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Possible Mechanism of Antispasmodic Action of *Garcinia kola* Seed Extract on Isolated Guinea Pig Ileum

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Authors' contributions

This work was carried out in collaboration between all authors. Author PMU identified the species of the plant, designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors DUO and OJO managed the literature searches and analyses of the study. Author LPT managed the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: *Garcinia kola* is used in West African countries for the treatment of various ailments such as cough, tooth decay, asthma and menstrual cramps. The inhibitory effect of *Garcinia kola* seed extract (GKE) on drug-induced contractions was studied on iliac smooth muscle preparations of guinea pig to ascertain the validity of the use of *Garcinia kola* in traditional medicine and to elucidate its possible mechanism of action.
Place and Duration of Study: The study was done in Post Graduate Laboratory, Department of Pharmacology, College of Medical Sciences, University of Calabar, Calabar-Nigeria, between November 2013 and April 2014.
Methodology: The antispasmodic influence of GKE (0.02 – 1 mg/ml) on acetylcholine, histamine and potassium chloride -induced contractions were carried out. The effect of GKE in a Ca^{2+}-free Tyrode medium and in the presence of adrenergic antagonists was also investigated.
Results: The results revealed that GKE inhibited or attenuated the spasmogenic effects of

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histamine and potassium chloride in a dose-dependent manner and shifted their log. dose-response curves to the right, with pA_2 values of 2.09±0.06 and 3.25±0.07 respectively. Pre-administration of propranolol, prazosin or labetalol had no attenuating influence on the antispasmodic effect of GKE. Iliac smooth muscle responses to cumulative increased [Ca^{2+}] in a depolarizing bathing medium and in a Ca^{2+}- free Tyrode solution were also blocked. Comparative antispasmodic potencies indicated that papaverine and aminophylline were more potent than the extract.

Conclusion: These findings suggest that *Garcinia kola* seed extract acts neither via cholinergic nor adrenergic receptor mediation, but may involve interference with Ca^{2+} mobilization, thus sharing with papaverine and/or aminophylline similar mechanism(s) of action.

Keywords: Garcinia kola; guinea pig; ileum; spasmogens; antispasmodic effect.

1. INTRODUCTION

Garcinia kola Heckle (Fam.: Clusiaceae or Guttiferae) is a tree that grows wild but sometimes cultivated near villages, mostly in moist environments. The tree is widespread throughout West Africa and is found up to an elevation of 900 meters [1]. The root and the seed parts of the tree are used in traditional medical practice. The root of *Garcinia kola* (*G. kola*) is a bitter chew stick and is used traditionally for the treatment of cough, tooth decay and gonorrhea. The mode of therapeutic application of the root is usually by oral administration of the cold alcoholic extract obtained by soaking *G. kola* root chippings in locally brewed gin. The seeds of *G. kola* are said to prevent or relieve intestinal spasm, act as curative for cold in the chest, relieve cough and hoarseness and to improve the voice [1-3]. The seeds are also used in traditional medicine for the treatment of asthma, bronchitis, gastroenteritis, rheumatism, menstrual cramps, cough, hepatitis and diarrhea [4,5].

Scientific investigation have been carried out to ascertain the biochemical, microbiological, pharmacological and physiological basis of therapeutic application of roots, seeds and leaves of *G. kola* in traditional medical practice [4-10]. The pharmacological activities of *G. kola* are many and include anti-oxidant [11], antibacterial [12-14], antiviral [3], antifungal [15], hepatoprotective and anti-inflammatory [16]. Naiho and Ugwu [17] reported on the efficacy of *Garcinia kola* in the reduction of blood pressure, an effect attributable to the presence of vasoactive substances in the plant herb. The activities of lactate dehydrogenase and glucose-6-phosephate dehydrogenase have been reported to be increased by aqueous extract of *Garcinia kola* [18]. The antioxidant potential of *G. kola* has been attributed to the presence of high content of ascorbic acid [19]. Oloyede and Afolabi [20] reported on the antioxidant potential and Fe^{2+} chelating ability of the methanolic extract of *Garcinia kola* leaf; they concluded that *G. kola* leaf extract possess a significant natural antioxidant activity. Phytochemical screening revealed that *G. kola* contains alkaloids, cardiac glycosides, phenols, tannins, saponins and flavonoids [21]. Biflavonoids, some flavonoid derivatives and other phytochemical components have the potential of inhibiting smooth muscle tone and intestinal peristalsis, enabling *G. kola* to be used in the treatment of asthma [19], hypertension [17], gastric ulcer [22] and as an antispasmodic agent and smooth muscle relaxants [4,17,23,24].

