

EVALUATION OF *IN-VITRO* VASORELAXANT EFFECT (POTENTIAL ANTIHYPERTENSIVE) OF *KIGELIA AFRICANA* FRUIT METHANOL EXTRACT ON POTASSIUM CHLORIDE AND PHENYLEPHRINE INDUCED TENSION IN WISTAR RAT AORTA

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ABSTRACT

This research on *Kigelia africana* was conducted in order to ascertain its ability to relax excited vascular smooth muscle in rat aorta. Preliminary investigation on whether the plant exhibits antihypertensive property was done before the evaluation of *in vitro* vasorelaxant effect. The vasorelaxant activity was determined using *in vitro* method on rat aorta with the aid of perfusion apparatus with a detachable organ bath. The administration of potassium chloride (KCl) raised the tension from 1.0 to 1.31 indicating that the aorta got to its peak of contraction. At 10 and 20mg/kg, the tension dropped significantly, showing relaxation of the smooth muscle while at 5mg/kg, drop in tension was insignificant at $p < 0.05$. However, at some of the doses, towards the end of experiment, there was steady resurgence in tension showing that the aorta resumed contraction. On the application of phenylephrine (PE), the tension rose to 1.18g. On administration of the extract, the tension dropped slightly showing mild vascular smooth muscle relaxation. From the results obtained, there was seeming similarity in the action of the *K. africana* compared to amlodipine/Ramipril in KCl and PE induced tension in aorta respectively. However, at 10 and 20mg/kg, a substantial decrease in tension was noted indicating that the extract action is dose dependent. Thus, from this *in-vitro* smooth muscle relaxation study in rats, the methanol extract of *K. africana* has depressant property that was likely expressed by enhancing the closing of voltage operated calcium channel and ACE inhibiting activity in KCl and Phenylephrine induced tension respectively.

Keywords: Evaluation, *In vitro*, Vasorelaxant, Effect, *Kigelia africana*.

INTRODUCTION

Cardiovascular diseases such as hypertension, atherosclerosis, cardiac disease, coronary heart disease, thrombosis are according to WHO considered the topmost killer diseases in the world today. Some synthetic conventional drugs such as amlodipine, ramipril, ACE inhibitors, Beta blockers, clopidogrel, warfarin, statins have been used in managing or treating these various forms of cardiovascular diseases. These therapeutic drugs have not been seen to be absolute cures but rather as managing agents. There are many medicinal plants some of which have been scientifically evaluated to be potential therapy for heart diseases. Despite these efforts, not enough data on clinical trials are available to ascertain and authenticate their level of efficacy and safety in human.

Medicinal plants have been used for treating various diseases including cardiovascular diseases since time immemorial across the globe. Drugs of plant origin and medicinal plant recipes are widely used today in managing or treating ailments for their potency, low cost and indeed their easy accessibility especially in rural areas. *K. africana* is one of such plants utilized in curing ailments. However, its efficacy, safety and mechanism of action especially as cardiodepressant, vasodepressant or antihypertensive agent are yet to be ascertained.

Kigelia africana (Lam.) Benth. (Bignoniaceae) is a plant that is commonly called sausage tree. It bears fruit with characteristic

shape like cucumber or yam. It is found in Nigeria and its fruit is traditionally used for treating arthritis, rheumatism, diuretics and as stimulant.

Many plants have been evaluated for their ability to reduce blood pressure or relax cardiovascular system (heart and vessels) in either mortensive or non-mortensive model. However, *K. africana* is one of those plants that are yet to be tested for the aforementioned property.

Several biological activity studies were already conducted and reported on *K. africana*. These include anti-inflammatory and analgesic activity conducted on *K. africana* bark ethanol extract using Carrageenan-induced and hot plate reaction/acetic acid induced writhing method respectively and reported by Owolabi and Omogbai (2007). In the test, factors such as prostaglandins and some other mediators were inhibited implying that the plant exhibited anti-inflammatory and analgesic activity respectively. The report of *in vitro* antioxidant property test carried out on the plant showed significant antioxidant property giving credence to its application locally in curing diseases associated with oxidative stress (Olaleye and Rocha, 2007). Its possession of caffeic acid derivatives could also be attributed to the plant antioxidant effect (Saini *et al.*, 2009).

