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Inhibitory effect of the methanol extract and fractions of *Garcinia kola* seeds on KCl-induced contractions of isolated guinea pig vas deferens

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Abstract

The objective of the study was to investigate the phytochemical constituents and pharmacological effects of fractions of a methanol crude extract of *Garcinia kola* seed on chemically induced contractions of isolated guinea pig vas deferens. The extract was obtained by Soxhlet extraction with methanol (80% v/v) at 65 ℃ for 25 h. It was then successively fractionated with solvents of different polarities including n-hexane, chloroform, ethyl acetate and acetone respectively. In the same vein, the fractions were tested individually at a pre-determined dose of 0.4 mg/mL alone and in the presence of KCl-induced contraction of an isolated guinea pig vas deferens at a tension of 0.5 g. Each of the fractions was also screened to determine its phytochemical constituents. The results showed that none of the fractions induced contraction of the isolated guinea pig vas deferens at the dose used. All the fractions demonstrated inhibitory action to KCl-induced contraction of the isolated guinea pig vas deferens, with the residual methanol fraction demonstrating the strongest inhibitory action. Nifedipine (8×10^{-3} mg/mL), a known calcium channel blocker, expectedly and completely blocked the KCl-induced contraction. The phytochemical screening revealed the presence of cardiac glycosides in all the fractions but the absence of anthraquinones in all. The presence of other phytochemicals including alkaloids, saponins, tannins, flavonoids, steroids and terpenes were separately present in all the fractions. Fractions of a methanol crude extract of *Garcinia kola* seeds show inhibitory action on KCl-induced contraction of isolated guinea pig vas deferens.

Keywords: KCl; Methanol extract; Vas deferens; Guinea pig; Phytochemical

INTRODUCTION

Various investigations have been carried out on different biological and physiological systems including the effects of acute and chronic consumption of *Garcinia kola* Heckel seeds. Most of these investigations were directed towards validating or refuting the claim of the aphrodisiac property of *G. kola*. In most parts of Nigeria, there has been incidence of chronic consumption of *G. kola* in an addictive manner with a claim that it has aphrodisiac property especially on male fertility. This property may be involved in inducing changes in the sex hormones levels, which include alterations in luteinizing hormone, follicle stimulating hormone and gonadotropin release hormone.

In addition, consumption of *G. kola* seeds may induce changes in the anatomical structures and physiological functions of various tissues involving the male

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reproductive system, which include the seminal vesicles, vas deferens, prostate glands and corpus carvernosum.

G. kola Heckel (family Clussiaceae) is also known as bitter kola, false kola and male kola (English), *orogbo* (Yoruba-Western Nigeria), *Namijin goro* (Hausa-Northern Nigeria), *Akuilu* or *Ugugolu* (Igbo-Eastern Nigeria), *Efiari* (Efik), and *Igoligo* (Idoma-Middle Belt). It is an evergreen, dicotyledonous plant found in moist forest, riverine and swampy areas [1]. It is a medium size tree that grows up to 12 meters high, cultivated and distributed throughout West and Central Africa. *G. kola* is endemic in the humid rain forest vegetation in the coastal areas and lowland plains up to 300 meters above sea level [2].

The plant has characteristic astringent, bitter and resinous taste and its seeds are chewed for medicinal and ceremonial purposes. Every part of the plant (bark, leave and root) is adjudged to be of medicinal importance [3]. *G. kola* is commonly used to treat cases of asthma, cough, poisoning and vomiting [4], for its supposed aphrodisiac activity and induction of insomnia [5] and improved bowel movement [6]. Some *in vivo* studies have shown that consumption of *G. kola* induced significant enhancement of sexual activity, which may serve as evidence for its aphrodisiac property. However, chronic consumption at relatively higher doses may not confer same sexual enhancing property as claimed by its local consumers [7].

This study intends to provide an analysis of the phytochemical constituents of various fractions of methanol extract of *G. kola* and an investigation to demonstrate the effects of these fractions on chemically induced contractile response of an isolated guinea pig vas deferens.

EXPERIMENTAL

Animals. Four adult male guinea pigs weighing between 351 and 368 g were purchased from a reputable breeder in Jos metropolis. They were housed in the Animal Experimental Unit of the University of Jos. They were approved and certified for the experiment by Committee on use of Experimental Animals of the Department of Pharmacology and Toxicology, University of Jos through ethical clearance protocol form number F17-00379 dated 26th April 2018. They were then handled under ethical conditions for the use and care of laboratory animals [8]. They were fed with standard solid nutritional pellets and water *ad libitum* until the commencement of the experiment.

