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Effects of methanolic leaf, bark and fruit extracts of *Kigelia africana* on haematology and erythrocyte membrane stability in rats

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Abstract
The toxicity, haematological and erythrocyte membrane stabilizing effects of different parts of *Kigelia africana* were evaluated. The different parts namely bark, leaf and fruit were subjected to phytochemical tests and flavonoids, saponnins, phlobatannin and tannins were found. Sub-acute toxicity study showed no adverse effect on erythrocytic indices at the doses tested for 28 days, *Kigelia africana* leaf (KAL) at 1000mg/kg caused leucopaenia in the rats. In vitro, *Kigelia africana* bark and fruit were able to protect red cell membrane against heat and hypotonicity induced lysis. Oral acute toxicity assays did not show any mortality at 5 g/kg of the plant extracts. The results indicated that the methanolic extracts of different plant parts *K. africana* had on adverse effect on haematology of rats at sub-acute dosing and is safe.

Keywords: Erythrocyte membrane, Haematology, *Kigelia africana*, Phytochemistry, Toxicity
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Introduction

Kigelia africana (*Lam Benth*) is a tropical African plant widely grown and distributed in Southern, Central and West Africa. It belongs to the family *Bignoniaceae* and commonly called the Sausage tree because of its huge fruits. In Nigeria, it is called 'pandoro' (Yoruba), 'uturubien' (Ibo) (Aiyeloja & Bello, 2006) and 'Hantsargiiwaa' (Hausa) (Blench, 2003). The tree is widely grown as an ornamental plant in tropical regions for its decorative flowers (Roodt, 1992). The sausage tree has a long history of use by rural African communities especially for its medicinal properties (Asekun *et al*., 2007). The tree can grow to more than 20 m tall and is found mostly in riverine areas. Different parts of this plant have been used for various purposes in different parts of the world (Burkill, 1985). The fruit pods are very fibrous with numerous seeds and tend to be inedible to humans as well as being poisonous when unripe. However, during famine in Malawi, the seeds are roasted and eaten. The Tonga applies powdered fruit as a dressing to wound (Gill, 1992). Unripe fruit is used in central Africa as a dressing for wounds, and in the treatment of haemorrhage and rheumatism (Sangita *et al.,* 2009). Venereal diseases are commonly treated with the extracts of *K. africana* usually in palm wine as oral medication (Walt & Breyer-Bradwijk 1962). The fruits and bark, ground and boiled in water are also taken orally or used in treating stomach ailments. The Shona people tend to use the bark as powder or infusion for application to ulcers, or applied in treatment of pneumonia (Pooley, 1993). Most commonly, traditional healers have used the sausage tree to treat a wide range of skin ailments from relatively mild complaints such as fungal infections, boils, psoriasis and eczema, through to the more serious diseases like leprosy, syphilis and skin cancer (Sangita *et al*., 2008). Previous studies of the fruits of *K. africana* showed some antibacterial activity (Grace *et al*., 2002).

The *Bignoniaceae* family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, saponins and caffeic acid (Sangita *et al*., 2009) in the fruits, stem, leaves and roots.
Considering the many medicinal purposes for which this plant is used, there is enormous scope for research need and further pharmacological investigation, hence this work compares and investigates the effect of administration of *Kigelia africana* fruit, bark and leaf extracts on haematology and erythrocyte membrane stabilisation in rats.

Materials and methods

Plant materials

Matured fruit, leaf and bark of *Kigelia africana* were obtained from the local market in Bode, Ibadan and authenticated at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife. The fruits were washed, cut into small pieces, oven dried at 40^{o}C and coarsely powdered. The leaves were air dried and powdered, the bark was oven dried and coarsely powdered. 500g of the plant parts were soaked separately in 1.5 litres of methanol for 72 hours and then filtered. The filtrates were concentrated using rotary evaporator. *Kigelia africana* fruit (KAF) yielded 40.8g (8.1%), *Kigelia africana* bark (KAB) yielded 25.2g (5.04%) and *Kigelia africana* leaf (KAL) yielded 30g (5.8%).

