ANTISPASMODIC AND SPASMOLYTIC EFFECTS OF METHANOLIC EXTRACT FROM SEEDS OF GARCINIA KOLA ON ISOLATED RAT SMALL INTESTINE

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Summary: The antispasmodic and spasmolytic effects of methanolic extract of seeds of *Garcinia kola* Heckel were studied on smooth muscle preparations *in vitro*. The influence of the extract on rat duodenum, jejunum and ileum was investigated using acetylcholine and barium chloride as agonists. The extract exhibited dose-dependent antispasmodic effects on contractions induced by acetylcholine, and dose-dependent spasmolytic effects on spasms induced by cumulatively increased concentrations of acetylcholine and barium chloride. The graded log concentration- response curves for acetylcholine were non-parallel but shifted to the right in the presence of the extract. It is concluded that the *Garcinia kola* extract inhibits smooth muscle activity via other mechanisms but not involving neither cholinergic nor adrenergic receptor interaction.
Key words: Garcinia kola, small intestine, acetylcholine, antispasmodic effect.

Introduction

The seeds of *Garcinia kola* Heckel (Fam-Guttiferae) were obtained from a tree which grows in the wild but are sometimes cultivated in the southern part of Nigeria. It is locally called "bitter kola". These seeds are edible and are consumed as adjuvant to the true kola (Cola nitida) and also for medicinal purposes (Daziel, 956; Braide, 1989). *Garcinia kola* seeds are used in Nigerian traditional medicine for the treatment of asthma, diarrhoea, gastroenteritis, menstrual cramps and as antidote for poisons (Braide, 1989; Orie and Ekon, 1993; Kabungu *et al,* 1987). *G. kola* seeds have bronchodilation effect on tracheal smooth muscle cells in humans (Orie and Ekon, 1993). Though preliminary phytochemical studies had indicated that the seeds of *G. kola* lack caffeine (Eka, 1983), it contains alkaloids and biflavonoids (Braide, 1989; Hussain and Waterman, 1982; Iwu, 1982). Alkaloid and biflavonoid fractions from these seeds exhibit antagonistic effects on drug-induced spasms on rat duodenum and uterus and on guinea pig ileum (Braide, 1989). Orie and Ekon (1993) have demonstrated the inhibitory effects of *G. kola* seeds on tracheal smooth muscle in humans. Other workers have investigated the effects of flavonoids of other plants and of synthetic flavonoids on some smooth muscle activity of experimental animals (Ogata *et al*, 1993; Belluco *et al*, 1993; Di Carlo *et al*, 1993). Biflavonoids from *G. kola* have anti-inflammatory properties (Braide, 1993), is a natural antioxidant (Olatunde *et al* 2002, Terashima *et al*, 2002; Olatunde *et al*, 2002b; Farombi *et al,* 2007) and is hepatoprotective (Adaramoye and Adeyemi, 2006; Farombi *et al*, 2009;

Adaramoye *et al*, 2008) Extracts from *G. kola* seeds have been reported to alter oestrous cycle, inhibit ovulation, induce teratogenicity (Akpantah *et al*, 2005), and to be non-toxic to erythrocytes even at high dose ranges (Esomonu *et al,* 2005). In the search for alternatives to synthetic hypoglycaemic agents, Adaramoye and Adeyemi (2006) reported anti-diabetic and hypolipidaemic effects of fractions of Kolaviron, (a *G. kola* seed extract) in streptozotocin (STZ)-diabetic rats. These reports are indicative of the ability of the active components of this species of kola and of flavonoids from other plants (Agil *et al,* 1994) to arrest inflammation and/or smooth muscle hypermotility and of other ailments when used in traditional medicine.

The objective of this study is to analyze the influence of the methanolic extract of *G. kola* seeds on the contractile responses of the rat intestine to spasms induced by acetylcholine and barium chloride in order to elucidate possible mechanism of action of this species of kola that is used in traditional herbal medical practice.