Preparation of extract. *G. kola* seeds were purchased from a reputable dealer in Terminus market in Jos metropolis. The seeds were re-authenticated by a taxonomist at the Federal College of Forestry, Jos, Plateau state and a herbarium voucher specimen (number FHJ 429-17) was prepared. The seeds were washed, de-husked and carefully cut in small pieces with a sharp knife to enhance drying. They were then dried under shade in the laboratory. Thereafter, they were grounded to powder and extracted according to the method described by Adegboye *et al* [9]. 250 g of the powdered seed was extracted continuously with methanol (80 %) in a Soxhlet extractor for 25 h at 65 ℃. The extract was evaporated to dryness in a vacuum evaporator at 50 ℃ until a constant yield of 52.65 g (representing 21.06 %) following repeated weighing was obtained. The extract was reconstituted in normal saline for the purpose of the experiment.

Fractionation of crude extract. The solvent partitioning method using partition coefficient of organic solvents with different polarities described by Otsuka [10] was used with slight modifications. A total of 24 g of the methanol crude extract was reconstituted with 80 % methanol and 500 mL of n-hexane was added followed by continuous stir for 1 h. The mixture was allowed to stand for 24 h after which it was decanted and filtered. The

filtrate was dried in an open air and thereafter stored as the n-hexane fraction. The residue was mixed with 500 mL of chloroform and stirred continuously for 1 h. The resulting mixture was allowed to stand for 24 h, and thereafter it was decanted and filtered. The filtrate was dried in an open air to obtain the chloroform fraction. The same procedure was successively used to obtain the ethyl acetate and acetone fractions. The percentage yield of each dried product of the fractionation and the crude residue was calculated. They were stored in a refrigerator maintained at a temperature of between 3℃ and 5℃ until the commencement of the experiment.

Phytochemical analyses of fractions. The phytochemical analyses were respectively conducted using specific methods relevant to each phytochemical of interest [10-14].

Effect of fractions on induced-contraction of isolated vas deferens. An adult male guinea pig was humanely sacrificed by exsanguination in accordance with rules and regulations guiding the use of animals [8]. The abdomen was cut open and the vas deferens was isolated and placed in a petri dish containing Tyrode's solution with double glucose concentration and without potassium chloride [composition (g/1000 mL): NaCl, 8.0; $CaCl_2$, 0.2; $NaHCO_3$, 1.0; $NaH_2PO_4 \cdot 2H_2O$, 0.05; $MgCl_2$, 0.1; Glucose, 2.0]. A section of 2 cm was cut and put in an organ bath of 25 mL that was aerated with 95 % O_2 and 5 % CO_2. One end of the tissue was tied to a hook and the other to the isometric transducer. The temperature was maintained at 37 ℃ and the resting tension was set at 0.5 g for calibration. It was then allowed to equilibrate for an incubation period of 15 min during which period the tissue was observed for any contraction. The contractions of the tissue with concentrations of 50 mM KCl from 1.2 mg/mL to 152.6 mg/mL were monitored and measured through a 3-channel student physiograph (Medicaid Systems, Chandigarh, India) at a speed of 2 mm/s. A maximum response of 3.0 cm was obtained. The same procedure was separately repeated using the submaximal dose of KCl in the presence of increasing concentrations of each fraction and the reduction in contractile responses monitored and measured. A similar procedure using nifedipine, a known calcium channel blocker was used. The percentage inhibitions for each dose of the separate fractions and that of nifedipine were calculated.

RESULTS

Fractionation of crude methanol extract of G. kola seed. The result of the fractionation of the crude methanol extract with some organic solvents showed that the residue methanol fraction was more in quantity (40.4%) while the chloroform fraction had the least quantity (12.8%) (Table 1).

Phytochemical screening. The presence of glycosides was found among all the fractions while that of anthraquinones was absent in all (Table 2). None of the fractions possessed all the phytochemical screened. The acetone fraction contained high saponins, flavonoids and carbohydrates while the n-hexane contained the highest steroids. The chloroform fractions possessed the least number of phytochemicals with only two of the nine screened as present. Similarly, the presence of alkaloids was found only in two of the five fractions, methanol and acetone.

KCl-induced contractions of isolated guinea pig vas deferens. The result showed corresponding increase in heights of contractions of the isolated guinea pig vas deferens with increase in concentration of KCl. The contraction reached a maximum of 3.0 cm with a concentration of 76.3 mg/mL. A further increase of the concentration to 152.6 mg/mL did not produce a further increase in magnitude of contraction (Figure 1 & 2).

Effect of each fraction on isolated guinea pig vas deferens. The results show that none of the fractions produced contractile or relaxant response on the isolated vas deferens at a controlled concentration of 0.4 mg/mL (Figure 3)

Inhibitory effect of each fraction on KCl-induced contraction. All the fractions produced inhibitory effects on KCl-induced contraction of an isolated guinea pig vas deferens. The residual methanol extract produced the strongest inhibitory effect while the chloroform fraction produced the least effect (Figure 4 & 5).

