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Anti-spasmodic activity of *Costus afer* leaves extract on an isolated rabbit jejunum

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ABSTRACT

The antispasmodic property of *Costus afer* ethanol leaves extract (CALE) was investigated on isolated rabbit jejunum in exclusively in vitro experimental models. About 2-3cm of the rabbit jejunum was mounted in a 35ml organ bath containing tyrode solution bubbled with 95% Oxygen and 5% carbondioxide and was allowed to equilibrate for 20 minutes before administrations of selected agonists, antagonists and CALE with proper washing after each administration. Results obtained indicate that all doses of Acetylcholine and histamine increased the amplitude of rhythmic contractions of the jejunum significantly ($P < 0.05$), while CALE caused significant ($P < 0.05$) inhibition of both basal rhythmic contractions and Acetylcholine and histamine induced contractions with 114.3µg/ml of CALE inhibiting 2.86µg/ml of acetylcholine and histamine by 69.37 and 50.34% respectively. The effects of CALE compared favourably with those of atropine and promethazine and suggest that the extract may contain principles with antispasmodic property and may be of value in the management of diseases like diarrhea, incontinence, peptic ulcer, gastrointestinal cramps, gastritis and muscular spasms.

Key words: Acetylcholine, Antispasmodic, *Costus afer*, Gastrointestinal, Histamine

INTRODUCTION

The gastrointestinal tract is under the control of the sympathetic and parasympathetic arms of the Autonomic nervous system. The interplay between these two arms brings about balance and helps maintain normal peristaltic functions responsible for the onward movement of intestinal contents [1]. Over activity of the parasympathetic arm causes increased peristalsis resulting in incontinence, gastrointestinal cramps, gastritis, peptic ulcers, diarrhea and ulcers due increased gastric secretions [2, 3]. These disorders indeed result from excessive involuntary muscle movement associated with excess release of Acetylcholine, a neurotransmitter which mediates parasympathetic functions [2].

Antispasmodic agents are substances that suppress muscle spasms. On the gastrointestinal tract their effect is to prevent spasms of the stomach and intestine mostly by blocking the action of neurotransmitter acetylcholine in the parasympathetic outflow and thereby inhibiting cholinergic nerve impulses by selectively blocking the receptors to

which acetylcholine binds. This anticholinergic property of the antispasmodic agents become useful in the management of disorders associated with over activity of the parasympathetic system on the gastrointestinal system [4]. The many side effects of existing antispasmodic agents mean a lot need to be done to develop safer ones. This, coupled with reasons of cost and tradition has led to renewed interest in herbal medicine and may be reasons why the exploitation of wild plants for medicinal purposes has continued to grow globally [5]. *Costus afer* is one of such plants that are being used.

Costus afer is commonly called Ginger lily or Bush cane in English, Kaki zuwa by the Hausas, Okpete or Okpoto by the Igbos and TeteOgun by the Yorubas, all of Nigeria is [6, 7], is a tall perennial semi-woody herb with leafy canes which may grow up to 3m high and belonging to family *costaceae*[8]. The plant is commonly found in the forest zones of most places including Senegal, Nigeria, South Africa, Guinea, Ghana, and Cameroun and in most regions in tropical Africa, particularly in higher rainfall areas [8, 6].

Phytochemical analysis of the leaf reveal the presence of alkaloids, saponins, tannins, flavonoids, phenols, glycosides and terpenoids [9, 6, 7]. The root extract is reported to be used as genital stimulant, laxative and treatment of leprosy and stomach troubles, while the stem juice/sap has been used to treat arthritis rheumatism and pharyngeal infections [8]. The hypoglycaemic property of ethanol leaf extract of *Costus afer* have been reported [9], while Ukpabiet *al.*, (2012) [6] reported its ability to alleviate carbon tetrachloride induced hepatic oxidative stress and toxicity. This current study was designed to investigate *Costus afer* ethanol leaf extract (CALE) for antispasmodic activity.

MATERIALS AND METHODS

2.1 Collection of Plant materials and preparation of extract

Fresh leaves of *Costus afer* were collected from a neighborhood in Umudike, Ikwuano Local Government Area, Abia State. The extract was prepared using a modified method of Akahet *al.*, (2009) [10]. The collected leaves were air dried at room temperature for 14 days after which they were ground to coarse powder using a manual blender. Thirty five (35) grams of the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 70°C for 48hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 4.85g and represented a yield of 13.86%.