In an anticancer experiment using dichloromethane and ethanol extract of fruit and stem bark respectively of *K. africana* tested on four melanoma cell lines and renal cell carcinoma line for

growth reduction impact, Lapachol, a viable component established by Hussain *et al.* (2007). The extract was reported to have played significant antineoplastic activity (Houghton *et al.*, 1994). In addition, Adoum, 2008 and Fafioye, 2005 reported that the fruit and root ethanol extract of *K. africana* showed moderate cytotoxicity at doses of (593, 495 and 1000) µg/ml.

Phytochemical screening and isolation using fractions of *K. africana* resulted in identification of some phytochemical constituents such as kigelinone, lapachol, isopinnatal, phenylpropanoids, ferulic and p-coumaric acid (Kolodziej, 1997; Houghton, 2007).

Plants with acclaimed cardiovascular effects either as cardio tonic, cardioprotective, fibrinolytic, vasodepressant, antihyperlipidaemic or antiplatelet aggregating include *Achillea santolina* (Al-Snafi, 2013), *Adonis vernalis* (Al-Snafi, 2015) *Agrimonia eupatoria* (Al-Snafi, 2015), *Agropyron repens* (Al-Snafi, 2016), Garlic (Breithaupt-Grögler *et al.*, 1997; Rahman *et al.*, 2006), Green tea (Hartley *et al.*, 2013; Zhang *et al.*, 2015), Hawthorn-*Crataegus* (Chang *et al.*, 2005)

As already known that vasorelaxant or vasodilator are possible antihypertensive agents, this research investigated the vasorelaxant property of *K. africana* as potential antihypertensive agent.

MATERIALS AND METHODS

Effect of Methanol Extract of *K. africana* Fruit on Isolated Aorta of Rat

The modified method of Amaechina and Omogbai (2007) was adopted and followed in this study. Healthy Wistar rats of 180g to 200g were obtained and kept in the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. They were heparinized to prevent blood clotting before being sacrificed by cutting their neck at anterior region with surgical pair of scissors. After sacrificing the rat, it was dissected with thoracic aorta identified, separated and cut. The cut aorta was immediately taken to a petri dish containing physiological salt solution (PSS).

Oxygenated Ringer-Locke solution is the perfusion fluid and its flow rate from reservoir was steady and kept at that throughout at 37°C by water circulated from thermostatic water bath.

The connective tissues associated with aorta were gently removed. The clean aorta was thereafter cut into ring form of 4 – 5mm long. For the data capsule to be ready for use, it was at first calibrated and the aortic ring taken into the organ bath containing PSS. Monitoring of happenings including recordings and display of tracing on the screen immediately commenced and PSS refreshed every fifteen minutes for three times. After forty-five minutes of monitoring, the PSS was once again refreshed (by replacing the old one with new one) and recording began for fifteen minutes.

The PSS was refreshed (by replacing the old one with new one) and its level in the organ bath was then adjusted to 4.5 ml (a little above the tissue). On the basis that from the preliminary study, the tissue exhibited no relaxation on unexcited condition, exciting (contractile) agents or agonist like Potassium chloride (KCl) or Phenylephrine (PE) was then applied and following contraction, significant reduction in tension was observed on the administration of the crude drug conventional drugs.

In the experiment proper, each contractile agent was administered to the tissue in the organ bath and

excitability/contraction as usual was observed. The basal contraction was recorded until the aorta stabilized. Thereafter, graded concentration of the crude extract at 5mg/kg was administered using micropipette into the organ bath at distinct concentration of 25, 50, 100, 250, 500 (µl) at 3-minute interval. Aorta contraction/relaxation was determined as difference between steady state and peak readings after injection (Fig. 1 and 3).

The same procedure was repeated for the extract at doses of 10mg/kg and 20mg/kg as well as the conventional drugs Amlodipine against KCl and Ramipril against Phenylephrine.

