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In vitro activity of methanol extract of *Citrullus lanatus* Thunb. (Curcubitaceae) fruit rind on isolated gastrointestinal tissues of rabbit and guinea pig

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Abstract

The small intestine undergoes segmental contractions and peristaltic waves causing onward movement of its contents [1]. Stimulation of parasympathetic division of the autonomic nervous system usually causes contractions by releasing acetylcholine, which increases activity of myenteric plexus, or by direct excitatory effect on smooth muscle of the gut [2]. This research evaluates effects of methanol extract of *Citrullus lanatus* (watermelon) fruit rind on isolated gastrointestinal tissues of rabbit and guinea pig. Phytochemical screening was evaluated for presence of chemical constituents. Flavonoids, steroids, alkaloids, carbohydrates, saponins and triterpenes were found to be present while anthraquinones were absent. The oral median lethal dose was established to be safe up to the highest dose of 5000 mg/kg in swiss albino mice. Intrinsic activities of MECL fruit rind on isolated guinea-pig ileum and rabbit jejunum were evaluated. MECL showed biphasic response on isolated gastrointestinal tissues where at lower log concentrations (-1.40×10^{-6} – 1.20×10^{-6} g/ml); spasmolytic effect was recorded on the microdynamometer, while higher log concentrations (1.51×10^{-6} – 1.60×10^{-6} g/ml) produced spasmogenic response. MECL was interacted with acetylcholine ($1x\ 10^{-5}$ mg/ml), histamine ($1x\ 10^{-5}$ mg/ml) and antagonists like atropine ($1x10^{-5}$ mg/ml) and mepyramine. The action of MECL on gastrointestinal tissue may be linked partly to its direct inhibitory effect on cholinergic receptor binding sites, propulsion of gastrointestinal muscle or due to activity of phytoconstituents present. Biphasic effects of MECL on isolated gastrointestinal tissue validates its use traditionally for treating diarrhoea and constipation in Northern Nigeria.

Keywords: Diarrhoea, *Citrullus lanatus* (watermelon) fruit rind, acetylcholine, atropine, biphasic response

INTRODUCTION

Crude drugs obtained from medicinal plants have been used to treat all manner of ailments in most traditional societies. The most important bioactive constituents of medicinal plants are alkaloids, tannins, flavonoids and phenolic compounds [3]. Many herbal plants used in the treatment of diarrhoea are effective in reducing gastrointestinal motility and gastric secretion, with minimal side effects. Several studies have evaluated the effectiveness of some traditional medicines in treating diarrhoea, in different continents [4,5]. However, the effectiveness of many of these anti-diarrhoeal traditional medicines is yet to be scientifically evaluated. Indigenous plants such as *Andrographis paniculata*, *Asparagus*

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racemosus, Butea monosperma, Cassia auriculata and others are widely used for the treatment of diarrhoea [6].

The study of gastrointestinal motility by *in vitro* techniques may be helpful in determining the therapeutic potential of newer drugs in motility disorders, alterations in motility secondary to physiological or pharmacological stimuli, evaluating the effect of pathological condition on gastrointestinal motility. Any substance affecting motility can disturb functionality of the gastrointestinal tract. For assay purposes, guinea pig ileum and rabbit jejunum is chosen because it yields steady baseline, for studying the effects of drugs [7]. The functional components of isolated intestines are terminal sympathetic and parasympathetic synapses as well as parasympathetic ganglionic synapse. Stimulation of sympathetic nerves inhibits peristaltic movements, while parasympathetic stimulation increases movement. Isolated gastrointestinal smooth muscle cells can also be used to assess the receptor binding sites. The parasympathetic system is responsible for maintaining normal intestinal motility through releasing acetylcholine (ACh). The aim of the study is to evaluate the effect(s) of methanol extract of *Citrullus lanatus* Thunb. (Curcubitaceae) fruit rind on intestinal motility of isolated gastrointestinal tissues of rabbit and guinea pig, validating its traditionally acclaimed antidiarrhoeal property [8].

