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The antispasmodic effect of aqueous root bark extract of *Carissa edulis* (Forssk.) Vahl on isolated rabbit jejunum is mediated through blockade of calcium channels

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ABSTRACT

Background: Spasms of the gut underlie hyperactive gut disorders. These conditions are highly prevalent and impart greater health care cost. Herbal antispasmodic remedies form a source of affordable, safe and easily available treatments in low resource areas. There is, therefore, a need to scientifically evaluate the therapeutic potential of these remedies. This study investigated the antispasmodic effect of aqueous root bark extract of *Carissa edulis*, herb used to manage hyperactive gut disorders such as abdominal colic and diarrhea.

Materials and methods: Pieces of jejunum were isolated from adult New Zealand White rabbits. They were mounted in an organ bath containing Tyrode's solution. The rate and force of contraction were recorded using Powerlab coupled to Chart5 Software. The effects of the extract (0.1-10.0 mg/ml) on spontaneous jejunal contraction were investigated. The effect of 1.0 and 3.0 mg/ml extract was investigated on acetylcholine, KCl and $CaCl_2$ induced contraction.

Results: *Carissa edulis* extract dose-dependently (0.1-10 mg/ml) significantly decreased the force but not the rate of spontaneous jejunal contraction. Extract (1 and 3 mg/) significantly decreased the magnitude of acetylcholine, KCl and $CaCl_2$ induced contraction.

Conclusions: Aqueous root bark extracts of *Carissa edulis* possess a significant antispasmodic effect on rabbit jejunum. This appears to be through calcium channel blockade. These results validate its use as a remedy for hyperactive gut disorders.

Keywords: *Carissa edulis*, diarrhea, antispasmodic, jejunum, motility.

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BACKGROUND

Gastrointestinal tract (GIT) spasm results in several gastrointestinal disorders including diarrhea and inflammatory bowel disease.[1] These conditions are highly prevalent: for example, 1·731 billion episodes of diarrhea occur annually and result in 700,000 deaths of children - especially those below 2 years of age;[2] while IBD is estimated to have a prevalence of over 1 million in the USA and 2.5 million in Europe.[3]

Antispasmodics are a group of compounds that are used to relieve spasm of the gastrointestinal tract. They consist of anticholinergics, calcium channel blockers and musculotropic agents.[4] Their use is associated with limited availability, side effects[4] and higher health care cost.[3] It is for these reasons that there is an increased interest in finding cheaper, safer and locally available compounds with potential use as an antispasmodic agent.

*Carissa edulis (*Forssk.) Vahl is a member of the Apocynaceae family. Its root decoction is used traditionally by communities in Kenya to relieve abdominal colic and diarrhea.[5,6] Aqueous root barks extract of this herb has been shown to cause relaxation of isolated rabbit jejunum possibly through activation of nitric oxide synthase.[7] This study, therefore, investigated other possible mechanisms of action of this herb by investigating its effect on isolated rabbit jejunum which had been pre-contracted by potassium chloride, acetylcholine, and calcium-chloride.

MATERIALS AND METHODS

Plant material and extract preparation

Roots of *Carissa edulis* were collected from Homa Bay area of Kenya in July 2017. Botanical identification of the plant was done at the Department of Botany, University of Nairobi and a voucher specimen deposited therein (Voucher No. LLO 2017/01).

The roots were cleaned with tap water, and their barks were peeled off and cut into smaller pieces. The material was then air-dried at room temperature for 7 days after which it was ground into a coarse powder using an electric mill. The

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powdered material (100 g) was boiled in distilled water (1000 ml) for 10 minutes. The mixture was then left standing for 4 hours. The resultant mixture was filtered using Whatman No.1 filter paper and the filtrate transferred into a round-bottomed rotary vapor flask where water was removed at a temperature between 35 and 40°C at a pressure of -760 mmHg.[7]

Study animals

New Zealand White rabbits of both sexes (1.5-2 kg) were used. They were housed singly in metallic cages measuring 46 × 48 × 36 cm at standard temperature (22–25°C) and humidity with a normal light/dark cycle. The animals were fed on standard rabbit pellets, vegetables, and water *ad libitum* and they were acclimatized for two weeks. This study was approved by Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (FVM BAUEC/2018/141)

