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Peace Doe
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana

Andaratu Iddrisu
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana

Peniel Lartey
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana

Kuupolo Elijah
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana

Samira Issaka
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana

Darko Ansah Enock
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana

Correspondence:
Peace Doe
Department of Pharmacology and Toxicology, School of Pharmacy, Central University Ghana, P.O. Box 2305, Tema Ghana
Email: pdoe[at]central.edu.gh

Evaluation of the Anti-Diarrheal activity of the ethanolic seed extract of *Annona muricata*

Peace Doe*, Andaratu Iddrisu, Peniel Lartey, Kuupolo Elijah, Samira Issaka and Darko Ansah Enock

ABSTRACT

Background: Diarrhea continues to be a public health problem in Ghana. The fruit and leaves of *Annona muricata* are used traditionally for the management of gastro-intestinal disorders. This study aims to investigate the ethanolic seed extract of *Annona muricata* for anti-diarrheal activity in rats. **Method**: Three different models were used to test for anti-diarrheal activity; gastrointestinal motility test, castor oil-induced diarrhea, and castor oil-induced gastro enteropooling. The observed effect of the extract at various doses (150mg/kg, 300mg/kg and 650mg/kg) was compared to both negative (distilled water 10ml/kg) and positive control (Loperamide hydrochloride 2mg/kg). **Results**: *Annona muricata* extract showed a dose-dependent reduction in diarrhea compared to the standard antidiarrheal drug, Loperamide hydrochloride and distilled water. The percentage inhibition in gastrointestinal motility calculated in reference to the controls was 58.98%, 67.34% and 14.69% for the highest concentration of the extract, Loperamide hydrochloride, and distilled water respectively. The percentage inhibition in defecation compared to the controls was 35.59% and 38.98% for 650mg/kg *Annona muricata*, and Loperamide hydrochloride respectively. The extract was able to inhibit intestinal fluid accumulation which was greater than the effect observed by Loperamide hydrochloride. **Conclusion**: The results obtained confirm the antidiarrheal activity of the seed extract of *Annona muricata* and thus provides the scientific justification for the traditional use of this plant for the management of diarrhea.

Keywords: *Annona muricata*, Ethanolic extract, Rats, Anti-diarrheal.

INTRODUCTION

Diarrhea is still the second principal cause of death among children below the age of five alongside respiratory infections [1]. Deaths in Ghana caused by diarrheal disease is 6630 or 3.15% of total deaths. The weighted death rate is 25.7% per 100000 population ranks Ghana number 49 in the world [2]. It has also been postulated that under-five children experience on an average 3.2 episodes of diarrhea every year and in developing countries, diarrheal disease accounts for about 21% of all deaths among under-five children [3, 4].

To alleviate this disease, traditional healers in Ghana make use of different medicinal plants including *Annona muricata*. *Annona muricata* is widely known as soursop due to the sour and sweet taste of its fruits [5]. *Annona muricata* has been used by various cultures as a natural remedy for a variety of ailments and has been reported to possess varied and interesting pharmacological properties. Different parts of the *Annona muricata* plant have been used to treat various ailments with the leaves and seeds being the main parts studied, perhaps because they are the most traditionally used [6].

The pharmacological activities of various parts of the plant include hypoglycemic [7, 8, 9], anti-cancer [10], hypotensive [11] wound healing [12, 13], gastroprotective [14], hepatoprotective [15], anti-inflammatory [16], anti-oxidant [17]. Furthermore, invivo studies indicate that the methanolic extract of the leaves of *Annona muricata* caused a 13.94% inhibition of gastrointestinal motility in mice [18]. Because of these reported pharmacological properties, there is the possibility that the seeds of the plant will also have antidiarrheal properties, though no experiments have been conducted to ascertain it. Therefore, this experiment is aimed at ascertaining the anti-diarrheal properties of the ethanolic seed extract of *Annona muricata*.

MATERIALS AND METHODS

Sample collection and identification

The fruits of *Annona muricata* were collected in Ashaiman in the Greater Accra region of Ghana in December 2018. It was then identified and authenticated by Miss Anna Kwarley Quartey of the Department of Pharmacognosy and Medicinal Chemistry, Central University Ghana. A sample was kept at the university's herbarium for further reference.

Sample preparation and extraction

The fruits were processed manually to de-pulp and extract the seeds, debris present on the seeds were cleaned. The seeds were then kept in the shade to dry. Drying was done for 14 consecutive days and was completed when consistent weights of the seeds were obtained. The seeds were milled to a coarse powder and the milled sample extracted with 80% ethanol using cold maceration method. The preparation was kept in the fridge and shaken intermittently for 7 days after which filtration was carried out. The rotary evaporator was used to evaporate the solvent. Evaporation was continued using a water bath to completely get rid of the liquid portion of the concentrate. The extract was collected quantitatively, packaged in an airtight container and stored in the fridge at 4^0C until use.

Experimental animals

Wistar albino rats weighing 95-105g were used for this experiment. The rats were obtained from the University of Ghana animal house and were kept in plastic cages at room temperature on a 12-hour light and dark cycle. They were allowed 7 days for acclimatization. The animals were provided standard pellet diet and water ad libitum. Care and handling of all animals before, during and after the experiments were according to internationally accepted guidelines [19]. Animals were fasted for 18 hours before the experiment.