Citrullus lanatus Thunb. (Curcubitaceae), commonly called watermelon and botanically classified as fruit [9] is a prostrate or climbing annual plant with several herbaceous, firm and stout stems up to 3 m long. It is cultivated across the world and it is mostly grown for fresh consumption of the juicy and sweet flesh of the mature fruit. In Nigeria, watermelon is called *"kankana"* (Hausa), *N la/ kekere, Egun* (Yoruba), *Ugu* (Igbo) [10]. Although several uses of *C. lanatus* fruit rind in traditional medicine have been documented, many of these claims are yet to be validated by researchers to establish the affirmation on its use scientifically as having antidiarrhoeal activity on pharmacological basis of laxative effect.

EXPERIMENTAL

Collection and identification of plant material. Fresh whole *C. lanatus* fruits and leaves were obtained from a farm in Gundutse–Dan Hassan Village, Kura L.G.A. of Kano State, Nigeria. Botanical identification was carried out by Mallam Namadi Sansui of Herbarium Unit of Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, where a voucher number 1266 was deposited for future reference.

Experimental animals. Two healthy male rabbits (800-1200 g) and two guinea pigs (400-500 g) obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, fed normal rat feed and provided with water *ad-libitum* were used. All experiments were conducted during daytime (08.00-18.00 h). All protocols approved by the Institutional Animal Ethical Committee according to the School's Academic Guidelines for use and care of experimental animals with approval number DAC/IW-IT/0711/16 were followed.

Laboratory equipment. Animal cages (locally made), porcelain pestle and mortar, deionised water, laboratory microwave oven, microdynamometer (Ugo Basile, Italy), water bath (HH-S Digital thermostatic water bath), weighing balance (Lab tech. BL 20001 and Mettler P162, USA), Isolated tissue apparatus, dissecting kit (Gold Cross, Malaysia), pipette, test tubes (Pyrex, France), syringes, stop watch, ruler

Drugs and chemicals. Methanol (AR JHD UN1230; Guangdong Guanghua Sci-Tech. Co., Ltd., China)**,** acetylcholine and

adrenaline (Sigma-Aldrich Inc., St. Louis, USA), atropine sulphate vial injection (Gland Pharma Ltd., Ameerpet, Hyderabad, India), histamine (Sigma-Aldrich Inc., St. Louis, USA), loperamide (Imodium® – Janssen Pharmaceutical, Beerse, Belgium), mepyramine (Sigma-Aldrich Inc., St. Louis, USA), propranolol (Sigma-Aldrich Inc., St. Louis, USA), Tyrode solution

Preparation and extraction of plant material. Fresh *C. lanatus* fruit rind (white and green exocarp) were peeled, sliced into thin smaller pieces, and air dried, under shade for twelve days until a constant weight was obtained. The air-dried rinds were reduced to powder using pestle and mortar and weighed. The powder 1.1 kg was extracted using cold maceration method with 70% methanol solvent (3.5 liters), with occasional shaking for three days. It was concentrated in flask evaporator under reduced pressure and subjected to drying under temperature (45- 50 ˚C) over a water bath. The sticky residue (extract) obtained was stored in labeled airtight container and placed in desiccator until it was to be used. Percentage yield was calculated as: (Weight of sticky extract / weight of dried powder) x 100

Preliminary phytochemical screening. The presence of steroids, phenols, triterpenes, flavonoids, alkaloids, saponins, tannins, glycosides, essential oils, of the methanol extract of *C. lanatus* fruit rind were determined using methods described by Sofowora [11] and Evans [12].

Acute toxicity study. The median lethal dose (LD_{50}) was determined using Lorke's method [13]. In the first phase, nine mice randomly divided into three groups of three mice per group were given 10, 100 and 1000 mg extract/kg orally (via cannula), respectively. Mice were observed for 4 h post administration for signs of adverse effects / toxicity and death after 24 h. There was no death recorded after 24 h for the whole groupings; thus, phase two was initiated. Three mice were administered the extract orally at doses of 1600, 2900 and 5000 mg/kg, to each mouse respectively. They were observed for signs of toxicity for the first four hours and mortality after 24 h. LD_{50} value was determined by calculating geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

