Spasmolytic and Anti-Secretory Activities of Water/Ethanol *Crinum jagus* Extract

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Abstract. The present study was undertaken to determine the potential anti-secretory and spasmolytic mechanism of *Crinum jagus* water/ethanol extract (25, 50 and 100 mg/kg p.o.). Anti-secretory activities were evaluated using castor oil and PGE₂-induced enteropooling models in rats. Spasmolytic activities were conducted on small intestinal transit, on carbachol, 5-HT- induced intestine motility as well as on naloxone-pretreated intestine transit. *C. jagus* extract 25, 50 and 100 mg/kg bw has significantly ($p < 0.01$) reduced in dose-dependent manner the castor oil-induced intestine secretion with inhibition rate of -59.7, -78.7 and -78.2% respectively. *C. jagus* 25, 50 and 100 mg/kg bw water/ethanol extract induced significant ($p < 0.01$) dose-dependent reduction of PGE₂-induced fluid accumulation, with inhibition rate of -16.9, -57.7 and -66.2% respectively. On intestine transit, as on carbachol and on serotonin-induced intestine motility, the extract significantly ($P < 0.01$) reduced the distance covered by the charcoal. The spasmolytic effect of *C. jagus* extract was inhibited by the naloxone. These findings show that *C. jagus* may reduce intestine secretion by the blockage of prostaglandin receptors. The spasmolytic effect could result from the blockage of muscarinic and/or serotonergic receptors, and by involving opiate receptors. These results support the traditional use of *Crinum jagus* in gastrointestinal disorders treatment.

List of abbreviations

C. jagus: *Crinum jagus*
WE: water/ethanol extract
WECj: water/ethanol extract of *Crinum jagus*
NC: normal control (group)
DC: diarrheic control (group)
bw: body weight
i.p.: intraperitoneal
p.o.: per os
5-HT: 5-hydroxytryptamine (serotonin)
Lop5: loperamide 5 mg/kg bw
PGE₂: prostaglandin E₂ (Prostacyclin).

Introduction

Intestinal spasms are uncontrolled contractions in the muscles of the small and large intestines. It can produce symptoms such as abdominal pain, colic, flatulence, constipation and diarrhea [1]. Diarrhea is the second leading cause of death in children under five and is responsible for 3 million child deaths per year [2]. It is the passage of more than three unformed stools in 24 hours [3] and can last several days, dehydrating and depriving the body of its mineral salts necessary for survival. Diarrhea is one of the major health threats to populations in tropical and subtropical poor countries. Healers and patients in many communities still rely on locally available phytomedicines. World Health Organization in Diarrheal Disease Control Programme has given a special emphasis on the use of traditional medicines in the control and management of diarrhea [3]. It is very important to find plants or plant products that relax the intestinal muscle and that may be used for treatment of diarrhea and intestinal cramps. Plants and other natural products like charcoal from traditional roasted banana and kaolin chalk are locally used in diarrhea management. Amongst those plants, *Crinum jagus* (Amaryllidaceae) is widely used in Africa as antitumor, antiviral, antiparasitary, immunostimulator, and against mental trouble and snake bite [4]. According to traditional medicine practitioners in the Western Region of Cameroon, this plant is used as anti-poison and for the treatment of many other diseases [5]. This study has been planned to explore the scientific basis for the medicinal use of *Crinum jagus* in hyperactive gut disorders, like abdominal spasms and diarrhea using *in vivo* assays.

Materials and Methods

Preparation of crude extract

Fresh *Crinum jagus* plants were collected from Batie (West Region, Cameroon) in August 2012. The specimen was confirmed at the National Herbarium of Yaounde, Cameroon, with the voucher number HNC 14049. The whole plant was cleaned, sliced into small pieces, shade dried and powdered. The plant powder was extracted with water/ethanol (4:1) mixture. 1000 g powder were macerated at room temperature in 5 L solvent for 48 hours (with occasional stirring), followed by filtration using Whatman filter paper N°1. The residue was macerated again in solvent for 48 hours and filtered. Likewise, the 2 filtrates obtained were pooled, concentrated in a rotary evaporator at 45°C to yield 291.6 g of dry dark hydro-ethanolic extract. The extracts were kept in a well-closed container at +4°C until use. Prior to the oral administration to rats or to *in vitro* assays, the extract was dissolved in distilled water.

