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Research Article
Intestinal Histological Evaluation and Antidiarrhoeal Effect of *Allium sativum* Juice in Experimentally Induced Diarrhoea in Rats

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Abstract

Background and Objective: Diarrhea is one of the leading causes of mortality in less developed countries yearly. Hence, it has become pertinent in modern medical research to search for new sources of antidiarrhoeal drugs. The present study evaluated the effects of *Allium sativum* cloves juice extract on lactose monohydrate and castor oil-induced diarrhoea in albino rats. **Materials and Methods:** Antidiarrhoeal effect of *Allium sativum* juice extract (ASJE) was evaluated using lactose monohydrate/castor oil-induced diarrhoea and gastrointestinal motility tests using two doses (5 mL and 10 mL kg^{-1} b.wt.) of ASJE. Body weight changes and faecal droppings were recorded and the caeca of rats were excised for necropsy and histological studies. The activity of ASJE was compared to loperamide (5 mg kg^{-1}) and atropine sulphate (3 mg kg^{-1}) which were used as standard reference drugs. **Results:** ASJE showed a marked antidiarrhoeal effect by inhibiting 69.23 and 76.92% of diarrhoea at doses of 5 and 10 mL kg^{-1}, respectively. The extract also significantly reduced intestinal transit ($p<0.05$) in GI motility test compared to the corresponding control. Caecum distention, goblet cell hyperplasia and inflammatory cellular were observed to be markedly prevented by ASJE treatment. The effects observed were comparable to those of the standard drugs. **Conclusion:** The findings from the present study indicated that the juice extract of *Allium sativum* cloves possess the ability to prevent experimentally induced diarrhoea in rats.

Key words: Intestine, histology, *Allium sativum*, diarrhoea, anti-diarrheal effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of herbal medicines by millions of people in developing countries is due to their local availability and ready prescription by traditional medicine practitioners who are a part of their community. Up to eighty percent (80%) of the world population rely on traditional medicines, which are predominantly plant-based[1]. A previous report documented that over 90 and 40% of Nigerians in the rural areas and urban areas, respectively, depend partly or wholly on traditional medicines for their health care needs[2]. The use of herbal medicines as complements or alternatives to orthodox medicines has been on the increase. The reasons, which have given rise to this trend, include: availability, cheapness and accessibility of these natural remedies[3]. However, their use is limited because many of the claimed medicinal values have not been scientifically evaluated and their safety profiles uncertain[4].

Allium sativum commonly called garlic is a bulbous perennial herb belonging to the family Liliaceae. The plant is also known as stinking rose, camphor of the poor and nectar of the gods[5]. It has its origin in antiquity and is widely recognized not only as a vegetable and seasoning for food but also for its medicinal properties. Chemical studies reveal that garlic contains a large spectrum of different compounds ranging from at least 33 sulfur compounds,17 amino acids and minerals such as selenium[6]. Allicin is a sulfur compound of garlic released when the garlic bulb is crushed giving the pungent characteristic odour which is believed to be responsible for some of the plant's pharmacological activity[7,8]. This concept is employed in native communities where garlic is consumed by chewing the bulb to access its medicinal effect. Claims in traditional folklore have been made concerning its medicinal effect such as protection against heart disease, lowering of cholesterol, epilepsy, whooping cough, treatment of diarrhoea and many others[9]. Pharmacological action of garlic includes antimicrobial, hypocholesterolaemic, hypotensive, antiplatelet activity and anticancer activities[10,11].

Diarrhoea is one of the leading causes of mortality in millions of children usually under the age of five[12]. Children living in unhygienic environment especially those in low and middle income countries mainly in Africa tend to suffer this disease[12,13]. Most people are affected by diarrhoea at some point in their lives and is commonly found among individuals living in crowded environment[14]. Diarrhoea presents various symptoms like dehydration, stomach pains, faecal urgency. The primary aim in treatment of diarrhoea is to reverse gastrointestinal hyper motility to normal, reduce faecal urgency, prevent dehydration and nutritional complications[12,15,16]. Despite the unified attempt made by international organizations to control this disease, its incidence still remains high[17]. Antidiarrhoeal drugs and therapies like oral rehydration solution and zinc supplement have been produced but there are accessibility challenges by those in rural settlement in developing countries[12]. The need to search for safe, cheap and readily available natural drugs as alternatives to conventionally used antidiarrhoeal agents is imperative. In traditional practice, garlic is believed to have antidiarrhoeal activity and has been used to control diarrhoea, particularly in children but a scientific validation to this claim is not yet documented. The present study therefore investigated the antidiarrhoeal effect of *Allium sativum* juice extract on experimentally-induced diarrhoea in rats.

