SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 2, pp. 405-410, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

SMOOTH MUSCLE RELAXANT ACTIVITY AND PROXIMATE EVALUATION OF *KIGELIA AFRICANA*

*Omonkhelin J Owolabi[1], Eric KI Omogbai[1], Anthony B Eledan[1] and Abiodun Falodun[2]
[1]Departments of Pharmacology and Toxicology and [2]Pharmaceutical Chemistry, Faculty of Pharmacy
University of Benin, Benin City, Edo State, Nigeria

ABSTRACT

The ethanolic extract of *Kigelia africana* at 400.0 and 800.0 µg /ml concentrations were tested on the isolated rat uterus. Both concentrations significantly ($p<0.0001$) exerted high smooth muscle relaxant activity on the uterus (a reduction of oxytocin and acetylcholine induced contractions as well as inhibition of the spontaneous contractions of the uterus were observed). Evaluation of the data also indicated that the relaxant effect was dose-dependent. Its relaxant activity was 80 and 90% of the inhibitory effects produced by salbutamol (0.002µg/ml) and atropine (0.02µg/ml) on oxytocin and acetylcholine induced contractions respectively. Proximate analysis of the powdered crude stem bark was also carried out. The results indicate the presence of active principles in the bark extracts of *Kigelia africana* which may be responsible for some of the applications in traditional medicines as remedy against threatened abortion and retained placenta. The proximate analysis carried out in this study is used to establish the identity of the crude drug sample. A moisture content of 5.55 ± 0.02 % was obtained. The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. The values of 3.26 ± 0.13 were obtained.

Keywords: *Kigelia africana;* rat uterus; tocolytic activity; bark extract.

INTRODUCTION

The use of medicinal herbs is as old as mankind and throughout ages, traditional medicinal plants have long contributed tremendously to alleviate the sufferings of the human race (WHO, 1995). Today there is no doubt that complementary medicines have gained wide acceptability and is increasingly attracting attention from scientific community world wide (Newal *et al.*, 1986).

Kigelia africana, a medium sized tree, 30 feet high or more is classified under the Bignoniaceae species. It's commonly referred to as the sausage tree and known locally by Cucasians as the cucumber tree. The sausage tree draws its name from its large sausage- shaped fruit, suspended from lengthy stalks (Cox and Balick, 1994).

It is abundant in the tropics and is widely used in Southern, Central and West Africa as a herbal remedy for various ailments such as eczema and psoriasis, diarrhoea, malaria, rheumatism, retained placenta and dizziness (Gill, 1992). The stem bark in particular has a wide reputation in folk medicine for the treatment of malaria, rheumatism, wounds, ulcers, retained placenta, venereal diseases, diarrhoea and to combat infections (Burkill, 1985). As hollow organs, uterus shares a number of similarities with the stomach, both belong to the group of smooth muscles that are spontaneously active and both maintain force as their volume increases and to expel its content, but even more differences exist between them (Bulletti *et al.*, 2000). Data on the effect of *Kigelia africana* on the contraction or relaxation of the uterine smooth muscle is lacking. Recent reports indicate the antibacterial activity of the fruits (Grace *et al.*, 2002), antimicrobial activities of the stem bark (Akunyili, 1991) and the antidiarrhoeal activity of the aqueous leaves extract (Akah, 1996). In the present study, we evaluated the ethanolic extract of the stem bark for possible activity on the isolated uterus of non-pregnant rats based on its use as a remedy for retained placenta.

*Corresponding author email: josphineomo@yahoo.com

MATERIALS AND METHODS

Plant material

The barks of *Kigelia africana* were collected in Okhoro Village, Egor Local Government Area of Edo State, Nigeria, between February and July 2005. The botanical identity of the plant and its bark were authenticated by Alhaji Alasa Abubakar (of blessed memory), a hebarium curator of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. Botanical authentication was confirmed at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria where a voucher specimen (No.FHI107654) was deposited for future reference. Immediately after collection, barks were cut into small pieces and dried under sunlight. The dried barks were pulverized into a fine powder using impact mill, weighed and kept for further analysis.

Extraction
The powdered material (500g) was macerated with absolute alcohol (2.5 litres) and left for 72 hours. The mixture was stirred at six-hourly intervals using a sterile glass rod. The extract was filtered and the filtrate evaporated to dryness with the aid of a rotary evaporator attached to a vacuum pump at 40^{o}C. The concentrated extract was stored in air tight containers, labelled and refrigerated at 4^{o}C prior to use.

