INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)
Platform for Pharmaceutical Researches & Innovative Ideas
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EVALUATION OF ANTI-DIARRHOEAL POTENTIAL OF METHANOL EXTRACT OF Garcinia kola STEM BARK

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ABSTRACT

The effect of methanol extract of *Garcinia kola* stem bark (GSB) on experimentally induced diarrhoea (small intestinal propulsion, castor oil induced diarrhoea and castor oil induced fluid accumulation models) was studied in rats. In the intestinal transit model, the propulsive movement of the small intestine was significantly ($p < 0.001$) inhibited by the extract; the degree of traversal of charcoal meal by the extract (18 - 72 mg/kg) relative to the mean intestinal length translated to 25.60% - 44.70% of inhibition of propulsive movement respectively. In the presence of yohimbine, an α_2-adrenoceptor antagonist, the effect of the extract was antagonized. The extract also caused a significant ($p<0.001$) decrease in the number of faecal matter passed by 43.50 – 81.20% respectively in castor oil induced diarrhoea; when yohimbine was combined with the extract however, there was an increase of 25.90% of faecal matter passed, relative to control. Furthermore, there was a dose-dependent decrease in intestinal fluid accumulation by 40.30 - 50.40%, with yohimbine however, the inhibitory effect of the extract was antagonized, causing increased fluid accumulation by 21.60%. The attenuation of the anti-diarrhoeal effects of the extract in the presence of yohimbine suggests a role for α_2-adrenergic receptor in the antidiarrhoeal effects of the extract. The presence of some phytochemical compounds in the extract that possess antidiarrhoeal properties, such as alkaloids, flavonoids, tannins and terpenes might be contributing to its antidiarrhoeal actions.

Key words: Anti-diarrhoeal, Castor oil, Charcoal meal, Stem bark, Garcinia kola

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INTRODUCTION

Diarrhoeal diseases are the major causes of illness and death all over the world [[1]]. Around 88% of diarrhoeal-related deaths are caused due to inadequate sanitation and poor hygiene. Diarrhoea

is considered as one of the leading causes of growth retardation and death in infants.

Diarrhoea, simply put, is an increase in frequency or decrease in consistency of bowel movements however; the medical definition correlates diarrhoea with an increase in stool weight (a stool weight above 300g/day generally indicates diarrhoea). This is mainly due to excess water, which normally makes up 60 - 85% of faecal matter.

Inflammatory diarrhoeas are generally accompanied by pain, fever, bleeding, or other manifestations of inflammation. The mechanism of diarrhoea may not only be exudation but, depending on lesion site, may include fat malabsorption, disrupted fluid/electrolyte absorption, and hypersecretion or hypermotility from release of cytokines and other inflammatory mediators [[5]]. The death rate due to diarrhoea in developing countries is about 1.5 – 2 million/year among children under five years of age. Despite the availability of several remedies to treat diarrhoea including botanicals and chemical agents, yet there is a great need for the evaluation of newer, economical and cost-effective agents to meet the challenges of upcoming era regarding disease burden. The use of traditional remedies in healthcare system is increasing day by day. It is well accepted that herbal remedies are relatively safe, affordable and easily accessible to layman, when compared with that of chemical drugs. Moreover, the plant remedies or naturally sourced products are known to contain synergistic and/or side effects neutralizing potentials, and usually offer their pharmacological actions mediated through multiple pathways.

OBJECTIVE

As mentioned earlier, diarrhoea has an inflammatory mechanism, therefore a highly valued medicinal plant that has proven analgesic and anti-inflammatory property such as *Garcinia kola* [[10]] may be of use in the treatment of diarrhoea. The objective of this study is to assess the methanol extract of *Garcinia kola* stem bark (GSB) for anti-diarrhoeal properties using standard experimental protocols.