Intestinal tone is present in normal conditions, a factor which renders stability to normal peristalsis [25]. This tone and peristalsis are regulated by acetylcholine and noradrenaline and their analogues which are elaborated by the autonomic nervous system (ANS) coupled with the availability of calcium ion (Ca^{2+}), which is a basic determiner of smooth muscle contraction [26-29]. While cholinergic stimulation and availability of Ca^{2+} result in increase intestinal activity, adrenergic stimulation inhibits intestinal peristalsis with increased tone of sphincters [29,30]. Certain drugs such as isoprenaline or adrenaline which stimulate adrenergic receptors and papaverine or aminophylline which cause increase in cyclic adenosine 3', 5' monophosphate (cAMP) interfere with Ca^{2+} fluxes during excitation-contraction coupling, also cause relaxation of smooth muscles [31,32]. Kolaviron, a biflavonoid complex from Garcinia kola, has been reported to induce vasodilation independent of endothelium in rats [23]. The pharmacological basis of *G. kola* in the treatment of asthma [19], hypertension [17], gastric ulcer [22] and other ailments have been linked to the presence of active phytochemical components

present in the plant herb [4,17,21-24]. Since *G. kola* has been shown to inhibit smooth muscle activity and is used in traditional medical practice to relieve intestinal spasms, cough and diarrhea, the present work was designed to investigate the effect of the methanolic extract of this species of kola on isolated tissue preparations, and the extract's possible mechanism of action.

2. MATERIALS AND METHODS

2.1 Preparation of Extract

The extract of *G. kola* seed (GKE) was prepared by modification of the methods previously described [4,24,33]. Briefly, *G. Kola* seeds were dried and grinded to a powder form. The dried seed powder was Soxhlet extracted twice with petroleum ether at 45ºC. The petroleum ether treated material was then extracted with methanol for 72 hours. The extract was slowly evaporated to dryness at 50ºC.

Starting material of 300g yielded 31.52g of the extract which was stored at -4ºC. Weighed sample of the methanol extract of *Garcinia kola* seed (GKE) was then used to prepare the test solutions.

2.2 Methods

Ileal smooth muscles were obtained from guinea pigs of both sexes weighing 400-450g and set up in an organ bath of 50ml capacity, following. The physiological salt solution (PSS) was Tyrode's solution (composition in g/ml: NaCl 8.00, KCl 0.20, $CaCl_2$ 0.02, $MgCl_2$ 0.10, NaH_2PO_4 0.05, $NaHCO_3$ and glucose 1.00). A high K^+-(Ca^{2+}-free) PSS was prepared by omitting $CaCl_2$, increasing KCl concentration by 50% and decreasing NaCl by 50% concentration. A Ca^{2+}-free Tyrode solution was prepared by omitting $CaCl_2$ in the preparation while NaCl and KCl concentrations remained unchanged. Each preparation was maintained at 37ºC under a resting tension of 1-1.5g and aerated with atmospheric air via an aerator (Type R.301, USA).

The preparations were allowed to equilibrate for at least 30 minutes before commencing experimentation while the PSS was changed every 15 minutes. Drug induced responses were recorded isotonically on graph papers of a slow-moving physiograph (Universal Oscillograph, Harvard Apparatus, Britain) with a horizontal writing lever which produced 10-12 fold magnifications. The physiograph was connected to an organ bath apparatus fitted with a heater (Double heated tissue bath, Harvard Apparatus, Britain). Drug or extract contact time with tissue was 0.5-2 minutes followed by 3-5 washings. An interval of 5-10 minutes was allowed between successive doses. In some experiments, drug-tissue contact time was 5-7 minutes. Under the mentioned conditions for each tissue preparation, the graded or cumulative concentration-response curves were registered. Progressive increased concentrations of the agonists were duplicated subsequently. The experiments were repeated later after addition of 2 $x10^{-5}$, $2x10^{-4}$ and $lx10^{-3}$ g/mL of *G. kola* extract.

Spasmolytic activity of *G. kola* extract was compared with reference drugs (aminophylline and papaverine) dose-dependently by calculating the percentage inhibition of ileal contraction induced by acetylcholine. Reference drugs and GKE were given three minutes before administration of acetylcholine at a dose of 5 x 10^{-7}M; a dose which has been previously found to cause appreciable ileal contraction. In another set of experiments, the preparations were washed with depolarizing solution [high K^+ -(Ca^{2+}- free) PSS or Ca^{2+}-free Tyrode]. Concentration - responses were obtained by cumulative addition of $CaCl_2$. Later, the effects of GKE on Ca^{2+}- induced contractions of the tissues were tested.