2.2 Animals

Thirty mice (25-30g), six Rabbits (1.8-2.5kg) obtained from the Animal production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the study. They were fed with standard feed, with water *ad libitum*, but starved for 12hours prior to commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised 1985), as expressed by Akahet *al.*, (2009) [10]. This study was carried out in the Physiology Laboratory of the Department of Physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.3 Acute toxicity study

Thirty mice of both sexes were divided into 6 groups of 5 mice each and were assigned graded oral doses of *Costus afer* ethanol leaf extract in the order 500, 1000, 2000, 3000, 4000 and 5000mg/kg body weight. After administration, the animals were allowed free access to feed and water and the number of deaths in each group was noted at the end of 24 hours. LD₅₀ was to be calculated using Karber's formula as expressed Enegideet *al.*, (2013) [11].

$$LD_{50} = LD_{100} \frac{\sum (Dd \times Md)}{N}$$

Where:

LD₅₀ = Dose that killed 50% of animals in a group

LD₁₀₀ = Dose that killed all animals in a group

$\sum (Dd \times Md)$ = Summation of all products of dose difference and mean deaths, and N = Number of animals in each group

2.4 *In vitro* study of the effect of CALE on isolated Rabbit jejunum

Each rabbit starved for 24hrs was killed by stunning and decapitation. The jejunum was carefully isolated and transferred into Tyrode solution that was continuously bubbled with oxygen (95%) and carbondioxide (5%) mixture, maintained at 37°C with pH value 7.4. The physiological salt solution (tyrode) had the following salts composition per liter of water: NaCl- 8g, KCl- 0.2g, CaCl₂-0.2g, NaHCO₃-1g, NaHPO₄-1g, MgCl₂-0.1g and Glucose-2g. The isolated jejunum, about 2-3cm in length was cut out and suspended vertically in a 35ml organ bath by means of ligatures attached at one end to a tissue holder and at the other end to an isometric force displacement transducer attached to a physiograph. The physiograph was connected to a computer screen for displaying isometric contractions. Resting tension on the muscle strip was readjusted, just sufficient to remove slack and the preparation was allowed to equilibrate within 20 minutes of mounting. In all the experiments, a 30 seconds time was allowed for individual tissue responses before being washed 2-3 times with tyrode solution.

All concentrations of test substances given in the text are final bath concentrations (FBC), except otherwise stated. Serial dilutions were made for reference drugs and CALE and administered in the following order:

1. Graded doses of Acetylcholine (Sigma, USA)(1.43, 2.86, 5.70, 8.5 and 11.40µg/ml)
2. Graded doses of Histamine (1.43, 2.86, 5.70, 8.50 and 11.40µg/ml)
3. Graded doses of CALE (14.29, 28.57, 57.14, 114.29 and 228.57µg/ml)
4. Graded doses of Acetylcholine in the presence of Atropine (Sigma, USA)
5. Graded doses of Acetylcholine in the presence of CALE
6. Graded doses of Histamine in the presence of Promethazine
7. Graded doses of Histamine in the presence of CALE

2.5 Statistical analysis

Results were expressed as Means $\pm$ standard error of mean (SEM) and analysed using one way Analysis of variance. P-values less than 0.05 at 95% level of significance were considered as being significant.

RESULTS

3.1 Acute toxicity (LD₅₀)

During the period of acute toxicity, all the mice administered CALE had normal disposition, were active and survived the 24 hours period of study with no signs of toxicity, even at an oral dose of 5000mg/kg body weight.

3.2 *In vitro* responses of an isolated rabbit jejunum to Acetylcholine, Histamine and CALE

All doses of Acetylcholine and Histamine significantly ($P < 0.05$) increased the amplitude of rhythmic contractions of the rabbit jejunum in a dose dependent manner, while all doses of CALE inhibited same with 228.6µg/ml reducing the amplitude of basal rhythmic contractions from 9.24 $\pm$ 1.12 to 2.00 $\pm$ 0.80 (table 1). The observed effects of Acetylcholine and Histamine were significantly ($P < 0.05$) blocked by Atropine and Promethazine respectively. CALE was observed to exert sufficient blocking effect on both Acetylcholine and Histamine induced contractions, with final bath concentrations of 114.3µg/ml exerting inhibitions of 46.82 and 73.09% for 11.40µg/ml respectively. The effect of CALE compared favourably with that of atropine and promethazine (tables 2 and 3)

Table 1: Effects of Acetylcholine, Histamine and CALE on the rhythmic contractions of the rabbit jejunum