Materials and methods
Plant extract

The seeds of the plant, purchased from local markets in Calabar, Cross River State of Nigeria, were chopped into small pieces after removing the testa, and ground to a paste. The ground material was dried in an oven at a temperature of 40°C – 60°C. The dried powder (300g) was defatted twice with two litres of petroleum ether and was then Soxhlet extracted using methanol. A brownish solid paste was obtained. The percentage yield of 10.5% was

obtained. The extract was stored at 4°C until further use.

Animals

Adult Albino rats weighing 180 –250g were obtained from the animal house of the Department of Pharmacology, University of Calabar for the study. They were kept in animal house at room temperature of 30 ± 2.0°C in a 12-hour light/dark cycle. They were fed *ad libitum* with normal rat chow obtained from Livestock feeds Aba, Nigeria and had access to tap water. The animals were fasted for 18-hour prior to experiment and were sacrificed by stunning. The abdominal region of each animal was opened by midline incisions and the duodenum, jejunum and ileum were quickly removed and placed in Tyrode solution (PSS), (composition in g/l: NaCl, 8.00; KCl, 0.20; $CaCl_2$, 0.20; $MgCl_2$, 0.10; NaH_2PO_4, 0.05; $NaHCO_3$, 1.00; glucose, 2.00), (Rodriguez *et al*, 1986). Tubular segments (2-3cm long) were cut from the duodenum, jejunum and ileum and were suspended in PSS at 37°C under the conditions mentioned in Table 1 and continually aerated with atmospheric air.

Each tissue preparation, under the conditions mentioned in Table 1, was allowed to equilibrate for a period of 45-60 minutes under a resting tension of 1 – 1.5g before it was challenged with the methanolic extract or *G. kola* seed (GKE) or agonist drug. The drug-induced responses or the spontaneous rhythmic contractions of the tissues were recorded isotonically on graph paper with a slow moving kymograph (C.F. Palmer LTD., England.)

Determination of antispasmodic effects

Various concentrations (1×10^{-5}–3×10^{-3} g/ml) of GKE were added to the bathing solution containing the tissue, and the responses of the tissue were recorded. In different tissue preparations, the experiments were performed using graded doses of acetylcholine (ACh) ($1 \times 10^{-6}M$ – $1 \times 10^{-4}M$). After obtaining responses to acetylcholine (ACh) stimulation, the experiments were repeated after pre-incubation with various concentrations of GKE (2×10^{-5} – 2×10^{-4} g/ml). From the results, log-concentrations against the amplitude of contractions (expressed as percentage of maximal control contractions) were constructed for ACh in the presence of two dose levels of GKE. From each of these curves, the EC_{50} and the E_{max} values of ACh were estimated for control

responses and in the presence of different concentrations of GKE.

Spasmolytic effects of the extract on drug induced contractions

Each tissue was prepared as described above and was bathed with normal Tyrode solution. The preparation was then challenged with cumulatively increased concentrations of ACh (5×10^{-7} – $2.5 \times 10^{-6}M$) or $BaCl_2$ (2×10^{-4} – $6 \times 10^{-4}M$) added to the bath fluid. The percentage inhibitions of drug-induced contractions were estimated for various concentrations of GKE and IC_{50} of GKE was calculated from the inhibition curve.

Drugs

Acetylcholine, barium chloride and propranolol were obtained from Sigma Chemical Co, MO, USA while prazosin was purchased from Allen and Hanburys LTD, London. Other chemicals were obtained from Sigma Chemical Co, MO, USA. All drugs were dissolved in deionized distilled water while stock solutions (100mg/ml) of the extract were prepared by dissolving 1g of the extract in 1ml of diethylether and the volume made up to 10ml with distilled water.