The effects of pretreatment with adrenergic antagonists - propranolol, prazosin and labetalol-on the GKE ileal relaxant activities were also determined. The sequence of drug administration in the presence of adrenergic antagonist was adrenergic antagonist, followed by GKE and lastly acetylcholine. The interval between each drug administration was 2-3 minutes.

The drugs used were acetylcholine, histamine and aminophylline (Sigma, USA.), prazosin and labetalol (Allen and Hanburys LTD., London), papaverine (L.I.R.C.Z.A., Synthelabo SPA Ltd., Italy), propranolol (Macclesfield, Great Britain), adrenaline and KCl (M&B, England). All chemicals were of analytical grade. Drugs were dissolved in the deionized distilled water while the extract was dissolved in 1ml of DMSO or diethylether and the volume made up to10ml stock solution.

2.3 Result Analysis

The determination of EC_{50} values were from the log dose-response curves [35] and confirmed by

means of the logit representation from a plot of log (E_A /E_{max} - E_A) against log concentration (M). Where E_A is the effect of agonist and E_{max} is maximal effect. The EC_{50} being the value on the abscissa when the log (E_A/E_{max} - E_A) equals zero [28]. The pA_2 values were determined by means of the Schild representation [36] from a plot of the reciprocal of the dose ratio (EC_xAg+Antagonist/EC_xAg) against antagonist concentration.

Where EC_xAg. is the concentration of agonist that produces x percent (30% or 50%) response on the tissue. The intersection on log antagonist concentration axis corresponds to pA_2 value [28].

2.4 pA_2 Determination

Data for the determination of pA_2 value were generated from log dose-response curves for agonist drugs and the presence of three different concentrations of GKE. The pA_2 is defined as the negative log of the molar concentration of antagonist drug which reduces the effect of double dose of agonist to that of a single dose. The Schild equation provides an experimental means of determining the pA_2 value [36].

2.5 Statistical Analysis

Results are expressed as mean values ± SEM based on at least four experiments. Significance was determined by student's t test. A probability level of 5% (*P*=.05) or better was considered significant.

3. RESULTS

3.1 Effects of GKE on Histamine and KCl –Induced Contractions

Graded additions of increasing concentrations of histamine ($4x10^{-10}$ – $6x10^{-7}$M) induced concentration-dependent contractions of the ileum. Pre-administration of GKE ($2x10^{-5}$g/mL, $2x10^{-4}$ g/ml and $1x10^{-3}$ g/mL) inhibited these contractions and shifted the graded log dose-response curves of histamine-induced contractions to the right in a dose-dependent manner (Fig. 1). Potassium chloride (KCl, $4x10^{-3}$ - $5x10^{-2}$M) induced contractions were inhibited dose- dependently by the three doses of the extract ($2x10^{-5}$ g/mL, $2x10^{-4}$ g/mL and $1x10^{-3}$ g/mL of GKE). The log. dose-response curves for KCl were shifted to the right in the presence of the extract in a dose-dependent manner (Fig. 2).

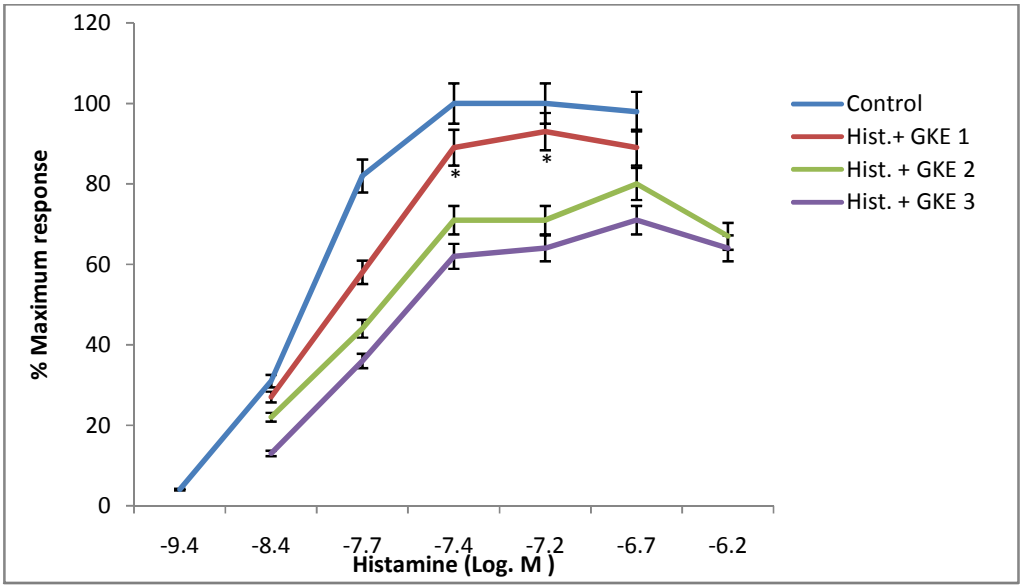

Fig. 1. Modification of responses of guinea pig ileum by *Garcinia kola* extract (GKE) to graded concentrations of histamine (Hist.). Results show mean ± SEM of four values.

