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Research Article

Effect of Atropine or Ranitidine on the Action of Aqueous *Garcinia kola* Seed Extract on Gastric Acid Secretion in Rats

[1]Anowi C. F., [2]Ononiwu I. M., [3]Utoh-Nedosa U. A.

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

[2]Department of Pharmacology, Faculty of Medicine, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

[3]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Abstract

A study found that crude extracts of *Garcinia kola* (guttiferae) seed and *Cola acuminata* (Sterculiaceae) produced dose dependent increases in gastric acid secretion in urethane-anesthetized rats. The present study evaluated the effect of aqueous *Garcinia kola* seed extract on gastric acid secretion in urethane-anesthetized rats and repeated the experiment in the presence of atropine (an acetylcholine muscarinic receptor antagonist) or ranitidine histamine H_2 receptor antagonist). The stomach of randomly selected urethane-anesthetized male and female albino rats weighing 150-220g (N= 5) were flushed with 13mg/kg; 26mg/kg; 40mg/kg; 53mg/kg or 67mg/kg aqueous *Garcinia kola* seed extract (which were equivalents of 2; 4; 6; 8 and 10mg/ml aqueous *Garcinia kola* seed extract. The stomach of control group rats (N=5) were flushed with 0.9% normal saline. The effluent fluids from the test and control rats were titrated against 0.001M sodium hydroxide to determine their concentrations of HCl in millimoles. A graph of acidity of the gastric effluents in millimoles against the employed doses of aqueous *Garcinia kola* seed extract was plotted. The graph showed that aqueous extract of *Garcinia kola* seed dose-dependently inhibited increases in gastric acid secretion beyond basal levels in test but not in control rats. Ranitidine and atropine each aqueous *Garcinia kola* dose-dependently and with a similar response curve antagonized gastric acid secretion in test rats and not in controls; the inhibition by atropine being greater than that of ranitidine. These results showed that it was not "muscarinic" or histamine "H_2" receptors but serotonin receptors that were involved in inhibition of gastric acid secretion in the rats. Each of aqueous *Garcinia kola* extract, atropine and ranitidine produced its effects by stimulating "β"-subunits and inhibiting "α"-subunits of serotonin receptor and atropine's production of this effect was greater than that of ranitidine.

Keywords:

INTRODUCTION

Garcinia kola seeds are eaten raw or employed in traditional medicine mixtures in West and Central Africa because of the medicinal qualities of their largely bitter and sometimes astringent liquid extract. In traditional African medicine, *Garcinia kola* plant extracts are used for the treatment of hoarseness of voice, cough, sore throat and respiratory tract inflammation; dysentery and diarrhoea; emesis; diabetes; post partum haemorrhage; cuts; parasitic skin diseases, Guinea worm infestation; liver

cirrhosis and as a snake repellent, bitter tonic / astringent tonic. These medicinal effects of *Garcinia kola* plant extracts have been authenticated in many animal studies[3, 4, 7, 8, 9, 11].

Since basal and histamine-stimulated gastric acid secretions were significantly ($P<0.01$), elevated in all groups of rats treated (fed) continuously for two weeks with low, medium and high dose *Garcina conrauana* (*cola*)-supplemented rat chow[5] and Intrperitoneal injection of the crude extracts of *Garcinia kola seed* and *kola acuminata seed* extracts each produced dose-dependent increases in gastric acid secretion in rats with the activity of *Garcinia kola* extract being greater than those of *Cola* acuminata[6], the present study investigated the potentials of *Garcinia kola* seed extract to stimulate gastric acid secretion in rats when infused alone into the stomach of albino rats and when similarly infused in the presence of the muscarinic anti-cholinergic agent, atropine or in the presence of the histamine H_2 receptor antagonist, ranitidine.

MATERIALS AND METHODS

Fifty three randomly selected previously acclamatized male and female albino rats weighing 150-220g were anesthetized with intramuscularly administered 0.6mg/kg urethane made by dissolving urethane crystals in normal saline. The point of achievement of anesthesia in the rats was taken as the point when the rats lost righting reflex. The test rats were put into five major groups each containing five test and 5 control rats in each sub-group.

The rats were incised midsection at the throat and the trachea shunted with a flexible tube to maintain respiration. A tube was inserted into the oesophagus at a point proximal to the cardiac sphincter of the stomach and another inserted just after the pyloric sphincter. An infusion tube was then connected to the oesophageal tube and its rate of discharge set at 15-20 drops per minute.

Two percent stock solution of the aqueous *Garcinia kola* seed extract (10g in 100ml) was made by dissolving it first in 5ml of dimethylsulphoxide (DMSO) and making up the volume with distilled water.

Normal saline was run into each animal for 20 seconds before the experiment to ensure that the tubing system was not blocked.

One of the test doses of *Garcinia kola* extract (13mg/kg, 26mg/kg, 40mg/kg, 53mg/kg and 67mg/kg) was infused alone into the stomach of each of a group of 5 rats at the rate of 15-20 drops per minute. The control animals were infused with 0.9% normal saline.