Experimental animal

Apparently healthy albino rats (130 – 170 g) of either sex obtained from the animal house of the Department of Animal Physiology, Faculty of Science of the University of Yaounde I were used. Animals were housed in clean metabolic cages placed in a ventilated house and allowed free access to standard rat diet: carbohydrates 50-55%, fats 15-20% and proteins 25-30% [6]. *In vivo* experiments were done according to the guidelines of the European Union on Animal Care (CEE Council 86/609) [7] that were adopted by the Institutional Committee of the Cameroon Ministry of Scientific Research and Innovation.

Extract activities on rat intestine

For each test, groups of five rats each were fasted for 18 h with free access to water and all treatments were done orally.

Castor oil-induced intestine secretion

Animals were given distilled water 1 mL/100g bw (Normal Control NC and Diarrheic Control DC groups), loperamide 5 mg/kg bw (Lop5), 25 (WE25), 50 (WE50) and 100 (WE100) mg/kg bw *C. jagus* water/ethanol extract. Then, one hour later animals of each group except the NC was administered the castor oil (1 mL/100g bw). 30 min later, animals were sacrificed, and their

small intestines were ligated from pyloric sphincter to ileo-caecal junction and the accumulation of intestinal fluid secretion induced by castor oil were assessed and expressed in reduction rate.

PGE₂-induced intestine secretion

Groups of rats received 2% gum acacia (Normal Control NC and Positive Control PC groups), 25 (WE25), 50 (WE50) and 100 (WE100) mg/kg bw *C. jagus* water/ethanol extract. One hour later animals of each group except NC received the PGE₂ (100 µg/kg in 2% gum acacia). Then 30 min later, animals were sacrificed, and their small intestines were ligated from pyloric sphincter to ileo-caecal junction and assessments of the accumulation of intestinal fluid secretion induced by PGE₂ were made and calculated in terms of percentage reduction.

Normal transit

Overnight-fasted rats were randomized into five groups of five rats each and were respectively administered per os: 1 mL/100 g bw distilled water for the NC, 25 (WE25), 50 (WE50) and 100 (WE100) mg/kg of *C. jagus* water/ethanol extract and the standard drug atropine sulphate 0.2 mg/kg bw i.p. (Gland Pharma. Pally. Dundigal. Post, Hyderabad, India) (AT0.2). Then each animal was orally given 30 min later 2 mL of charcoal meal (10% activated charcoal in 5% gum acacia) as diet marker, and was sacrificed 30 min after administration of the charcoal. The distance covered by the charcoal meal in the intestine was expressed as the percentage of the total distance travelled from the pylorus to the caecum [8].

Carbachol-induced transit

Six groups of five normal rats each respectively received 1 mL/100 g bw distilled water (Normal Control NC and Carbachol Control groups), 25 (WE25), 50 (WE50) and 100 (WE100) mg/kg bw *C. jagus* water/ethanol extract and the standard drug atropine sulphate 0.2 mg/kg bw i.p. (AT0.2). Thirty minutes later animals except NC received 0.5 mg/kg bw i.p. carbachol (Sigma Aldrich, RT 66223-622PX). After carbachol administration, all the animals orally received 2 mL charcoal meal and were sacrificed 30 min later. The distance covered by the charcoal meal in the intestine was expressed as the percentage of the total distance traveled from the pylorus to the caecum.

Naloxone-pretreated intestine transit

Six groups of five normal rats each, except NC were intraperitoneally pretreated with 0.4 mg/kg bw naloxone (Merck A7658; 8297YN). Thirty minutes later, different groups respectively received 1 mL/100 g bw, p.o. distilled water (Normal Control NC and Naloxone Control groups), 5 mg/kg loperamide (Lop5), or 25 (WE25), 50 (WE50) and 100 (WE100) mg/kg bw *C. jagus* extracts. Thirty minutes after, all the animals orally received 2 mL of charcoal meal and were sacrificed 30 min later. The distance covered by the charcoal meal in the intestine was expressed as the percentage of the total distance traveled from the pylorus to the caecum.

Serotonin-induced transit

Six groups of five normal rats each respectively received 1 mL/100 g bw distilled water (Normal Control NC and 5-HT Control groups), 25 (WE25), 50 (WE50) and 100 (WE100) mg/kg bw *C. jagus* water/ethanol extract, and the standard drug loperamide 5 mg/kg bw (Lop5). Thirty minutes later each animal except NC received 1 mg/kg bw i.p. serotonin (Sigma Aldrich, H9523; 441TO). Just after serotonin administration, all animals orally received 2 mL charcoal meal and were sacrificed 30 min later. The distance covered by the charcoal meal in the intestine was expressed as the percentage of the total distance traveled from the pylorus to the caecum.