Isolated rabbit jejunum preparation

The animals fasted for 24 hours before experiments but they had access to water *ad libitum*. After they were killed by cervical dislocation, their abdomens were cut open and the jejunum portion (6-8 cm) from the ligament of Treitz was isolated out.[8]

Two-centimeter segments of rabbit jejunum were mounted individually in an 80 ml tissue bath containing Tyrode's solution which was maintained at 37°C and aerated with 95% O_2 and 5% CO_2. The composition of the Tyrode's solution in mM was: KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, Na_2HPO_4 0.42, $CaCl_2$ 1.8, glucose 5.55 and a pH 7.4.[9]

The upper end of the segment was hooked to an isometric force transducer (ML500/A, ADInstrument) coupled with a Power Lab data acquisition system (Power Lab 8/30). The force (g) and rate (number per minute) of contraction were recorded and analyzed using Chart5 software for Windows. The tissues were allowed to equilibrate for 30 minutes before the addition of any drug. The bath fluid was replaced with normal Tyrode's solution at an interval of 20 minutes. Only segments of jejunum that showed spontaneous contractions and those that produced reproducible contraction on addition of ACh or KCl were selected for the study.

Pharmacological evaluation

The effect of the extract on the basal tone of rabbit jejunal contraction was determined by studying its effect on spontaneous contractions. After the stabilization period, spontaneous contractions of the jejunum were recorded for 2 minutes. Extract at concentrations of 0.1, 0.3, 1.0, 3.0, and 10.0 mg/ml was added cumulatively into the organ bath at an interval of 3 minutes before washing out. The results were expressed as a percentage of the spontaneous contractions of the rabbit jejunum.

To evaluate the antispasmodic effect of the extract, its effect on ACh (100 μM) and KCl (80 mM) induced contraction were investigated as follows: extract (1.0 mg/ml or 3.0 mg/ml) was added 3 minutes prior to the addition of ACh followed by a 1-minute record of activity. It was also added 5 minutes after the addition of KCl followed by a 3-minute record of activity. The effect of atropine (0.01 μM) on ACh induced contraction was also investigated. The inhibition of ACh or KCl induced contractions by the extract was expressed as a percentage of the maximal ACh or KCl induced contractions respectively.

To further evaluate calcium channel antagonism, the isolated jejunum was stabilized in normal Tyrode's solution. This solution was replaced with Ca^{2+} -free Tyrode's solution containing EDTA (0.1 mM) for 15 minutes. After confirmation of no or minimal spontaneous contraction, control response to calcium was obtained by the addition of $CaCl_2$ (10 mM) into the organ bath followed by a 5-minute record of jejunal activity. Calcium chloride was also added after 3- minutes of tissue incubation with the extract (1.0 mg/ml and 3.0 mg/ml) in calcium-free Tyrode's solution. This protocol was adapted with modification from.[10] $CaCl_2$ induced contraction in the absence and presence of extract was expressed as a percentage of the spontaneous contractions in normal Tyrode's solution.

Data and statistical analysis

The results were expressed as Mean ± SEM; n represents the number of experiments. Data were analyzed using SPSS version 20, while graphs were generated using GraphPad Prism version 7. One-way ANOVA, followed by the Bonferroni post hoc test was used to compare between groups. The difference was considered significant only if $p < 0.05$.

RESULTS

Extract yield

100 grams of powdered root barks produced 8.21 grams of aqueous extract. This represents an extract yield of 8.21 %.

Spontaneous contraction

The isolated rabbit jejunum showed rhythmic spontaneous contractions that did not change with time. Addition of the extract caused a dose-dependent decrease in the force of spontaneous jejunal

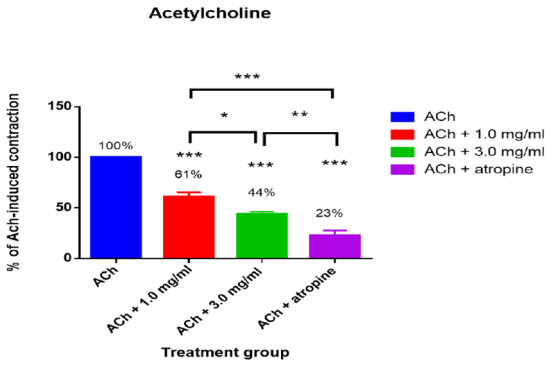

Figure 1 Bar graph showing the effect of the extract and atropine on acetylcholine induced contraction. Results are shown as mean ± SEM, (n= 5), *p < 0.05, **p<0.01, ***p<0.001