Effect of methanol crude extract on KCl-induced contraction. The results showed that the methanol crude extract caused a dose-dependent inhibitory effect on KCl-induced contractions of the isolated guinea pig vas deferens (Figure 6 & 7)

Effect of ethyl acetate fraction on KCl-induced contraction of isolated guinea pig vas deferens. The results revealed a dose-dependent inhibitory effect of the ethyl acetate fraction on KCl-induced contractile responses of the isolated guinea pig vas deferens (Figure 8)

Effect of nifedipine on KCl-induced contraction of isolated guinea pig vas deferens. The results expectedly showed the strong inhibitory effect of nifedipine (8×10^{-3} mg/mL) on the KCl-induced contractile response of the isolated guinea pig vas deferens. There was complete inhibition just on one trial (Figure 9).

DISCUSSION

In all living cells, the resting membrane potential (RMP) is said to be governed by many ions. However, for many of such cells, the main determinant ions are K^+, Na^+, Cl^- and Ca^{2+} ions [15]. Therefore, the transmembrane movements of these ions particularly K^+, Na^+ and Cl^- through their respective channels collectively contribute to the RMP. When more than one channel is present (as it is always the case), the RMP is then determined by using the Goldman-Hodgkin-Katz equation which reveals the relative contribution of each ion. This contribution depends on both the electrochemical gradient and relative permeability for each of the ions.

Table 1: Yield (%) of each fraction of the methanol extract of *Garcinia kola* seeds

Fraction	Quantity (g)	%
Methanol	10.1	40.4
n-Hexane	3.8	15.2
Chloroform	3.2	12.8
Acetone	2.7	14.8
Ethyl acetate	4.2	16.8
Total	25	100.0

Table 2: Phytochemical Compositions of the fractions of methanol crude extract of *Garcinia kola* Seeds

Constituents	Methanol	n-Hexane	$CHCl_3$	Ethyl acetate	Acetone
Alkaloids	+	-	-	-	++
Saponin	++	-	-	++	+++
Tannins	++	-	-	++	++
Flavonoids	+	-	-	++	+++
Carbohydrates	+	+	-	+++	+++
Anthraquinones	-	-	-	-	-
Steroids	+	+++	-	-	++
Terpenes	+	-	+	-	+
Glycosides	+	+	++	++	++

+ = Present, ++ = more present, +++ = highly present, - = absent

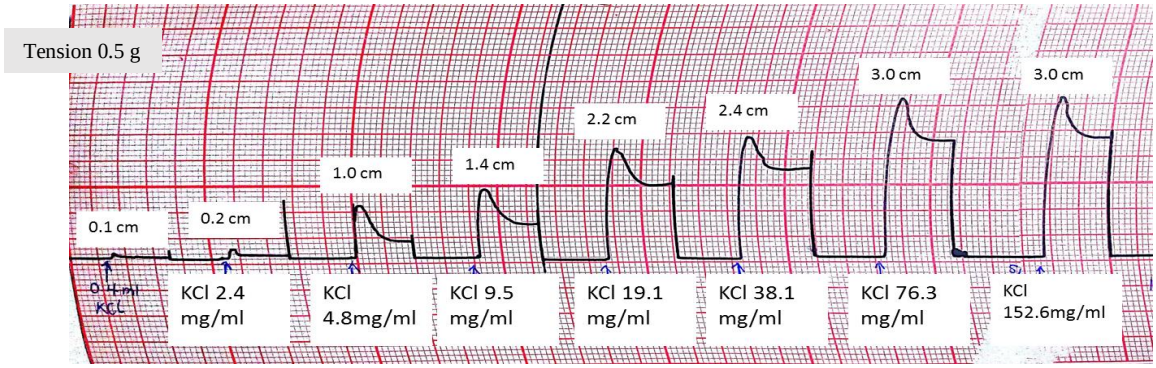


Figure 1: Effect of a control trial of KCl-induced contraction of an isolated guinea pig vas deferens