Statistical analysis

Data were expressed as mean ($\bar{X}$) $\pm$ standard error of mean (S.E.M) ($\bar{X} \pm$ S.E.M) of the group. The statistical significant differences were determined by one-way analysis of variance (ANOVA), followed by Dunnett's t-test. A value of $p < 0.05$ was considered significant.

Results

Castor oil-induced intestine secretion

The diarrheic control (DC) rat intestinal fluid content was 2.81 ± 0.03 mL against 0.80 ± 0.06 mL for the normal control (NC). *Crinum jagus* 25, 50 and 100 mg/kg bw extract, dose dependently and significantly ($P < 0.01$) inhibited castor oil-induced intestinal accumulation: -59.7%, -78.7% and -78.2% respectively (Fig. 1).

Figure 1: Enteropooling in castor oil-induced diarrheic rats treated with *C. jagus* water/ethanol extract 25 (WECj25), 50 (WECj50), 100 (WECj100) and Loperamide 5 (Lop5) mg/kg bw. Values are mean $\pm$ S.E.M, (n=5). Significant difference: ** $p < 0.01$ compared with Diarrheic Control (DC); ^a $p < 0.05$, ^b $p < 0.01$ compared with Normal control. (): inhibition rate.

PGE₂-induced intestine secretion

In PGE₂-induced enteropooling assay, PGE₂ administration caused a substantial increase ($p < 0.01$) of fluid accumulation in rats. Administration of 25, 50 and 100 mg/kg bw water/ethanol extract of *C. jagus* produced significant ($p < 0.01$) dose dependent reduction of fluid accumulation with inhibition rate of -16.9%, -57.7% and -66.2% respectively (Fig. 2).

Figure 2: Enteropooling in PGE₂-induced diarrheic rats treated with *C. jagus* water/ethanol extract 25 (WECj25), 50 (WECj50), and 100 (WECj100) mg/kg bw. Values are mean $\pm$ S.E.M, (n=5). Significant difference: ** $p < 0.01$ compared with Positive Control (PC); ^b $p < 0.01$ compared with Normal Control (NC). (): inhibition rate.

Normal transit test

In normal control rats one hour after charcoal feeding, the black meal moved up to 59% of the intestine total length. All the tested compounds significantly ($P < 0.01$) reduced the distance

moved by the charcoal (Table 1). The inhibitory effects of water/ethanol extract 25, 50 and 100 mg/kg on charcoal transit were -46.32%, -60.38% and -64.55% respectively (Table 1).

Table 1: Effect of *Crinum jagus* water/ethanol extract 25 (WECj25), 50 (WECj50), 100 (WECj100) and Atropine (0.2 mg/kg bw) on rat intestine transit.

	ITL (cm)	CCL (cm)	TLCR (%)	Covered Rate (%/NC)
NC	90.20±2.97	53.2±2.63	58.88±1.46	100
Atropine	94.40±1.08	22.00±0.71	23.3±0.63 ^{**}	-60.43±1.07
WECj25	94.80±2.27	29.90±0.87	31.62±1.25 ^{**}	-46.32±1.42
WECj50	97.80±2.06	22.80±0.86	23.34±0.92 ^{**}	-60.38±1.05
WECj100	94.48±1.59	19.70±0.44	20.80±0.56 ^{**}	-64.55±1.54

Values are mean ± S.E.M. (n=5). Significant difference: ^{**}P < 0.01 compared with normal control group (NC). ITL: Intestine total length; CCL: Charcoal covered length; TLCR: Total length covered rate.

Carbachol-induced intestine transit

Table 2: Effect of *Crinum jagus* water/ethanol extract: 25 (WECj25), 50 (WECj50), 100 (WECj100) and Atropine (0.2 mg/kg bw) on Carbachol-induced rat intestine transit.

