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The Effect of *Kigelia Africana* on the Uterus: Spotlight on Antioxidant Status and Cytoarchitecture.

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ABSTRACT

Numerous medicinal plants are of global interest today because of their therapeutic and economic significance. *Kigelia africana,* is a multipurpose medicinal plant with many attributes and considerable potentials. It is a powerful antioxidant which is rich in flavonoids and steroids. The study was carried out to investigate the effect of ethanolic extract of *kigelia africana* on the uterus of the non-pregnant Sprague-Dawley (S-D) rats. Twenty female S-D rats were used and they were divided into four groups (A-D). Each group contained five rats per group. Groups B-D received *kigelia africana* extract at 100 mg/kg, 300 mg/kg and 500 mg/kg doses respectively while group A served as the control and received distilled water. The animals were weighted daily, and the experiment was for duration of 28 days. At the end of the experiment, the animals were sacrificed and a ventral laparotomy was performed; the uterine horns were removed and dissected into two parts. The right horn was processed for histology, while the left horn was assayed for biochemical markers of oxidative stress (catalase, lipid peroxidation and superoxide dismutase). A significant increase ($p<0.05$) in the activities of SOD was observed compared to the control. MDA activities reduced significantly in a dose-dependent manner. No significant difference was observed in the catalase activities of the treated and control rats. Histological sections of the treated and control uterus appeared normal. *kigelia africana* increases the antioxidant status of the uterus thus preserving the cytoarchitecture of the uterus.

Keywords: *kigelia africana,* uterus*,* lipid peroxidase (MDA), catalase, superoxidase dismutase (SOD)

INTRODUCTION

According to the World Health Organization (WHO), approximately 80% of the world's population currently uses herbal medicines directly as teas, decocts or extracts with easily accessible liquids such as water, milk, or alcohol [1]. The use of herbs is very common in developing countries, particularly in rural settings [2]. The medicinal use of plants in the treatment of many diseases has gained popularity over the years [3, 4] according to WHO most of the people living in developing countries rely on medicinal plants for their primary health care [2,5].

Kigelia africana (Lam Benth), a tropical African plant that is widely grown and distributed in West Africa belongs to the family of *Bignoniaceae*. Due to its huge fruits it is called the sausage tree. In Nigeria, it is called 'pandoro' by the Yorubas, 'uturubien' by the Ibos [6] and 'Hantsar giiwaa' by the Hausas[7] *Kigelia africana* fruit has been used commonly in folk medicine to energise and improve sexual performance in men as well as treat sexually transmitted diseases like syphilis. In Kenya, the roasted seeds mixed with beer cause enlargement of sexual organs [8]. In South eastern Nigeria, the fruits and flowers are mixed with alcohol or water and used by traditional healers for fertility treatment among women and men of child bearing age [9].

The Bignoniaceae family is noted for the occurrence of flavonoids, terpenes, tannins, steroids, saponins and caffeic acid in the fruits, stem, leaves and roots [10]. Consequently, *Kigelia africana* is rich in tannins, flavonoids, steroids, phlobatannins, cardiac glycoside, terpenoids and saponins [11].

kigelia africana has been reported to restore the cytoarchithecture of the testis [12] and to boast the male reproductive function [13], However, there is a dearth of literature on the effect of *kigelia africana* on the non-pregnant uterus. This study is designed to investigate the effect of ethanolic extract of *Kigelia africana* on the antioxidant status and cytoarchitecture of the uterus in Sprague-Dawley rats.

MATERIALS AND METHODS

Plant Materials

Mature and ripe fruit of *Kigelia africana* were harvested from the forest of Badagry town in Lagos. The taxonomic identification was done at the Department of Botany in University of Lagos, Nigeria

and voucher specimen number- LUH 6426 was recorded for ease of identification.