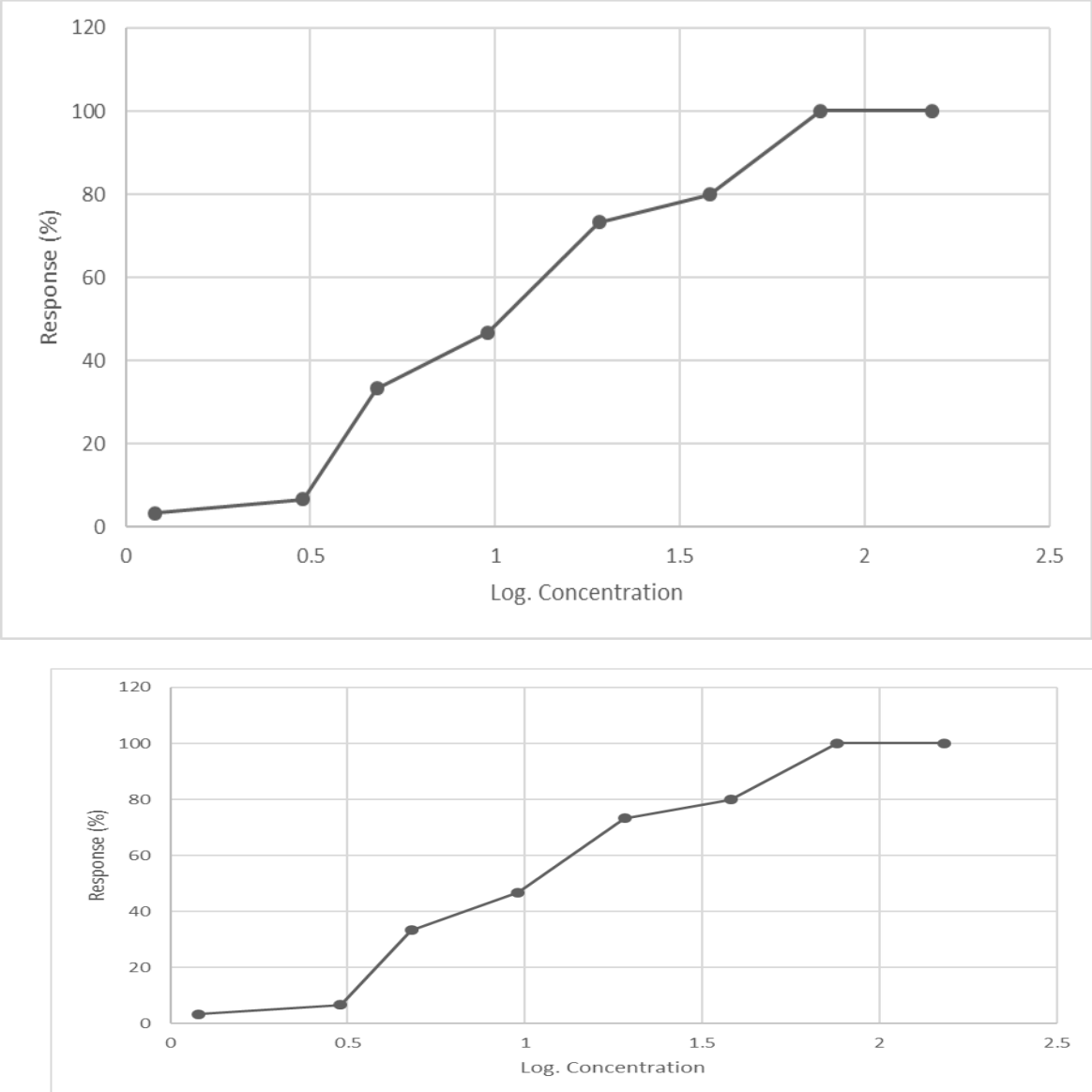


Figure 2: A Plot of the effect of control trial of KCl-induced contractions on an isolated guinea pig vas deferens

