

Full Length Research Paper

Effects of *Xylopia aethiopica* fruit extract on isolated rabbit ileum

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In this study, pharmacological reactivity of rabbit ileum to crude extract of fruits of *Xylopia aethiopica* in the presence of other agonist and antagonist agents was evaluated. The contractility of the isolated rabbit ileum was recorded using kymograph. The effects of the crude extract of *X. aethiopica* on rabbit ileum were independently determined using a dose range of 10^{-8} to 10^{-4} mg/ml. However, to determine the effects of the extract on rabbit ileum in the presence of other agonist and antagonist a constant dose of 10^{-4} mg/ml of the extract was used. The result showed that the crude extract of *X. aethiopica*, nifedipine only and serotonin only produced a significant ($p < 0.001$) dose dependent relaxation of the ileum muscle. Addition of the extract to nifedipine shifted the graph to the right. Administration of CaCl_2 alone produce a dose dependent contraction of the rabbit ileum with negative correlation coefficient ($p < 0.001$). Co-administration of CaCl_2 with the extract shifted the CaCl_2 response curve to the left. Addition of extract to serotonin caused a significant ($p < 0.01$) decreased in the relaxation of the rabbit ileum. In conclusion, this study suggests that *X. aethiopica* has smooth muscle relaxant effects on rabbit ileum and as such may reduce gastrointestinal motility.

Key word: *Xylopia aethiopica*, smooth muscle, Ileum, motility, agonist.

INTRODUCTION

Xylopia aethiopica, has both nutritional and medicinal uses. It is a member of the custard apple family, Annonaceae, and is used as a spice in various traditional dishes of Western and Central Africa (Choumessi et al., 2012). It is one of the plants species man has discovered in the search for food and health care (Okwari et al., 2014).

Phytochemical analysis shows that the plant contain alkaloid (Ekong and Ogan, 1968), proteins, carbohydrate,

lipids, crude fibers in various concentrations, some minerals such as calcium, potassium and iodine (Berminas et al., 1999).

X. aethiopica has been applied in ethnomedicine in the treatment of cough, bronchitis, dysentery and female sterilization. It is believed to aid uterine contraction and is applied as an abortifacient agent (Iwu, 1993). Some of its investigated uses include antibacterial and antiseptic properties (Okwari et al., 2014).

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Nwafor and Kalio (2006) reported that aqueous extract of *X. aethiopica* caused a significant reduction in the gestation period, number of litters lost after seven days and weight of litters. In line with this, Ebong and Shode (2000) reported that the aqueous and petroleum ether extract of *X. aethiopica* possesses potent spasmolytic properties on the smooth muscles of the rat uterus. However, contrary to this claim, the study on the effect of aqueous and methanol extract of the fruits of the plant on gastrointestinal motility shows that, it has a smooth muscle relaxant effects in the gastrointestinal tract (Ebong et al., 1995). The contradiction in this report may be due to differences in the receptor activated by the extract of the plant in the uterine muscle and gastrointestinal tract. Hence, this study aims at evaluating the effect of aqueous extract of fruit of *X. aethiopica* on the motility of the rabbit intestine in the presence of other agonist and antagonist agents.

METHODOLOGY

Chemicals used

The chemical and drugs used for this study were obtained from sigma (United Kingdom) and prepared freshly by dissolving in distilled water.

Preparation of plant extract

Dried fruit of *X. aethiopica* were bought from Watt market in Calabar Cross River State. It was authenticated at Department of Botany University of Calabar. It was then grounded to a coarse powder using an engine grinder. The ground *X. aethiopica* was then subsequently weighed and 100 g of dried ground fruit was dissolved in 500 ml of deionized water (Ebong and Shode, 2000). The solution was left for 24 h before filtration with Whatman filter paper Number 1. The filtrate was then dried in an oven at about 40°C.

Preparation of isolated tissue

The animal for this study was fasted for 24 h prior to the beginning of the study to ensure complete emptying of the small intestine. The rabbit was then killed by cervical dislocation to ensure that gastrointestinal tract (GIT) reflexes were not inhibited. A midline incision was made on the abdomen along the *linea alba* to expose the small intestine. As soon as possible, a piece of the proximal ileum free from the mesentery near the duodenum was removed as it shows greater spontaneous activity as compared to the distal ileum. This part was then immersed in ice-cold saline and cut into small segments of about 3 to 5 cm long. The tissues were continually aerated with the aid of an aerator.

