African Journal of Pharmacology and Therapeutics Vol. 5 No. 3 Pages 169-173, 2016
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Research Article

The safety of *Kigelia africana* on pregnancy and pregnancy outcomes in Sprague-Dawley rats

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Background: *Kigelia africana*, belongs to the family of *Bignoniaceae*. It has been used commonly in folk medicine to energise and improve fertility in both males and females. Decoctions of *Kigelia africana* are taken as abortifacients. Therefore, there is need to scientifically substantiate and validate these claims.

Objective: This study was carried out to determine the effect of *Kigelia africana* on pregnancy and pregnancy outcomes in Sprague-Dawley rats.

Methodology: Twenty female adult Sprague-Dawley rats divided into 4 groups (N=5) were used. Rats were mated on proestrus with males of proven fertility. Spermatocytes in the vaginal smear confirmed pregnancy. *Kigelia africana* was given at 100, 300 and 500 mg/kg bodyweight daily from the 1st to 20th day of pregnancy while control rats received distilled water. Rats were anaesthetized with ketamine on day 20 of pregnancy and a ventral laparotomy was performed. Foetuses were removed and parameters taken. The uterus was excised and assayed for antioxidant activities of superoxide dismutase, catalase and malondialdehyde, an index of lipid peroxidation.

Result: Significant increases were observed in crown-rump length, tail length and litter size. Superoxide dismutase activities increased significantly while significant reductions were recorded in malondialdehyde levels. In addition, uterine weights increased in the treated groups compared with the control.

Conclusion: *Kigelia africana* is uterothrophic and is safe with no deleterious effect on pregnancy and the foetuses in Sprague-Dawley rats.

Keywords: *Kigelia africana,* pregnancy, malondialdehyde, catalase, superoxide dismutase.

Received: May, 2016
Published: November, 2016

1. Introduction

In spite of great advances observed in modern medicine in recent decades, plants still play an important contribution to healthcare (Shu, 1998). In Nigeria, traditional medicine has become well acknowledged and established as an alternative to conventional medicine (Kafaratu, 1994).

Kigelia africana (syn. *K. pinnata*), a tropical plant, belongs to the family of *Bignoniaceae*. It is a semi-deciduous-to-deciduous tree that grows up to 25 m tall. It can be found all over sub-Saharan Africa, but its native range extends from Tanzania in the north to KwaZulu–Natal in South Africa in the south. Its habitat includes open woodlands and moist places such as riverbanks on alluvial soils, but is widespread throughout the savannah areas of tropical Africa (Watt and Breyer-Brandwijk, 1962). *Kigelia africana* is rich in tannins, flavonoids, steroids, phlobatannins, cardiac glycoside, terpenoids and saponins (Atolani et al, 2011)

Among other uses, there are many folkloric uses of *Kigelia africana* in the treatment, enhancement and proper functioning of the reproductive systems in both males and females. For instance, the fruit of *Kigelia africana* is used by the Vhavenda men of the Limpopo province of South Africa to increase penis size (Mabogo, 1990) while in Kenya, the fruit is commonly added to beer as an aphrodisiac (Kokwaro, 2009). The leaves of *K. pinnata* are consumed by lactating women in various parts of sub-Saharan Africa as they are thought to enhance the volume and quality of breast milk (Glew et al, 2012). Also in Ghana, fruit and roots are boiled with

ISSN 2303-9841

the "tassels" of plantain flowers as a "woman's remedy," while the fruit is rubbed on the breasts of young girls in Cape Verde to enhance their development (Oliver-Bever, 1986). In addition, decoctions made from the bark and leaves are taken as abortifacients.

Kigelia africana fruit extract has been reported to proffer cytoprotection on the uterus by increasing the antioxidant status in the uterus of Sprague-Dawley (S-D) rats (Gbotolorun et al, 2015). There is a dearth of literature to ascertain the folkloric claims of the *Kigelia* plant on the female reproductive system and function. This study was carried out to determine the effect of *Kigelia africana* on pregnancy and pregnancy outcomes in S-D rats.

2. Materials and Methods

2.1 Plant materials

Mature fruits of *Kigelia africana* were harvested from the forest of Badagry town in Lagos. The fruits were authenticated at the Department of Botany in University of Lagos, Nigeria. Voucher specimen number- LUH 6426 was given and the specimen was kept at the herbarium.