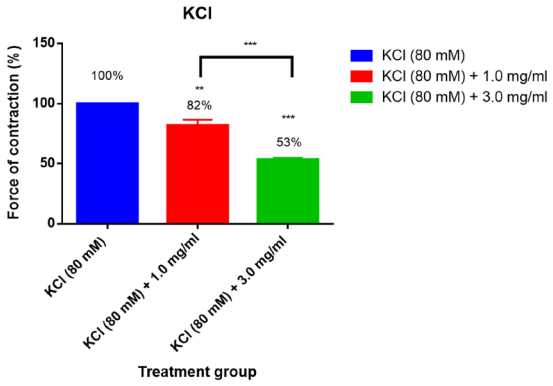

Figure 2 Bar graphs showing the effect of extract on potassium chloride induced contraction. Results are shown as mean ± SEM, (n= 5), *p < 0.05, **p<0.01, ***p<0.001

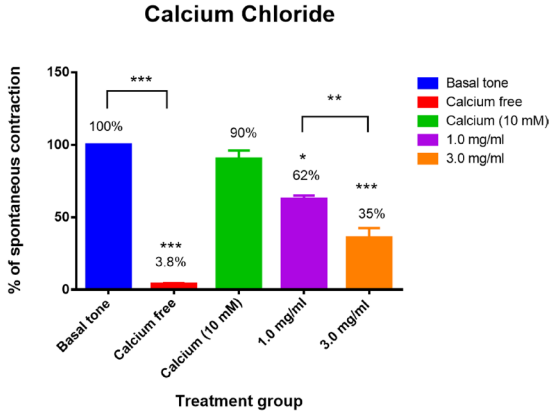

Figure 3 Bar graph showing effect of extract on $CaCl_2$ induced contraction. Results are shown as mean ± SEM, (n= 5), *p < 0.05, **p<0.01, ***p<0.001 vs. Calcium (10 mM)

contractions. Doses of 0.3, 1.0, 3.0 and 10.0 mg/ml of the extract caused a significant decrease in the force of spontaneous jejunal contraction whereas 0.1 mg/ml of the extract had no significant effect. The extract had no significant effect on the rate of contraction as shown in table 1. The effect of the extract on the force of contraction was partially reversible on washing out (Data not shown).

Acetylcholine induced contraction

Extract and atropine significantly diminished the magnitude of acetylcholine-induced contraction and amounted to [100 ± 00% (control) vs. 61.16 ± 4.40 % (1.0 mg/ml) vs. 43.85 ± 2.26 % (3.0 mg/ml) vs. 22.81 ± 4.93 % (atropine); one-way ANOVA $p < 0.001$]. Post hoc analysis showed that there was a significant difference between all the treatment groups as shown in figure 1.

Potassium chloride-induced contraction

The extract significantly inhibited KCl-induced contraction [100% (80 mM KCl) vs. 81.97 ± 4.56% (80 mM KCl +1.0 mg/ml extract) vs. 53.46 ± 1.21% (80 mM KCl +3.0 mg/ml extract); one-way ANOVA $p < 0.001$]. Post hoc analysis showed significant differences between all the treatment groups as shown in figure 2.

Calcium chloride-induced contraction

There was significant difference between the experimental groups, [100% (basal tone) vs. 3.84 ± 0.70% (calcium free) vs. 90.30± 5.80% (10 mM calcium chloride) vs. 62.50 ± 2.43 % (1.0 mg/ml) vs. 36.69 ± 6.85 % (3.0 mg/ml); one-way ANOVA, $p < 0.001$].

The addition of the extract significantly inhibited $CaCl_2$ induced contraction 1 mg/ ml ($p < 0.05$) and 3.0 mg/ml ($p < 0.001$). Replacement of normal Tyrode's with calcium-free Tyrode's caused a significant decrease in the force of jejunal contraction ($p < 0.001$). The addition of $CaCl_2$ in the Calcium-free Tyrode's solution significantly restored the force of contraction (calcium-free vs. 10 mM calcium chloride; $p < 0.001$). There was, however, no significant difference between the 10 mM calcium-induced contraction in calcium-free Tyrode's and the basal tone (Basal tone vs. 10 mM calcium chloride; $p > 0.05$) as shown in figure 3.