MATERIALS AND METHODS

This study was performed at Panacea Diagnostic and Research Laboratories, Enugu, Enugu State from September, 2015-August, 2016.

Plant collection: Fresh bulbs of garlic (*Allium sativum* L.) were purchased from a retail food store in Ogbete Main market, Enugu, Enugu State, Nigeria in the month of March, 2016. Plant material identification and comparison with the voucher specimen deposited at the Herbarium Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka was done.

Preparation of fresh juice extract: Two hundred grams of fresh garlic cloves were peeled, washed and crushed in a fruit juice extracting machine. The resultant juice homogenate was further filtered twice using a muslin cloth. The juice was placed in a storage leak-proof container and kept in a refrigerator (4±2°C) until needed.

Animals: Thirty-six male rats (150-180 g) of the Wistar strain were obtained from the Animal house of the Department of Physiology, University of Nigeria. The animals were kept in clean cages in the Animal House of Panacea Diagnostic and Research Laboratories, College Road, Enugu State. The animals were kept under standard environmental conditions, a 12:12 h light/dark cycle and temperature of 25±2°C. Pyrogen free water and commercially available rat pellets (Guinea Feed®, Benin Nigeria) were provided for the animals *ad libitum*. The animals were allowed to acclimatize for 1 week at the laboratory condition prior to the experimentation.

Animal handling was in accordance with Institutional and International guidelines for care and use of Animals in Scientific Research[18].

Drugs and chemicals: Loperamide and atropine sulphate (Sigma Chemical Co., St. Louis, MO, USA), normal saline (NaCl 0.9%), lactose monohydrate [$C_{12}H_{22}O_{11}.H_2O$], castor oil and charcoal meal (10% active charcoal in 100ml of 5% aqueous tragacanth) were used.

Induction of diarrhoea in the rats: The method described by Mitra *et al.*[19] was used but with slight modification. The rats were fasted for 18 h prior to the induction. Diarrhoea was induced in the animals by feeding with Lactose mixed diet [50% lactose and 50% commercial rat chow][20] for 7 days. However, further aggravation of the diarrhoea was achieved using castor oil on day 7 only. The control rats were fed the commercial chow and water only. The passage of loose or watery stool indicated diarrhoea in the animals.

Experimental design: Twenty rats were divided into 5 groups with each group comprising of 4 animals. Rats in group 1 (normal control) and 2 (diarrhoeal control) were administered with distilled water. Groups 3, 4 and 5 comprised diarrhoeal rats which were administered with 5 mg kg^{-1} b.wt.) of loperamide, 5 and 10 mL kg^{-1} b.wt. of *Allium sativum* juice extract [ASJE] respectively from day 3-7 once daily. All drug administration throughout this study were done orally using an oral cannula. The body weights of all rats before and after the experiments were measured and the body weight differences were determined. Faecal droppings were collected after 4 h on the first day of drug treatments and weighed[19].

Gross and histopathological studies: At the end of the treatments, the rats were weighed and euthanized using chloroform. They were cut open and necropsy was conducted to determine macroscopical changes in the gastrointestinal tract and other visceral organs. The relative organ weight of some visceral organs (liver, kidney, spleen and caecum) was estimated. The caeca and colons were also closely examined for signs of distension (gas bloating). Caeca lengths and widths were further measured using a measuring thread and tape. Caeca of all rats were subsequently rinsed in normal saline and fixed in 10% formal saline prior to histological processing for microanatomical examination. Haematoxylin and Eosin (H and E) staining procedure was employed to stain the tissue sections for light microscopical examination[21]. The sections were examined using Olympus Binocular microscope with in-built lighting system and areas of interest were photomicrographed using an AmScope digital microscope-eyepiece-camera.