Drugs and Chemicals
The following drugs were used: diethylstilbestrol (Merck), Oxytocin (Rotex Medica), Salbutamol (Glaxosmithkline), Acetylcholine (Sigma Aldrich), and Atropine (Sisbu Xierkang Pharm Co.Ltd), Absolute ethanol (Sigma Aldrich).

Animals
Female non-pregnant Wister rats weighing 120-150g and swiss albino mice (20-40g) of either sex were obtained from the Animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. Animals were given two weeks acclimatization time before being subjected to the experimental protocol. The animals were maintained on a standard diet (Ladokun feeds, Ibadan, Oyo State, Nigeria) and had access to food and water *ad libitum.* Animals were housed in a cage with a twelve hour light-dark cycle. [The ethical committee on the use of animals, Faculty of Pharmacy, University of Benin, Edo State. Nigeria, approved all animal experiments].

Proximate analysis of the powdered crude bark
The following quantitative parameters were carried out using standard methods (African Pharmacopoeia, 1986, British Pharmacopoeia 1988, and AOAC, 2003).

Moisture content /water loss on drying
The powdered drug (2.0 g) was weighed into a clean crucible of known weight. After oven drying at 105^{o}C for 5 hours and cooled. The crucible was weighed to determine weight loss in the powdered drug. The average percentage weight loss, with reference to the air dried powdered drug was determined for four replicates.

Total Ash Determination
The crucibles were washed thoroughly, dried in hot oven at 100^{O}C, cooled in desiccators and weighed. A 2.0 g portion of each of the samples were weighed into the crucible and put in the furnace. Heating was started gradually until temperature of 600^{o}C was reached. This temperature was maintained for 6 hours. The crucible was then put inside the desiccators and cooled. After cooling the sample was reweighed and the percentage ash calculated.

$$\% \text{ Ash} = \frac{W - Z \times 100}{N}$$

W=Weight of the crucible and ash
Z=Weight of empty crucible
N=Weight of Sample.

Acid Insoluble Ash Value Determination
The crucible with the ash of the stem bark from the experiment above was transferred into a beaker containing 25 ml of dilute HCl. The beaker and its contents were boiled for 5 minutes and the boiled contents filtered through an ashless filter paper. The washings were then passed through the filter paper in a manner as to allow the collection of the residue at the tip of the cone of the filter paper. The weight of the clean and heated porcelain crucible was accurately determined. The filter paper with the residue was folded with a small cone and transferred into the crucible. The crucible was gently heated until the filter paper was completely ashed, and then heated strongly for few minutes. The crucible and its contents were cooled, weighed and the final weight was noted. The weight of the residue (ash) was then calculated. This was done by subtracting the constant weight of the crucible and ash. The weight of the ash was divided by the initial weight of the drug multiplied by a hundred was taken as the acid insoluble ash value.

Water Soluble Ash Value Determination
The crucible with the total ash as in acid insoluble ash was transferred into a beaker containing 25 ml of distilled water. The beaker and its contents were boiled for 5 minutes and filtered through an ashless filter paper. The filter paper containing the residue was folded and placed in a weighed porcelain crucible. The crucible was then heated in the muffle furnace, until the filter paper was completely ashed. The crucible and its content were cooled and weighed and the final weight noted. The weight of the residue was then calculated by subtracting the constant weight of the second crucible and its ash. This is the water insoluble ash. The weight of the water soluble ash was obtained by subtracting the weight of the water insoluble ash from the total ash. The weight of the water soluble ash divided by the initial weight of the crude drug was multiplied by 100 and was taken as the water soluble ash value.

Determination of Extractives
(a) Alcohol Soluble Extractive Value
Powdered leaf drug (5.0 g) was weighed into a 250 ml stopper conical flask. Ethanol 90 % (100 ml) was added and the conical flask and stoppered. The flask was shaken in a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The extract was filtered by suction filtration using a Buckner funnel. The weight of a heated cooled flat bottom porcelain crucible was accurately determined. The filtrate (20 ml) was poured into weighed crucible and evaporated to dryness at 100^{o}C. The residue

was dried to constant weight and the final weight noted. The weight of the residue obtained from the extract (20 ml) was determined by subtracting the constant weight of crucible from the residue. The alcohol extractive was then calculated with reference to the initial weight of the powdered drug and expressed as percentage.

(b) Water Soluble Extractive Value

The above experiment was repeated using chloroform: water 400:1. The water soluble extractive value was done for the powdered drug.