Amlodipine (a calcium channel blocker or calcium ion influx antagonist) was applied against KCl as calcium influx stimulator and Ramipril (an alpha adrenergic agonist) or angiotensin converting enzyme (ACE) inhibitor was applied against Phenylephrine as ACE stimulator.

Statistical analysis

Statistical analysis was done using one-way ANOVA and Dunnett's multiple comparison test with the aid of Graphpad prism 6.

RESULTS

In vitro vasorelaxant effect of methanol extract of *K. africana* fruit on induced tension in isolated Wistar rat aorta.

The various responses of rat aorta when treated firstly with potassium chloride (KCl) followed by the methanol extract of *K. africana* in one experiment and Phenylephrine (PE) followed by the methanol extract of *K. africana* in another experiment are as presented (fig. 1 - 4).

The vasorelaxant effect of methanol extract of *K. africana* fruit on potassium chloride induced tension in isolated Wistar rat aorta.

Signals from the tension signal transducer were amplified and sent to data acquisition and analysis system where the steady state and peak readings were recorded in traces (fig. 1). The differences between the steady state and the peak reading were determined and used in plotting the graph (fig. 2).

From fig. 2, the administration of KCl raised the tension from 1.0g to 1.31g in each treatment, indicating that the aorta got to its pre-contraction peak. At a concentration of 0.11 mg/ml on a dose of 5mg/kg of crude drug, the tension came down slightly to 1.24g due to the relaxation of smooth muscle. Reduction in tension was yet again observed down to 1.18g at concentration of 0.49 mg/ml implying a gradual relaxation but all not significant from the statistical analysis. At a concentration of 0.59mg/ml, a steady rise in tension to 1.19g was noted implying the resumption in aortic smooth muscle contraction. Other doses at 10mg/kg and 20mg/kg of the extract showed significant reduction in tension. The rats treated with KCl and double distilled water (ddw) of course as expected did not show relaxation. This mechanism of action is comparable to what was obtained in amlodipine.

From the graphical illustration (Fig. 2), the upper limit concentration of this crude drug could be deduced to be 1.0 mg/ml for at least experimental purpose.

Statistical analysis was done using One-way ANOVA and Dunnett's multiple comparison test in statistical software package known as Graphpad prism 6. Values are expressed as mean ± SD (n=6) with statistical significance at p<0.05.

In vitro vasorelaxant effect of methanol extract of *K. africana* fruit on phenylephrine induced tension in isolated Wistar rat aorta.

Signals from the tension signal transducer were amplified and sent to data acquisition and analysis system where the steady state and peak readings were recorded in traces (fig. 3). The differences between the steady state and the peak reading were determined and used in plotting the graph (fig. 4).

From fig. 4, phenylephrine raises the tension from 1.0g to 1.19 g. The crude drug at concentrations of 0.11 and 0.15 mg/ml slightly lowered the tension from 1.19g to 1.17g and 1.15 respectively. As from concentration of 0.32 to 0.91mg/ml, the tissue relaxed gradually to 1.3g and further to 1.4g from where

contraction resurfaced again. Other doses of 10mg/kg and 20mg/kg of the extract showed similar insignificant relaxation when compared to 5mg/kg extract. The control groups, KCl and ddw showed no relaxation as expected. For ramipril at 5mg/kg, there was substantial decrease in tension at various graded concentrations.

By the graphical illustration of the results (Fig. 4), the upper limit concentration of this crude drug could be deduced to be 1.0 mg/ml for at least experimental purpose.

Statistical analysis was done using One-way ANOVA and Dunnett's multiple comparison test in statistical software package known as Graphpad prism 6. Values are expressed as mean ± SD (n=6) with statistical significance at p<0.05.