**P=.05 vs control, GKE 2 and GKE 3. GKE 1, GKE 2 and GKE 3 = Garcinia kola extract 2 x 10^{-5}, 2 x 10^{-4} and 1 x 10^{-3} g/mL respectively.*

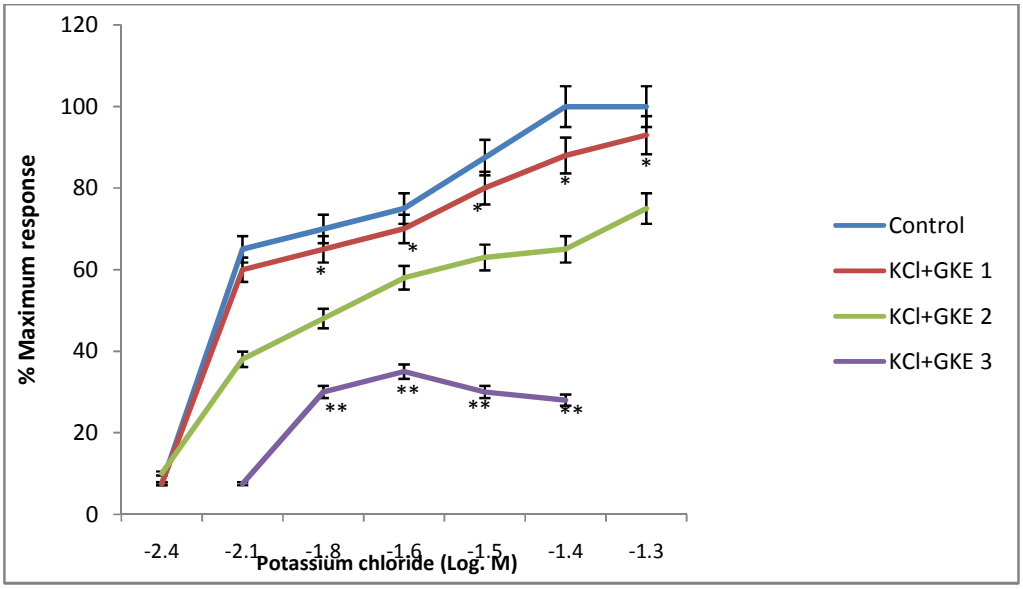

Fig. 2. Effect of Garcinia kola extract (GKE) on the responses of isolated guinea pig ileum to graded concentrations of potassium chloride (KCl). Results show mean ±SEM of four values.
**P=.05 vs control and GKE 2, **P<-.001 Vs GKE 1 and GKE 2. GKE 1, GKE 2 and GKE 3 = Garcinia kola extract 2 x 10^{-5}, 2 x 10^{-4} and 1 x 10^{-3} g/mL respectively*

The effect *G. kola* extract on EC_{50} and E_{max} values of acetylcholine, histamine and KCl -induced contractions indicate increased EC_{50} and decreased E_{max} values of agonist drugs with increased concentration of GKE (Table 1). The pA_2 values, as calculated from the plot of Schild equation [36] were 2.69±0.06 and 3.25±0.07 (Table 1).

3.2 Comparative Antispasmodic Potencies of GKE and Reference Drugs

Pre-administration of GKE resulted in antispasmodic effect on acetylcholine ($5x10^{-7}$ M) -induced spasm on the guinea pig ileum in a dose-dependent manner. At a dose of 0.3 mg/ml and 0.6 mg/ml, GKE significantly ($P<0.01$) reduced acetylcholine-induced contractions. Papaverine and aminophylline equally exhibited significant ($P<0.01$) dose-dependent antispasmodic effects on acetylcholine-induced contractions on the guinea pig ileum when compared with control. There was no significant ($P=.05$) difference between GKE antispasmodic effect and those of reference drugs at low dose levels but significant ($P=.05$ and $P<0.01$) difference occurred at higher dose levels. The effect of GKE at a dose of 0.6 mg/ml was comparable to that of papaverine at a dose of $8.0x10^{-3}$ mg/ml (Table 2).