Animal preparation

Female non-pregnant Wister rats were pretreated intraperitoneally with 1 mg/kg of Diethylstilbestrol 24 hours prior to the actual experiment (Veale, 1989). The rats were killed by cervical dislocation and exanguinations. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1 cm piece was mounted in a 50 ml organ bath containing De Jalon physiological salt solution having the following chemical composition: NaCL, 9 g/l, $NaHCO_3$, 0.5 g/l, D-glucose, 0.5 g/l, KCL, 0.402 g/l, $CaCL_2.2H_20$, 0.08 g/l. Each uterine strip was suspended vertically between two parallel stainless steel hooks for measurement of isometric tension.

The tissue was aerated with 95% oxygen 5% carbon (IV) oxide and temperature maintained at 37^oC, with a PH of 7.4. The spontaneous contraction of the uterus was recorded with FT 03 transducer connected to an Ugo Basile recorder (7075). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500 mg).

The tissue was allowed to equilibrate for 30 minutes before the commencement of the experiment.

The dose- response curves of oxytocin and acetylcholine induced contractions were first obtained, the effect of the ethanolic extract (400.0 and 800.0 µg/ml) and that of two positive controls (salbutamol and atropine) were also determined.

Statistical analysis

All results are expressed as the mean of five experiments ± SEM. The statistical package used was SAS, 1994 Users guide, Version 8.2. SAS Institute Inc., Cary, NC, USA. The statistical significance ($p < 0.0001$) of differences between means was assessed by an analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS

The plant extraction gave a yield of 3.78%. On the proximate analysis shown in table 1, a moisture content of 5.55 ± 0.02 % was obtained. The total ash which is a measure of the non-volatile inorganic constituents remaining after ashing was 3.26 ± 0.13. The results showed that various concentrations of oxytocin (0.2 to 0.8ml of 0.1 and 1.0 I.U) produced a significant contraction of the rat uterus, with maximum response been produced at 0.8 ml of 1.0 I.U. This was also noted for acetylcholine (0.2 to 0.8 ml of 1.0, 10.0 and 100.0µg/ml) which also produced significant contraction of the uterus with the maximum been produced by 0.8 ml of 100.0 µg/ml.

Table 1. Percentage (%) values of proximate analysis of the stem bark of *Kigelia Africana.*

Parameter	Values ± SEM (%)
Moisture content	5.55 ± 0.02
Total ash	6.42 ± 0.01
Acid insoluble ash	3.26 ± 0.13
Water soluble ash	2.88 ± 0.18
Alcohol extractive	8.68 ± 0.50
Water extractive	9.42 ± 0.18

Evaluation of the data indicates that there was a significant ($p<0.0001$) dose-dependent reduction in oxytocin and acetylcholine induced contractions by the ethanolic extract at 400.0 and 800.0µg/ml concentrations tested (Figs. 1 and 2).

It is noteworthy that a complete blockade of acetylcholine induced contraction was observed by both doses of the extract against the first four doses of acetylcholine, this was also observed for atropine that completely blocked the response to the first three doses of acetylcholine (Fig. 2).

The results in figure 1 also shows a comparative inhibitory activity produced by the extract and salbutamol which is used clinically in the treatment of threatened abortion in gravid uterus, while figure 2 shows a comparative inhibitory activity produced by the extract and atropine on acetylcholine induced contraction.

The activity of the two concentrations of the extract compares well with salbutamol and atropine, two positive controls that significantly ($p<0.0001$) relaxed the uterus (Figs. 1 and 2 respectively), producing about 80% and 90% of the inhibitory effects of salbutamol and atropine.

DISCUSSION

The proximate analysis carried out in this study is used to establish the identity of the crude drug sample. The moisture content shows the susceptibility of crude drug samples to microbial attack especially fungi, and also to degradation due to hydrolysis of the crude powdered drug. A moisture content of 5.55 ± 0.02 % (Table 1)