	ITL (cm)	CCL (cm)	TLCR (%)	Covered Rate (%/NC)
NC	90.20±2.97	53.20±2.63	58.88±1.46	100
Carbachol	77.60±1.26	69.20±1.88	89.14±1.53 ^b	+51.40
Atropine+Carbachol	83.80±1.85	27.80±1.07	33.18±1.06 ^{**b}	-48.84
WECj25+Carbachol	85.00±2.02	39.20±1.02	46.18±1.30 ^{**b}	-21.6
WECj50+Carbachol	78.70±2.29	27.80±1.16	35.85±1.29 ^{**b}	-40.00
WECj100+Carbachol	79.20±1.69	25.60±1.57	32.24±1.50 ^{**b}	-45.2

Values are mean ± S.E.M. (n=5). Significant difference: ^{**}P < 0.01 compared with Carbachol control; ^b P < 0.01 compared with normal control group (NC). ITL: Intestine total length; CCL: Charcoal covered length; TLCR: Total length covered rate.

Carbachol has increased the intestine propulsion in rats compared with the normal control (NC) (89.148% versus 58.88%). Atropine, 25, 50 and 100 mg/kg bw *C. jagus* extract significantly (p<0.01) inhibited carbachol-induced intestine transit by -48.8%, -21.6%, -40% and -45.2% respectively (Table 2).

Naloxone-pretreated intestine transit

Pretreatment with naloxone reversed the ant motility effect of *C. jagus* water/ethanol extract. Charcoal meal progression in plant treated rat was comparable to that of normal animals as well as that of naloxone or loperamide rat (Table 3).

Table 3: Effect of *Crinum jagus* water/Ethanol extract 25 (WECj25), 50 (WECj50), 100 (WECj100) and Loperamide (Lope, 5 mg/kg bw) on Naloxone-pretreated rat intestine transit.

	ITL (cm)	CCL (cm)	TLCR (%)	Covered Rate (%/NC)
NC	90.20±2.97	53.20±2.63	58.88±1.46	100
Naloxone	77.40±2.71	51.20±2.08	66.17±1.80 [*]	+12.40
Naloxone + Lope5	97.00±1.64	56.80±1.53	58.61±1.69	-0.46
Naloxone + WECj25	92.20±1.43	54.20±1.16	58.91±2.10	+0.06
Naloxone +WECj50	83.40±1.66	48.80±1.46	58.49±1.03	-0.70
Naloxone +WECj100	78.80±2.48	45.40±2.06	57.51±0.88	-2.31

Values are mean ± S.E.M. (n=5). Significant difference: ^{*}P < 0.05 compared with normal control group (NC); ^b P < 0.05 compared with Naloxone control. ITL: Intestine total length; CCL: Charcoal covered length; TLCR: Rate of total length covered.

Serotonin-induced intestine transit

Serotonin has increased the intestine propulsion in rats compared with the normal control rats (NC) (70.05% versus 58.88%). Water/ethanol *C. jagus* extract and loperamide slightly reduced serotonin-induced gastro-intestinal motility in rats (Table 4).

Table 4: Effect of *Crinum jagus* water/ethanol extract 25 (WECj25), 50 (WECj50), 100 (WECj100) and Loperamide (5 mg/kg bw) on 5-HT-induced rat intestine transit.

	ITL (cm)	CCL (cm)	TLCR (%)	Covered Rate (%/NC)
NC	90.2±2.97	53.20±2.63	58.88±1.46	100
5-HT	89.00±1.80	55.60±1.47	70.05±1.33**	+18.98
Lope +5-HT	67.40±1.29	33.20±1.98	51.38±1.40**b	-21.23
WECj25+5-HT	76.80±2.15	37.00±1.79	48.09±1.40**b	-18.32
WECj50+ 5-HT	79.20±1.62	38.00±1.73	47.93±1.63**b	-18.59
WECj100+5-HT	87.00±0.71	41.20±1.16	47.34±1.17**b	-19.59

Values are mean ± S.E.M. (n=5). Significant difference: **P< 0.01 compared with normal control group (NC); ^bP< 0.01 compared with Serotonin control. ITL: Intestine total length; CCL: Charcoal covered length; TLCR: Total length covered rate.

Discussion

This study was designed to determine the antisecretory and antispasmodic activities of *Crinum jagus* water/ethanol extract. In the Western Region of Cameroon *C. jagus* is used against poison and in hyperactive gut disorders, like diarrhea and abdominal colic.