Preparation of Extract
The fruits of *Kigelia africana* were washed, cut into small pieces, air-dried and ground into powder form using a grinding machine. The extraction was done using the Soxhlet apparatus with ethanol as the solvent as described by Abioye *et al,* (2003)[14]. Briefly 1.5 Kg of the powder was packed into the thimble of the Soxhlet apparatus containing 1 L of ethanol. At the end of the extraction, the extract was oven-dried at 38°C. A yield of 155.33 g was obtained and was stored in sterile bottles and kept in the refrigerator at 4°C until use. Three different sub-lethal doses- 100 mg/kg, 300 mg/kg and 500 mg/kg of *Kigelia africana* were used since the LD_{50} was well over 3900 mg/kg body weight of rat.

Experimental Animals
Twenty female Sprague-Dawley rats, weighing between 130-150 g were used for the experiment. The animals were obtained from Peter's Farm (Nig.) Enterprises in Badagry. They were kept in wire mesh plastic cages in the animal house of the Department of Anatomy College of Medicine of the University of Lagos, Nigeria under standard condition of temperature (27-30°C), with a 12 hours light and 12 hours dark cycle. They were left to acclimatize for two weeks before the commencement of the experiment. The animals were given water and commercial rat chow (Boar feed Ikeja, Lagos) ad libitum

Experimental Design
Animals were divided into 4 groups (A-D) with each group containing five rats. Group A served as control and received 1ml of distilled water. Groups B-D received 100 mg/kg, 300 mg/kg and 500 mg/kg extract of *kigelia africana* respectively. *Kigelia africana* was administered orally for 28 days. At the end of the treatment regime, the animals were sacrificed by cervical dislocation. All procedures involving animals were approved by the Departmental Committee on the use and care of animals and tissue collection.

Histological Procedures
A ventral laparotomy was performed, the uteri were dissected, weighed, and the right horn was transferred into a universal bottle containing boins fluid for at least 72 hours. Tissues were processed for microscopic examination using a standard protocol and 5 µm thick paraffin sections were made. Slides were stained with routine hematoxylin and eosin stains and photomicrographs were made at a magnification of 100 and 400 using Olympus and leica microscopes.

Biochemical Analysis
Briefly, the left uterine horns from each group were washed in an ice cold 1.15% KCL solution, blotted and weighed. They were then homogenized with 0.1M phosphate buffer (pH 7.2). The tissues were introduced into the mortar and laboratory sand was added to it (acid washed sand). This was blended together using a pestle. The resulting homogenate was centrifuged at 2500 rmp speed for 15 minutes. Thereafter, it was removed from the centrifuge and the supernatant was decanted and stored at -80°C until analysis.

Superoxide dismutase was assayed utilizing the technique of Leung and Armstrong, 1980. A single unit of enzyme was expressed as 50% inhibition of Nitroblue tetrazo-lium (NBT) reduction/min/mg/protein.

Catalase was assayed colorimetrically at 620 nm and expressed as µmoles of H_2O_2 Consumed/min/mg/protein as described by (Gernigon-Spychalowicz *et al*, 2009)[15].

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of (Buege and Aust, 1978). MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56 × 105 M-1CM-1.

Statistical Analysis
All data were expressed as Mean ± SD, one way analysis of variance (ANOVA) was used to analyse the experimental data. LSD multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant at $p < 0.05$.

RESULTS
No mortality was recorded during the experiment and no signs of toxicity symptoms were experienced by the animals as a result of the administered extract.

Effect on Body Weight
The study showed a significant increase in weight ($p>0.05$) in all the groups when the initial weight was compared to the final weight during the course of the experiment (Table 1). The highest weight gain was experienced in the group that received 100 mg of *kigelia africana* followed by the group that received 300 mg. The percentage weight gain was the same for 500 mg and the control group.