Experimental procedure

With the aid of threaded suture needle, a piece of thread was attached to each end of the tissue segment. At one end, the thread was made into a small loop and attached to the hook in the organ bath. The other piece of thread was attached to the writing lever of the kymograph with plasticine. The tissue was bathed with tyrode

solution (the solution composed of NaCl: 0.8%, KCl: 0.02%, NaHCO₃: 0.01%, NaHPO₄: 0.005%, CaCl₂: 0.02%, Glucose: 0.1%, Deionized water to make up 100 ml) and allowed to equilibrate for about 20 to 30 min. The bathing solution was flushed out at intervals of 10 min. The kymograph drum was set to a rotating velocity of 0.01 revolution/s with a speed of 0.1×0.5 mm/s. After equilibration period, the basal equilibrium response was obtained after which graded doses of the extract was added to the tissue and its result obtained. The tissue was flushed 3 to 4 times before the addition of other drugs. Each drug effect was considered alone using their graded doses and thereafter with 10⁻⁴ mg/ml of extract. The procedure was repeated five times using new ileal strips from different rabbit. The temperature of the organ bath was maintained at 37°C.

Statistical analysis

Results were expressed as Mean ± Standard Error of Mean (SEM). In all cases, the comparison of the different sets of data was done by using the unpaired student t-test. P-value of <0.05 was considered statistical significant. Correlation and regression analysis was done to show association.

RESULTS

The plant extract independently elicited dose-dependent relaxation of the rabbit ileum (correlation coefficient = 0.9990). The lowest concentration of the extract (10⁻⁴ mg/ml) produce 8.90±0.19% decrease in contraction of the smooth muscle. While the highest dose (1 mg/ml) produce 44.51±0.95% relaxation of the rabbit ileum (Figure 1). Nifedipine and serotonin also independently produce relaxation of the rabbit ileum (Figures 2 and 4). The correlation coefficient between dose and response was found to be significant at p<0.01 for both nifedipine and serotonin. Introduction of CaCl₂ alone evoked contraction of the smooth muscle in a dose dependent manner (p<0.01). The response of the smooth muscle to graded doses of nifedipine and CaCl₂ has a negative correlation coefficient (-0.9660 and -0.9766, respectively).

Addition of 10⁻⁴ mg/ml of the extract to nifedipine increased the relaxation of the smooth muscle with maximal response obtained at a dose of 10⁻⁴ mg (Figure 2). Co-administration of CaCl₂ with the 10⁻⁴ mg/ml of extract reduced the contractile response of the smooth muscle (Figure 3). Serotonin administration alone produces dose dependent increase in relaxation of the rabbit ileum. Addition of extract (10⁻⁴ mg/ml) to serotonin caused a significant decrease in the relaxation of the rabbit ileum (Figure 4).

DISCUSSION

The results obtained from this study showed that the extract of *X. aethiopica* produced a dose dependent relaxation of the rabbit ileum. This is in line with the report of Ebong et al. (1995), who reported that the aqueous and methanol extracts of the fruit of *X. aethiopica* has a

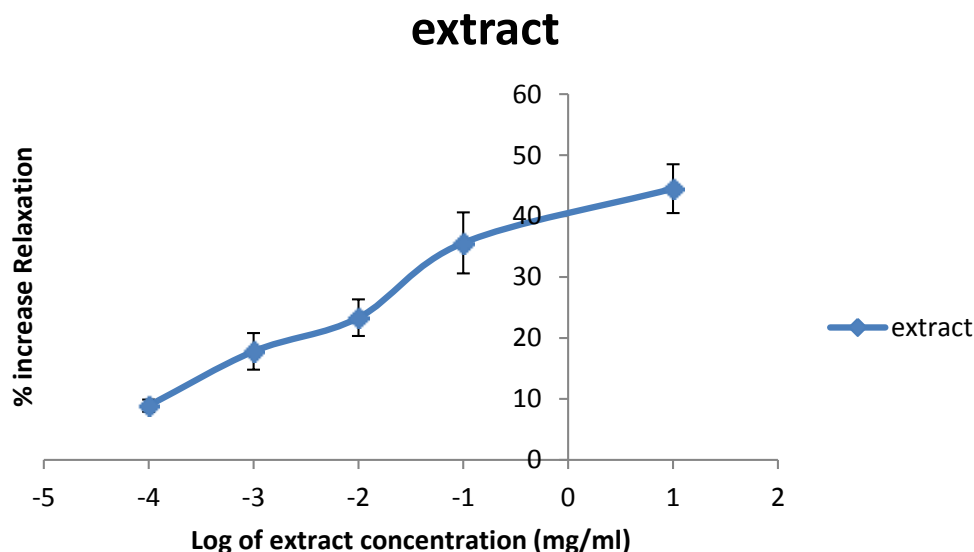


Figure 1. Effects of graded doses of the extract of *Xylopiya aethiopia* on motility of the rabbit ileum.

