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EFFECTS OF AQUEOUS KOLA NUT (*COLA NITIDA*) EXTRACTS ON THE CONTRACTION OF ISOLATED RABBIT ILEUM

[1]Alagwu E.A, [2]Osim E.E, [3]Nwozor C.M and [2]Edom O.T
[1]Department of Physiology, Imo State University, Owerri, Imo State, Nigeria.
[2]Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.
[3]Department of Physiology, Anambra State University, Uli, Anambra State, Nigeria.
E-mail: corneliusnwozor@gmail.com

Abstract: Kola nut (*Cola nitida*) is a an edible fruit employed over a century ago by folk medicine for the treatment of various human ailments such as digestive disorder, migraine headache, hyperkinetic disorders in children, heart failure etc. Its effect on the intestinal motility of isolated rabbit ileum (3 - 4cm) was studied alongside other known adrenergic agonist (adrenaline) and blockers (phentolamine and propranolol) at different concentrations made from the stock solution (2g/10ml) and administered in incremental doses of 0.1ml, 0.2ml, 0.4ml, 0.6ml, corresponding to the following concentrations 2mg/ml, 4mg/ml, 8mg/ml and 12mg/ml using Magnus method (1904) as modified by Osim (2002). Adrenaline (AD) was administered at different concentrations and motility of the isolated rabbit ileum recorded. Effects of phentolamine and propranolol each on the water extract and adrenaline actions on the isolated rabbit ileum were also observed and recorded. Results showed that water extract of *Cola nitida* demonstrated dose-dependent decrease in contraction that was statistically significant ($p<0.05$). Adrenaline (AD) also showed dose-dependent decrease in contraction ($p<0.05$) on the isolated rabbit ileum. It was also observed that phentolamine and propranolol drugs both reduced the inhibitory effects due to adrenaline. The reductions were statistically significant ($p<0.05$). With water extract of kola nut, both drugs (phentolamine and propranolol) did not reduce the inhibitory effect on the isolated rabbit ileum due to water extract ($p>0.05$). It is therefore concluded that water extract of kola nut decreased intestinal motility on the rabbit ileum. This decrease was dose-dependent and did not use adrenergic receptor pathway to effect its action as adrenergic blockers (phentolamine and propranolol) did not reduce the effect of water extract when used together.

Keywords: Kola Nut, Intestinal Motility, Rabbit Ileum.

INTRODUCTION

Kola nut is a popular masticatory in Nigeria and other West African countries (Russel, 1955; Umoren *et al.,* 2009). It is a member of the tropical family sterculiaceae and it grows as a tree form. Kola is mostly produced in Africa and is cultivated to a large extent in Nigeria, Ghana, Ivory Coast, Brazil and West Indies (Eijnatten, 1973; Oludemokum, 1983; Opeke, 1982). It played important role in the traditional medicine over a century but suffered set back in modern medicine due to lack of systematic scientific study. Its compositions have been reported which include: water, cellulose, sugar, ash, theobromine, caffeine, fat, protein, polyphenols, tannins (Somorin, 1973; Jayeola, 2001).

Cola nitida has been found to be useful in the production of beverages, flavouring material, alkaloids, caffeine, laxatives, heart stimulants and sedatives (Oluyole, 2009). Students chew it to help keep awake and withstand fatigue (Purgesleve, 1977). Some of the effects of *C. nitida* on the reproductive system have been documented. Some authors reported that consumption of *C. nitida* had little or no effect on semen quality (Adisa *et al.,* 2010; Oldereid *et al.,* 1992). However others showed that caffeine impaired semen quality (Barkay *et al.,* 1977; Harrison 1978; Aitken *et al.,* 1983; Hammit *et al.,* 1989).

It has been found that most of the Physiological actions of *C. nitida* are due to caffeine (Eijnatten, 1973). Caffeine is a bronchodilator that increases minute ventilation in patients with chronic obstructive lung disease and has been utilized as a respiratory stimulant in neonates with recurrent apneic episodes (Murat *et al.,* 1981; Gong *et al.,* 1986). Caffeine stimulates gastric secretion of acid and pepsin (Gerber *et al.,* 1985). Lipid metabolism may be affected by caffeine indirectly through an effect of increased circulating catecholamines stimulating lipolysis and release of free fatty acids (Williams *et al.,* 1985). Caffeine is known to be a fat burner and is beneficial in assisting weight loss (Blades, 2000).