Result Analysis

The EC_{50} values were calculated from the log concentration-response curves and confirmed by means of logit representation from a plot of log ($E_A/E_{max} – E_A$) against log concentration (M). The EC_{50} is the value in the abscissa when the log ($E_A/E_{max} – E_A$) equals zero (Rodriguez *et al*, 1996).

Statistical analysis

All values were expressed in mean ± SEM. The data were statistically analyzed using student's t-test. A P-value of 0.05 was considered significant.

Results

The extract of *G. kola* elicited contraction of the isolated rat ileum at concentrations of 2×10^{-6} – 2×10^{-4} g/ml. Increased concentrations of the extract in these preparations at these dose ranges produced decreases in heights of contractions, such that 1×10^{-5}, 2×10^{-5} and 4×10^{-5} g/ml produced contractions in a descending order (Fig. 1). These effects were abolished by pre-incubation with 2×10^{-6} g/ml of GKE. However, the extract produced antispasmogenic and antispasmodic effects at higher concentrations in the presence of spasmogens, acetylcholine or barium chloride (Figs.2, 3; Tables 2 and 3).

Table 1: Experimental conditions in the rat tissue preparation

Experimental condition [a]	Duodenum	Jejunum	Ileum
Bath volume (ml)	10	10	50
Speed of recording [b] paper (mm/sec)	0.063	0.063	0.016
Equilibration time (min)	45	45	60
Interval between doses (min)	1.5	1.5	5
Dosing method	Cumulative	Cumulative	Graded
Agonist (concentration range)	$BaCl_2$ ($2-3x10^{-4}$M), Ach ($5x10^{-7} – 2.9 x10^{-6}$M)	$BaCl_2$ ($2-6x10^{-4}$M)	ACh ($5x10^{-6} – 2.9 x10^{-4}$M)

(a): All tissues were maintained at 37 ºC in Tyrode solution and aerated with atmospheric air. (b): Isotonic recording, Ach = acetylcholine, BaCl2 = Barium chloride

Table 2: Effect of adrenoceptor antagonists on the relaxant effect of GKE on Ach-induced contractions on guinea pig ileum

Drugs[+] (Max. conc.)	Tissue response (mm)	t-value	% Inhibition of contraction
ACh ($5x10^{-7}$M)	38.8 ± 0.6	-	-
GKE ($3x10^{-4}$ g/ml) +ACh ($5x10^{-7}$M)	35.3 ± 0.5	4.481*	9.0 ± 1.3
Pro.($1.6x10^{-2}$ mg/ml) +GKE ($3x10^{-4}$ g/ml +ACh ($5x10^{-7}$M)	34.3 ± 0.4	6.241*	11.6 ± 1.3
Praz. ($4x10^{-4}$ mg/ml) + GKE (($3x10^{-4}$ g/ml) +ACh ($5x10^{-7}$M)	34.9 ± 0.7	4.229*	10.1 ± 1.8

*+ Listed in order of introduction. Figures in brackets represent drug concentrations in organ bath. * = P< 0.01 (Compared with control). ACh = acetylcholine, GKE = G. kola extract, Pro. = Propranolol, Praz. = Prazosin Results show mean ± SEM of 4 observations.*
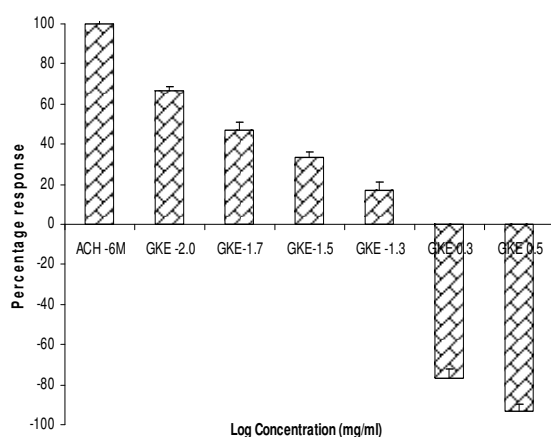