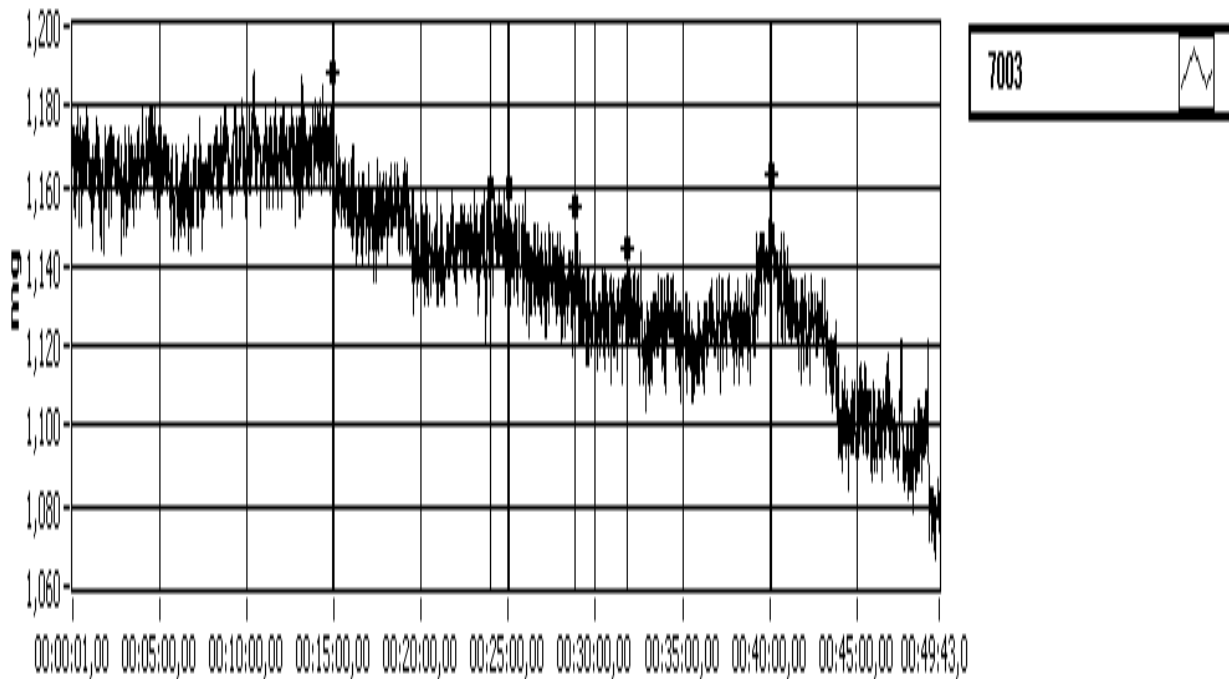


Fig. 1: *In vitro* vasorelaxant effect of methanol extract of *K. africana* fruit on Potassium chloride induced tension in isolated Wistar rat aorta (sample of tracings).

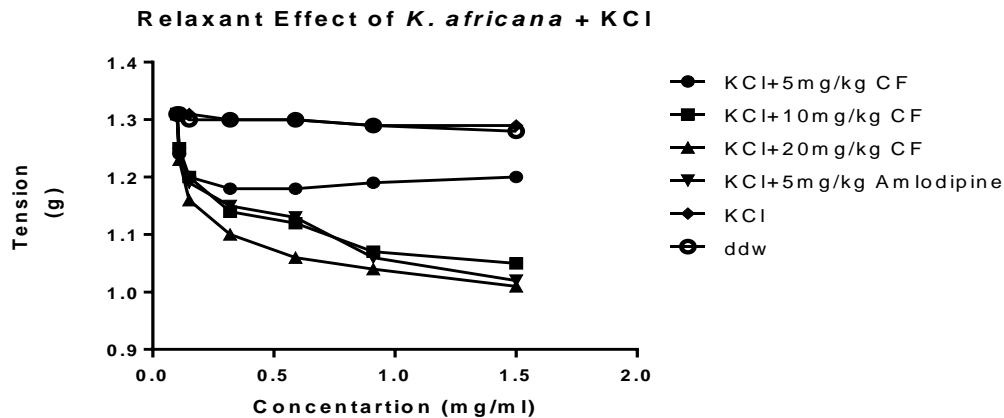


Fig 2: *In vitro* vasorelaxant effect of methanol extract of *K. africana* fruit on Potassium chloride induced tension in isolated Wistar rat aorta