Phytochemical screening

Sample of the crude extract was investigated for the presence of the following phytochemical constituents in the seeds of *Annona muricata*: tannins, saponins, phlobatannins, alkaloids, flavonoids, cardiac glycosides following standard procedures [20].

Acute Toxicity Test

Acute toxicity studies were also carried out on *Annona muricata*. Doses up to 2000mg/kg were administered orally to the animals according to guidelines stated by the Organization for Economic and Co-operative Development (OECD) [21]. The rats after administration of *Annona muricata* were observed continuously for the first 6 hours, and the following 24 hours for delayed toxicity.

Gastro-Intestinal Motility Test

This test was carried out according to standard methods [22] with slight modifications. The rats were fasted for 18 hours and were put randomly into groups of 5, each group having 5 rats each, after which their weights were taken. Group 1 was the negative control and rats were pretreated with distilled water (10ml/kg). Group two was positive control which was also pretreated with Loperamide hydrochloride (2mg/kg). Group 3, 4 and 5, were pretreated with increasing doses of *Annona muricata* (150mg/kg, 300mg/kg and 650mg/kg respectively). One hour after, 1ml of activated charcoal meal which served as a marker was administered to the rats in each group. The animals were sacrificed by cervical dislocation after an hour of the charcoal meal. The small intestines were harvested and the distance traveled by the charcoal meal from the pylorus to the caecum measured for each rat.

Castor oil-induced Diarrhea Test

The method for castor oil-induced diarrhea test as reported by Uddin *et al*. [23] was used. Rats were fasted for 18 hours, put randomly into groups of 5 and their respective weights taken. The rats were pretreated as described in the gastro-intestinal motility test above. 30 minutes after the pretreatment, 1ml of castor oil was administered to each animal. Each rat was carefully placed in an individual cage lined with A4 paper. Observation started 15 minutes after the administration of castor oil. The nature of stools and frequency of defecation was recorded for each rat every hour, for a total of 6 hours.

Castor oil-induced gastroenteropooling Test

The method described by Robert *et al*. [24] was employed with slight variations. Rats were fasted for 18 hours and distributed randomly into groups of 5. Pretreatment was done as described in the gastro-intestinal motility test above, and 1ml of castor oil administered after an hour. The rats were sacrificed after an hour upon administration of the castor oil by cervical dislocation. The small intestines, from the pylorus to the ceacum, were harvested. The weight of the small intestines with the content was weighed and recorded, then the content of the small intestines was pooled into a measuring cylinder and the volume recorded, the empty small intestines were weighed.

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM). One-way ANOVA test with Turkey's multiple comparisons test was used to analyze and compare the data using Graph pad prism 8 software, while $p<0.05$ was considered statistically significant.

RESULTS

Table 1: Showing the results of phytochemical screening

Phytochemical constituents	Present (+)
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Alkaloids	+
Proteins	+
Amino acids	+
Steroids	+
Phenols	+
Oils	+

Acute toxicity test

No mortality was observed at 2000mg/kg of *Annona muricata* for the initial 7 hours and subsequent 10 days after administration. This indicates that the ethanolic seed extract of *Annona muricata* has an LD50 value of greater than 2000mg/kg in rats.

Gastrointestinal motility test

The ethanolic seed extract of *Annona muricata* reduced normal gastro-intestinal motility significantly at 150mg/kg, 300mg/kg and 650mg/kg ($p<0.0001$) compared to the control as shown below.

Table 2: Showing the percentage inhibition of motility of rats by increasing doses of *Annona muricata*

Treatment groups	Mean Total length of small intestine (cm)	Mean Distance travelled by charcoal mean±SEM	Inhibition of intestinal motility (%)
Distilled water (10ml/kg)	77.60	66.2±4.980	14.69
Loperamide HCl (2mg/kg)	76.54	25.0±2.350****	67.34
Annona muricata (150mg/kg)	71.48	38.6±0.510****	50
Annona muricata (300mg/kg)	63.80	30.5±0.791****	52.35
Annona muricata (650mg/kg)	67.24	27.58±1.820****	58.98

Results are expressed as mean ± SEM (n=5). ****P<0.0001

Castor oil-induced diarrhea

The ethanolic seed extract of *Annona muricata* caused a significant reduction in diarrheal episodes with 650mg/kg of the extract producing the maximum effect (90.3%).

Table 3: Showing mean number of watery stools induced by the different treatment groups

Treatment groups	Mean number of watery stools±SEM	Inhibition of diarrhea (%)
Distilled water (10ml/kg)	6.2±1.36	0
Loperamide HCl (2mg/kg)	1.0±0.77**	83.87
Annona muricata (150mg/kg)	1.6±0.60**	74.19
Annona muricata (300mg/kg)	1.4±0.24**	77.42
Annona muricata (650mg/kg)	0.6±0.40***	90.3

Results are expressed as mean ± SEM (n=5). **P<0.01, ***P<0.001

Castor oil-induced gastroenteropooling

The volume and weight of small intestinal content were reduced significantly (p<0.0001) in a dose-dependent manner compared with the negative control as shown below. The highest reduction in volume of intestinal content was obtained by 650mg/kg of the extract.