FBC for Ach. and Histamine(µg/ml)	Basal amplitude of contraction (mm)	Amplitude in response to Acetylcholine (mm)	Amplitude in response to Histamine (mm)	Amplitude in response to graded doses of CALE (mm)
1.43	8.47 $\pm$ 0.90	15.20 $\pm$ 0.72*	11.05 $\pm$ 0.50*	5.21 $\pm$ 0.45*
2.86	9.50 $\pm$ 0.65	18.64 $\pm$ 0.81*	12.89 $\pm$ 0.86*	3.05 $\pm$ 0.72*
5.70	9.00 $\pm$ 1.05	22.60 $\pm$ 0.92*	14.70 $\pm$ 1.05*	3.50 $\pm$ 1.05*
8.50	8.90 $\pm$ 0.76	25.87 $\pm$ 1.08*	17.30 $\pm$ 0.98*	2.83 $\pm$ 0.91*
11.40	9.24 $\pm$ 1.12	25.95 $\pm$ 1.78*	19.40 $\pm$ 2.13*	2.00 $\pm$ 0.80*

* = $p < 0.05$ when compared with basal

Graded doses of CALE =14.29, 28.57, 57.14, 114.29 and 228.57µg/ml

Table 2: Percentage inhibitions of atropine and CALE on Acetylcholine induced contractions

FBC of Acetylcholine (µg/ml)	% Inhibition offered by Atropine (0.029µg/ml)	% Inhibition offered by CALE (114.3µg/ml)
1.43	89.50	53.82
2.86	60.46	69.37
5.70	57.65	53.75
8.50	46.00	53.92
11.40	41.13	46.82

Table 3: Effect of Promethazine and CALE on Histamine induced rhythmic contractions of the rabbit jejunum

FBC (µg/ml)	% Inhibition offered by promethazine	% Inhibition offered by CALE
1.43	150.94	61.09
2.86	144.92	50.34
5.70	104.42	50.14
8.50	88.21	58.38
11.40	77.84	73.09

DISCUSSION

With the potential toxicity associated with active plants ingredients, there was the need to evaluate the safety of *Costus afer* leaves extract. Results obtained indicate that *Costus afer* ethanol leaves extract (CALE) is safe and could be well tolerated after oral consumption since no death was recorded even at a high dose of 5000mg/kg body weight, with all animals looking physically and emotionally healthy. In the *in vitro* experiment, administered Acetylcholine and histamine induced contractile responses. Acetylcholine achieved this effects by binding the numerous muscarinic receptors present in the smooth muscles of the gastrointestinal tract [4], while histamine did same via the histamine receptor pathway. Uchiyama *et al.*, (2004) [12] had reported that Acetylcholine mediates gastrointestinal tract contractions via a mixed M₂/M₃ receptor population with M₃ acting via phospholipase C and M₂ via inhibition of adenylatecyclase, such that despite the predominance of M₂-receptors, direct contraction of intestinal muscles is mediated via M₃-receptor subtype and only this subtype is involved in contraction *in vitro*. On the other hand histamine induced contractions of the jejunum by binding to histamine receptors including the H₄ subtype which is reported to play role in regulation of gastric acid secretion, gastric mucosal defense, intestinal motility and secretion [13]

Costus afer ethanol leaves extract (CALE) inhibited the normal rhythmic contractions and significantly blocked Acetylcholine and histamine induced contractions in a manner similar to the blocking effects of atropine and promethazine. CALE may have achieved these effects by binding to the muscarinic and histaminergic receptors present in the smooth muscles of the intestine, antagonizing the activity of Acetylcholine and histamine and thereby inhibiting intestinal peristaltic contractions. These results suggest that CALE contain principles with both anticholinergic and antihistamine properties and as such capable of exerting antispasmodic effects on the gastrointestinal tract. Momohet *al.*, (2011) [9] had reported that *Costus afer* contain phytochemical substances including alkaloids, flavonoids, tannins, phenols, glycosides and terpenoids, some of which have been implicated in the inhibition of intestinal motility and control of diarrhea by increasing colonic water and electrolyte reabsorption [14].

CONCLUSION

The inhibitory effect of *Costus afer* ethanol leaf extract on Acetylcholine and histamine induced intestinal contractions in the *in vitro* experiments attest reveals the antispasmodic property of the extract and means that the extract may be of value in the search for new antispasmodic agents with minimal side effects for use in the management of diseases like diarrhea, incontinence, peptic ulcers, muscular spasms and other conditions associated with increased intestinal motility and secretions.

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