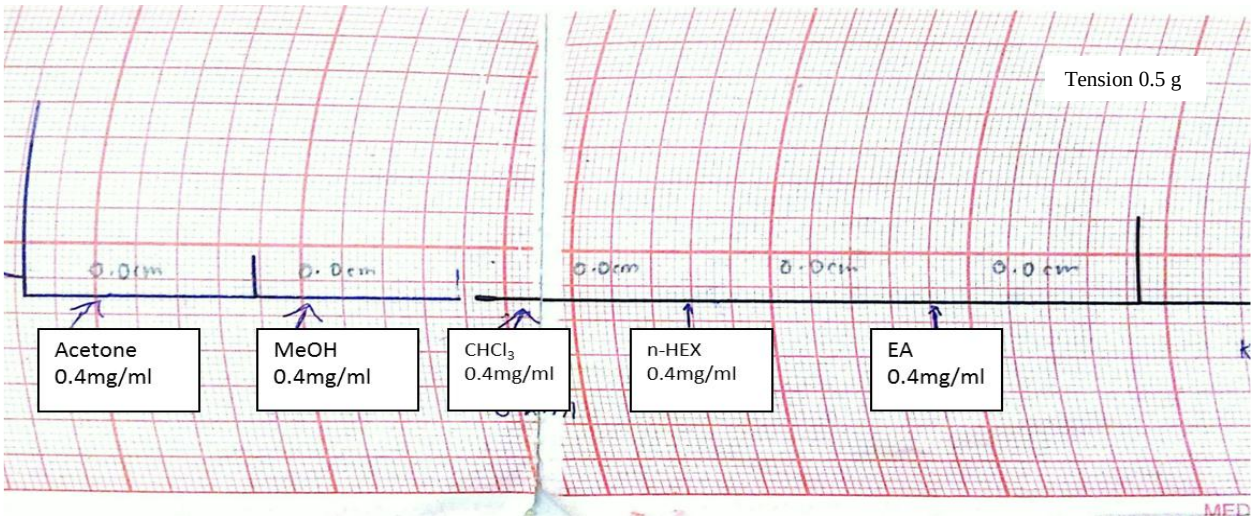
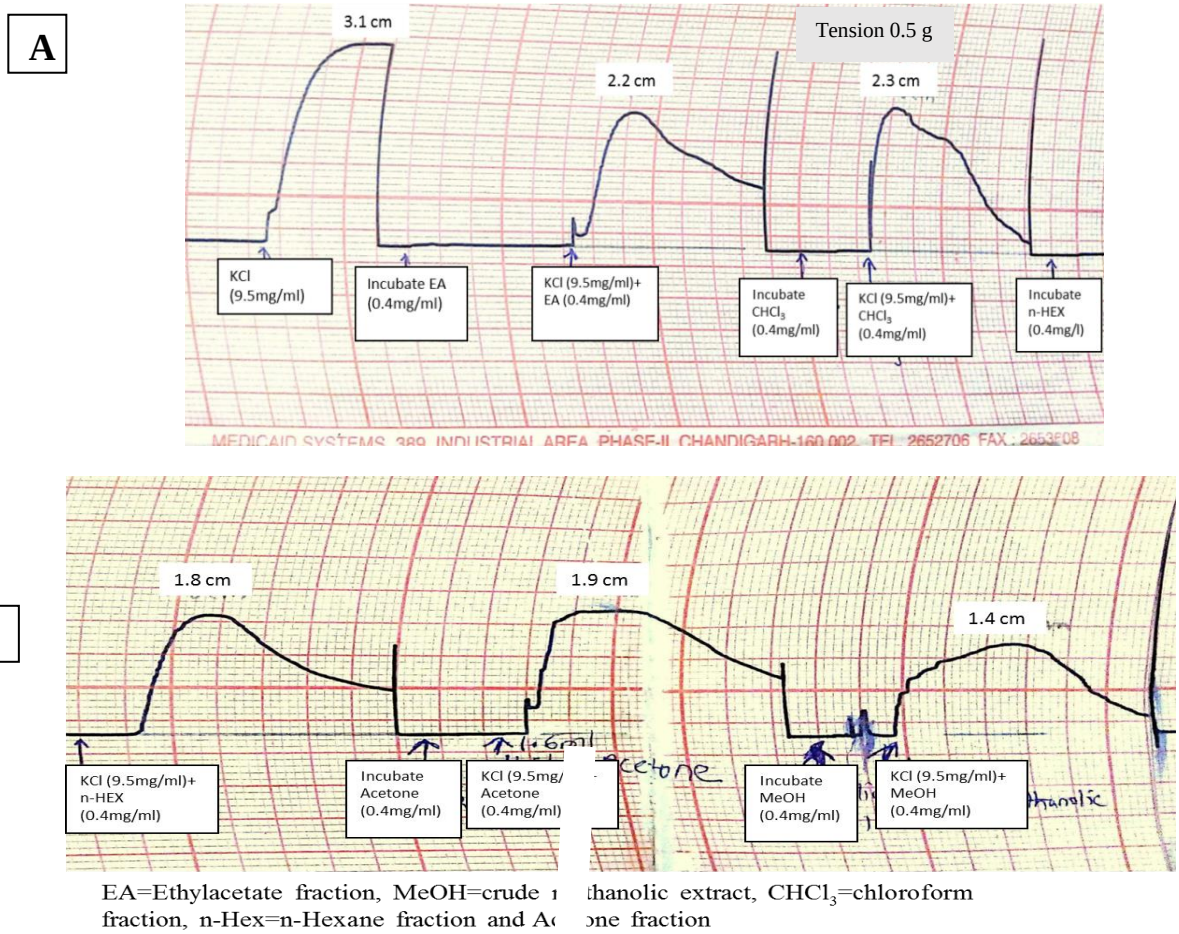


Figure 3: Effect of each reaction on the isolated Guinea pig vas deferens. None of the fractions caused contraction of the vas deference EA = Ethyl acetate; MeOH = Methanol; $CHCl_3$ = Chloroform; n-Hex = n-Hexane