***In Vitro* Isolated Tissue Studies**

Effect of methanol extract of *C. lanatus* fruit rind on isolated rabbit jejunum. Segments of the jejunum of exsanguinated rabbit, about 3 cm long, were removed and dissected free of adhering mesentery. The intestinal content was removed by flushing with Tyrode's solution. The tissue was mounted in a 25ml organ bath containing Tyrode's solution maintained at 37°C, aerated with a 95% O_2 and 5% CO_2 mixture. The tissue was allowed to equilibrate for 60 minutes during which the physiological solution was changed every 15 min. At the end of equilibration period, dose response relationship was established using acetylcholine (0.008×10^{-6} – 0.064×10^{-6}g/ml) and repeated in the presence of atropine (0.1×10^{-6} g/ml). Methanol extract of *C. lanatus* fruit rind (MECL) at concentrations (0.04×10^{-6} – 40×10^{-6}g/ml) and adrenaline (0.02×10^{-6} – 0.04×10^{-6}g/ml) were also tested on the jejunum. The tissue was allowed to equilibrate for 30 minutes before drug administrations. Effects of MECL and acetylcholine were also tested. The contact time for each concentration was 5 min, followed by washing three times, before the next drug administration. Isolated tissue was allowed a resting period of 15 min before the next drug administration. Responses were recorded isometrically using microdynamometer at sensitivity of 2.0-3.0 mV and a speed of 24 mm/min [14]. Furthermore, the effects of various doses of the extract in the presence of antagonists, like

atropine (0.04×10^{-6} – 0.08×10^{-6}g/ml) and propranolol (0.08×10^{-6} – 0.32×10^{-6}g/ml) which were incubated for 3 minutes prior to the introduction of the drug, were determined. Contact time for each drug concentration is 1 min, which was followed by washing three times [14].

Effect of methanol extract of *C. lanatus* fruit rind on isolated guinea pig ileum. Similar protocol as for that of the effect of methanol extract of *C. lanatus* on isolated rabbit jejunum was followed, using guinea pig ileum. The effects of histamine (0.02×10^{-6} - 0.16×10^{-6} g/ml), acetylcholine and methanol extract of *C. lanatus* fruit rind (0.04×10^{-6} – 40×10^{-6}g/ml) were tested on guinea pig ileum. Effects of antagonists like atropine (0.04×10^{-6} - 0.16×10^{-6} g/ml), histamine and mepyramine (0.08×10^{-6}–0.16×10^{-6}g/ml) were tested [14].

RESULTS

Percentage yield of methanol extract of *C. lanatus* fruit rind. Powdered dried weight of fruit rind of 1.1 kg using cold maceration method produced 262.76g of methanol crude extract, giving a percentage yield of 23.89 % $^{w}/_{w.}$

Preliminary Phytochemical Screening. Preliminary phytochemical screening of the methanol extract of *C. lanatus* fruit rind revealed the presence of alkaloids, carbohydrates, saponins, cardiac glycosides, flavonoids, steroids and triterpenes. However, anthraquinones and tannins were absent.

Acute toxicity study. Acute toxicity studies carried out showed no lethality or toxic reactions observed at any of the doses of methanol extract of *Citrullus lanatus* fruit rind used in the study upon oral administration even at the highest dose of 5000 mg/kg in mice.

Effect of methanol extract of *C. lanatus* fruit rind on isolated rabbit jejunum. Isolated tissue studies showed relaxatory activity of the MECL fruit rind on baseline contractions of rabbit jejunum, but at higher concentrations of 32×10^{-6} and 40×10^{-6} g/ml, a sudden spike of smooth muscle tissue contractile effect was produced. MECL fruit rind (16.0×10^{-6} g/ml) when interacted with acetylcholine (0.016×10^{-6}g/ml) blocked the effect of the latter, causing relaxation of the tissue (Figure 1 and Plate I). Atropine, when interacted with the extract produced synergistic effect by further relaxing the tissue (Figure 2).

Effect of methanol extract of *C. lanatus* fruit rind on isolated guinea pig ileum. There was no observable response on guinea pig ileum with lower concentrations of the extract administered, but at higher concentrations (4×10^{-6} g/ml – 40×10^{-6} g/ml) and its interaction with histamine, relaxatory effect was elicited (Figure 3, Plate II). Mepyramine produced a blocking effect on histamine contractile effect too. Other drug effects were also produced on the tissue (Figure 4).