DISCUSSION

In this study, the frequency and the amplitude of the isolated rabbit jejunum spontaneous rhythmic contraction remained constant with time. This is

due to slow waves generated by the Interstitial Cells of Cajal.[11]

C.edulis, in a dose-dependent manner significantly inhibited the force but not the rate of spontaneous jejunal contraction. This suggests that the extract did not modify the frequency of pacemaker cells.[12] The effects of the extract on the spontaneous jejunal contraction were partially reversible on washing out. This is advantageous as an irreversible effect is likely to cause paralytic ileus.[13] In fact, antidiarrheal drug loperamide is contraindicated for use in children and those with severe diarrhea due to its higher risk of causing paralytic ileus.[1]

Contraction of smooth muscle cells, including those of rabbit jejunum, is dependent on the increase in free cytoplasmic calcium. This occurs either through the influx of extracellular calcium or through the release of intracellular calcium.[14] Therefore, the inhibitory effect of the extract on spontaneous rabbit jejunal contraction may have been due to its interference with calcium release or influx or by the stimulation of release of inhibitory transmitter substances.

The extract significantly inhibited ACh-induced contraction. In the gut, ACh activates M_3 receptors. These receptors are coupled to $G_{q/11}$ which activates PLC-β resulting in the generation of DAG and IP_3. IP_3 triggers the release of calcium from the sarcoplasmic reticulum. Activation of muscarinic receptors also stimulates non-selective cation channels.[15] Therefore, the inhibitory effect of the extract on ACh-induced contraction is mediated either through the inhibition of muscarinic receptors or through the inhibition of the influx of extracellular calcium.

The extract significantly inhibited KCl-induced contraction. High concentrations of KCl greater than 30 mM depolarized the cell membrane resulting in the activation of voltage-gated calcium channels.[14] Substances that inhibit high KCl-induced contractions are considered to be Ca^{2+} channel blockers.[16] This suggests that the extract possibly mediates its effect by interfering with the influx of extracellular calcium through calcium channels. This is further supported by the finding that the extract significantly inhibited calcium chloride-induced contraction in calcium-free Tyrode's solution. Several medicinal plants have also been shown to exert their antispasmodic effect through inhibition of calcium influx via calcium channels.[10] The magnitude of spontaneous contraction was significantly decreased in calcium-free Tyrode's solution but was significantly restored on the addition of calcium chloride. This indicates the importance of extracellular Ca^{2+} in the spontaneous contraction of isolated rabbit jejunum,[10] indeed extracellular calcium has been shown to play a major role on the spontaneous contraction of isolated rabbit jejunum compared to release of intracellular calcium.[17]

CONCLUSION

The findings of this study show that *Carissa edulis* has an antispasmodic effect on isolated rabbit jejunum. The effect of *C. edulis* is probably mediated through interference with the influx of release of calcium. This supports its use by some African communities to manage hyperactive gut disorders such as abdominal colic and diarrhea. However, further investigations are recommended in order to determine its other possible mechanisms of action and to identify the active compounds responsible for its effects.

WHAT IS ALREADY KNOWN ON THIS TOPIC

The aqueous root bark extract of Carissa edulis has been shown to have a myorelaxant effect on isolated rabbit jejunum through activation of nitric oxide synthase.

WHAT THIS STUDY ADDS

This study investigates other possible mechanisms of action of this extract by investigating its effect on acetylcholine, potassium chloride, and calcium-chloride induced contraction.

COMPETING INTEREST

The authors declare no conflict of interest. The study was fully funded by the authors.

AUTHOR'S CONTRIBUTION

Oluoch Linus Lincone, Boniface Mwang Chege, Mbugua Paul Mungai, Muriithi Anne Wangechi designed the study. Boniface Chege Mwangi, Siringo Cyril George, Oluoch Linus Lincone did the experimental work. Oluoch, Mbugua Paul Mungai, Muriithi Anne Wangechi, Siringo, Boniface Mwangi Chege analyzed the experimental data and wrote the paper. All the authors reviewed the manuscript and approved its submission.

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