EA=Ethylacetate fraction, MeOH=crude methanolic extract, $CHCl_3$=chloroform fraction, n-Hex=n-Hexane fraction and Acetone fraction

Figure 4: Effects of each fraction on KCl-induced contraction of an isolated guinea pig vas deferens. Contraction was with KCl (9.5 mg/ml alone) and in the presence of KCl (9.5 mg/ml in the presence of 0.4 mg/ml of each fraction) EA=Ethyl acetate, MeOH=methanolic extract, $CHCl_3$=chloroform fraction, n-Hex=n-hexane fraction. Panel **A** shows the inhibitory effects of ethyl acetate and chloroform fractions **(**both at a controlled dose of 0.4 mg/ml). Panel **B** shows the inhibitory effects of n-hexane, acetone and methanol fractions (each at the dose of 0.4 mg/ml)

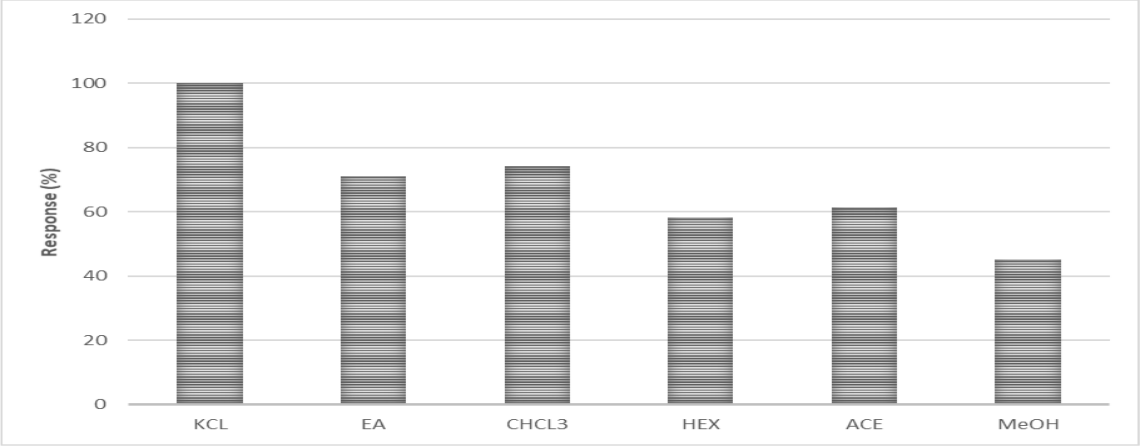


Figure 5: Effects of each fraction (%) on KCl-induced contraction of an isolated guinea pig vas deferens. Contraction was with KCl (9.5 mg/ml alone) and in the presence of KCl (9.5 mg/ml in the presence of 0.4 mg/ml of each fraction)

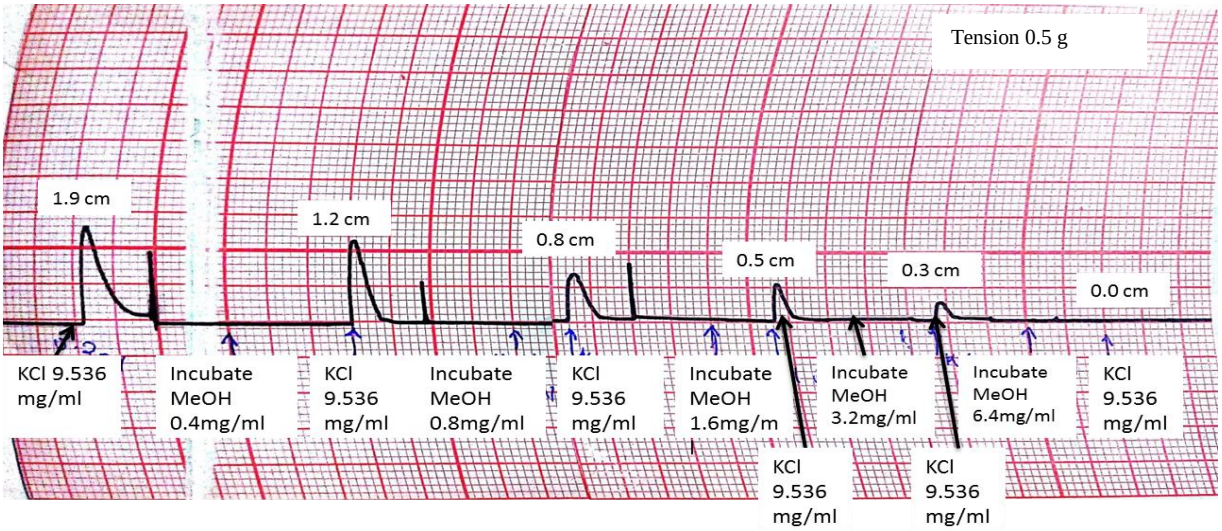


Fig.6. Effect of the methanol crude extract on KCl-Induced Contraction of an Isolated Guinea Pig Vas Deferens. The concentration of KCl was 9.5 mg/ml while that of the extract varied from 0.4-6.4 mg/ml.