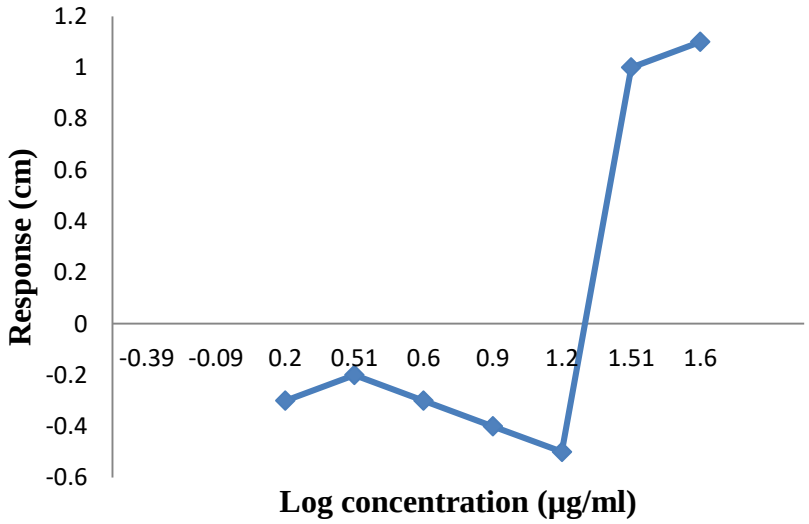

Figure 1: Log-concentration effect of methanol extract of *Citrullus lanatus* fruit rind on isolated rabbit jejunum

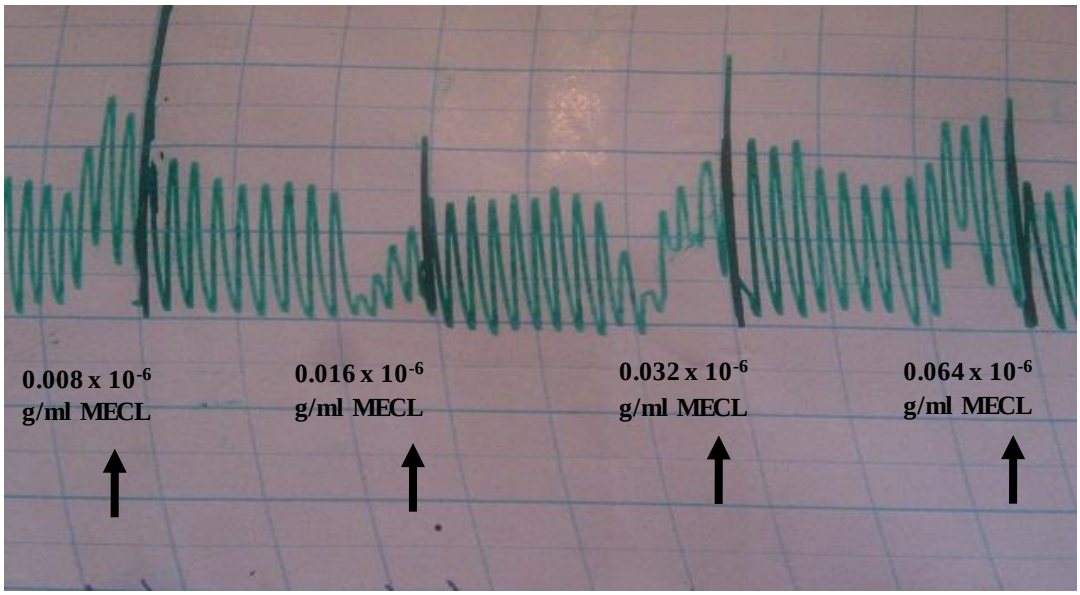

Plate I: Effect of graded concentrations of MECL (0.008×10^{-6} – 0.064×10^{-6}g/ml) on isolated rabbit jejunum