Fig. 1: Effect of Garcinia kola extract on the motility of rat ileum

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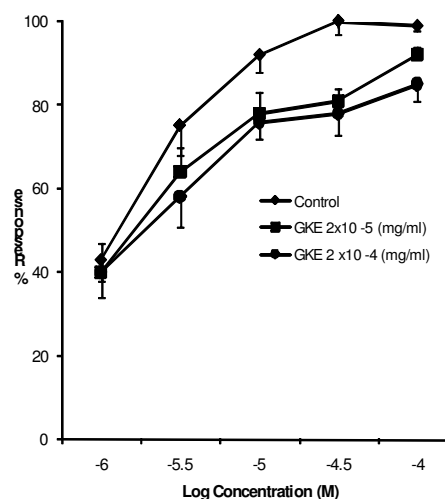

Fig. 2: Effect of various doses of GKE on Ach-induced contraction on rat ileum.

*Table 3: BaCl$_2$ induced contractions on rat duodenum and the dose-dependent
Spasmolytic effect of GKE on BaCl$_2$ induced contractions*

BaCl$_2$ (M)	Tissue response *	GKE (g/ml)	% Inhibition of max.Responses *
2×10^{-4}	30.0 ± 4.0	1×10^{-4}	30.1 ± 7.2
4×10^{-4}	54.6 ± 2.2	2×10^{-4}	52.6 ± 4.6
6×10^{-4}	72.9 ± 4.0	1×10^{-3}	76 ± 5.6
8×10^{-4}	100 ± 4.0	2×10^{-3}	100 ± 4.0

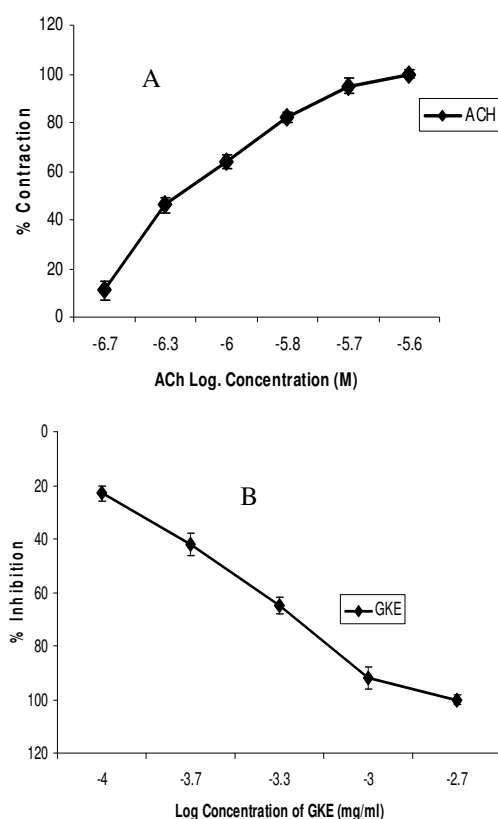* Results show mean ± SEM of 6 observations

Fig. 3: (a) Cumulative log. concentration.-response curve for Ach on rat duodenum (b) Inhibition curve of GKE on Ach-induced contractions on rat duodenum.

Antispasmodic action

Addition of the *G. kola* extract (GKE: $2 \times 10^{-5} – 2 \times 10^{-4}$ g/ml) to the isolated rat ileum preparation shifted the log dose-response curves of acetylcholine (Ach) induced contractions to the right in a dose-dependent manner (Fig. 2). Within the dose ranges of ACh used (Table 1), GKE affected both the EC$_{50}$ and the E$_{max}$ values. The EC$_{50}$ control was 1.3×10^{-6}M of ACh, the EC$_{50}$ in the presence of 2×10^{-5} g/ml of GKE was 1.6×10^{-6}M and the corresponding E$_{max}$ was 92%.