Gastrointestinal motility test: This test was conducted as described by Chitme *et al.*[22]. Sixteen experimental rats were used and divided into four groups of 4 rats each. The animals were fasted for 18 h before the experiment but were allowed free access to water. The rats in the 1st group were given normal saline orally and served as negative control while those in the 2nd group received Atropine sulphate (3 mg kg^{-1}) intraperitoneally. The 3rd and 4th groups received 5 mL kg^{-1} and 10 mL kg^{-1} b.wt., of the ASJE, respectively. After 1 h, 1 mL of castor oil was administered orally to the 4 groups followed by 1 mL of the marker [charcoal meal in aqueous tragacanth] which was administered orally 1 h after castor oil treatment. The rats were sacrificed 1 h later and the distance travelled by the charcoal meal from the pylorus to the caecum was measured using a measuring tape calibrated in centimeter. The full length of the intestine was equally measured and the percentage motility was calculated thus[23]:

$$\text{Motility (\%)} = \frac{\text{Distance travelled by charcoal meal}}{\text{Total length of the small intestine}} \times 100$$

Statistical analysis: Data obtained from the study were expressed as Mean±SEM Statistical Package for Social Sciences software program (SPSS, Chicago, IL, version 20.0) was used for the analysis. Data were subjected to one-way analysis of variance (ANOVA). This was followed by Tukey-highest significant difference (HSD) post-hoc test and student's t-test to determine the statistical significance of the differences in the parameters among the groups. The level of significance was considered at $p<0.05$.

RESULTS

Effect of ASJE treatment on body weight: Rats in all groups (control and treatment groups) decreased in weight by the end of the study (Fig. 1). The highest and least weight losses were observed in 5 and 10 mL kg^{-1} ASJE treated rats, respectively. However, no significant difference was observed in the body weight differences ($p>0.05$).

Effect of lactose/castor oil induced diarrhoea: Figure 2 show the weight of faecal droppings of the experimental rats in diarrhoea-induced groups. There was a significant decrease ($p<0.05$) in the faecal droppings' weight between the

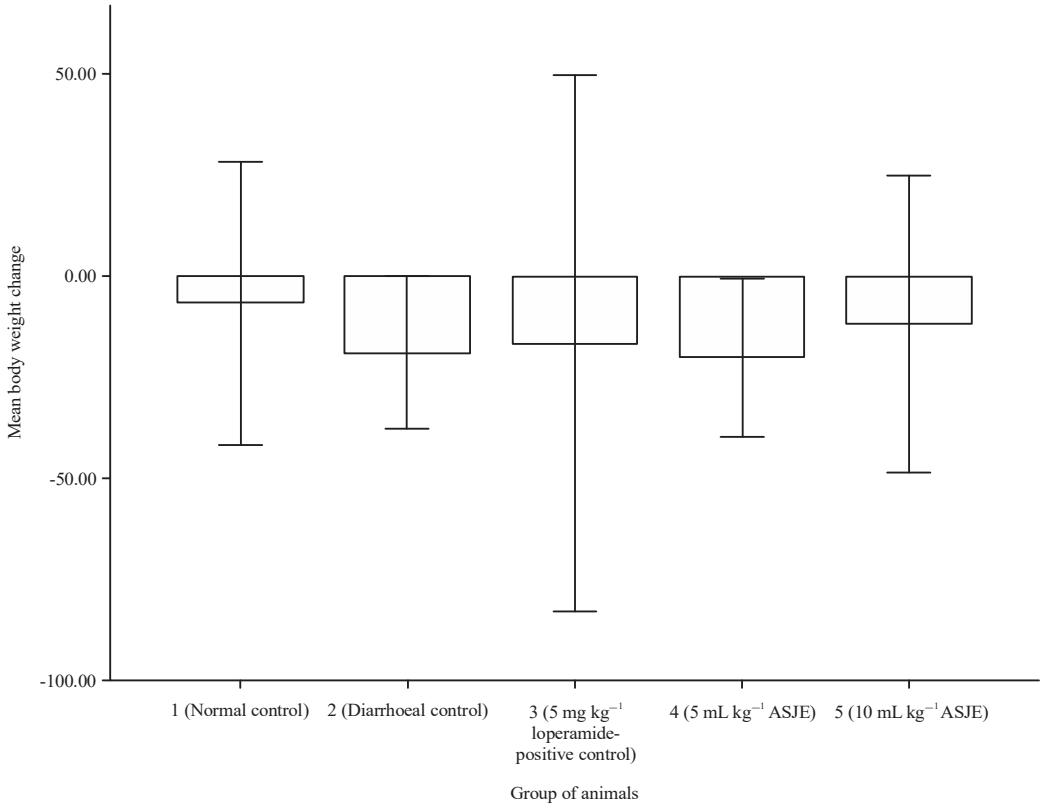

Fig. 1: Bar charts showing the effects of treatment with ASJE on body weight change