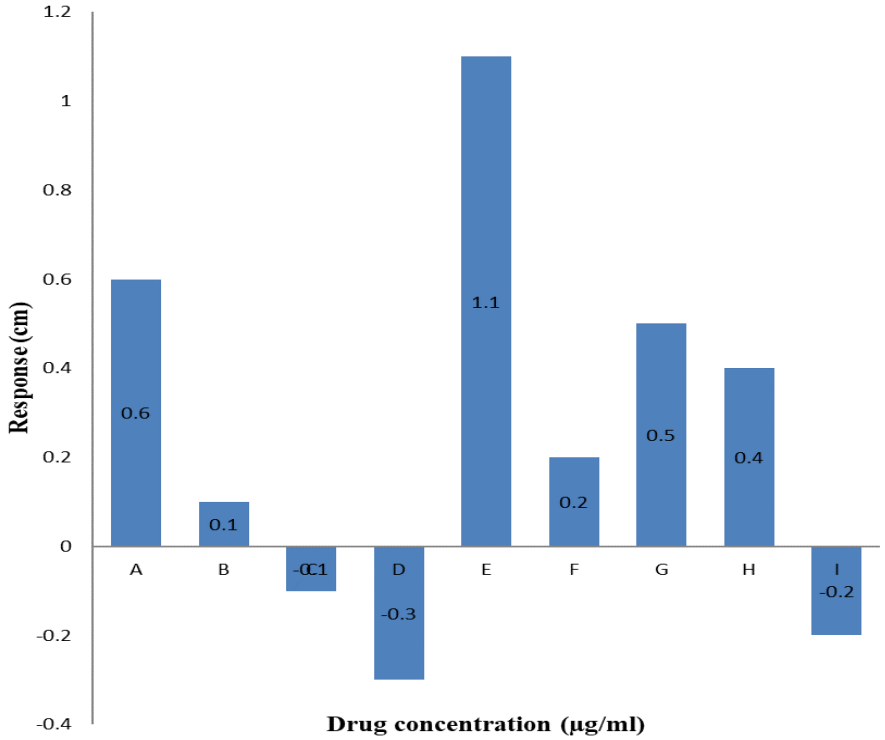

Figure 2: Effect of methanol extract of *C. lanatus* fruit rind and some drugs on isolated rabbit jejunum
A - Adrenaline (0.02) B - Propranolol (0.04) C – Propranolol (0.08), adrenaline (0.04) D –Propranolol (0.16) MECL (16) E - Acetylcholine (0.016) F - Atropine (0.08) G - Atropine (0.08), Acetylcholine (0.016) H - Atropine (0.08), MECL (16) MECL Where; MECL- methanol extract of *C. lanatus* fruit rind

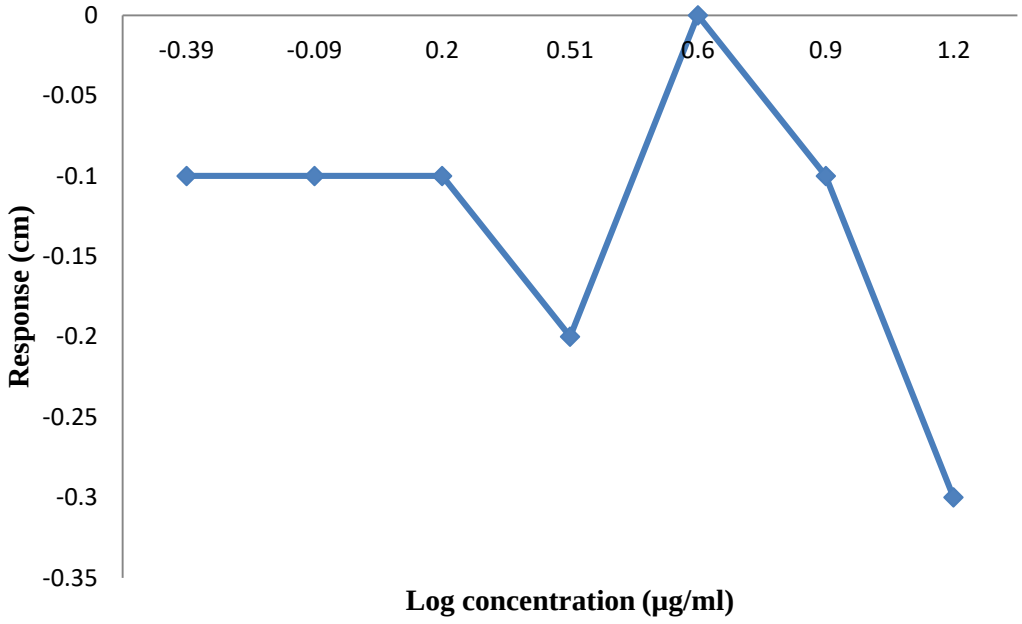

Figure 3: Relaxatory effect of graded concentrations of methanol extract of *Citrullus lanatus* fruit rind on isolated guinea pig ileum