Experimental animals

Sprague-Dawley rats weighing 180-200g were used for the experiments. The animals were obtained from the Animal House of the Faculty of Pharmacy, Obafemi Awolowo University Ile-Ife. They were kept in the Animal Room of the Department of Animal Science to acclimatize for two weeks prior to the commencement of the experiment. All animals were allowed unrestricted access to water and commercial rat pellets and properly housed in wire mesh cages.

Phytochemical screening

Dry solid samples of the crude methanolic extracts obtained were subjected to phytochemical screening methods as previously described (Trease & Evans, 2002). Mayer, Dragendoff, Wagner and picric reagents were used to test for Alkaloid. Frothing test for saponin, ferric chloride test for tannin while Salkowski test for cardiac glycosides.

Acute toxicity test (LD_{50})

Sixty five female Sprague-Dawley rats (150-180g) obtained from the Animal House of the Faculty of Pharmacy of the ObafemiAwolowo University, Ile-Ife, Nigeria, were randomly divided into thirteen groups of 5 animals per group. The animals were fed with pelleted rat feed and had free access to drinking water but were starved for 12 hours prior to testing. The extracts were orally administered at 100, 500, 2000mg/kg methanolic extract of *Kigelia africana* bark, leaf and fruit (KAB, KAL, KAF) respectively. Group one was administered distilled water (10 ml/kg) as control. General symptoms of toxicity and mortality were observed for 24 hours for any sign of delayed toxicity.When no signs of toxicity were observed another set of animals (n=5) were administered the extracts at 5000mg/kg (Lorke, 1983).

Sub acute toxicity test

Sixty five rats were randomized into groups, consisting of six animals in each group. Group 1: control rats were administered drinking water (0.5 ml) on a daily basis for 28 days. Groups 2, 3, 4, 5, 6, 7, 8, 9 and 10 received 100, 500 and 1000 mg/kg body weight of methanolic extract of KAB, KAL and KAF respectively. Determination of haematological parameters that is erythrocyte count and total leucocyte count were by the haemocytometer method as described by Jain (1986) at the end of the experiment.

Membrane stabilization activity

Preparation of erythrocyte suspension - Fresh whole ox blood (10 ml) was collected, transferred into heparinized centrifuge tubes, centrifuged at 3000 rpm for 5 min, and washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). The composition of the buffer solution (g/L) was NaH_2PO_4 (0.2), Na_2HPO_4 (1.15) and NaCl (9.0) (Shinde, *et al*., 1999).

Heat induced haemolysis - The isotonic buffer solution (5 ml) containing 250 and 500 µg/ml of the methanolic extracts of KAB, KAL and KAF were put in 4 sets each (per concentration) of centrifuge tubes. Control tubes contained 5 ml of the isotonic buffer solution or 5 ml of 100 ug/ml of hydrocortisone. Erythrocyte suspension (0.05 ml) was added to each

tube and gently mixed. A pair of the tubes was incubated at 54°C for 20 min in a regulated water bath. The other pair was maintained at 0–4°C in a freezer for 20 min. At the end of the incubation, the reaction mixture was centrifuged at 1300 g for 3 min and the absorbance (OD) of the supernatant measured at 540 nm using Spectronic 2ID (Milton Roy) spectrophotometer. The level of inhibition of Haemolysis was calculated using the relation (Shinde *et al*., 1999): Inhibition of Haemolysis (%) = 100 [1-{(OD_2-OD_1)/(OD_3-OD_1)}]

Where OD_1 = absorbance of test sample unheated; OD_2 = absorbance of test sample heated; OD_3 = absorbance of control sample heated