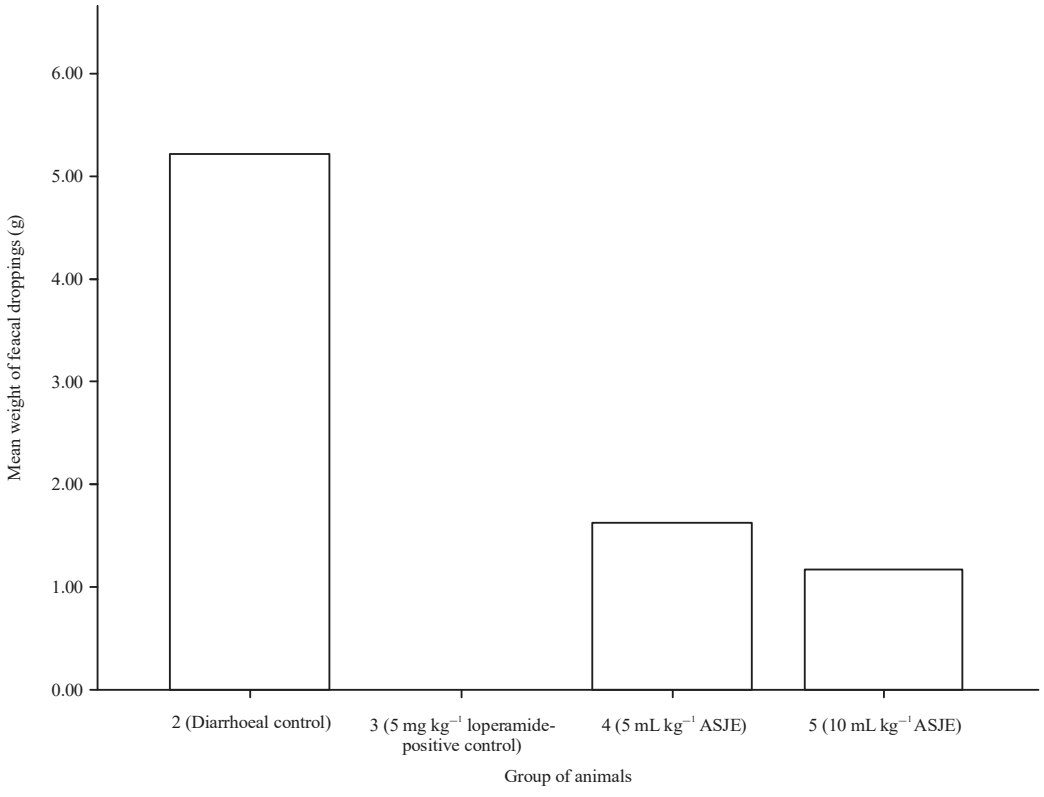

Fig. 2: Bar charts showing the effects of treatment with ASJE on weight of faecal droppings