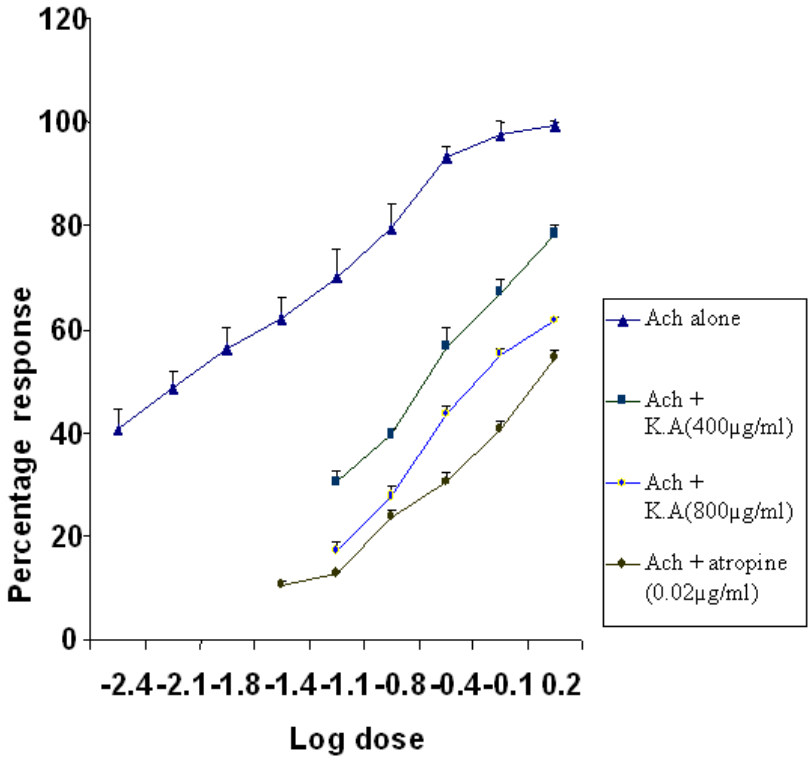

Fig. 1. Inhibitory activity of the ethanolic stem bark extract of *Kigelia africana* on oxytocin induced contraction of the isolated rat uterus.
Values are mean percentage responses ± SEM; (n = 5 animals); K.A stands for the ethanolic extract of *Kigelia africana.*
The extract significantly (P<0.0001) reduced the percentage response due to oxytocin.

obtained from this study is indicative of the storage quality for some time without microbial degradation or hydrolytic break down of the chemical constituents. The maximum range is between 6-8% in African Pharmacopoeia (1986).

The total ash is a measure of the non-volatile inorganic constituents remaining after ashing. It is made up of two parts, the physiological and the non physiological ash. The physiological ash consists of carbonates, phosphates, nitrates, sulphates, chlorides and silicates of metals which the plant took up when it was growing. The non-physiological ash represents ash from extraneous matter. The acid insoluble ash is residue obtained when total ash is boiled with 10% HCl. It is a measure of the sandy matter in the crude drug samples. The values of 3.26 ± 0.13 were obtained.

The uterus is spontaneously active, which means that, without any nervous or hormonal stimulation, a piece of isolated, pregnant or non-pregnant, uterus will produce regular spontaneous contractions (Brenninkmeijer *et al.*, 1999). Our results showed that the ethanolic extract at concentrations of 400.0 and 800.0 µg/ml dose-dependently produced significant inhibition of oxytocin and acetylcholine induced contractions of the uterine smooth muscle in non-pregnant rats (Figs. 1 and 2). The 800.0 µg/ml gave the higher percentage inhibition. A complete blockade of acetylcholine induced contraction was also observed by both doses of the extract against the first four doses of acetylcholine. It can thus be inferred that the extract might act via muscarinic receptors since there was complete blockade at lower doses and significant reduction in acetylcholine induced contractions at higher doses of acetylcholine. In addition, interestingly the extract was found to inhibit the normal spontaneous contraction of the uterus.

The results suggest that the ethanolic extract of *Kigelia africana* has a potential tocolytic effect that can be explored for therapeutic advantage as an alternative treatment for threatened abortion and dysmenorrhoea.

On the basis of its acclaimed folkloric use in the treatment of retained placenta, a contractile or oxytocin like effect on the uterus was expected. However the results points to a relaxant rather than a contractile effect.

CONCLUSIONS

The findings of our study indicate that the ethanolic extract of *Kigelia africana* stem bark possess inhibitory