This study is aimed at evaluating the effect of this nut on the intestinal motility of rabbit ileum.

MATERIALS AND METHODS

Materials used in this experiment included: kymograph, drum and paper, organ bath apparatus, tyrode solution, dissecting board, scissors, forceps, suture, needle, retort stand and clamps, syringes (1ml and 10ml), measuring cylinders (250ml and 100ml), sample bottles (5ml), aerator, phentolamine (Sigma, UK), propranolol (Sigma, UK), adrenaline (Yanzhou Xierkangtai Pharmaceutical Factory, Jinguan Bei, China).

Experimental Animals

Four New Zealand white rabbits obtained from the University firm, housed in the department of Physiology animal house, acclimatized for two weeks and fed with rabbit feeds/pellets. They were allowed access to water *ad libitum*, starved 18hours prior to the experiment.

Kola Nut and Water Extract

The fresh kola nuts were purchased from a local market. It was identified as *Cola nitida* by Frank Apejoye, a botanist from the Botany Department of the University. Water extract was prepared by slicing the kola nuts after thorough washing with clean water and drying the slices in the sun for two days and later dried in the oven in the department (Astel Hearson. No 950IRC) at 40^{o}C for three days. The dried slices were ground and weighed with an electronic scale (250g). The ground material was mixed with three liters of distilled water and kept overnight for 18hours. It was thereafter filtered using white satin first and finally with filter paper. The filterate was dried in oven into powdery form. The weight of the dried extract was 28g, the percentage yield was 11.2% calculated as,

Weight of powdery kola nut = 250g.
Weight of dried extract = 28g.

$$Percentage\ yield = \frac{28}{250} \times \frac{100}{1} = 11.2\%$$

Methods

Magnus method (1904) as modified by Osim (2002) was used for the experiment. Animals were killed by cervical dislocation, midline incision made quickly via the linea alba to expose the intestine. The proximal and distal ends of the exposed intestine were identified, cut and immersed in tyrode solution bubbled with oxygen prior to the experiment. The cut segments (3-5cm) of the isolated ileum were mounted vertically in 10ml organ bath containing pre-bubbled tyrode solution. One end tied to a fix support in the organ bath while the other end was attached to a writing lever held in place with plasticine, a resting tension of 1g maintained throughout the experiment with temperature of 37^{0}C monitored through a thermometer. The

kymograph drum wrapped with paper, was set to rotate at a velocity of 0.01 x 5mm/minute where contractions of the tissues were recorded. 0.2ml of each serial dilution was administered (serial dilutions were made from stock solution of 2g/10ml, by drawing up 0.1ml of stock solution and then making it up to 1ml with deionised water, each preceding dilution serving as a stock solution for the next dilution). Tyrode solution was usually washed off after each administration allowing 3–5 minutes for the tissue to recover before the succeeding administration. The tissue response was recorded in incremental doses of 0.2 units for 5 minutes, allowing a minute between each addition. Response to extract in combination with other standard drugs was also recorded. Drugs used were phentolamine, propranolol and adrenaline.

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). A computer package, SPSS Plus and Excel analyzer were used for the computation. P-value less than 0.05 were considered significant.

RESULTS

Graded concentrations of water extract of *Cola nitida* produced dose-dependent decreases in contraction of intestinal smooth muscle in the rabbit ileum. The final bath concentration (FBC) ranged from 2g/ml to 12mg/ml of the *C. nitida* extract which produced percentage relaxation of the maximum dose ranging from 69.5–100%. The relaxations were statistically significant when compared with the control (initial height) Table 1.

Table 2 shows the effect of first dose (2mg/ml) of the water extract of *C. nitida* (4 trials) on intestinal motility with 5.00±0.00 mean decrease in contraction. Table 3 shows the effect of 4mg/ml of water extract of *C. nitida* (4 trials) on the intestinal motility with 6.00±0.00 mean decrease in contraction. Tables 4 and 5 show the effects of 8mg/ml and 12mg/ml of water extracts of *C. nitida* on the motility of the small intestine with 6.5±0.5mm and 7.75±0.25mm decreases in contractions respectively. Tables 7, 8, 9, 10 show graded concentrations of adrenaline (tried 4 times) on the intestinal motility of rabbit with 5.00±0.00, 6.00±0.00, 7.00±0.00, and 12.00±0.00 decreases in contractions respectively.