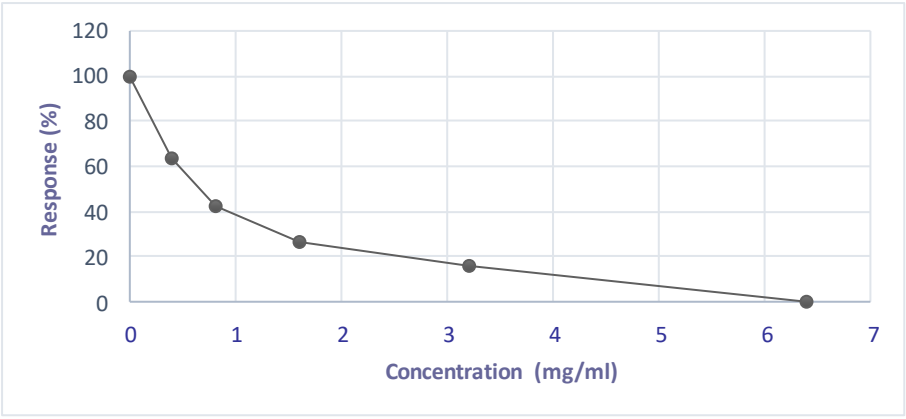


Figure 7: A graphical plot of the inhibitory effects of the crude methanol crude extract of *Garcinia kola* seeds on KCl-induced contractions of an isolated guinea pig vas deferens. Concentration of KCl = 9.5 mg/ml

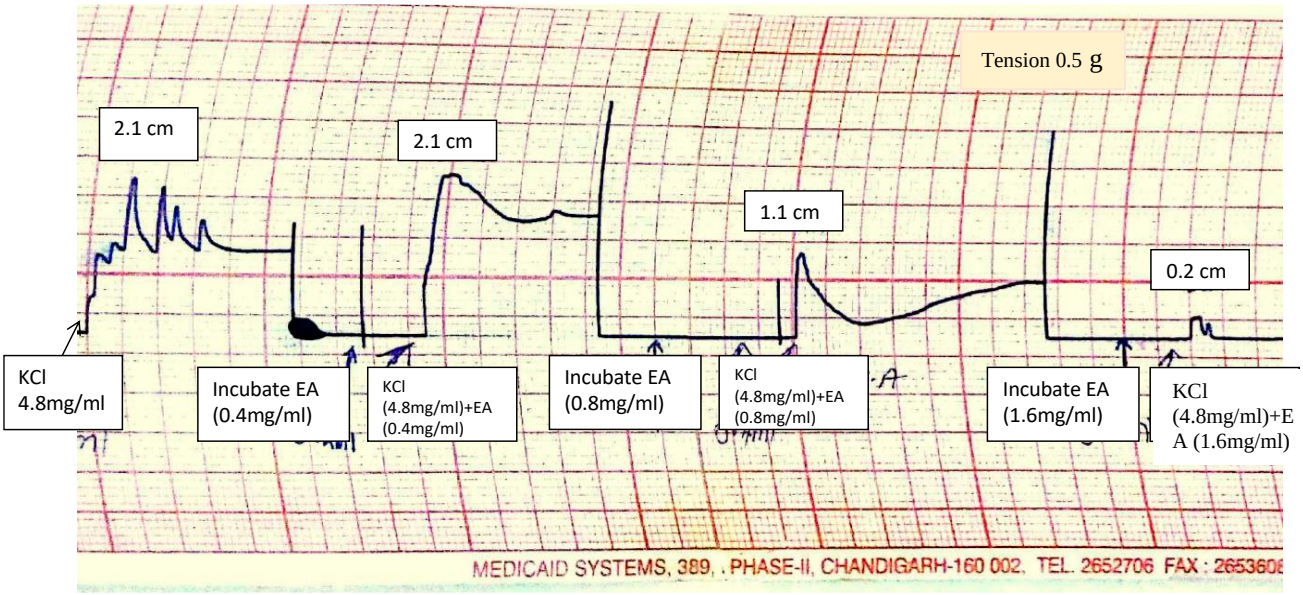


Figure 8: Effects of ethyl acetate fraction on KCl-induced contractions of an isolated guinea pig vas deferens. KCl concentration was maintained at 4.8 mg/ml.