In castor oil-induced intestinal fluid accumulation models, *C. jagus* dose-dependently showed antisecretory activity with the effect of the 100 mg/kg dose, comparable to that of loperamide, which is known for its spasmolytic, antidiarrheal and antisecretory activities [9]). Castor oil is known to cause increased intestinal fluid contents and promotes diarrhea indirectly through the effect of its active constituent, ricinoleic acid, formed by the hydrolysis of oil, which changes the electrolytes and water transport by reducing active Na⁺ and K⁺ absorption as well as Na⁺ and K⁺ ATPase activity [10]. The effect of water/ethanol *C. jagus* extract on castor oil-induced intestinal fluid accumulation was reported to be highly significant at all tested doses compared to control group. This may be attributed to inhibition of active secretion of ricinoleic acid via the reduction of Na⁺, K⁺ ATPase activity. The retardation in castor oil-induced fluid accumulation was well supported by PGE₂-induced enteropooling study which demonstrated an antienteropooling effect of *C. jagus*. PGE₂ stimulates gastrointestinal motility and secretion of water and electrolytes [11], thus inducing an increase in the peristalsis and an intestinal hyper secretion of fluid. The varied biological functions exerted by PGE₂ are through the pharmacologically distinct, G-protein coupled plasma membrane receptors [12]. Activation of EP₂ and EP₄ prostaglandins receptors leads to an increase of cAMP, and thus increased intestine secretion [13]. Water/ethanol *C. jagus* extract reduction of PGE₂-induced intestinal secretion may have been through a decrease of cAMP level via the blockage of EP₂ and EP₄ prostaglandins receptors.

The role of multiple types of physiological mediators, such as, acetylcholine, substance-P, cholecystokinins and 5-hydroxytryptamine, is well established in regulatory function of gastrointestinal system [9]. It has also been documented that most of the spasmolytic agents have therapeutic potential in diarrhea by causing relaxation of the smooth muscle of the gut. Carbachol unlike acetylcholine in various gastrointestinal smooth muscles produces contractions by activating M₃ muscarinic receptors. The M₃ receptor is coupled preferentially to Gq-type G proteins, resulting in the activation of phospholipase C (PLC) and the formation of inositol trisphosphate (IP₃ which causes Ca²⁺ release from intracellular stores and can also mobilize Ca²⁺ secondarily through Ca²⁺-sensitive or store-dependent mechanisms [14]. *C. jagus* extract substantially reduced charcoal meal propulsion compared to carbachol control. This may be due to anticholinergic effect. Naloxone, a μ-

opioid competitive antagonist, works by competing with other relevant drugs on the receptor [15]. *C. jagus* extract on naloxone-pretreated intestine did not show any significant effect. The action was almost comparable to loperamide, a well-known peripheral opioid agonist. Activation of μ receptors by loperamide inhibits release of acetylcholine and thus relaxes smooth muscular tone in the gut wall. The physiological consequence of this is to enhance phasic colonic segmentation and inhibit peristalsis, thus increasing intestinal transit time [16]. Pretreatment with naloxone reversed the antispasmodic effect of *C. jagus* extracts confirming that its action may occur by opioid receptors. 5-HT₃ serotonin receptor elicits contraction of ileum via acetylcholine [17]. *C. jagus* extract antagonized intestine motility induced by 5-HT. 5-HT₃ receptors are located on pre- and postganglionic autonomic neurons of enteric nervous systems where they mediate effects in the control of intestinal motility [17]. *C. jagus* extract as such behave like 5-HT₃ receptor antagonist.

Phytochemical studies revealed that water/ethanol extract of *Crinum jagus* contains volatile oils, fatty acids, catechic tannins, carbohydrates, alkaloids, sterols, triterpenes, flavonoids, coumarins, anthocyanidines, polyphenols and saponins [5]. Amongst these phytochemical constituents, alkaloids have been reported to possess antidiarrheal activity, tannins as a protein denaturing agent producing protein tannates which make intestinal mucosa more resistant reducing secretion [10]. Thus, the pharmacological activities obtained may be attributed to the conjugation action of these phytoconstituents.

Conclusion

The present study shows that the antidiarrheal activity of *Crinum jagus* may be due to a possible antisecretory effect via prostaglandins receptors, to its antispasmodic compounds which may act by blocking muscarinic or serotonergic receptors on smooth muscles and which may reduce gastrointestinal secretion by decreasing diarrheal faeces. This work also confirmed the involvement of opiate receptors in the anti-diarrheal action of *Crinum jagus*.

Competing interests

The authors declare that there is no conflict of interests.

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