MATERIALS AND METHODS

Effect of extract on rat small intestinal propulsion

The rat small intestinal propulsion method [[11, 12]] was adopted in this study to assess small intestinal transit. Adult rats were randomized into 6 groups of 6 animals each and fasted for 24h; they were allowed access to water *ad libitum*. Group 1 was given 10ml/kg normal saline orally (p. o.) through an oro-gastric tube. Groups 2 – 4 were pre-treated with 18, 36 and 72mg/kg; (p. o.) respectively of the methanol extract of GSB. Group 5 received yohimbine (1 mg/kg; s. c.) and 10 min later 72mg/kg; (p. o.) of extract was given, while group 6 received only diphenoxylate (5 mg/kg; p. o.). After 1h each was given 1ml charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth) orally. 30 min later the rats were anaesthetized with light ether and sacrificed by cervical dislocation and bled. The small intestine was ligated at both pyloric sphincter and the ileocaecal junctions and the small intestine rapidly dissected out and placed on a clean surface. The intestine was carefully inspected and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured.

The peristaltic index and percentage inhibition were calculated with the formulae below:

$$\text{Peristaltic index} = \frac{\text{Distance moved by the suspended charcoal head}}{\text{Whole Length of Small Intestine}}$$

$$\text{Percentage Inhibition} = \frac{A - B}{A} \times 100$$

Where:

A = Distance moved by the charcoal head (cm) in the control group.

B = Distance moved by the charcoal head (cm) in the treated group.

Effect of extract on castor oil-induced diarrhoea in rats

In this experiment, diarrhoea was induced with castor oil [[13, 11]]. Rats of both sexes were used. The rats were fasted for 24h but allowed free access to

water. They were randomized and placed in cages of 6 rats per cage. Group 1 was administered with normal saline (10ml/kg). Groups 2 – 4 were given 18, 36 and 72mg/kg; (p.o.) of GSB extract respectively, group 5 was administered with yohimbine (1mg/kg; s.c.), then 10min later 72mg/kg; (p.o.) of the extract was given while group 6 was given 5mg/kg; (p.o.) diphenoxylate. After 1h, all the rats received 2ml castor oil orally and were observed for consistency of faecal matter and the frequency of defecation for 5h

Effect of extract on castor oil-induced intestinal fluid accumulation in rats.

The procedure of castor oil-induced enteropooling [14, 11] was adopted in this study.

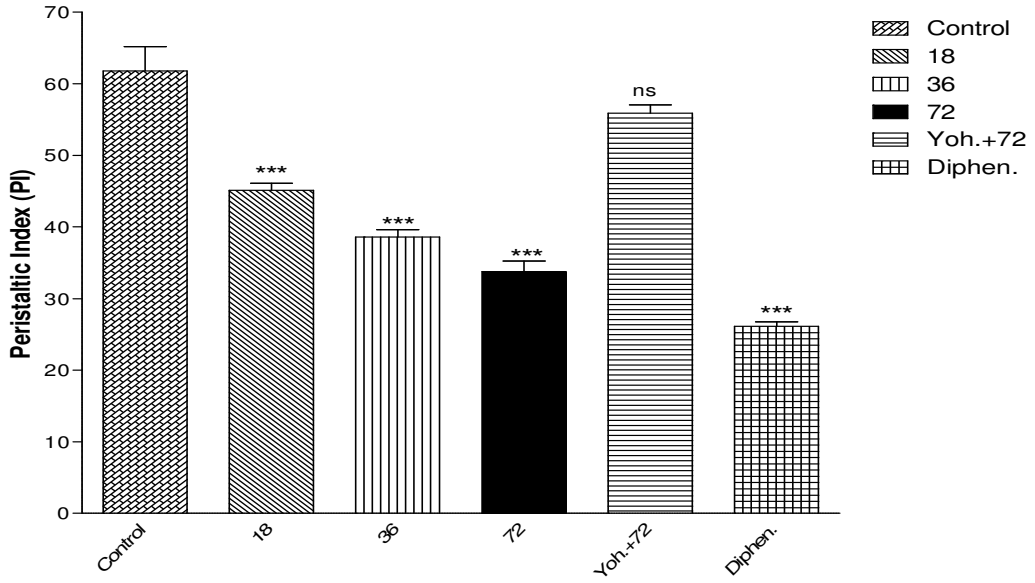
Rats were randomized into 6 groups of 6 rats each and fasted for 24h but allowed access to water *ad libitum*. Group 1 received castor oil (2ml/rat), groups 2 – 4 were treated with GSB extract (18, 36 and 72mg/kg; p.o. respectively), group 5 was administered with yohimbine (1mg/kg s.c.), then 10min later 72mg/kg; (p.o.) of the extract was given, while group 6 received only diphenoxylate (5mg/kg; p.o.). After 1 h, each rat in groups 2 – 6 was given 2 ml of castor oil (p. o.). 30 min later, the rats were anaesthetized with light ether and sacrificed by cervical dislocation and exsanguinated; the small intestine was ligated at both pyloric sphincter and the ileo-caecal junctions. The entire small intestine was dissected out and its contents expelled into a graduated measuring cylinder and the volume recorded.