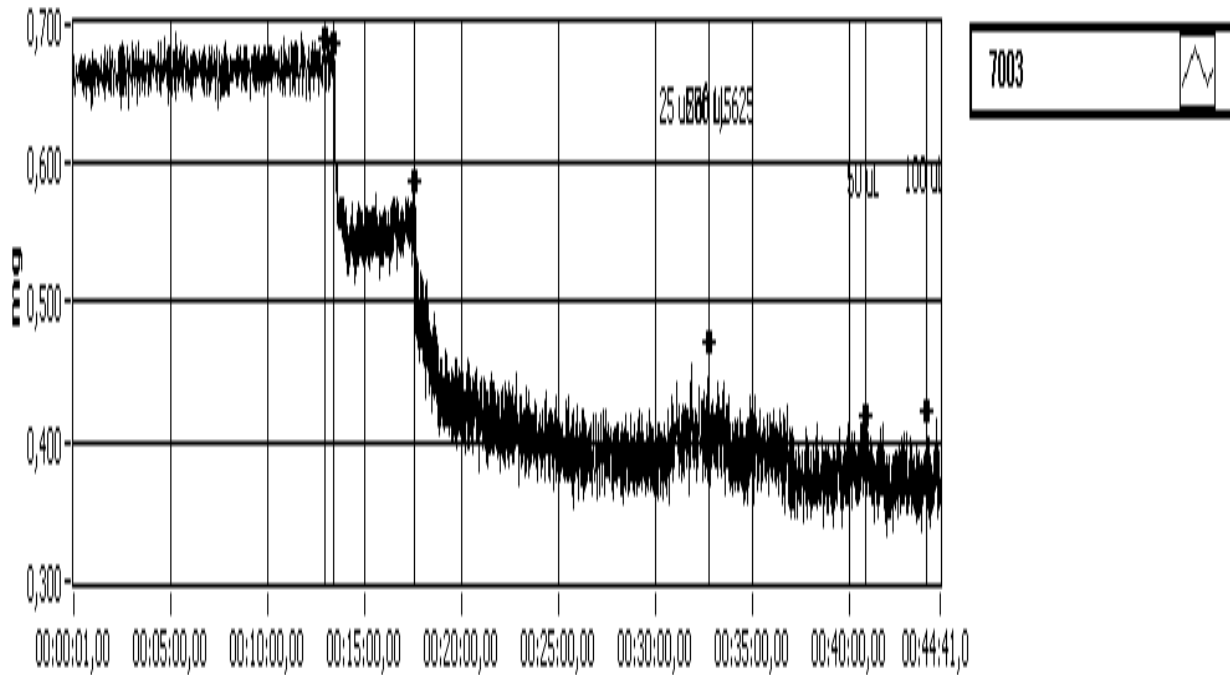


Fig. 3: *In vitro* vasorelaxant effect of methanol extract of *K. africana* fruit on phenylephrine induced tension in isolated Wistar rat aorta (sample of tracings).

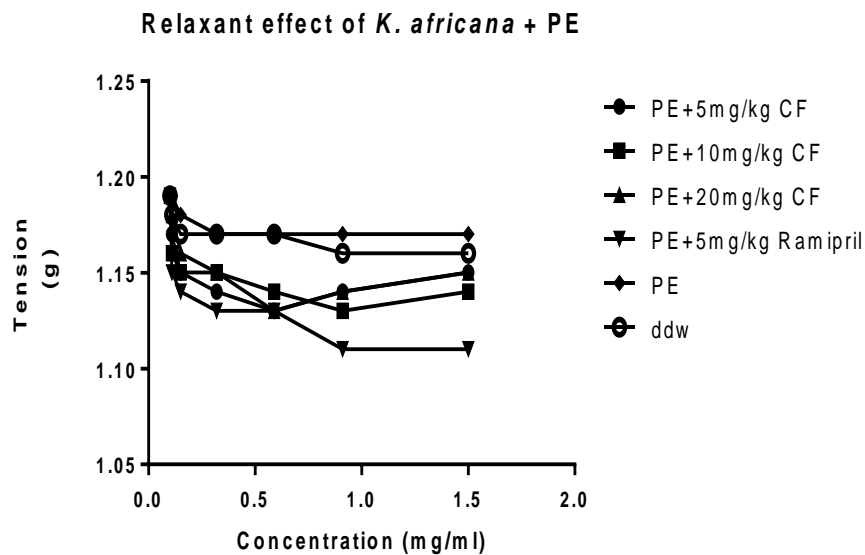


Fig 4: *In vitro* vasorelaxant effect of methanol extract of *K. africana* fruit on phenylephrine induced tension in isolated Wistar rat aorta