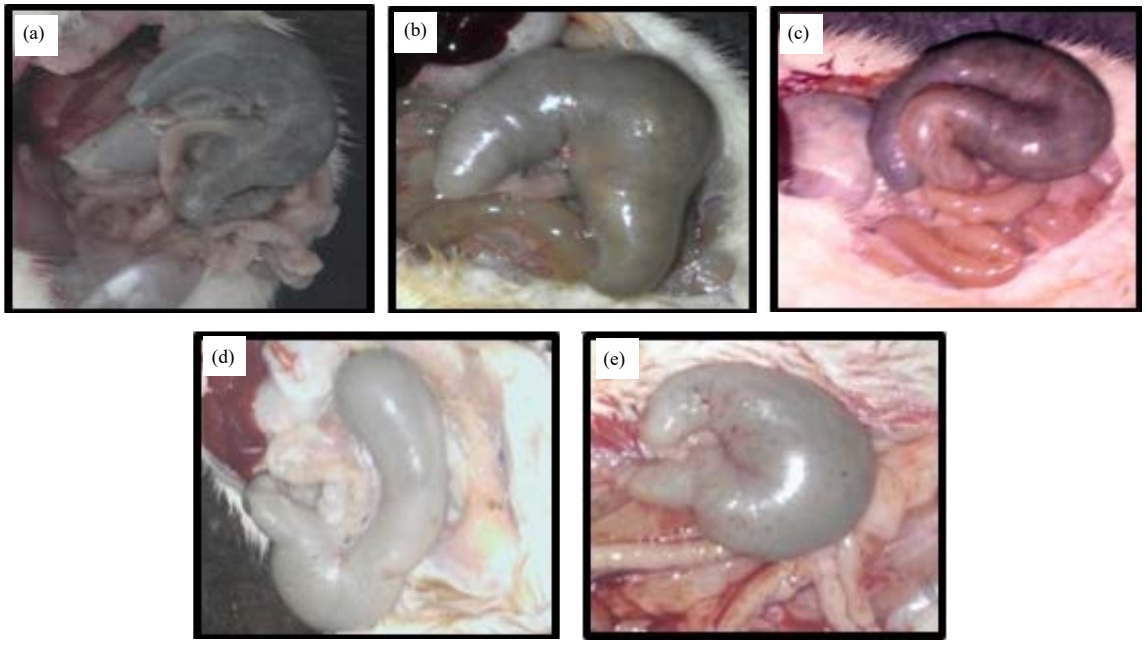

Fig. 3(a-e): Photomacrographs of rats' Caeca demonstrating the effects of ASJE and loperamide on lactose-induced caecum distension, (a) Normal control (group 1) shows relatively normal small shaped caecum, (b) Diarrhoeal control (group 2) marked caecum distension is observed, (c) Loperamide control, (d) 5 mL kg^{-1} and (e) 10 mL kg^{-1} ASJE groups (groups 3, 4 and 5, respectively)
All shows reduced caeca distension, caecum of group 4 being the least bloated

Table 1: Effect of treatment with *Allium sativum* juice extract on relative organ weighs of diarrhoeic rats

	Relative organ weights			
Groups	Liver index (%)	Right kidney index (%)	Left kidney index (%)	Spleen index (%)
Normal control	5.15±0.10[b]	0.39±0.02	0.41±0.01	0.48±0.08
Negative control (Diarrhoea model)	4.11±0.36[a]	0.39±0.02	0.42±0.02	0.51±0.07
Positive control (Loperamide)	3.66±0.11[a]	0.44±0.02	0.42±0.00	0.48±0.08
Low dose ASJE (5 mL kg^{-1})	3.83±0.24[a]	0.46±0.01[ab]	0.46±0.03	0.51±0.09
High dose ASJE (10 mL kg^{-1})	4.05±0.25[a]	0.46±0.02[ab]	0.46±0.02	0.44±0.04
F-ratio	6.260	4.235	1.146	0.167
Significance	0.004	0.017	0.373	0.952

Data expressed in Mean±SEM, [a, b]$p<0.05$ when compared to the normal control and diarrhoea control respectively, ASJE-*Allium sativum* juice extract

diarrhoeal control and ASJE-treated groups. Between the 2 doses of ASJE, group 5 treated with 10 mL kg^{-1} of ASJE had the higher percentage of inhibition (76.92%) than that offered by 5 mL kg^{-1} of ASJE (69.23%). No significant difference was observed between the rats given Loperamide at 5 mg kg^{-1} and the 2 extract doses.

Gross anatomical findings: The excised tissues (liver, kidney and spleen) showed no obvious signs of tissue degeneration, necrosis or haemorrhage macroscopically. The results of the relative organ weights determination are shown in Table 1. Liver indices of all diarrhoea-induced rats (groups 2-5) were significantly decreased ($p<0.05$) when compared to control. Right kidney index of rats in ASJE-treated rats were observed to be significantly increased ($p<0.05$) when compared to both the normal and diarrhoea controls. No change was observed in left kidney and spleen indices in all the treatment groups when compared with the controls ($p>0.05$).