RESULT

Effect of extract on rat small intestinal propulsion

The result from this experiment is as shown in Fig. 1. The effect of extract was investigated on intestinal propulsive movement in rats. The extract (18 - 72mg/kg) caused the charcoal meal to traverse 38.33±1.39 - 28.5±2.40cm respectively, relative to the mean intestinal length (85.03±2.90 – 84.32±1.96cm). This degree of traversal translated to 25.60% - 44.70% of inhibition respectively. Similarly, diphenoxylate, an opioid derivative, caused 52.2% transit inhibition. In the presence of yohimbine, an α_2-adrenoceptor antagonist, the effect of the extract was antagonized.

Fig. 1: Effect of extract on intestinal propulsion in rats



Values represent Mean ± SEM (n = 6)

Significance relative to control: $^{ns=}$Not Significant; *** p<0.001

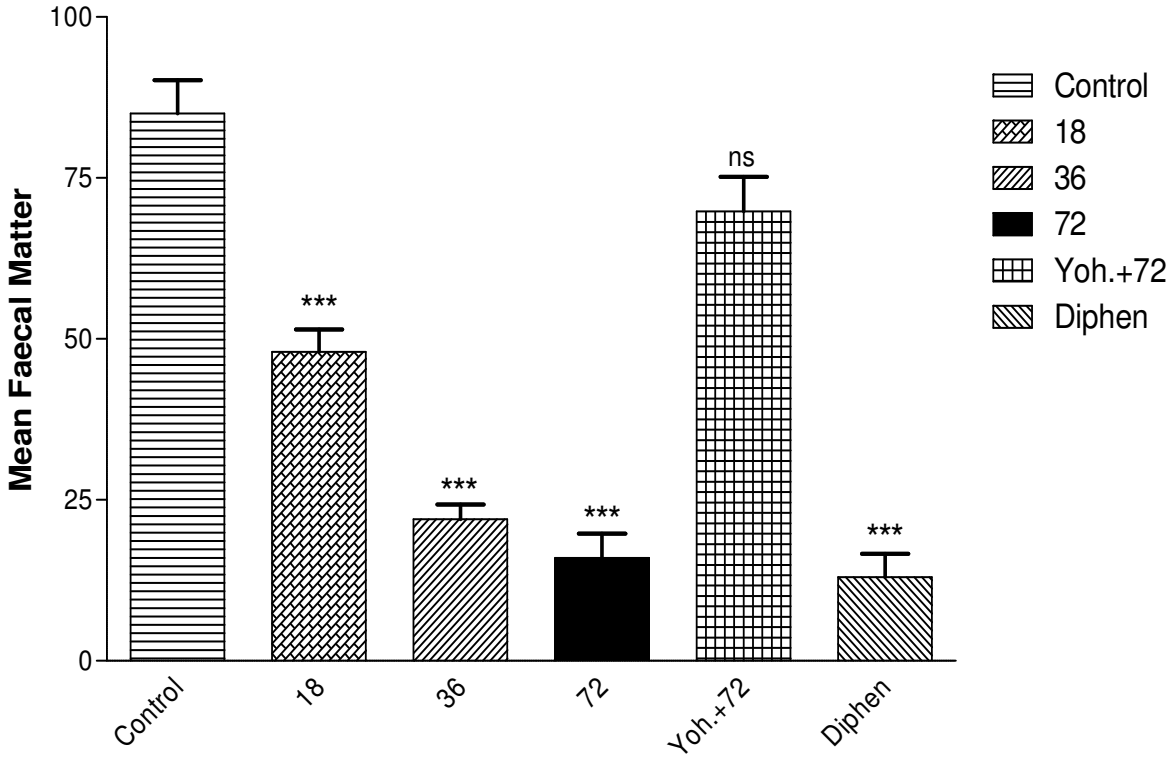
Yoh. = Yohimbine

Diphen. = Diphenoxylate

Effect of extract on castor oil-induced diarrhoea in rats

The effect of the extract was studied on castor-oil induced diarrhoea in rats, and the result is as shown in Fig. 2. The extract (18 – 72mg/kg) significantly ($p<0.001$) decreased the number of faecal matter passed by 