Phentolamine did not have any significant effect on water extract activity on the rabbit ileum ($p>0.05$). However, it significantly affected adrenaline inhibitory action on the rabbit ileum ($p<0.001$) (fig.1). Propranolol did not cause any change in effect when compared with water extract of *C. nitida.* But, when compared with adrenaline, propranolol significantly caused a decrease in relaxation ($p<0.05$) (fig.2).

Table 1: Effect of Graded Concentrations of Extract *(C. nitida)* on Intestinal Motility

Extract Concentration (mg/ml)	Extract Volume (ml)	Log FBC Concentration of Extract	Decrease in Height (Concentration)	% Max. Decrease
20	0.1	0.30	5.00 ± 0.00	69.89
40	0.2	0.60	6.00 ± 0.00	83.87
80	0.4	0.90	6.50 ± 0.50	89.25
120	0.6	1.08	7.75 ± 0.25	100

FBC: Final Bath Concentration

Table 2: Effect of First Dose (2mg/ml) of the Water Extract of *C. nitida* on the Intestinal Motility

No. of Trials	Extract Concentration (mg/ml)	Extract Volume (ml)	FBC of Extract (mg/ml)	Log Concentration (mg/ml)	Initial Height (mm)	Decrease Height (ht) (mm)
1	200	0.1	2	0.30	4	5
2	200	0.1	2	0.30	4	5
3	200	0.1	2	0.30	3	5
4	200	0.1	2	0.30	4	5
Mean	200	0.1	2	0.30	3.75	5
SEM	0	0	0	0	0.25	0

Table 3: Effect (Using 4mg/ml) of Water Extract of *C. nitida*, Four Trials on the Intestinal Motility

No of Trials	Extract Concentration (mg/ml)	Extract Volume (ml)	FBC of Extract (mg/ml)	Log Concentration (mg/ml)	Initial Height (mm)	Decrease in Height (mm)
1	200	0.2	4	0.60	4	6
2	200	0.2	4	0.60	4	6
3	200	0.2	4	0.60	3	6
4	200	0.2	4	0.60	4	6
Mean	200	0.2	4	0.60	3.75	6
SEM	0	0	0	0	0.25	0

Table 4: Effect (Using 8mg/ml), Four Trials on the Motility of the Small Intestine

No. of Trials	Extract Concentration (mg/ml)	Extract Volume (ml)	FBC of Extract (mg/ml)	Log Concentration (mg/ml)	Initial Height (mm)	Decrease in Height (mm)
1	200	0.4	8	0.90	4	7
2	200	0.4	8	0.90	4	7
3	200	0.4	8	0.90	3	5
4	200	0.4	8	0.90	4	7
Mean	200	0.4	8	0.90	3.75	6.5
SEM	0	0	0	0	0.25	0.5

Table 5: Effect (Using 12mg/ml), Four Trials on the Motility of the Ileum

No. of Trials	Extract Concentration (mg/ml)	Extract Volume (ml)	FBC of Extract (mg/ml)	Log Concentration (mg/ml)	Initial Height (mm)	Decrease in Height (mm)
1	200	0.6	12	1.08	4	8
2	200	0.6	12	1.08	4	8
3	200	0.6	12	1.08	3	7
4	200	0.6	12	1.08	4	8
Mean	200	0.6	12	1.08	3.75	7.75
SEM	0	0	0	0	0.25	0.25

Table 6: Effect of Graded Concentrations ($2x10^{-8}$ to $2x10^{-5}$mg/ml) of Adrenaline on Intestinal Motility

Adrenaline Concentration	Volume of Adrenaline (ml)	FBC	Decrease in Height of Contraction	% Max. Decrease
10^{-6}	0.2	$2x10^{-8}$	5.0 ± 0.00	41.67
10^{-5}	0.2	$2x10^{-7}$	6.0 ± 0.00	50.00
10^{-4}	0.2	$2x10^{-6}$	7.50 ± 0.00	58.33
10^{-3}	0.2	$2x10^{-5}$	12.00 ± 0.00	100

Table 7: Shows the Effect of the First Dose ($2x10^{-8}$mg/ml), Tried Four Times on the Motility of the Small Intestine of the Rabbit