As shown in Fig. 3, macroscopically, the caeca and colons of diarrhoea control (group 2) rats showed obvious signs of distension (bloating) when compared with normal control (group 1). Caeca and colons of loperamide and ASJE-treated rats showed signs of bloating but not as that observed in diarrhoea control (group 2). Caecum length and width of diarrhoea-control group were significantly increased ($p<0.05$) when compared to the normal control (Table 2).

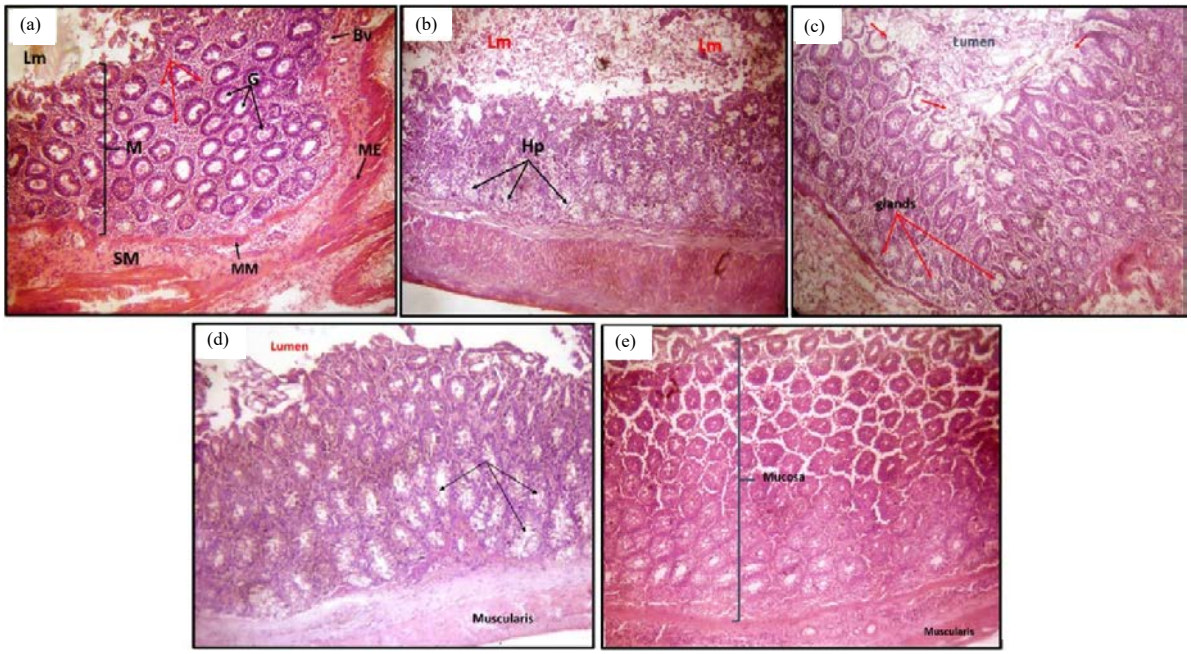

Fig. 4(a-e): Photomicrographs of caecum transverse sections of rats from control, loperamide and ASJE-treated groups, [Stain: Haematoxylin and Eosin/Magnification×100], (a) Normal control group 1 shows normal histoarchitecture, normal mucosa (M) bearing glands (G), lamina propria (red arrows), submucosa (SM), muscularis mucosa (MM), muscularis externa (ME), lumen (Lm) and blood vessels (Bv), (b) Diarrhoeal control group 2 show extensive histomorphological alteration, the glands show increased goblet cell hyperplasia (Hp), cellular debris and inflammatory cellular infiltration are observed within the lumen (Lm), (c) Loperamide group 3 shows evidence of tissue preservation, glands show marked reduction of goblet cell hyperplasia, mild erosion of superficial mucosal region is noted (red arrows), (d) 5 mL kg^{-1} ASJE treated group 4 shows evidence of tissue preservation, marked reduction of goblet cell hyperplasia is noted, cellular infiltration is not evident and (e) 10 mL kg^{-1} ASJE treated group 5 marked preservation of caecum histoarchitecture is observed

Table 2: Effect of treatment with crude *Allium sativum* on caecum weight, index, length and width of diarrhoeic rats