Hypotonicity-induced haemolysis - The hypotonic solution (distilled water) (5 ml) containing 250 or 500 µg/ml of the extracts was put in 2 pairs (per dose) of centrifuge tubes. Control tubes contained 5 ml of the vehicle (distilled water) or hydrocortisone (0.5 mg/5 ml). Erythrocyte suspension (0.05 ml) was added to each tube and after gentle mixing, the mixtures were incubated for 1 h at room temperature (31°C). After incubation, the reaction mixture was centrifuged for 3 min at 1300 g and the absorbance (OD) of the supernatant measured at 540 nm using Spectronic 21D (Milton Roy) spectrophotometer. The inhibition (%) of haemolysis was calculated using the relation (Shinde *et al*., 1999):

Inhibition of haemolysis (%) = 100 [1-{(OD_2-OD_1)/(OD_3-OD_1)}]

Where OD_1 = absorbance of test sample in isotonic solution; OD_2 = absorbance of test sample in hypotonic solution; OD_3 = absorbance of control sample hypotonic solution

Statistical analysis

Data were expressed as means ± standard error of five replicates and were statistically analyzed using Analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SAS. Values were considered significant at $P<0.05$.

Results

LD_{50} Determination

The extracts were well tolerated by the animals as there were no observable signs of acute toxicity effects like restiveness, seizures or dizziness after the administration of 5000mg/kg. The LD_{50} of the different parts of *Kigelia africana* is therefore greater than 5000mg/kg orally in rats.

Phytochemical studies

The extracts were positive for saponins, cardiac glycosides, phlobatanins, flavonoids and tannins (Table 1).

Effect of extracts on haematological parameters

The results of this study with respect to the haematological changes showed that there was no significant change ($P<0.05$) in the erythrocyte indices for all the different extracts and doses tested when compared with the control group. KAB at 1000mg/kg showed a significant decrease in the WBC values when compared with the control, there was no difference for all the other groups for the WBC value (Table 2).

Heat and hypotonicity induced haemolysis

The extract protected the erythrocytes against heat- and hypotonicity-induced haemolysis in a concentration-related manner. The protective effect was greater on haemolysis induced by heat than that caused by hypotonicity (Table 3).

Table 1: Results of Phytochemical screening of *Kigelia africana* plant parts

Compounds	Tests	KAB	KAF	KAL
Alkaloids	Mayer and Dragendoff Test	Absent	Absent	Absent
Combined Anthraquinone	Borntrager's test	Absent	Absent	Absent
Free Anthraquinone	Borntrager's test	Absent	Absent	Absent
Saponins	Frothing test	+	+	+
Cardiac glycosides	Salkowski test	+	+	+
Plobatannins	Plobatannin test	+	+	+
Flavonoids	Shinoda's test	+	+	+
Tannins	Ferric chloride test	+	+	+

Key: KAB: *Kigelia africana* bark, KAF: *Kigelia africana* fruit, KAL: *Kigelia africana* Leaf

Table 2: Effect of sub acute administration of extracts on haematology of rats

EXTRACT	Dose rate (mg/kg)	PCV (%)	RBC(10^9/l)	HB (g/dl)	WBC (10^6/l)
KAB	100	32.7±2.0^{b}	8.5±5.1^{a}	11.4±0.3^{b}	6.0±1.5^{a}
KAB	500	36.7±2.8^{ab}	7.0±1.1^{a}	12.8±0.1^{ab}	5.0±1.6^{a}
KAB	1000	36.7±1.8^{ab}	7.0±0.8^{a}	12.8±0.1^{ab}	3.4±0.7^{b}
KAL	100	36.8±2.6^{ab} 37.2±1.1^{ab}	9.1±1.1^{a}	12.9±0.02^{ab}	4.8±0.5^{a}
KAL	500	41.5±1.8^{a}	7.2±0.9^{a}	13.0±1.5^{a}	6.6±0.7^{a}
KAL	1000	40.5±1.7^{a}	7.7±2.2^{a}	14.5±1.0^{a}	7.0±0.9^{a}
KAF	100	40.8±3.2^{a}	7.6±0.7^{a}	14.2±0.4^{a}	6.3±1.3^{a}
KAF	500	41.3±0.9^{a}	10.1±1.8^{a}	14.5±0.4^{a}	6.2±1.5^{a}
KAF	1000	36.0±1.5^{ab}	9.2±1.4^{a}	14.3±0.3^{a}	4.4±1.7^{a}
CONTROL	2ml/kg		6.25±0.3^{a}	11.5±0.4^{b}	7.1±1.2^{a}