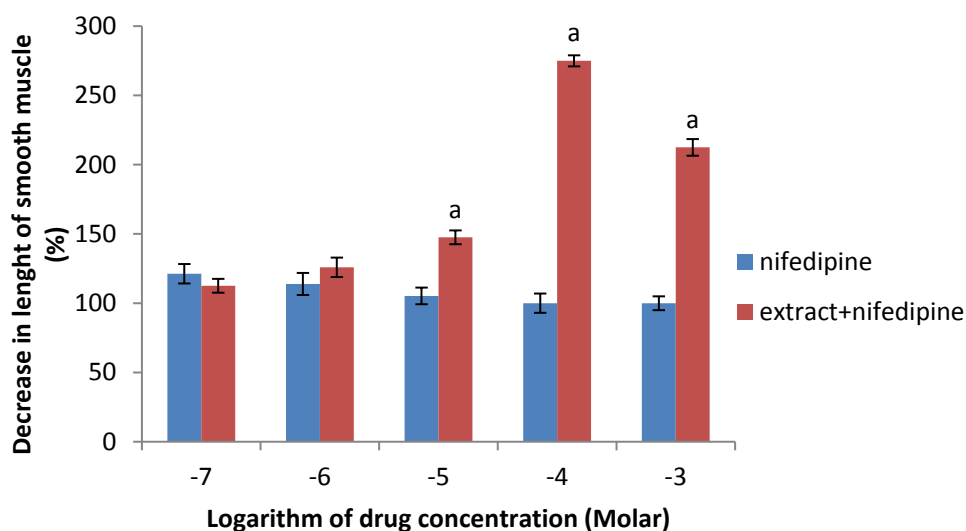


Figure 2. Effects of graded doses of Nifedipine + 10^{-4} mg/ml of *Xylopiya aethiopia* on motility of rabbit ileum (a= $p < 0.05$).

smooth muscle relaxant effect on gastrointestinal smooth muscle. The lowest concentration of the extract produced a decrease in contraction, but at high concentration, the extract produce strong relaxation. The smooth muscle relaxant effects of the extract may be due to the presence of alkaloids in the extract, since alkaloids have been previously implicated in the relaxation of smooth muscle (da Silva et al., 1999). Administration of nifedipine, a non-competitive calcium channel blocker produced relaxation of the rabbit ileum. As the concentration of nifedipine

increased, the relaxation of the rabbit ileum decreased dose dependently. Addition of extract to graded doses of nifedipine increases the relaxation of smooth muscle of ileum. This may suggest that the extract and nifedipine are both acting through different receptor.

Administration of CaCl_2 produces contraction of the smooth muscle. This contractile response is reduced with increase concentration of CaCl_2 . Addition of extract to graded doses of CaCl_2 further reduced the contractile response of the smooth muscle of ileum. It may be