3.3 Effects of GKE on Ca^{2+}- Induced Contractions

Cumulative addition of Ca^{2+} to a high K^+-(Ca^{2+} free) physiological solution resulted in cumulative increases in the amplitudes of phasic contractions in the guinea pig ileum. This effect was inhibited by the addition of GKE (0.3mg/ml) to the bath fluid. In a normal K^+- (Ca^{2+}- free) Tyrode solution, increasing [Ca^{2+}] ($CaCl_2$ $2x10^{-4}$ – $7x10^{-3}$ M) resulted in increases in contractile responses of the guinea pig ileum. This effect was totally abolished when the tissue was pre-incubated with GKE (0.3mg/mL). Following washout of the extract from the preparations, there was complete tissue recovery and response to Ach ($5x10^{-7}$ M) was normal.

3.4 Effect of Pretreatment with Adrenergic Antagonists on the Actions of GKE

The methanolic extract of *G. kola* (GKE 0.3 mg/mL) significantly ($P<0.01$) inhibited contractions induced by acetylcholine (ACh, $5x10^{-7}$ M) on the guinea pig ileal muscle Pre-administration of propranolol ($1.6x10^{-2}$ mg/mL), a β-adrenergic antagonist or prazosin ($4x10^{-4}$ mg/mL), a α-adrenergic antagonist, failed to

Table 1. Influence of methanolic extract of *Garcinia kola* (GKE) on EC_{50} and E_{max} values of acetylcholine, histamine and potassium chloride

Tissue	Agonist	GKE (g/mL)	EC_{50}(M)	EC_{50}Ag+GKE/EC_{50}Ag	1/(x-1)	pA_2	-Log GKE (Log[A])	E_{max} (%)
Rat ileum	Acetylcholine	--	$1.3x10^{-6}$	--	--		--	100
	($1x10^{-6}$ – $1x10^{-4}$ M)	$2x10^{-5}$	$1.6x10^{-6}$	1.26	3.85		4.7	92
		$2x10^{-4}$	$2.0x10^{-6}$	1.5	1.75		3.7	85
Guinea pig ileum	Histamine	--	$7.0x10^{-9}$	--	1.17		--	100
	($4x10^{-10}$ - $6x10^{-7}$M)	$2x10^{-5}$	$1.3x10^{-8}$	1.857	0.41		4.7	93
		$2x10^{-4}$	2.4x10-^{8}	3.429	0.29	2.69±0.06	3.7	80
		$1x10^{-3}$	3.1x10-^{8}	4.429	--		3.0	71
Guinea pig ileum	Potassium chloride	Nil	$7.0x10^{-3}$	--	--		--	100
	($4x10^{-3}$ – 5x 10^{-2}M)	$2x10^{-5}$	$7.3x10^{-3}$	1.04	27.8		4.7	93
		$2x10^{-4}$	$9.6x10^{-3}$	1.37	4.3	3.25±0.07	3.7	75
		$1x10^{-3}$	--	--	0.5		3.0	35

Results are as calculated from log. concentration-response curves for agonists and in the presence of different concentrations of GKE. 1/(x-1) is the reciprocal of the dose ratio at 50% response for acetylcholine and histamine, but at 30% response for potassium chloride induced responses

Table 2. Comparative antispasmodic potencies of *Garcinia kola* extract (GKE), aminophylline and papaverine

[+] Drug/conc.	Height of contraction (mm)[++]	% inhibition of maximal contraction	[a]P<	[b]P<
ACh ($5x10^{-7}$M, Control)	38.3 ± 0.6	--		
GKE (0.3mg/mL) + Ach	35.3 ± 0.5	9.0 ± 1.2	0.01	N.S.
PAP ($0.8x10^{-3}$mg/mL) + ACh	33.3 ± 0.9	14.2 ± 1.2	0.01	N.S.
AMP ($0.5x10^{-3}$mg/mL) + ACh	34.5 ± 0.8	11.1 ± 1.3	0.01	N.S.
GKE (0.6mg/mL) + Ach	33.0 ± 1.2	14.9 ± 3.1	0.01	N.S.
PAP ($1.6x10^{-2}$mg/mL) + ACh	29.8 ± 1.0	23.2 ± 2.6	0.001	0.01
AMP ($1x10^{-2}$mg/mL) + ACh	32.2 ± 0.7	16.2 ± 1.8	0.001	0.05

[++]Results show Mean ± SEM of four experiments. [+]Listed in order of introduction if more than on drug in organ bath. Figures in brackets represent final organ bath concentrations. ACh = acetylcholine, GKE = Garcinia kola extract, PAP = papaverine, AMP = aminophylline, N.S. not significant (P = .05), [a]P = compared with control, [b]P = compared with GKE (0.3mg/mL)

attenuate GKE inhibitory effect, while tissue response to Ach.