43.50 – 81.20%. Diphenoxylate caused 84.00% inhibition of castor oil-induced diarrhoea. In the presence of yohimbine, the extract caused an increase of 25.90% faecal matter passed relative to control.

Fig. 2: *Effect of Extract on castor oil-induced diarrhoea in rats*

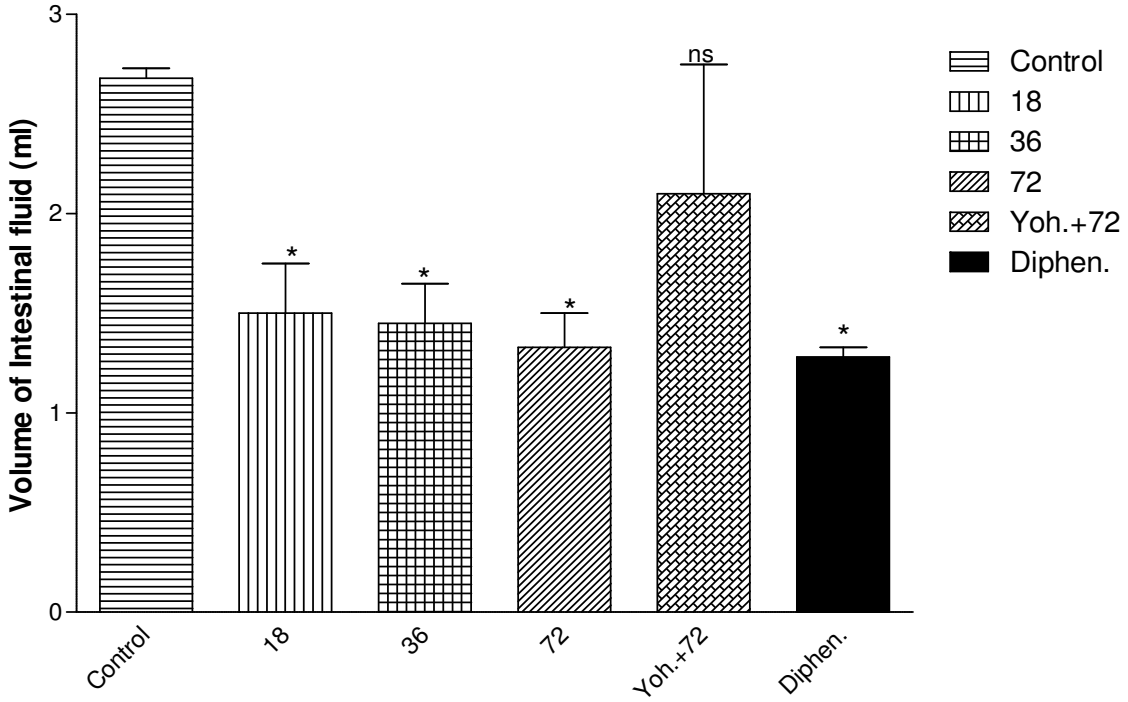


Values represent Mean ± SEM (n = 6)
Significance relative to control: $^{ns=}$Not Significant; *** $p<0.001$
Yoh. = Yohimbine
Diphen. = Diphenoxylate

Effect of extract on castor oil-induced intestinal fluid accumulation in rats

The results obtained from the experiments are as shown in Fig. 3. There was a dose-dependent decrease in intestinal fluid accumulation. The intermediate and high doses (36 and 72mg/kg) respectively, of the extract significantly ($p<0.05$) inhibited intestinal fluid accumulation by 45.90% and 50.40% respectively, while the lowest administered dose (18mg/kg) inhibited intestinal fluid accumulation by 40.30%, however, the inhibition was not statistically significant, relative to control. In the presence of yohimbine, the inhibitory effect of the extract was antagonized; hence, it increased the fluid accumulation by 21.60%.