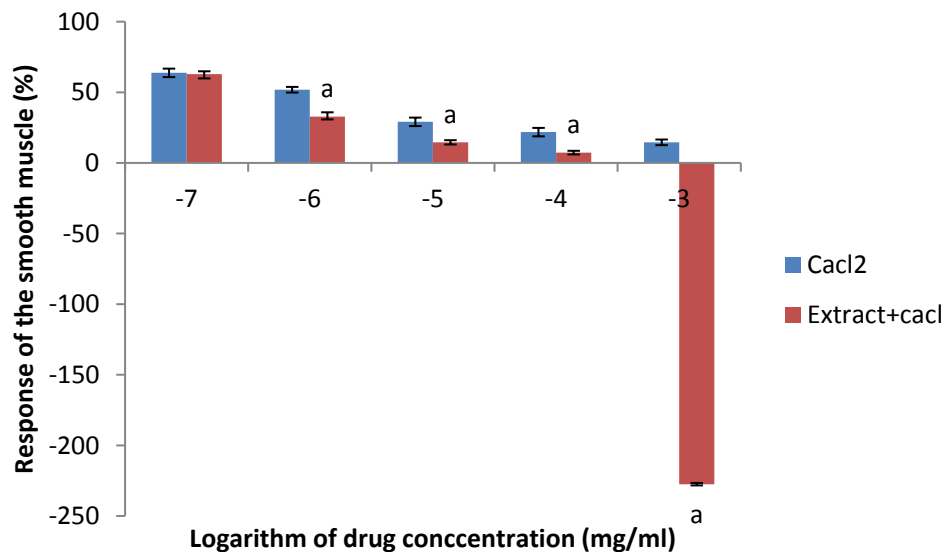


Figure 3. Effects of graded doses of $\text{CaCl}_2 + 10^{-4}$ mg/ml of *Xylopiya aethiopia* on motility of rabbit ileum ($a = p < 0.05$).

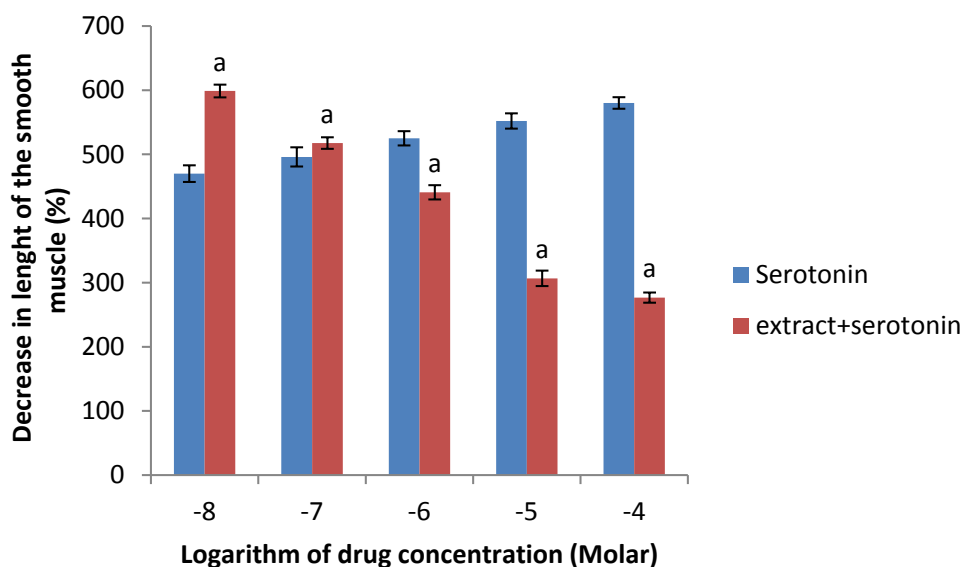


Figure 4. Effects of graded doses of Serotonin + 10^{-4} mg/ml of *Xylopiya aethiopia* on motility of rabbit ileum ($a = p < 0.05$).

inferred here that the extract potentiated the relaxant effect on the smooth muscle in the presence of CaCl_2 . This may also indicate that both are acting independently to exert their effects.

Serotonin alone produced a dose dependent increase in relaxation of the rabbit ileum. Simultaneous administration of serotonin and extract produced a dose dependent decrease in relaxation of the rabbit ileum rather than the expected increase in relaxation by two

significant relaxants. This may suggest that the two substances may be using the same pathway and competing for receptor sites. Different types of serotonin receptors are expressed on different gastrointestinal cells, enteric nerves, smooth muscle and interstitial cells of cajal (Wouters et al., 2007). Expression and function of these receptors differ, some of them mediate relaxation, others only inhibition and activation of smooth muscle. This diversity makes it difficult to establish the clear

function of serotonin in the gastrointestinal system (Wouters et al., 2007). It is therefore possible that the extract of *X. aethiopica* employed this difference in serotonin receptor action to exert contractile effects in some smooth muscle such as uterus (Nwafor and Kalio, 2006), while exerting smooth muscle relaxant effects on rabbit ileum.

In conclusion, *X. aethiopica* has a smooth muscle relaxant effect on rabbit ileum and as such may reduce gastrointestinal motility.

Conflicts of interest

The authors have not declared any conflict of interest.

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