Effect on Biochemical Markers of Oxidative Stress
The study showed a dose-dependent significant reduction ($p < 0.05$) in lipid peroxidation when the treated groups were compared to the control. Furthermore, superoxide dismutase activities were significantly increased ($p < 0.05$) in all the treatment groups compared with the control group. Catalase activities did not follow any particular order. It was highest at 300 mg where it was comparable with the control value, and was reduced at 100 and 500 mg with lowest value at 500 mg. However, no significant difference was observed when the treated group was compared with the control (Table 2).

Effect on Uterine Histology

Histological sections of the control rat appeared normal. The surface epithelium (E) were of the simple columnar variety, with many cells having cilia. The connective tissue stroma (S) had abundant ground substance and fibroblastic cells. Uterine glands (UG) and arteries (A) are seen in the endometrium extending from the functional to the basal layer. The myometrium (M) consists of multiple interwoven layers of well vascularized smooth muscle. A clear lumen (Lm) is shown (plates 1A & 2A). Microscopic examination of sections from rats that were administered *kigelia africana* at doses of 100, 300 and 500 mg/kg body weight showed no difference from the control. The uterine sections appeared normal. Numerous endometrial glands (UG) lined with pseudostratified columnar cells and many small arteries are shown. The myometrium (M) and perimetrium (PM) appear normal (plate's 1B-D & 2B-D).

Table 1: Effect of *Kigelia africana* fruit extract on body weight in S-D rats.

Treatment	Initial body weight (g)	Final body weight(g)	% weight gain
Control	150.20 ± 6.5	159.40 ± 10 *	6.13
100 mg/kg	146.60 ± 10.3	163.20 ± 8.8 *	11.3
300 mg/kg	150.40 ± 6.6	160.60 ± 5.8 *	6.78
500 mg/kg	150.20 ± 6.5	159.40 ± 10.0 *	6.13

Values are expressed as Mean ± S.D; N=5.*= P<0.05

Table 2: Effect of Administration of *Kigelia africana* fruit extract on oxidative stress markers in S-D rats.

Treatment	SOD (µ/mg protein)	CAT (µ/mg protein)	MDA (µ/mg protein)
Control	285.66 ± 24.5	2.09 ± 0.1	12.49 ± 3.8
100 mg/kg	356.10 ± 191.7 *	1.98 ± 1.1	7.84 ± 0.9 *
300 mg/kg	338.74 ± 124.0 *	2.08 ± 1.2	8.78 ±0.5 *
500 mg/kg	383.15 ± 110.1 *	1.80 ± 0.6	9.83 ± 1.2 *

Values are expressed as Mean ± S.D; N= 5.*= P <0.05.