Fig. 3: Effect of extract on castor oil-induced intestinal fluid accumulation in rats



Values are expressed as Mean ± SEM; (n=6)
Significance relative to control: ns = Not Significant; * = $p < 0.05$.
Yoh. = Yohimbine
Diphen. = Diphenoxylate

DISCUSSION

Diarrhoea is evoked by hyperpropulsive motility of gastrointestinal tract and hypersecretion throughout the intestinal mucosa. It is known that stimulation of the α_2-adrenergic receptor causes transit delay [15, 16].

The extract significantly inhibited small intestinal propulsive movement in rat. Inhibition by the highest dose was however; lower than that of the standard drug, diphenoxylate, a muscarinic blocker. The data suggest that the effect on gastrointestinal propulsive movement is mediated via the α_2-adrenergic receptor, since yohimbine, an α_2-adrenoceptor antagonist inhibited the transit delay induced by the extract.

The use of castor oil for the induction of diarrhoea has been widely studied [17], and it is known that the most active component is ricinoleic acid, which causes changes in electrolytes and water transport and generates enormous contractions in transverse and distal colon [18] thereby producing permeability changes in the intestinal mucosal membranes that result in watery luminal content that flows rapidly through the small and large intestines [19, 20].

The extract showed a dose-related inhibition in all diarrhoeal parameters: onset of diarrhoea, total number of stools, number of wet stools, and measured frequency and severity of diarrhoea.

Drugs affecting motility, frequency, and consistency of diarrhoea also affect secretion [16, 21, 12]. The intraluminal fluid accumulation induced by castor oil was blocked by the extract in a dose-related manner. Involvement of the α_2-adrenergic receptor mechanism was further confirmed by the antagonistic action of yohimbine in the enteropooling test.

Clinically, diarrhoea may result from disturbed bowel function, in which case there is impaired intestinal absorption, excessive intestinal secretion

of water and electrolytes, and rapid bowel transit [[22]]. The antidiarrhoeal index (ADI) is a measure of the combined effects of these different components of diarrhoea such as purging frequency and onset of diarrheal stools, as well as intestinal frequency. The extract produced a dose-dependent antidiarrhoeal index, although its greatest effect was lower than that produced by diphenoxylate. Activation of the sympathetic innervations of the intestines result in the inhibition of peristaltic activity and a reduction in tone. This inhibitory effect is mediated mainly by α_2-adrenergic receptor. Activation of the pre-junctional α_2-adrenergic receptor on the parasympathetic terminals may also play an important role in the inhibitory action of sympathetic nerve stimulation of gastrointestinal motility by inhibiting acetylcholine release [[23]]. The sympathetic nervous system also controls the balance between absorption and secretion in the ileum through activation of the mucosal α_2-adrenergic receptor. Stimulation of these receptors in the ileum results in a decrease in ion fluxes, consistent with the ability of α_2-adrenergic receptor agonists to inhibit intestinal fluid secretion. The extract, in the gastrointestinal propulsive movement and enteropooling tests exhibited effects similar to those of α_2-selective agonists, and the attenuation of these effects in the presence of yohimbine, an α_2-adrenergic receptor antagonist, suggests a role for α_2-adrenergic receptor in the antidiarrhoeal effects of the extract.

Overall, the presence of different phytochemical classes in *G. kola* stem bark such as, alkaloids, flavonoids, tannins, terpenes possessing spasmolytic and antidiarrheal activities [[24, 25, 26, 27, 28, 29,12]], might be contributing towards its antidiarrheal, antisecretory and spasmolytic actions, possibly acting through multiple target sites.

CONCLUSION

This study shows that the crude extract of *Garcinia kola* possesses antidiarrhoeal, antisecretory and antispasmodic effects mediated through dual blockade of muscarinic receptors and Ca^{2+} channels. Thus, this study provides a sound mechanistic support to its medicinal use in hyperactive gastrointestinal disorders like diarrhoea, and is a step forward towards the evidence-based use of phytomedicine.

ACKNOWLEGEMENT

The authors gratefully acknowledge the assistance of Ms. Sifonobong John Akpan of Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo.

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