The same test dose of aqueous *Garcinia kola* seed extract was infused into the same group of rats after the rats had been given intra-peritoneal 0.02mg/kg atropine or intra-peritoneal ranitidine of 0. 15mg/kg. The control group animals received atropine and ranitidine in the same doses as their test group but were flushed with 0.9% normal saline.

The gastric contents flushed out (the effluent fluids) at the end of administration of each dose of aqueous *Garcinia kola* extract to test rats (and 0.9% normal saline to controls) were titrated against 0.001M sodium hydroxide in the presence of phenolphthalein as indicator.

The concentrations of HCl in millimoles secreted in the stomach of the test rats during the experiments were calculated using the equation:

$$C_1 V_1 = C_2 V_2$$

C_1 = Concentration of acid in effluent
C_2 = Concentration of Base
V_1 = Volume of acid
V_2 = Volume of base

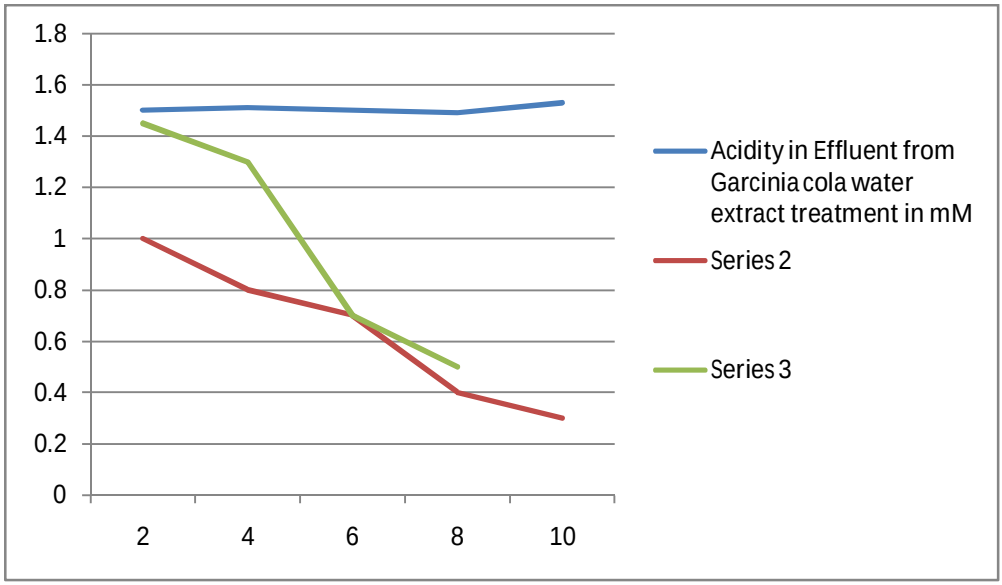
Graphs of the different dilutions of aqueous *Garcinia kola* extract employed in the study in mg /ml were plotted against the concentrations of secreted acid in the effluent fluids.

RESULTS

Treatment of rats with 2mg/mi, 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml of aqueous *Garcinia kola* seed extract did not produce any appreciable change in acidity of the gastric contents of the treated rats (from 1.5mM to 1.53mM) [figure 1).

Both atropine and ranitidine produced antagonism of basal gastric acid secretion which increased with increase in the dose of the aqueous *Garcinia kola* seed extract. Atropine and ranitinine produced the same degree of antagonism of intrinsic gastric acid secretion in the presence of 6mg/ml (40mg/kg) aqueous *Garcinia kola* seed extract (figure 1).

Atropine generally produced a stronger antagonism of the basal gastric acid secretion in the test rats than ranitidine. Atropine caused the acidity in the stomach effluents of the test rats which was at 1.5mM in the presence of 2mg/ml of the extract to reduce to 1mM (while ranitidine reduced it to 1.45) and the acidity of the effluents which was at 1.53mM in the presence of 10mg/ml of the extract to drastically to the value of 0.3mM (while ranitidine reduced it to 0.04mM) [figure 1]. The antagonism of gastric acid secretion in the test rats by atropine increased as the dose of the aqueous *Garcinia kola* extract increased. The steepest gradient of the slope of inhibition of gastric acid secretion was produced by atropine in the presence of 4mg/ml and 6mg/ml doses of *Garcinia kola* extract (figure 1).



→ CONCENTRATION OF AQUEOUS GARCINIA COLA SEED EXTRACT IN Mg/Ml

↑ Series 1: Acidity in Effluents of *Garcinia kola* seed Extract Treatments (on its Own) in Millimoles (mM).
Series 2 Acidity in Effluents of *Garcinia* kola seed Extract Treatments in Millimoles (mM) in the Presence of Atropine.
Series 3: Acidity in Effluents of *Garcinia kola* seed Extract Treatments in Millimoles (mM) in the Presence of Ranitidine.

Figure 1: Graph of Dose-Response Relationship of Aqueous *Garcinia kola* seed Extract and Gastric Acid Secretion.