Increasing the concentration of GKE to 2×10^{-4} g/ml produced an EC$_{50}$ of 2.0×10^{-6}M and E$_{max}$ value of 85% when compared with control.

In another set of experiments using guinea pig ileum, pre-administration of propranolol (1.6×10^{-2} mg/ml), a beta-adrenergic receptor antagonist, or prazosin (4×10^{-4} mg/ml), and alpha-adrenergic receptor antagonist, failed to abolish the relaxant effect of GKE on Ach induced contractions (Table 2).

Spasmolytic effects of GKE on Ach and BaCl$_2$ –induced spasms

GKE exhibited dose-dependent spasmolytic effects on spasms induced by cumulative increased concentrations of Ach on rat duodenum (Fig. 3). At a dose of 1×10^{-4} g/ml, GKE exhibited $23 \pm 3\%$ inhibitory effect on Ach (2.5×10^{-6}M) induced contraction; at a dose of 1×10^{-3} g/ml, the inhibitory effect was $92 \pm 4\%$ (n = 6).

GKE equally suppressed, in a dose-dependent manner, contractions induced by cumulative addition of non-specific smooth muscle stimulant, barium chloride (BaCl$_2$), on the rat duodenum (Table III). On rat duodenum, the addition of 1×10^{-4} g/ml of GKE resulted in $30.1 \pm 7.2\%$ inhibitory effect on BaCl$_2$ induced contraction, whereas a dose of 2×10^{-3} g/ml GKE inhibited BaCl$_2$ induced contractions by $100 \pm 4\%$ (Table III; n = 6). A similar trend was observed in experiments involving rat jejunum.

Discussion

The results obtained in the present investigation show that the methanolic extract of the seeds of Garcinia kola (GKE) has a biphasic action on contractility of intestinal smooth muscle cells. It induces contraction at low doses and causes relaxation at high doses. This spasmogenic effect at low doses may be through direct action on intestinal mucosa, but not a partial agonist effect or a depolarizing blockage action. GKE diminished the responses of ACh and BaCl$_2$ on rat duodenum,

jejunum and ileum in a dose-dependent manner. It has been reported that G. kola contains biflavonoids (Adaramoye and Adeyemi, 2006b) and plants containing biflavonoids/flavonoids possess inhibitory effects on smooth muscle activity (Kabangu *et al*, 1987; Orie and Ekon 1993 and Di Carlo *et al* 1993). The fact that the EC_{50} value of ACh was significantly increased in the presence of the extract, while its E_{max} diminished coupled with non-parallel shift of the dose-response curves, suggests that some components (alkaloids or flavonoids) of the extract are non-specific inhibitors of smooth muscle activity. There was no evidence that the extract was interacting with specific autonomic receptors. Further evidence supporting non-specific interaction was based on the observation that on the already contracting gastrointestinal smooth muscle in response to agonist drugs, GKE exhibited rapid dose-dependent spasmolytic effect on spasms induced by ACh and $BaCl_2$, which are specific and non-specific smooth muscle spasmogens, respectively. Adrenergic blockade by prazosin (an alpha-adrenergic receptor antagonist) or propranolol (a beta-adrenergic receptor antagonist) failed to attenuate the inhibitory effect of GKE on ACh induced contractions. The failure of enhancement of ACh induced contractions in the presence of adrenergic receptor antagonists, when GKE was administered, indicated that GKE was not eliciting its smooth muscle relaxant effect via activation of any adrenergic receptors.

Braide (1989), Orie and Ekon (1993) and Di Carlo *et al* (1993) had suggested that *G. kola* seeds and/or extracts from it (alkaloids or biflavonoids) reduced tone and strength of contraction of smooth muscles in the presence of ACh, $BaCl_2$ or histamine. It is concluded that the antispasmodic and spasmolytic effects of methanolic extract of the seeds of *G. kola* (GKE) on smooth muscle activity of gastrointestinal tract are mediated via non-specific mechanisms.

Acknowledgement

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