Adrenaline ($1x10^{-5}$ M) significantly (P<0.01) inhibited contractions induced by ACh ($5x10^{-7}$ M) on the ileum. Pre-administration of labetalol ($5x10^{-3}$ mg/ml), a drug which blocks both α- and β- adrenoceptors, significantly (P<0.01) attenuated the effect of adrenaline on the ileum. Administration of GKE (0.5mg/ml) resulted in significant (P<0.01) antispasmodic effect on ACh ($5x10^{-7}$ M) induced spasm. Pre-administration of labetalol ($5x10^{-3}$ mg/mL) did not attenuate the antispasmodic effect of GKE (0.5mg/ml) on ACh ($5x10^{-7}$ M) induced spasm on the ileum (Fig. 4). The inhibitory effect of adrenaline ($1x10^{-5}$ M) on ACh ($5x10^{-7}$ M) -induced contraction was 26.5±4% (an effect that was attenuated by labetalol ($5x10^{-3}$ mg/mL). The inhibitory effect of GKE (0.5 mg/mL) alone on ACh ($5x10^{-7}$ M) -induced contraction was 31.7±2.6%, and was 30.2±0.4% following pre-administration of labetalol ($5x10^{-3}$ mg/mL). The effect of adrenaline was not significantly (P=.05) different when compared with that of GKE at the dose levels used.

($5x10^{-7}$ M) decreased by 9.0±1.3% in the presence of GKE (0.3 mg/ml) alone, it decreased by 11.6±1.0% and 10.0±1.8% when the tissue was pre incubated with propranolol ($1.6x10^{-2}$ mg/mL) and prazosin ($4x10^{-4}$ mg/mL) respectively, prior to GKE and ACh sequential administration (Fig. 3). There was no significant (P=.05) difference between the effects in the presence of adrenergic antagonists when compared with the effect of GKE alone.

4. DISCUSSION

The methanolic extract of *Garcinia kola* (GKE) exhibited does-dependant antispasmodic effects on spasms induced by acetylcholine, histamine and potassium chlorine (KCl). The dose-response curves for histamine and KCl were non-parallely shifted to the right in the presence

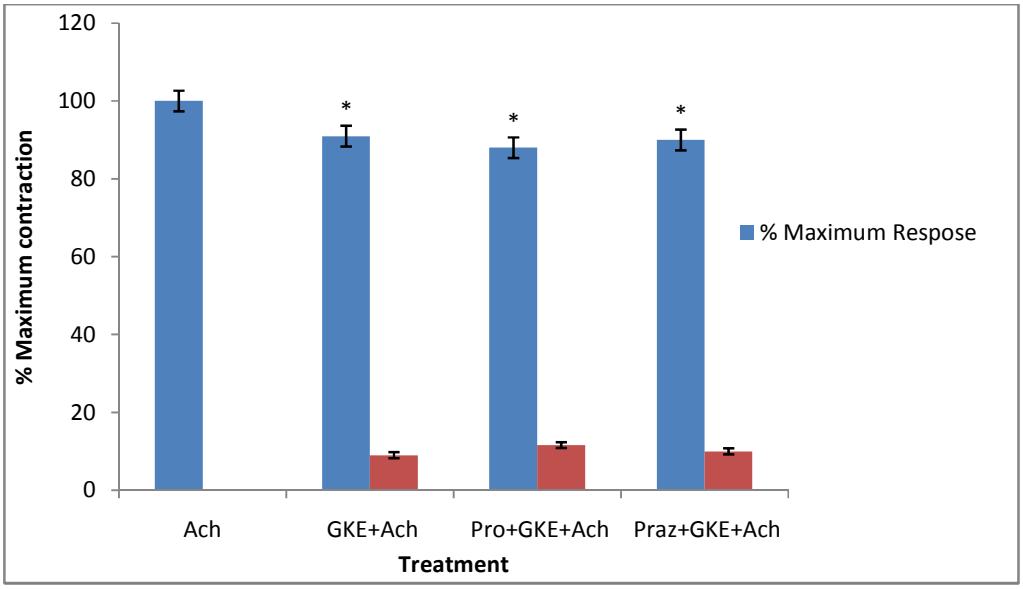

Fig. 3. Relaxant effects of *Garcinia kola* extract (GKE, 0.3 mg/mL) on acetylcholine (Ach, 5x10^{-7}M) - induced contractions on guinea pig ileum and the influence of adrenoceptor antagonists. Results show mean ± SEM of four values.