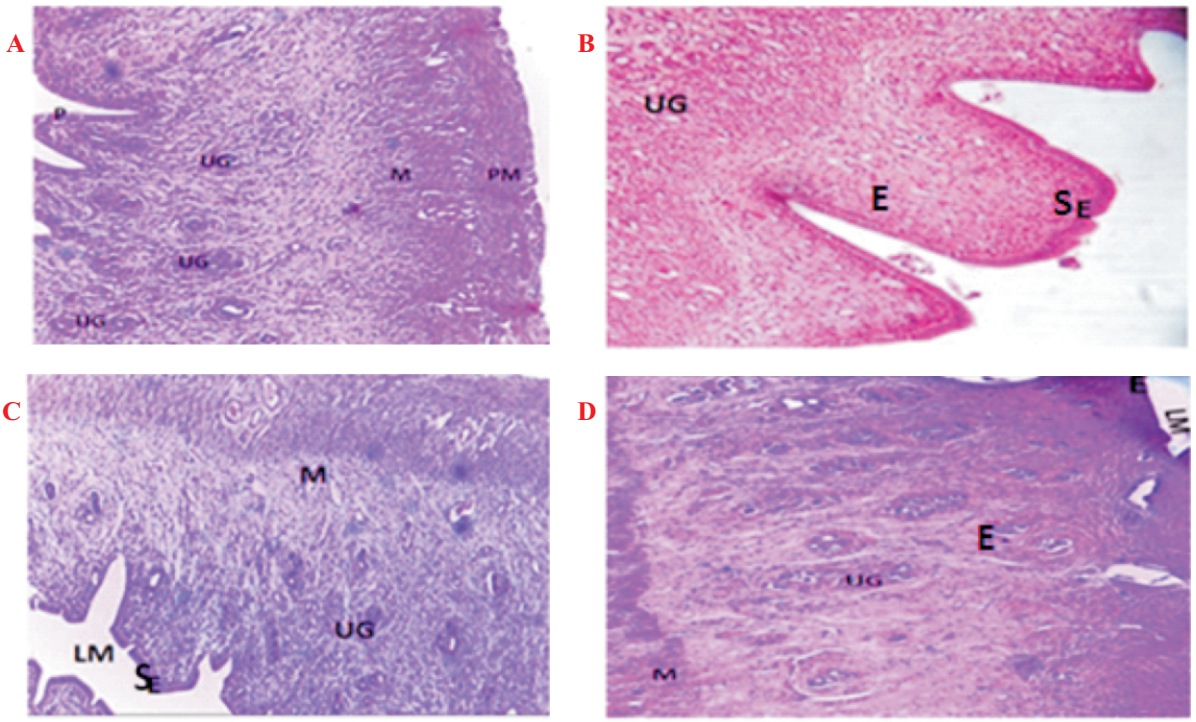

Plate 1: Photomicrograph of uterus of rat showing the perimetrium (PM), uterine glands (UG), myeometrium (M), surface epithelium (SE), endometrium (E) and lumen (LM). **A** =Control, **B** =100 mg/kg, **C** =300 mg/kg, **D** = 500 mg/kg. H&E X100