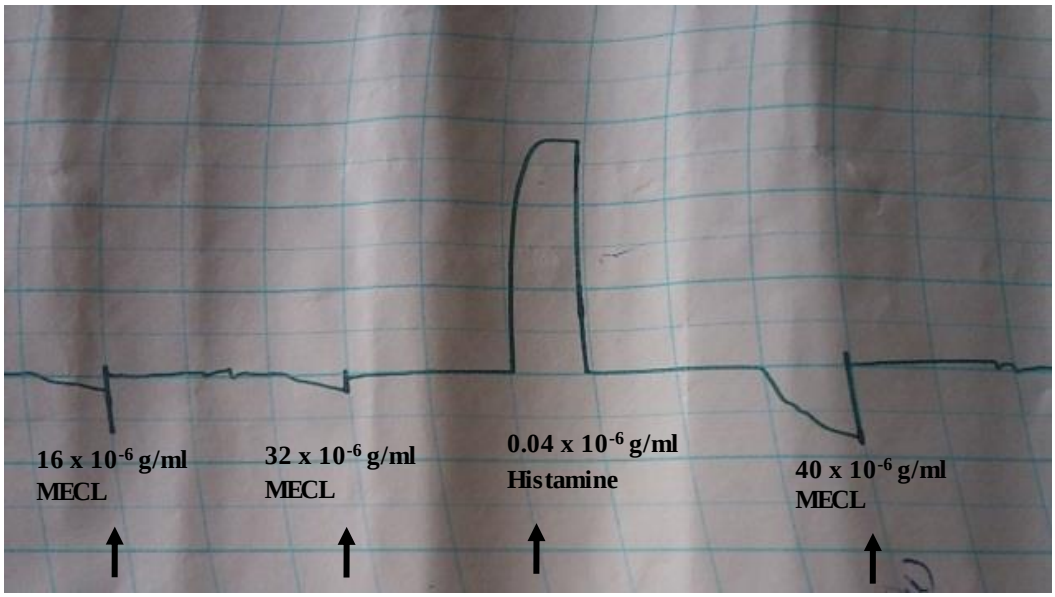

Plate II: Effect of graded concentrations of MECL (16×10^{-6} – 40×10^{-6}g/ml) on isolated guinea pig ileum