Groups	Caecum weight (g)	Caecum index (%)	Caecum length (cm)	Caecum width (cm)
Normal control	9.28±0.43^{b}	6.79±0.53	5.00±0.23^{b}	4.20±0.24^{b}
Negative control (Diarrhoea model)	6.68±0.80^{a}	5.20±0.52	9.65±0.24^{a}	6.70±0.21^{a}
Positive control (Loperamide)	6.80±0.22	5.46±0.38	8.67±0.10^{ab}	6.00±0.58^{a}
Low dose ASJE (5 mL kg^{-1})	7.13±1.68	5.61±1.47	6.65±0.26^{ab}	4.50±0.44^{b}
High dose ASJE (10 mL kg^{-1})	3.30±0.09^{ab}	2.94±0.11^{a}	7.95±0.56^{ab}	5.15±0.30^{b}
F-ratio	6.209	3.432	33.009	7.493
Significance	0.004	0.035	0.000	0.002

Data expressed in Mean±SEM, $^{a,b}p<0.05$ when compared to the normal control and diarrhoea control respectively, ASJE: *Allium sativum* juice extract

ASJE treatment significantly reduced these parameters in a manner better than that of the standard drug (Loperamide).

Histological examination: Figure 4 shows the light microscopical findings of groups 1 -5. In Caecum section from Control rats (group 1), normal tissue architecture with prominent features are shown. In the Diarrhoeal control (group 2), the caecum section showed extensively increased goblet cell hyperplasia and infiltration of inflammatory cells. The histological features of rats treated with ASJE (group 4 and 5) showed a marked reduction in goblet hyperplasia and good preservation of the caecum histoarchitecture in the manner comparable with that offered by loperamide (group 3).

Gastrointestinal motility: As shown in Table 3, castor oil increased the gastrointestinal motility in the untreated diarrhoeal rats. However, treatment with ASJE significantly

Table 3: Effect of treatment with crude *Allium sativum* on gastrointestinal motility

Groups	Treatments	Parameters		
		Total length of intestine (cm)	Distance travelled by marker (cm)	Intestinal transit distance (%)
Negative control	Castor oil+Charcoal meal+Normal saline	105.12±4.11	62.75±6.00	59.38±4.31
Positive control	Castor oil+Charcoal meal+Atropine sulphate	108.25±2.89	47.50±4.90	44.28±5.77
Low dose ASJE	Castor oil+Charcoal meal+5 mL kg^{-1} of ASJE	94.12±4.85	7.00±0.91*	7.43±0.89*
High dose ASJE	Castor oil+ Charcoal meal+10 mL kg^{-1} of ASJE	101.37±1.67	2.00±1.41*	1.91±1.34*
F-ratio		2.86	56.98	57.45
Significance		0.81	0.00	0.00

Data expressed in Mean±SEM, *p<0.05 when compared to the negative control (group I), ASJE: *Allium sativum* juice extract

decreased gastrointestinal movement in a dose-dependent manner better than that offered by the standard drug (Atropine sulphate).

DISCUSSION

Diarrhoea is usually considered a result of altered motility and fluid retention or accumulation within the intestinal tract[24]. Many antidiarrhoeal agents act by reducing the GIT motility and/or secretion. The present study examined the effectiveness of *Allium sativum* on GIT motility and treatment against diarrhoea using a model of lactose monohydrate/castor oil-induced diarrhoea.

Castor oil is a laxative which has been used extensively in pharmacological tests for screening and evaluation of antidiarrhoeal agents in experimental models[25]. Its co-administration with lactose monohydrate in the present study produced a severe diarrheic state visually evidenced by defecation of loose stools by the treated rats. Castor oil exerts laxative effect due to its active metabolite, ricinoleic acid, which causes irritation and inflammation of the intestinal mucosa, leading to a release in prostaglandins resulting in an increase in peristaltic activity. Thus as motility is stimulated, changes in electrolyte permeability of the intestinal mucosal membrane ensues[26,27]. Lactose monohydrate, on the other hand, is being broken down to form short fatty chain acids, carbon dioxide and hydrogen, which result in bloating and osmotic diarrhoea[19].