No. of Trials with 10^{-6}	Initial Height of Contraction (mm)	Height After Adrenaline (mm)	Decrease in Contraction (mm)	% Decrease
1	9	4	5	55.56
2	9	4	5	55.56
3	10	5	5	50.00
4	9	4	5	55.56
Mean	9.25	4.25	5	54.17
SEM	0.25	0.25	0	1.39

Table 8: Effect of Second Dose of Adrenaline $2x10^{-7}$ mg/ml, Tried Four Times on the Motility of the Small Intestine of the Rabbit

No. of Trials with 10^{-5}	Initial Height of Contraction (mm)	Height After Adrenaline Admin. (mm)	Decrease in Contraction (mm)	% Decrease
1	9	3	6	66.67
2	9	3	6	66.67
3	10	4	6	60
4	9	3	6	66.67
Mean	9.25	3.25	6	65
SEM	0.25	0.25	0	1.67

Table 9: Shows Mean Decrease in Contraction of 7.00±0.00 After Four Trials with $2x10^{-6}$ mg/ml of Adrenaline

No. of Trials	Initial Height of Contraction (mm).	Height After Adrenaline (mm)	Decrease in Contraction (mm)	% Decrease
1	9	2	7	77.78
2	9	2	7	77.78
3	10	2	7	70
4	9	3	7	77.78
Mean	9.25	2.25	7	75.83
SEM	0.25	0.25	0	1.94

Table 10: Effect of Adrenaline on Intestinal Motility at $2x10^{-5}$mg/ml

No. of Trials	Initial Height of Contraction (mm).	Height After Adrenaline (mm)	Decrease in Contraction (mm)	% Decrease
1	9	-3	12	133.33
2	9	-3	12	133.33
3	10	-2	12	120.
4	9	-3	12	133.33
Mean	9.25	-2.75	12	130.
SEM	0.25	0.25	0	3.33

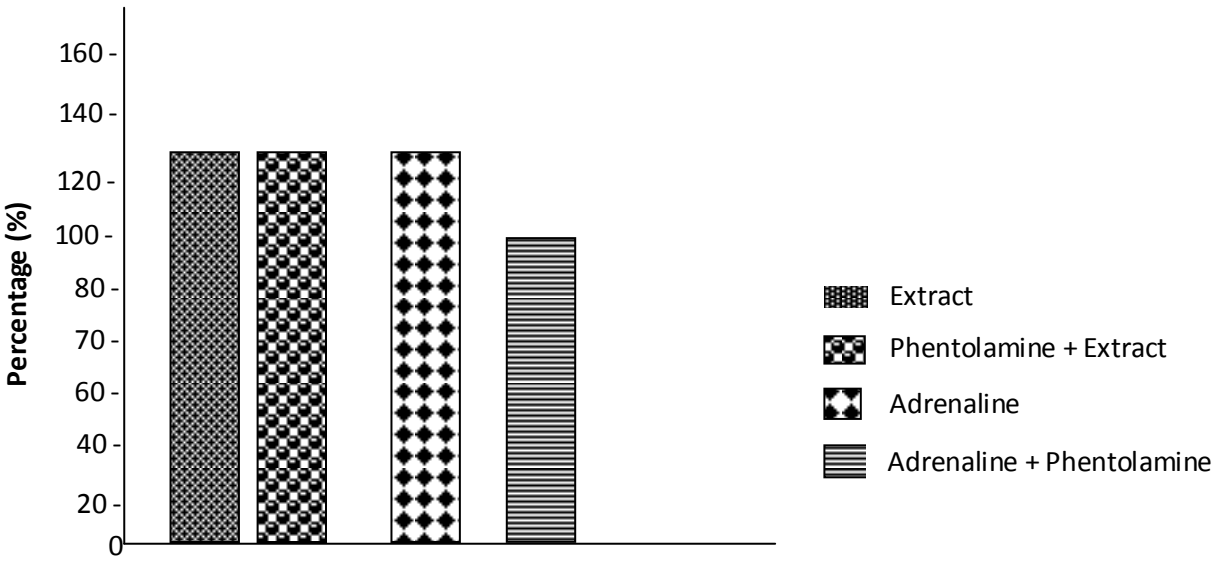

Fig.1: Effect of Phentolamine on Extract and Adrenaline on Intestinal Motility in Rabbit