**P<0.01 compared with control. Pro = propranolol 1.6x10^{-2}mg/mL, Praz = prazosin 4x10^{-4}mg/mL.*

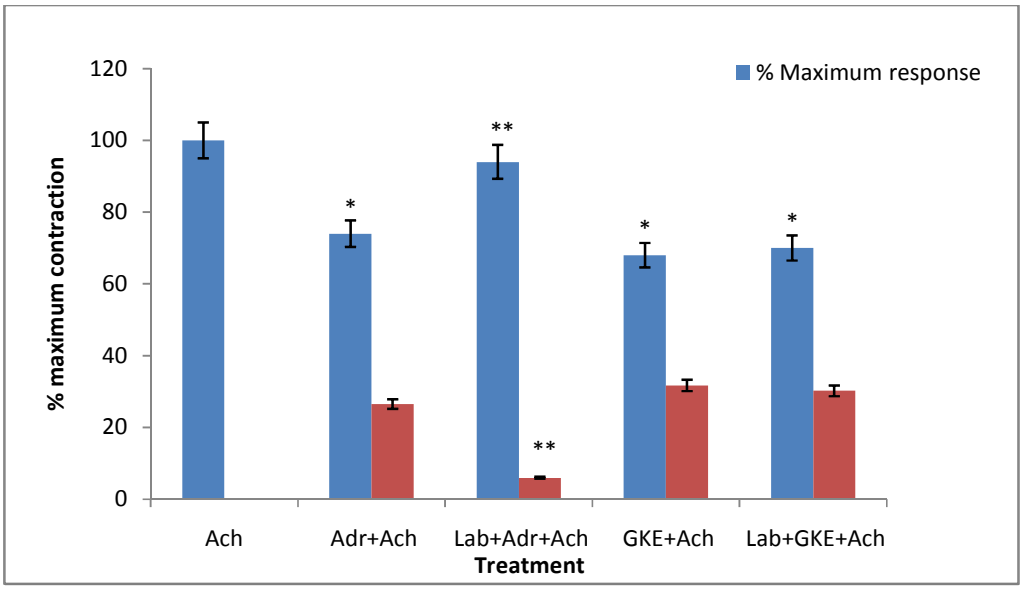

Fig. 4. Influence of adrenaline (Adr, 1x10^{-5}M), labetalol (Lab, 5x10^{-3}M) and *Garcinia kola* extract (GKE, 0.5mg/mL) alone and in combination on acetylcholine (Ach, 5x10^{-7}M)- induced contractions in guinea pig ileum. Results show mean ± SEM of four values.

**P<0.01 compared with control, **P<0.01 compared with Adr + Ach, GKE +Ach and Lab + GKE + Ach combinations*

of increasing concentrations of GKE, with increasing EC_{50} values but with decreasing E_{max} values. This result is in agreement with our earlier observation that GKE exhibited potent inhibitory effects on drug induced spasms on isolated rat intestine [24], and with other reports that *G. kola* (Heckle), plant flavonoids and some plant biflavonoids possess smooth muscle relaxant potentials [4,5,17,19,23,37]. Propranolol, a β-adrenergic antagonist and

prazosin, a α-adrenergic antagonist could not block the inhibitory effects of GKE. Labetalol, a drug which blocks both α- and β-adrenoceptors, blocked the effect of adrenaline on the ileum but failed to attenuate the inhibitory effect of GKE. These results are in line with the reports of Naiho and Ugwu [17] and indicate that GKE exert its smooth muscle relaxant effect by actions which are not due to neither cholinergic nor adrenergic receptor mediation.

Braide [4] suggested that it was unlikely that *G. kola* extracts acted by selectively blocking muscarinic, histaminic or serotoninergic receptors indiscriminately to cause the observed inhibition of the agonistic actions of these agents. The fact that the EC_{50} values of histamine and KCl were significantly increased in the presence of the extract and that the E_{max} values were diminished, lead us to believe that some components of the extract behave as non-specific non-competitive antagonists of the said agonists at the level other than their respective receptors. It is known that agents which act on specific receptors to elicit their effects (agonist or antagonist) do so in very small concentrations [31], which can be ascertained experimentally from the values of pA_2 ($-\log IC_{50}$). A high pA_2 value indicates that low concentration of the antagonist is needed to reduce the agonistic effect by 50%, whereas a low pA_2 value indicates that a high concentration of the antagonist is needed. The pA_2 values for GKE, as calculated from the plot of Schild equation [36] were 2.69±0.06 and 3.25±0.07 in the presence of the agonistic action of histamine and KCl respectively. These low pA_2 values indicate that GKE acts via non-specific, non-competitive inhibition.