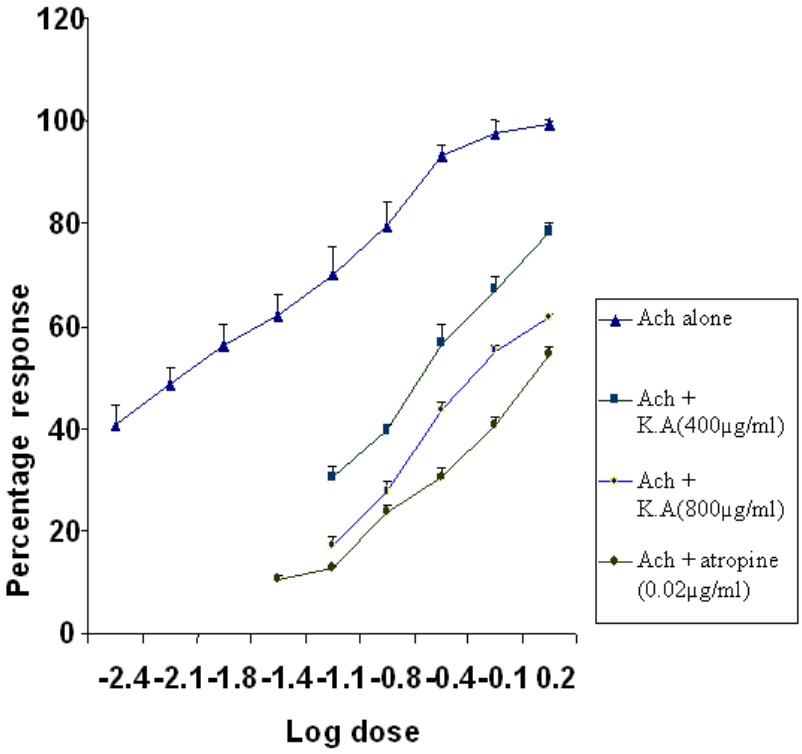

Fig. 2. Inhibitory activity of the ethanolic stem bark extract of *Kigelia africana* on acetylcholine induced contraction of the isolated rat uterus.
Values are mean percentage responses ± SEM; (n = 5 animals).
K.A stands for the ethanolic extract of *Kigelia africana.* The extract significantly (P<0.0001) reduced the percentage response due to acetylcholine. The first four doses of acetylcholine were completely blocked by both doses of the extract.

activity on the uterine smooth muscles in non-pregnant rats, which is inconsistent with the literature report (of its use in the treatment of retained placenta). Rather it's been envisaged that the active ingredients (compounds) will have a potential for being added to the present list of tocolytic agents used clinically. Further work is in progress to determine the mechanism of action at the receptor site.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Dr Z.A.M Nworgu and Pharm F.C Amaechina.

REFERENCES

African Pharmacopoeia. 1986. Vol. 2. 1st Ed. OAU/STRC Publications. pp.128-144.

Akah, PA. 1996. Antidiarrhoeal activity of the aqueous leaf extract of *Kigelia africana* in experimental animal. Journal of Herbs, spices and medicinal plants. 4(2): 31-38.

Akunyili, DN. 1991. Antimicrobial Activities of the Stembark of *Kigelia Pinnata*. Journal of Ethno-pharmacology. 35: 173-177.

AOAC. 2003. 14th Ed. Official Method of Analysis Association of Official Analytical Chemists. Washinghton DC, USA. 1112 -1114.

Brenninkmeijer, CB., Price, SA., Lopez, BA. and Phaneuf. 1999. Expression of G-protein-coupled receptor kinases in pregnant term and non-pregnant human myometrium. Journal of Endocrinology. 162: 401-408.

British Pharmacopoeia BP. 1988. Vol.II. Her Majesty's Stationary Office, London. P.1022.

Bulletti, C., De Ziegler, D., Polli V., Diotallevi L., Del Ferro E. and Flamigni, C. 2000. Uterine contractility during the menstrual cycle. Human Reproduction, 15: 81-89.

Burkill, HM. 1985. The useful plants of West Tropical Africa. Planta West Africa. 1: 254-257.

Cox, PA. and Balick, MJ. 1994. The ethnobotanical approach to drug discovery. Sci Am. 270(6):82-87.

Gill, LS. 1992. Ethnomedical uses of plants in Nigeria. Uniben Press, Benin City. pp 143.

Grace, OM., Light, ME., Lindsey, KL., Moholland, DA., Staden, JV. and Jager, AK. 2002. Antibacterial activity and isolation of antibacterial compounds from the fruit of

the traditional African medicinal plant *Kigelia africana*. South African journal of Botany. 68: 220-222.

Newal, CA., Anderson, LA. and Phillipson, JD. 1986. Herbal medicine, a guide for health care professionals. Pharmaceutical press. 291-296.

Veale, DJH., Oliver, DW., Arangics, NS. and Furman, KJ. 1989. Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. Journal of Ethnopharmacology. 37: 341-346.

World Health Organization (WHO). 1995. The World Health Report, Bridging the gap. Geneva 1, p.118.