrease of 10% HCT can raise the BP of 8.6mmHg [26]. In this work, the HCT for the hypertensive group was increased and the treatment with the standard drug or the extract did not alter any change. The administration of the extract alone gave similar results as well as with RBC and Hb, indicating that in this research, there was no obvious effect of hypertension on these haematological indices. This pattern is repeated for other haematological indices such as TWBC, LYMP, and MID. The absolute red blood cell indices viz MCV, MCH and MCHC did not show any alteration by hypertension. However, platelet level was significantly decreased in the hypertensive and increased in the treatment groups. Epidemiological studies have confirmed the high risk of stroke or myocardial infarction in multiple sclerosis that are ischaemic incidents, strictly associated with incorrect platelet function [28]. Platelets are essential in the formation and maintenance of blood and lymphatic vessels [29]. It plays an essential role in sensing and responding to perturbations in the blood and

vasculature [29]. These various properties make platelet count an important diagnostic tool for the determination of hypertension. In this current research, since the period of hypertensive induction lasted for only two weeks, it can be assumed that platelet count can be used as a simple haematological tool to deter mine undiagnosed or early hypertension.

CONCLUSION

The study suggests that platelet count could be useful in the early diagnosis of hypertension and can serve as a simple diagnostic prognosticator. It also suggests that the extract of *Kigeliaafricana* serves to relieve these hypertensive complications.

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