2.2 Preparation of extract

The fruits of *Kigelia africana* were washed, cut into small pieces, air-dried and ground into powder form by using a grinding machine. The extraction was done using the Soxhlet apparatus with methanol as the solvent as described in Abioye et al, (2003). Briefly 1.5 kg of the powder fruit was used for the extraction. The extract was dried in an oven regulated at 40 °C and a yield of 10.36% was obtained, stored in sterile universal bottles and kept in the refrigerator for further use. Three different doses (100, 300 and 500 mg/kgbody weight) were administered.

2.3 Experimental animals

Twenty female S-D rats, weighing between 130-150 g were used for the experiment. The animals were obtained from Peter's Farm Nigeria Enterprises in Badagry. They were left to acclimatize for two weeks before the commencement of the experiment. The animals were maintained at 12 hours light: 12 hours darkness and were given standard rodent chow and water ad libitum. All procedures involving animals were approved by the Departmental Committee on the use and care of animals and tissue collection.

2.4 Experimental design

The animals were randomly divided into 4 groups of 5 rats per group. Rats on proestrous were mated with males of proven fertility. The presence of spermatozoa in the vagina smear was a confirmation of pregnancy and was counted as day one of pregnancy. *Kigelia africana* extract was administered daily for 20 days beginning from the first day of confirmation of pregnancy, at doses of 100, 300 and 500 mg/kg. Control animals received distilled water. At the end of the treatment regime, the animals were sacrificed by cervical dislocation and a ventral laparotomy was performed; foetuses were removed and foetal parameters (crown-rump length, tail length, umbilical cord length, placental weight, foetal weight and foetal size) were recorded. The uterine horns were excised, trimmed of fat, weighed and was assayed for antioxidant activities of superoxide dismutase, catalase and malondialdehyde.

2.5 Biochemical analysis for antioxidant activities

Briefly, the 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.

2.6 Uterine superoxide dismutase activity

Superoxide dismutase was assayed utilizing the technique of Kakkar et al, (1984). A single unit of enzyme was expressed as 50% inhibition of Nitroblue tetrazolium (NBT) reduction/min/mg/protein

2.7 Uterine catalase activity

Catalase was assayed colorimetrically at 620 nm and expressed as μmoles of H_2O_2 consumed/min as described by Sinha (1972). The reaction mixture (1.5ml) contained 1.0ml of 0.01M pH 7.0 phosphate buffer, 0.1ml of tissue homogenate and 0.4ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5% Potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

2.8 Uterine malondialdehyde activity

Uterine malondialdehyde levels were determined using the modified thiobarbituric acid (TBA) method of Buege and Aust (1978). Malondialdehyde reacts with thiobarbituric acid to give a red compound absorbing at 532 nm. The stock reagent contains 2 ml 15%w/v trichloroacetic acid, 0.375%w/v thiobarbituric acid and 0.25mol/L hydrochloric acid. A 0.5 g testicular tissue sample was homogenized in 5 ml of 0.15 M KCl and the homogenate centrifuged at 1000 g for 10 min in a Uniscope laboratory centrifuge and the supernatant collected. An aliquot of 2 ml of the stock reagent was added to 1 ml of testicular homogenate supernatant and mixed thoroughly and placed in an Equitron water bath (80 - 90°C) for 15 min. It was then cooled and the flocculent precipitate removed by centrifugation at 1000 g for 10 min and the absorbance of the supernatant determined with a spectronic spectrophotometer at 532 nm against blank containing all the reagents. Concentration of malondialdehyde was calculated using the molar absorptivity coefficient of MDA which is 1.56 x 10^5 $M^{-1}cm^{-1}$.

2.9 Statistical analysis

All data were expressed as mean ± SD, one way analysis of variance (ANOVA) was used to analyse 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 $p < 0.05$.

ISSN 2303-9841

3. Results

No mortality was recorded during the experiment and no signs of toxicity were observed in the pregnant dams. Pregnancy period was without complications: no bleeding, resorption, preimplantation or postimplantation losses were observed as a result of the administered extract. All the foetuses were alive and viable.