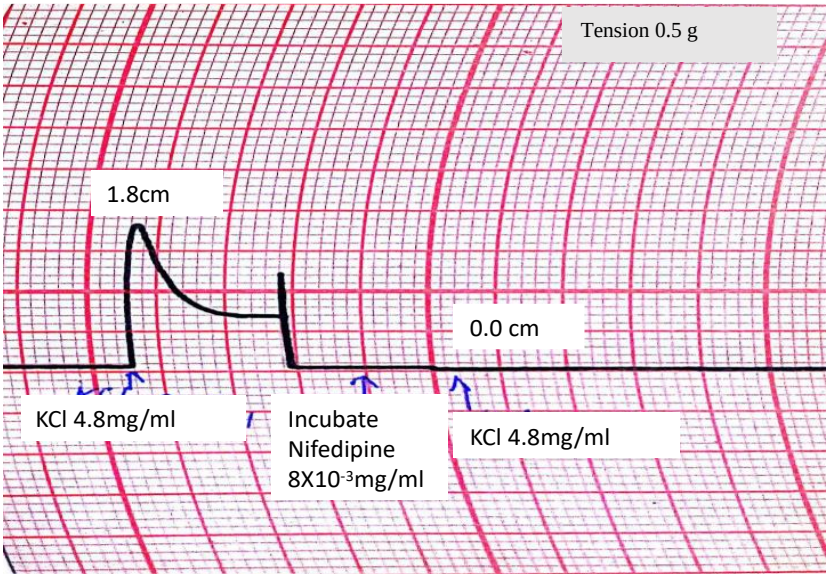


Figure 9: Effect of Nifedipine (8 x 10^{-3} mg/ml) on KCl-induced contraction of an isolated guinea pig vas deferens. Nifedipine completely blocked the contractile response of KCl (4.8 mg/ml).

In many cells during their resting periods, the comparative permeability of K^+ ions is more than those of Na^+ and Cl^-. Consequently, for positive driving force, most of the cations have the tendency of moving outside the cell along their electro-chemical gradient (efflux) and on the other hand, most anions move into the cell (influx). This is believed to maintain the RMP and in extreme cases, it results in hyperpolarization with decreased responsiveness to all kinds of stimulation.

Expectedly, administration of KCl produced dose-dependent contractile responses of the guinea pig vas deferens. However, none of the fractions of the extract

produced a similar contraction; but on the contrary, each blocked the responses due to KCl, suggesting possible inhibitory mechanism. The residual methanol extract produced the strongest inhibitory effect while the chloroform fraction produced the least effect. In a similar but more pronounced manner, nifedipine, a calcium channel blocker, produced an inhibitory effect. The inhibitory effect of the fractions on the KCl-induced contraction of the guinea pig vas deferens smooth muscle did not follow their polarity pattern or their phytochemical constituents. Anthraquinones were not present in any of the extracts while cardiac glycosides were found in all the fractions. The methanol fraction that produced the strongest inhibitory effect possesses all the phytochemical constituents except the anthraquinones, which were found absent in all the fractions.

KCl has long been used as a convenient stimulant to bypass *G*-protein-coupled receptors and activate smooth muscle by a highly reproducible and relatively simple mechanism involving activation of voltage-operated calcium channels [16]. Increase in extracellular K^+ is often a way to depolarize neurons in experiments mainly because the membrane has low permeability for Cl^- ions. Activation of Ca^{2+}-sensitive K^+ channels also allows the conductance of Ca^{2+} into the cell down its electrochemical gradient leading to muscle contraction. Ca^{2+}-sensitive K^+ channels are also sensitive to the blockage effect of nifedipine and other similar blockers [17].

Based on the aforementioned, it can be suggested that the fractions of the methanol extract of *G. kola* may possess inhibitory property similar to that exhibited by nifedipine, a calcium channel-blocker. Although a substantial component of the contractile response of the vas deferens is mediated by different mechanisms, the adrenergic mechanism appears to dominate [18]. It is therefore not surprising that many substances are capable of altering the contractility of the vas deferens by modulating the release of endogenous chemicals including ions such as that of Ca^{2+} capable of altering its RMP profile [19-22]. Blockage of such contractile responses has implications on ejaculation, most often resulting in male infertility [23].

In conclusion, this study is able to establish a preliminary pharmacological action of the fractions of methanol extract of *G. kola* seeds on KCl-induced contractions of isolated vas deferens. The inhibitory effect of the fractions on vas deferens may suggest possible implications on ejaculation with attendant male infertility effect of *G. kola* perhaps if use for a long term. On the other hand, it may suggest its benefits in smooth muscle contractile disorders such as those of the vascular system that can result in hypertension.

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