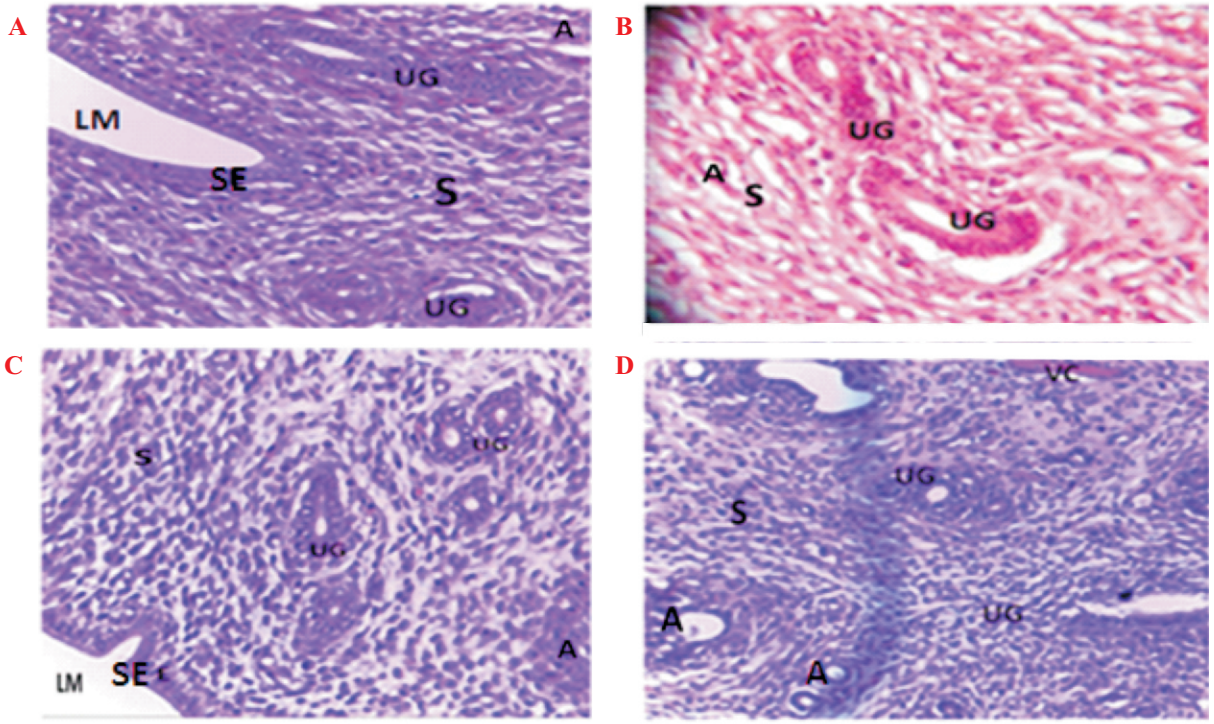

Plate 2: Photomicrograph of uterus of rat showing the stroma (S), surface epithelium (SE), uterine glands (UG), arteries (A), endometrium (E) and lumen (LM). **A** =Control, **B** =100 mg/kg, **C** =300 mg/kg, **D** = 500 mg/kg. H&E X400

DISSCUSION

The animals experienced a significant increase in weight during the course of the experiment. Percentage weight gain was highest in the group that received 100 mg/kg body weight of *kigelia africana*. From the result of our study, *Kigelia africana* did not impair appetite, digestion or nutrient uptake and this correlated positively with weight gain. The effect of *kigelia africana* on body weight may be attributed to the multiple physiological effects of the micronutrient and phytochemical composition present in the plant[16]. This result is in consonance with the study of other investigators who have reported significant weight gain with *kigelia africana* extracts [17-19]. Oxidative stress results if the availability of free radicals in the body greatly supersedes available antioxidants. Natural antioxidants such as plant polyphenols play a vital role in scavenging and inhibiting free radicals, and are responsible for the antioxidant potentials of plants[20,21]. In this present study, SOD activities were significantly increased in the groups that received *Kigelia africana* extract indicating a strong antioxidant defence mechanism against free radicals production.

Superoxide radical is involved in diverse physiological and pathophysiological processes[22]. The superoxide radical is formed when electrons leak from the electron transport chain[23]. SOD is present in high concentrations in all tissues and has a high catalytic efficiency, providing the cell with a high degree of cellular protection against superoxide anion under normal condition. SOD decomposes superoxide anion into hydrogen peroxide and oxygen at very high rates. Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen. Catalase activities were slightly reduced when the experimental groups were compared with control. Although the reduction was not dose dependent, however, catalase activities were comparable to control values at the 300 mg dose. In this study, the slight reduction in the antioxidant status of catalase is suggestive of a defence system that is being compromised as a result of the rapid production of hydrogen peroxide generated from the decomposition of superoxide anions. High concentration of hydrogen peroxide is deleterious to cells such as DNA, proteins, and lipids, leading to mutagenesis and cell death[24].

Therefore, it can be adduced that catalase activities are best at 300 mg/kg body weight. The reason for this selective performance is not known. Furthermore, MDA levels showed a dose dependent decrease in activities, indicating the antioxidant effect of *kigelia africana* in mopping up the production of free radicals/reactive oxygen species. *Kigelia africana* contains flavonoids and tannins[15]. Flavonoids and tannins are phenolic compounds, and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Our study is supported by other studies in which *kigelia africana* was reported to have strong antioxidant activities against free radical generation [25,16].

Histological sections from treated rats showed no appreciable difference in the cytoarchitecture of the uterus when compared with control sections. Histology showed healthy endometrium with uterine glands lined with pseudostratified columnar cells. The columnar cells of the epithelial surface appeared normal with no alterations. The result of this study demonstrates that *Kigelia africana* has no deleterious effect on the uterus. This report is in consonance with the studies of Dosumu *et al.*[12] and Azu et al.[25] who reported on the cytoprotective effect of *Kigelia africana* after cotton seed and cisplatin induced toxicity in the testis respectively.

CONCLUSION

This study demonstrates that *kigelia africana* fruit extract is non-toxic in the uterus and can proffer cytoprotection through its rich antioxidant properties.

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