Effect of *Kigelia africana* fruit extract administered on day 1-20 of pregnancy on body weights of pregnant S-D rats:

There was 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 however, it was not dose dependent. The percentage weight gain was highest in the 500 mg/kg dose of *Kigelia africana* group and lowest in the control group (**Table 1**).

Effect of *Kigelia africana* fruit extract on uterine weight of pregnant S-D rats:

At the dose of 100 mg/kg body weight of *Kigelia* extract, the weight of the uterus was comparable to the control value. However, as dose increased, uterine weight increased but not significantly and was highest at 500 mg/kg body weight compared to the control (**Table 2**).

Effect of *Kigelia africana* fruit extract administered on day 1-20 of pregnancy on oxidative stress markers in S-D rats:

A dose-dependent significant reduction ($p < 0.05$) in malondialdehyde levels was seen when the treated groups were compared to the control. Superoxide dismutase activities recorded a dose dependent significant increase ($p < 0.05$) in all the treatment groups compared with the control group. Catalase activities showed a slight increase however, no significant difference was observed when the treated groups were compared with the control (**Table 3**).

Effect of administration of *Kigelia africana* fruit extract on foetal parameters in S-D rats:

Umbilical cord length, placental and foetal weights were not significantly different from control values. Crown rump length increased in all the treatment groups and was significant at both 300 and 500 mg/kg doses but was not dose dependent. Tail lengths recorded significant increases in all the treatment groups compared to the control. However, increasing the dose beyond 300 mg/kg did not produce any additional effect on tail length. Litter size was increased significantly at 100 and 500 mg/kg doses but was comparable to control values at 300 mg/kg of *Kigelia africana* (**Table 4**).

Table 1: Effect of *Kigelia africana* fruit extract administered on day 1-20 of pregnancy on body weights of S-D rats.

Treatment	Initial weight (g)	Final weight (g)	% weight gain
Control	156.60 ± 08.60	177.40 ± 06.10*	13.28
100 mg/kg	148.00 ± 14.30	173.40 ± 13.00*	17.16
300 mg/kg	154.20 ± 22.70	176.20 ± 20.20*	14.27
500 mg/kg	155.40 ± 11.80	183.00 ± 14.10*	17.76

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

Table 2: Effect of *Kigelia africana* fruit extract on uterine weights of pregnant S-D rats**.**

Treatment	1-20 day of pregnancy (g)
Control	0.76 ± 0.50
100 mg/kg	0.75 ± 0.45
300 mg/kg	0.88 ± 0.35
500 mg/kg	0.92 ± 0.43

Values are expressed as Mean ± S.D; N=5.

Table 3: Effect of *Kigelia africana* fruit extract administered on day 1-20 on oxidative stress markers in S-D rats.

Treatment	SOD (min./mg protein)	CAT (Mmol/min./mg protein)	MDA (µ/mg protein)
Control	229.98 ± 13.6	2.22 ± 0.1	11.55 ± 0.9
100 mg/kg	258.03 ± 66.1*	2.57 ± 0.5	10.38 ± 2.5*
300 mg/kg	282.16 ± 42.2*	2.53 ± 1.0	8.66 ± 1.7*
500 mg/kg	323.79 ± 43.3*	2.62 ± 0.1	7.67 ± 1.1*

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

SOD- superoxide dismutase, CAT- catalase, MDA- malondialdehyde.

 ISSN 2303-9841

Table 4: Effect of administration of *Kigelia africana* fruit extract on fetal parameters**.**

Treatment	CRL (cm)	TL (cm)	UCL (cm)	PW (g)	FW (g)	LS
Control	3.25 ± 1.0	1.36 ± 0.1	3.28 ± 0.9	0.70 ± 0.1	4.37 ± 1.0	5.20 ± 1.7
100 mg/kg	3.88 ± 0.1	1.45 ± .07*	3.40 ± 0.4	0.73 ± 0.1	3.98 ± 0.2	5.40 ± 2.3*
300 mg/kg	4.37 ± 0.5*	1.47 ± .04*	2.74 ± 0.4	0.66 ± .05	4.08 ± 0.4	5.20 ± 1.9
500 mg/kg	4.33 ± 1.0*	1.47 ± 0.1*	3.28 ± 0.9	0.66 ± 0.1	4.10 ± 1.0	6.00± 2.3*

Values are expressed as Mean ± S.D; N=5.= P<0.05*
CRL-Crown rump length, TL-Tail length, UCL-Umbilical cord length, PW-Placenta weight, FW-Foetal weight, LS- Litter size.