The graph of "Series 1" shows that water extract of *Garcinia kola* seed does not *stimulate* but rather dose-dependently inhibits increases in gastric acid secretion beyond normal levels. The inhibition of gastric acid secretion by atropine (Series 2 graph) and ranitidine (Series 3 graph) each increased with increasing doses of aqueous *Garcinia kola* extract which demonstrated a synergistic inhibition of gastric acid secretion by aqueous *Garcinia kola* extract and atropine or ranitidine. This result confirms the observation that *Garcinia* kola water extract had a dose-dependent inhibitory action on increases in gastric acid secretion beyond normal levels, in the rats.

DISCUSSIONS

Estimation of the secondary metabolites in *Garcinia kola* seed showed the presence of tannin (0.69±0.01; Saponnin 15.79±0.28; oxalate 1.707±0.13; cryogenic glycosides 59.56±0.05 and cardiac glycosides 67.10±0.03mg/ 100g dry matter[7].Other phytochemical studies showed the abundance of flavonoids in *Garcinia kola* seed extracts and their involvement in antispasmodic and organ protective activities[2, 3, 9, 10] Administration of 100mg/kg *Garcinia kola* seed extract 3 times a day for 5 consecutive days, reduced 800, 1000, 1200mg/kg paracetamol-induced lethality in rats from 50, 90 and 100% respectively to 0, 20 and 40% respectively[8]. Intraperitoneally-administered 100mg/kg kolaviron, a mixture of C-3/C-8 biflavonoids from *Garcinia kola* seed extract reduced the fasting blood sugar of normoglycaemic rats from 115mg/100ml to 65/100ml after 4 hours and reduced the blood sugar of alloxan-induced diabetic rats from 506mg/100ml to 285mg/ml at 12 hours[9]. Kolaviron also inhibited rat lens aldose reductase (RLAR) activity with an IC_{50} value of 5.4 x 10^{-6} [9]. Flavonoids and coumarin derivatives have been shown to possess spasmolytic, diuretic and anti-ulcer activity[10].

The results of the present study showed that aqueous extract of *Garcinia* kola seed had no effect on basal or normal gastric acid secretion in the stomach of the test rats and actually produced synergistic inhibition of gastric acid secretion with atropine and ranitidine. No effect on gastric acid secretion was observed in the control groups that received 0.9% normal saline in the absence or presence of atropine or ranitidine. The inhibitory effect of aqueous *Garcinia kola* seed extract is in line with inhibitory effects it demonstrated in its anti-diabetic effect[9]; in its antagonism of paracetamol-induced lethality in rats[8] and in its inhibition of rat lens aldose reductase activity[9].

Since ranitidine and atropine each independently antagonized gastric acid secretion in test rats (and not in controls) and their antagonism of gastric acid secretion increased with increase in the dosage of aqueous *Garcinia kola* seed extract in a similar pattern, this is regarded as a demonstration of the same mechanism of action by ranitidine and atropine in inhibition of gastric acid secretion in the rats. Since neither atropine nor ranitidine exclusively abolished gastric acid secretion in the test rats,

neither acetylcholine muscarinic nor histamine H_2 receptors was exclusively involved in inhibition of gastric acid secretion in the rats. We suggest that Serotonin receptors were used to by atropine and ranitidine to produce their inhibition of gastric acid secretion and that the results showed that the acetylcholine "muscarinic" and Histamine "H_2" receptor are located at the same subunits of the serotonin receptor given the similarity of the curves produced by atropine and ranitidine in this study. We suggest that each of aqueous *Garcinia kola* extract, atropine and ranitidine produced its effects by stimulating "β"-subunits and inhibiting "α" subunits of serotonin receptors of the gastric acid secretary cells of rat stomach. The inhibitory effects of atropine on the serotonin receptors of rat gastric secretary cells were greater than those of ranitidine. By extension, these results suggest that serotonin is the intrinsic mediator of gastric acid secretion in rat stomach.

Atropine was more efficacious than ranitidine in inhibition of gastric acid secretion in the test rats (reducing the acidity of the gastric effluent produced under the effect of 2mg/ml aqueous *Garcinia kola* seed extract from 1.5mM to 1mM (while ranitidine reduced it to 1-45mM) which demonstrated that an "anti-muscarinic" drug atropine was more efficient in inhibition of gastric acid secretion in rats than a histamine "H_2" receptor antagonist ranitidine.

CONCLUSIONS

From the results of this study it is concluded that *aqueous Garcinia kola* seed extract inhibited increases in gastric acid secretion beyond basal levels on its own and produced a dose dependent marked synergistic inhibition of gastric acid secretion with atropine, a muscarinic antagonist and ranitidine, histamine- (H_2)-receptor antagonist. Partial inhibition of gastric acid secretion by atropine or ranitidine ruled out cholinergic muscarinic and histamine-H_2 receptors and suggested serotonin receptors as the site of inhibition of gastric acid secretion by atropine, ranitidine and aqueous *Garcinia kola* seed extract. Atropine, ranitidine and aqueous *Garcinia kola* seed extract inhibited gastric acid secretion by stimulating β-subunits and inhibiting α-subunits of serotonin receptors of the gastric acid secretary cells of rat stomach.

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