KCl induced dose-dependent ileal muscle contractions in normal Tyrode solution were attenuated by pre-administration of GKE. In a Ca^{2+} -free Tyrode medium, no response to depolarization by KCl was observed. This result confirms the report that KCl induced contraction is dependent on extracellular Ca^{2+} entry [38]. Responses of the guinea pig ileum to cumulative increased Ca^{2+} concentration in a depolarizing bathing medium and in Ca^{2+} -free Tyrode were also blocked. Given that the contraction of the ileum previously depolarized is proportional to the addition of Ca^{2+}, and since Ca^{2+} is a basic determiner of muscle contraction, it can be proposed that the antispasmodic activity of the extract of *G. kola* is either by blocking the release of intracellularly bound Ca^{2+} or by preventing the entry of extracellular Ca^{2+} into the smooth muscle cell. Adesuyi et al. [21] and Mazi et al. [39] on commenting on the phytochemical, proximate composition and nutritive properties of *Garcinia kola* concluded that the nutritive and health benefits of this plant drug is attributable to the abundant active phytochemical constituents present in it. The blood pressure lowering effect [17], the anti-asthmatic effect [19] and the antimalarial activity [40] of *Garcinia kola* were all attributable to the presence of pharmacologically active components present in the plant. In a related study, Townsend et al. [41] indicated that the airway relaxant effect of Ginger and its isolated active components relax airway smooth muscles in part by altering intracellular Ca^{2+} regulation, an effect mediated by the plant's active components.

Braide [4] proposed that *G. kola* alkaloid and flavonoid fractions produced muscle relaxation by interference with various calcium pools responsible for excitation - contraction coupling, such as chelation of extracellular Ca^{2+} and inhibition of Ca^{2+} influx. Adaramoye and Medeiros [23] indicated that the vasorelaxant effects of kolaviron, a biflavonoid complex from *Garcinia kola*, involve extracellular Ca^{2+} influx blockade, inhibition of intracellular Ca^{2+} release and the opening of K^+ channels which resulted to membrane hyperpolarization/repolarization. In their report, Ebomoyi and Okojie [19] concluded that the anti-asthmatic mechanisms of *Garcinia kola* include, among others, "inhibition of Ca^{2+} influx by acting as a blocker of both receptor-operated and voltage-dependent Ca^{2+} channels". In smooth muscles, increase in cAMP concentration alters cell membrane permeability to ions with resultant muscle relaxation, an effect which could be brought about by β-adrenergic agonist, aminophylline and papaverine [32]. Relaxation of smooth muscle occurs when free (unbound) Ca^{2+} in the cytoplasm is reduced as a result of inhibition of Ca^{2+} influx, reuptake into cellular stores, active extrusion across cell membrane or a combination of all these processes [25,26]. Papaverine has been proposed to act via reuptake and active extrusion processes whereas aminophylline acts mainly by inhibiting Ca^{2+} influx [4,32]. The present study suggests a link with the mechanism of action of aminophylline and GKE via inhibition of Ca^{2+} influx, since KCl induced contraction, which depends on extracellular Ca^{2+} entry [38] was also blocked by GKE. However, if GKE is acting as a chelator of Ca^{2+} as propose to be a possible mode of action of flavonoids on divalent cation

sensitive steps in the regulation of cellular secretions and smooth muscle activity [4], then it is possible that GKE interacts with Ca^{2+} via more than one process that link Ca^{2+} with smooth muscle relaxation.

5. CONCLUSION

Smooth muscle relaxation may result from inhibition of cholinergic receptors or stimulation of adrenergic receptors. In addition, interference with Ca^{2+} mobilization results in inhibition of smooth muscle contraction. We demonstrated that GKE exert its smooth muscle relaxant effect by actions which are not due to neither cholinergic nor adrenergic receptor mediation and the low pA_2 values indicate that GKE acts via non-specific, non-competitive inhibition. The results from the present study also showed that responses of the guinea pig ileum to cumulative increased Ca^{2+} concentration in a depolarizing bathing medium and in Ca^{2+} -free Tyrode were also blocked. Given that the contraction of the ileum previously depolarized is proportional to the addition of Ca^{2+}, it is proposed that the antispasmodic activity of the extract of *G. kola* is either by blocking the release of intracellularly bound Ca^{2+} or by preventing the entry of extracellular Ca^{2+} into the smooth muscle cell. In summary, the methanolic extract of *Garcinia kola* exhibit antispasmodic activity via nonreceptor interaction, but may involve interference with Ca^{2+} mobilization with subsequent impairment of excitation-contraction coupling.

CONSENT

Not applicable.

ETHICAL APPROVAL

We declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed; the laws governing the handling of laboratory animals in College of Medical Sciences of this University were strictly adhered to. All experiments have been approved by the Departmental and Faculty Boards of the College.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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