4. Discussion

The pregnant rats experienced a significant increase in weight during the course of the experiment in all the treatment groups. Percentage weight gain was highest in the group that received 500 mg/kg body weight of *Kigelia africana*. From the result of this study, *Kigelia africana* correlated positively with weight gain during pregnancy. Atolani et al*,* (2011) reported that the effect of *Kigelia africana* on body weight may be attributed to its micronutrient and phytochemical composition. Therefore, it can be deduced that *Kigelia africana* promotes appetite, digestion or nutrient uptake and this correlated positively with weight gain. The result of this study is in consonance with the study of other investigators who have reported significant weight gain with *Kigelia africana* extracts (Azu et al*,* 2010; Gbotolorun et al*,* 2015).

In this present study, there was a dose dependent increase in the weight of the uterus in the treatment animals however; it was not significantly different from the control. Phytochemical study has shown that *Kigelia africana* contains flavonoids (Atolani et al*,* 2011). Flavonoids have been reported to have high affinity toward estrogen receptors, and may participate in increasing the number and sensitivity of estrogenic receptors (Katzenellenbogen and Gorski 1975; Trout et al*,* 1992). The increase in the weight of the uterus in the treatment groups may be attributed to the direct effect of flavonoids on cell division and proliferation induction of the uterine cells (Al-Dujaily, 2001; Buhler, 2003). Therefore, it may be deduced from this study that *Kigelia africana* has uterotropic effect (Padilla-Banks et al*,* 2001). The report of this study is consistent with the reports of other investigators who recorded increases in uterine weights (Al-Bekari et al*,* 1990; Makaverich et al*,* 1997; Collins-Burrow et al*,* 2000; Al-Dujaily, 2001; Marbut et al*,* 2007).

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 (Lu and Foo, 2001). Superoxide dismutase 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 anions under normal condition. In this present study, Superoxide dismutase activities were significantly increased in the *Kigelia africana* treated groups indicating a strong antioxidant defence mechanism against free radicals production. The superoxide radical is formed when electrons leak from the electron transport chain (Halliwell et al*,* 1992). Superoxide dismutase decomposes superoxide anion into hydrogen peroxide and oxygen at very high rates. Catalase is vital when H_2O_2 concentrations are raised by catalysing the decomposition of H_2O_2 to water and oxygen. In this current study, catalase activities were slightly increased when the experimental groups were compared with control and the increase was dose dependent. The slight increase in catalase activities is indicative of its efficiency in catalysing the decomposition of H_2O_2 to water and oxygen. The result of this study is consistent with the results of previous studies in which catalase activities increased after administration of *Kigelia africana* to S-D rats (Azu et al*,* 2010; 2011; Gbotolorun et al*,* 2015). The study also showed that malondialdehyde levels decreased in a dose dependent manner. This clearly suggests that *Kigelia africana* provides the cells with a high degree of cellular protection against lipid peroxidation.

There was no observable gross abnormality in the foetuses from the treated dams compared with the control. Crown rump length increased in all the treatment groups and was significant at both 300 and 500 mg/kg. This correlated positively with significant increases in tail lengths in all the treatment groups compared to the control. In addition, the study recorded significant increments in litter size at both 100 and 500 mg/kg doses. From this study, it can be deduced that the foetuses from the treated groups were taller and more in number than the control foetuses. However, other parameters recorded such as umbilical cord length, placental, and foetal weights were not significantly different from control values.

5. Conclusion

This study demonstrates that *Kigelia Africana* fruit extract possesses notable antioxidant properties. The extract was also observed to be safe during pregnancy in S-D rats, with no teratogenic effects on the foetuses.

Conflict of Interest Declaration

The authors declare no conflict of interest.

Acknowledgements

The authors sincerely appreciate Mr Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy of the University of Lagos, who assisted with the extraction process of *Kigelia africana*. Also, we send our warm regards to Mr Samuel of the Biochemistry Department of Nigerian Institute of Medical Research (NIMR) for assisting with the antioxidant analysis.

 ISSN 2303-9841

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 ISSN 2303-9841