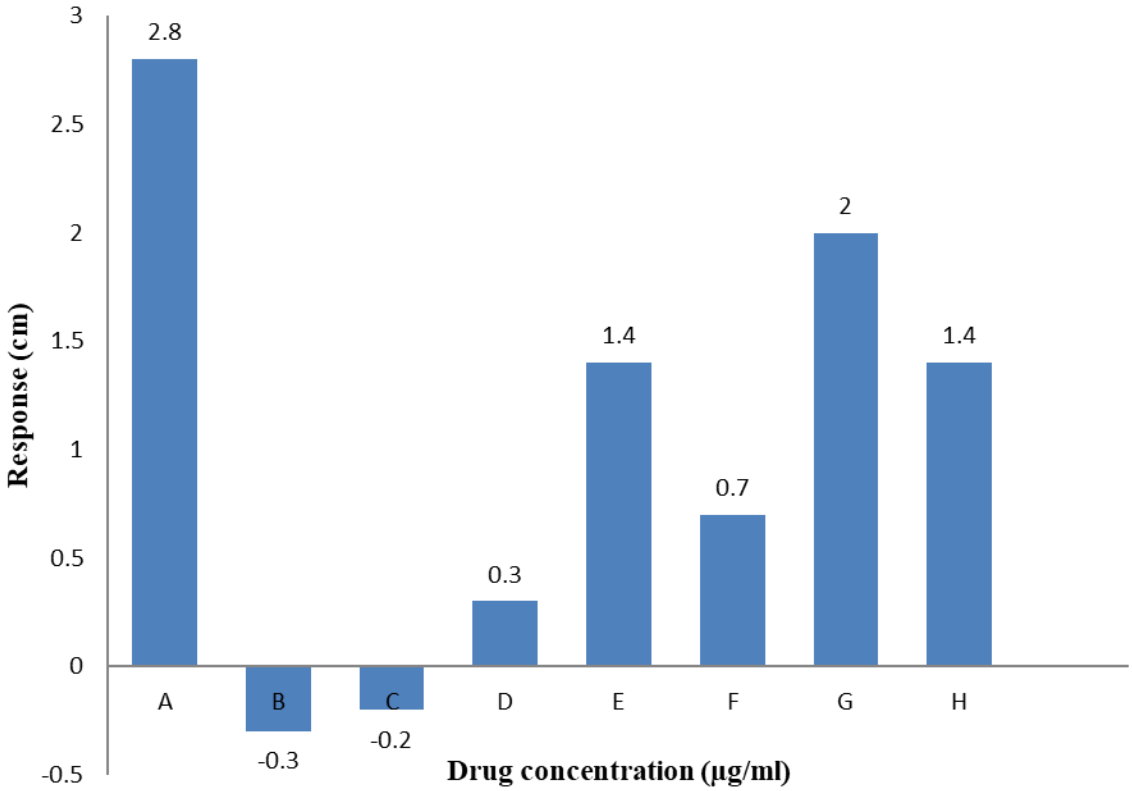

Figure 4: Effect of methanol extract of *C. lanatus* fruit rind and some drugs on isolated guinea pig ileum
A - Histamine (0.04) B - mepyramine (0.08) C - mepyramine (0.16) D - mepyramine (0.16), histamine (0.04) E - histamine (0.04) F - MECL (32), histamine (0.04) G - histamine (0.04) H- MECL (32), histamine (0.04) MECL- methanol extract of *C. lanatus* fruit rind

DISCUSSION

The phytoconstituents found in the methanol extract of *C. lanatus* fruit rind partly describes the probable spasmolytic activity elicited on isolated gastrointestinal tissue. Antidiarrhoeal and anti-dysenteric investigations of medicinal plants carried out by various researchers, showed that

antidiarrhoeal activity was due to the presence of alkaloids, tannins, saponins, flavonoids, steroids and triterpenoids/triterpenes [15-17]. Anti-diarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretions, which are known to be altered in diarrhoeic conditions [18,19]. Sesquiterpenes, flavonoids and terpenoid derivatives are known for inhibiting release of autacoids and prostaglandins, thereby inhibiting gastrointestinal motility and secretion induced by castor oil [20]. The sesquiterpene lactones have the ability to relax smooth muscles, thereby relieving gastrointestinal distress [21].

Both endogenous and exogenous acetylcholines usually achieve contractile effects on the gastrointestinal tract by binding to the numerous muscarinic receptors present in the smooth muscles of the gut [22]. Any agent therefore that can block the effect of acetylcholine by binding to these muscarinic receptors like atropine causes reduction in the contractions of the gastrointestinal tract leading to delayed movement of its content [23]. The methanol extract of *C. lanatus* fruit rind on isolated tissue preparations, produced relaxatory response with the lower concentrations, while higher concentrations produced increased contractile effect of the tissue. MECL inhibited acetylcholine induced contractions *in vitro*, suggesting that MECL fruit rind contain active principles with anticholinergic property which acted by blocking the effect of acetylcholine by acting on the cholinergic receptors of the gastrointestinal tract. These results agree with the findings of Sharma *et al.,* [24], who reported that *Citrullus lanatus* fruit has spasmolytic effect on rabbit jejunum collaborating the claim in traditional medicine that the extract may be used to treat diarrhoea. The biphasic activities (spasmolytic and spasmogenic) activities elicited on isolated tissue preparations (rabbit jejunum and guinea pig ileum) confirmed partly, the extract's antidiarrhoeal activity by delaying intestinal motility and indicate similar mechanism of action to the standard drugs (atropine sulphate and mepyramine) respectively.

Conclusion. A biphasic activity (spasmogenic and spasmolytic) of the methanol extract of *Citrullus lanatus* fruit rind on isolated gastrointestinal smooth muscle tissue was established.

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