In this study, oral administration of the juice extract of *A. sativum* showed significant antidiarrhoeal activity comparable to the reference drug by decreasing the diarrhoea induced by the lactose-castor oil treatments. This was evidenced by the profound dose-dependent decrease in the frequency and weight of defecation. This yielded an increased percentage inhibition of diarrhoea up to 76.92% for the higher dose of 10 mL kg^{-1} of ASJE producing a better effect than the low dose (5 mL kg^{-1}). It is therefore suggestive that the antidiarrhoeal activity of *A. sativum* revolves around the higher dose used in this study. A possible mechanism to this activity may be by anti-prostaglandin activity or by stimulation of water and electrolyte re-absorption from the intestinal lumen.

Microscopically, castor oil treatment produced histoarchitectural alterations in the intestinal mucosa of treated rats in the present study. This finding is supported by previous works on cytotoxicity of castor oil on intestinal epithelial cells causing mucosal structural changes consistent with enhanced mucosal permeability[28]. In addition, treatment with lactose monohydrate in this study, caused caecum distension (bloating) as observed at necropsy whereas microscopically, inflammatory cellular infiltration and goblet cell hyperplasia were noted. These findings corroborate with previous studies by Mitra *et al.*[19] and Tellez *et al.*[29], however, upon treatment with the juice extract of *A. sativum,* a better preservation of the intestinal mucosa was observed with significant reduction in bloating, goblet cell hyperplasia and no evidence of cellular infiltration in the lamina propria.

The assessment of effect of laxatives in GIT motility test model using activated charcoal as marker have been used for many years. This method indicates the maximum distance travelled by the marker in the small intestine in a given time interval after its administration[30]. In the present study, administration of the doses of the juice extract caused a statistically significant reduction in the distance covered by the marker. This inhibitory effect of the extract on gastrointestinal motility is better than that offered by the standard drug, atropine, an antimuscarinic agent which is known to inhibit peristalsis[31]. *A. sativum* extract in the present study seems to have exerted its effects by a similar action on peristalsis enabling the effective inhibition of intestinal motility. Most antidiarrhoeal agents have demonstrated profound abilities to reduce intestinal transit[26,32].

Antidiarrhoeal activity have been found in plants possessing tannins, alkaloids, saponins, flavonoids, steroids and terpenoids[33,34]. Phytochemical screening of *Allium sativum* in a previous study revealed the presence of

alkaloids, terpenoids, saponins, flavonoids, tannins, anthraquinones and glycoside[35]. Presence of tannins in a plant extract denatures proteins which form protein tannates that make the intestinal mucosa more resistant to chemical alteration thereby reducing secretion[36]. More so, the antidiarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility and hydroelectric secretions which are known to be altered in diarrhoeic conditions[27]. It is therefore possible that the tannins and flavonoids content of the extract among others may be the responsible principles for the antidiarrhoeal effect of *A. sativum*.

Antidiarrhoeal activities of some agents have been associated with their antimicrobial action[37]. It is well known that bacteria and viruses are causative agents of diarrhoea[38] and research has shown that *A. sativum* has demonstrated effective antibacterial activity against some diarrhoea-causing bacteria[39]. This implies that the extract may prove to be a potent antidiarrhoeal agent in treatment of diarrhoea cases caused by such organisms in infected individuals.

This study indicated that the juice extract of *A. sativum* could possibly prevent diarrheal caused by laxatives, thus supporting its use in the treatment of diarrhoea in traditional settings. Findings from this study also suggests that no adverse health effect would be expected following treatment with ASJE up to 10 mL kg^{-1}. However, further toxicological studies using the juice should be confirmed. Further studies are also required to identify and characterize the active principles and also establish the mechanisms responsible for the antidiarrhoeal activity demonstrated in this study.

CONCLUSION

Data from the present study undoubtedly demonstrated that *A. sativum* possesses significant antidiarrhoeal activity in experimentally-induced diarrhoea in rats. In this study, it was observed that ASJE protected the intestinal histomorphology, decreased intestinal transit distance and number of faecal droppings. These findings indicated that ASJE could be a potential drug for the treatment of diarrhoea.

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SIGNIFICANCE STATEMENT

The study discovers the antidiarrhoeal effect of *Allium sativum* juice extract in experimentally induced diarrhoea that can be beneficial to decrease the risks of diarrhoea especially among children. The study will help researchers to find the active compound which is responsible for the antidiarrhoeal effect that many researchers were not able to explore. Thus a new idea on preparation of extract from *A. sativum* for use against diarrhoea-causing agents may prevent diarrhoea in vulnerable individuals.

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