KAB = *Kigelia africana* bark KAL = *Kigelia africana* leaves KAF = *Kigelia africana* fruit

Means with different superscript within a row are significantly different at p<0.05

Table 3: Effect of extracts on heat and hypotonicity induced haemolysis of red blood cells

Extract	Concentration (µg/ml)	Heat-induced haemolysis (Inhibition %)	Hypotonicity-induced haemolysis (Inhibition (%)
KAB	250	48±0.87^{c}	21±0.92^{e}
KAB	500	104± 4.62^{a}	58±1.08^{b}
KAL	250	0±0^{f}	12±0.91^{f}
KAL	500	5±0.41^{e}	32±1.47^{d}
KAF	250	64±2.27^{d}	12±0.82^{f}
KAF	500	91±1.82^{b}	37±1.08^{c}
Hydrocortisone	50	50±0.91^{c}	97.8±1.04^{a}

KAB—*Kigelia africana* bark, KAL--- *Kigelia africana* leaf, KAF--- *Kigelia africana* fruit

Means with different superscript within a row are significantly different at p<0.05

Discussion

In vitro assessment of the effect of the extract on membrane stabilization showed that *Kigelia africana* bark and fruit extracts inhibited heat- and hypotonicity-induced lysis of ox red blood cells. *Kigelia africana* bark showed a dose dependent inhibition of membrane lysis and the highest percentage inhibition of the parts while *Kigelia africana* leaf exhibited very weak activities. Direct interaction of constituents of the extract with membrane components such as proteins seems to be the most probable mechanism of action for these extracts. Membrane proteins are largely responsible for the physical properties of the cell membrane and may contribute to the regulation of the volume and water content of cells by controlling the movement of sodium and potassium ions (Rowman, 1996) through the protein channels which make up ion channels in the cell membrane (Guyton and Hall, 2000). Since pathological conditions can alter surface-volume ratio of the cell through loss of membrane surface or gain in volume (Rowman, 1996) the physical integrity of the treated cell membranes may have been enhanced by the extract (through a direct protective interaction with the membrane proteins) to hinder cell lysis including that caused by products such as those of the complement system involved in the inflammatory response cascade and hypotonic solutions that cause the cell to swell and rupture (Rowman, 1996). The stability of biological membranes is also affected by reactive oxygen species and the presence of phytochemicals like flavonoid, phlobatannin, saponnins which are able to react with these reactive species may be a factor in the membrane stabilisation ability of these extracts (Anam, 1997). The oral LD_{50} of the extracts were greater than 5000mg/kg hence the possibly of acute intoxication is remote. The subacute toxicity study showed that the extracts at the highest doses tested did not produce significant reduction in erythrocyte indices hence these extracts are not toxic to red blood cells. The study showed that there was a significant decrease in total white blood cell count in the group dosed with 1000 mg/kg. Toxic plants do not produce a direct effect on the white blood cells (Swenson & Reece, 1993), however Jain (1986) reported that toxic substances could cause decrease in TWBC through either bone marrow depression or competition with folic acid utilization to cause leucopenia, hence KAB at 1000mg/kg is able to cause immunosuppression in rats when administered over a long period of time. In conclusion, the

methanolic extracts of different parts of *Kigelia africana* are safe and the use of the fruit in arthritis is justified, but the *K. Africana* at higher dose should be used with caution since it could result in immunosuppression.

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