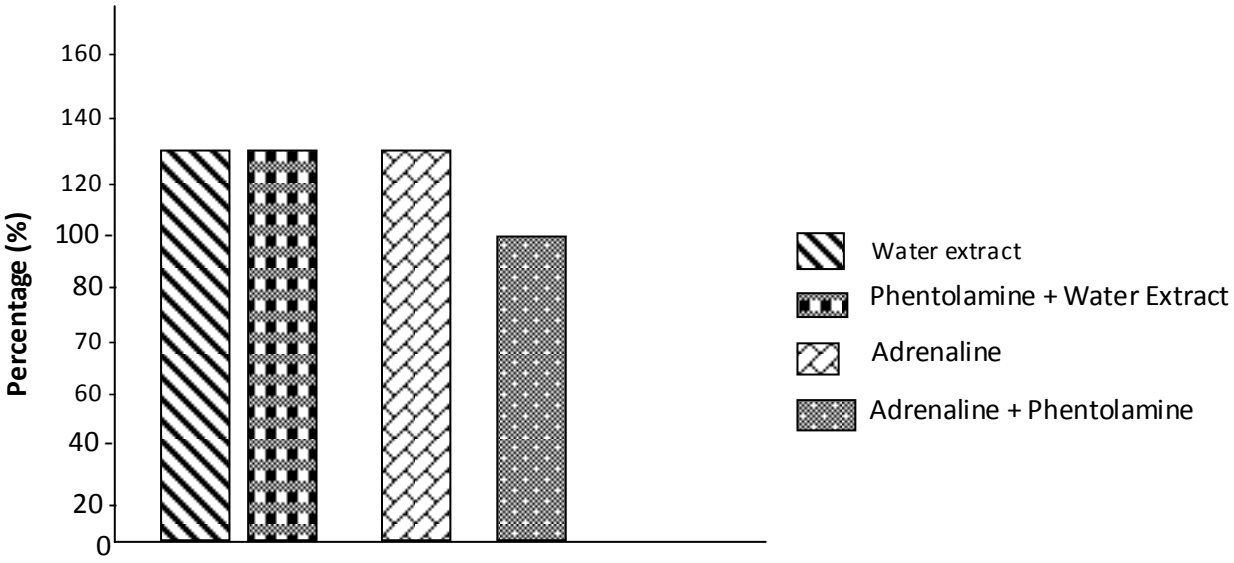

Fig 2: Effect of Propranolol on Water Extract and Adrenaline on Intestinal Motility in Rabbit

DISCUSSION

The effects of water extract of kola nut and adrenaline were studied on the intestinal motility of isolated rabbit ileum. The results obtained showed that the water extract and adrenaline drug decreased the contraction of rabbit ileum in dose-dependent manner. Increasing the concentrations of both water extract and adrenaline produced greater decrease in intestinal contraction ($p<0.05$) of the rabbit ileum.

When phentolamine (alpha-blocker) and propranolol (beta-blocker) each was administered with water extract and adrenaline, the results obtained showed that the decreased contractions were statistically significant when compared with the initial contraction. On the water extract of

kola nut, the decrease in contraction caused by water extract was not affected by the administration of phentolamine and propranolol ($p>0.05$). The mechanism by which phentolamine and propranolol countered the decrease in contraction caused by adrenaline could be explained as phentolamine and propranolol are adrenergic receptor blockers. Adrenaline acts through adrenergic receptors and when the receptors are blocked by these drugs, little sites are left for adrenaline to manifest its action. The reduction of adrenaline action is as a result of the blockage of the receptor sites by these drugs (phentolamine and propranolol).

On the water extract, it is likely that the extract's activity is not dependent on adrenergic receptors. It appeared that the water extract of kola nut has no action on the gastrointestinal smooth muscles adrenergic receptors like adrenaline. However other mechanisms may be involved which were not investigated in the course of this research.

CONCLUSION

It is therefore concluded that the water extract of kola nut caused significant decrease in intestinal contraction of rabbit ileum and this action does not utilize adrenergic receptor pathway. If this result is applicable to man, consumption of much quantity of kola nut may result in constipation. Its moderate intake may be beneficial in treatment of intestinal hurry.

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