DISCUSSION

Potassium chloride as a known smooth muscle cell membrane depolarizer, usually by its nature opens voltage gated channel to allow influx of extracellular calcium ions through the channel. This leads to the stimulation of contractile machinery of the aorta thereby increasing the smooth muscle tone that eventually causes the contraction of the aorta. Thereafter, an interface occurred between calcium ion and camodulin to form

calcium-camodulin coupling complex. The complex resulting from this interface eventually activates the smooth muscle fibres to contract. Thus, the relaxation of the aorta noted during the study was perhaps due to the ability of *K. africana* crude extract to have interfered by blocking the voltage operated calcium channel in the cell membrane and so preventing the extracellular calcium ions from entering the cell. The depression in tension was as a result of the decrease in the extracellular calcium ions entering the cell which according to

some previous works is perhaps due to the ability of the crude drug to inhibit adenylate cyclase (Hanf *et al.*, 1993; Jurevičius and Fischmeister, 1996; Han *et al.*, 1998). Furthermore, the pattern of action in vascular smooth muscle relaxation resulting from the cumulative administration of *K. africana* fruit methanol extract on KCl induced tension was dose-dependent. This discovery is in tandem with the previous revelation made by Udopa *et al.* (1991), Taddel and Rosas (2000).

On the other hand, phenylephrine (a selective alpha adrenergic agonist) is known to activate the release of ACE that convert angiotensin I to angiotensin II which causes vasoconstriction and subsequently rise in blood pressure. Therefore, relaxation as little as it is, may have been due to the ability of *K. africana* to inhibit ACE, therefore preventing the production of angiotensin II from angiotensin I.

The significant depressant property of *K. africana* on KCl induced contraction and mild depression exhibited on phenylephrine-induced contraction in rat aorta, therefore might be due to certain constituents with calcium channel blocking and ACE inhibiting property respectively of the plant extract.

Though, there is no chemical that has direct link with the vasorelaxant activity of *K. africana* fruit, the pharmacological activity may be due to one or some of the phytochemical constituents already established in fruit among which are 7-Hydroxyviteoid II, 7-Hydroxyeucommic acid, 7-Hydroxy-10-deoxyeucommiol, 10-Deoxyeucommiol, Jiofuran Jioglutolide, 1-Dehydroxy-3,4-dihydroxy-3,4-dihydroaucubigenin, des-p-hydroxybenzoyl, kisasagenol, 3-(2'-hydroxyethyl) 5-(2'' hydroxypropyl), Dihydrofuran-2-(3H), Ajugol, 6-trans caffeoyl ajugol, 6-p-Coumaroyl sucrose ((Gouda *et al.*, 2003), β -Sitosterol (Desai *et al.*, 1971), Quercetin, Luteolin (Grace *et al.*, 2002).

Although the extract was not tested in isolated heart, it is difficult to draw conclusion of not having cardiodepressant property, as this effect is shared by certain conventional vasodilators such as hydralazine and minoxidil (VanZwieten, 2001). Since the methanol extract of *K. africana* fruit proved to be an active vasorelaxant, it could be a potential antihypertensive agent.

CONCLUSION

In line with the results obtained from the *in-vitro* smooth muscle relaxation studies in rat aorta, the methanol extract of *K. africana* has tension depressant property that is perhaps expressed by blocking of voltage operated calcium channel (VOCC) and ACE inhibiting activity. Therefore, this smooth muscle relaxant ability of the plant fruit extract indicates that it could be a potent antihypertensive agent if subjected to clinical trials and is successful.

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