Table 4: Showing the percentage reduction in volume of small intestinal content

Treatment groups	Mean volume of intestinal content ± SEM	Mean weight of intestinal content ±SEM	Reduction in volume of intestinal content (%)
Distilled water (10ml/kg)	1.08±0.02	1.354±0.071	0
Lopermaide HCl (2mg/kg)	0.8±0.105*	1.374±0.071	25.9
Annona muricata (150mg/kg)	0.68±0.037***	0.6200±0.070***	37.04
Annona muricata (300mg/kg)	0.48±0.004****	0.5760±0.103***	56.76
Annona muricata (650mg/kg)	0.28±0.025****	0.4960±0.086****	75.93

Results are expressed as mean ± SEM (n=5). *P<0.05, ***P<0.001, ****P<0.0001

DISCUSSION

This study was carried out to determine the antidiarrheal properties of the ethanolic seed extract of *Annona muricata* using three experimental models for diarrhea in rats. Diarrhea was induced in all models by administering castor oil to each rat. Castor oil is known to produce diarrhea through ricinoleic acid, its active metabolite released by the effect of lipases in the superior portion of the small intestines [25]. Ricinoleic acid activity in castor oil is mediated by binding to prostaglandin E2 receptor 3 (EP3) on the gastrointestinal smooth muscle cells. This action promotes fluid build-up in the intestines by impeding the absorption and increasing the secretion of fluids and electrolytes. Phytochemical evaluation of the ethanolic seed extract revealed the presence of flavonoids, tannins, and alkaloids (Table 1). Flavonoids have been reported to alter the synthesis of cyclooxygenase 1 and 2 (COX 1 and 2) and lipoxygenase (LOX) consequently blocking the synthesis of prostaglandin [26]. The tannins in the extract cause a precipitation of the proteins present in the mucosa of the intestine by making tannates, which make the mucosal layer of the small intestine more resistant to chemical modification and therefore cause a decrease in intestinal peristalsis and secretion [27, 28].

Most antidiarrheal agents act by inhibiting or reducing fluid secretion and decreasing the propulsive movement of gastrointestinal smooth muscles [29]. Therefore, to appropriately postulate its anti-diarrheal effect, *Annona muricata* was evaluated using gastrointestinal motility and castor oil-induced enteropooling tests. The extract was able to inhibit intestinal motility and this effect was dose-dependent (Table 2). The gastro-intestinal motility test was employed to observe the transit of intestinal content. The extract was able to reduce gastro-intestinal transit with percentage reduction in distance traveled by charcoal meal at 150mg/kg and 650mg/kg of *Annona muricata* were 50% and 58.98% respectively, compared to 14.69% and 67.34% for the negative and positive control respectively. A reduction in motility of the gut muscles extends the time substances spend in the intestines which in turn enables more time for fluid absorption [30]. The decrease in the distance travelled by the charcoal meal can be hypothesized to be as a result of the anti-motility agents present in the extract. Studies have shown that medicinal agents that are capable of inhibiting intestinal transit in pathophysiological states are effective in relieving diarrhea [31]. Phytochemical constituents such as flavonoids and tannins are proven to have antidiarrheal properties as a result of their anti-motility activity [32]. Thus, the significant anti-diarrheal activity observed by *Annona muricata* could be due to the anti-motility effect of flavonoids as well as tannins.

In the castor oil-induced diarrhea model, the extract produced a significant decrease in the number of watery stools (Table 3). The percentage inhibition of diarrhea exhibited by *Annona muricata* 650mg/kg was 90.3% compared to Loperamide hydrochloride at 83.67% (Table 3.0). The antidiarrheal properties exhibited by *Annona muricata* may be said to be through the inhibition of the synthesis of endogenous prostaglandin. This is because castor oil induces diarrhea by stimulating the biosynthesis of prostaglandins [33].

In the castor oil-induced enteropooling test, *Anonna muricata* caused a significant reduction in the build-up of intraluminal fluid compared to distilled water (negative control) and positive control (Table 4). The maximal effect was greater than the standard drug, Loperamide hydrochloride. Ricinoleic acid, an active metabolite of castor oil, causes irritation and inflammation of the mucosal wall of the small intestine, which in turn will result in the liberation of prostaglandins. The liberated prostaglandins increase fluid production by inhibiting the reabsorption of water and sodium chloride [34]. It can therefore be postulated that the *Annona muricata* extract caused a significant inhibition of hypersecretion and enteropooling in the gastrointestinal tract by increasing water and electrolyte reabsorption or by blocking the accumulation of intestinal fluids induced by castor oil. The anti-gastro enteropooling activity may be due to the presence of flavonoids, steroids, and tannins [35, 36].

CONCLUSION

The findings of this study provide convincing evidence that the ethanolic seed extract of *Annona muricata* possesses significant antidiarrheal activity. However, further studies are needed to isolate the

active compound(s) and to determine the precise mechanisms responsible for the observed pharmacological activity.

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