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Effect of zinc treatment on intestinal motility in experimentally induced diarrhea in rats

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Summary: Zinc supplementation is a critical new intervention for treating diarrheal episodes in children. Recent studies suggest that administration of zinc along with new low osmolarity oral rehydration solutions / salts (ORS) can reduce the duration and severity of diarrheal episodes for up to three months. Several mechanisms of action of zinc has been proposed, however there is dearth of information about the effect of zinc on intestinal motility during diarrhea. Male albino Wistar rats (80-100g) were used. The effect of different doses of zinc sulphate (25, 50, 100, 150mg/Kg) on the number of wet faeces was investigated. Intestinal motility during castor oil induced diarrhea was assessed using activated charcoal meal and the mechanisms of action of zinc sulphate on motility were investigated. The effective dose of zinc sulphate (100mg/Kg) significantly reduced ($p< 0.001$) the number of wet faeces (3.0 ± 0.00) compared with control (6.8 ± 0.25) during diarrhea. This antidiarrheal effect of zinc was abolished by propranolol and nifedipine. Zinc sulphate significantly reduced ($p< 0.05$) intestinal transit time (60.7 ± 7.13%) compared with control (85.7 ± 2.35%). It is concluded that zinc sulphate reduces the frequency of wet faeces output and intestinal motility during diarrhea via activation of β adrenergic receptor and L-type Ca^{2+} channel.

Keywords*:* Diarrhea, Zinc, Intestinal motility, Adrenergic receptor, Calcium channel.

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INTRODUCTION

Diarrhea is the passage of three or more unformed stool per day, often in addition to other enteric symptoms, or the passage of more than 250 g of unformed stool per day (DuPont 2004). It includes loss of electrolytes and water and decreased absorption of fluid from the gastrointestinal tract. It also involves increased frequency of bowel movements (Lakshminarayana *et al*., 2011). Diarrhea has long been recognized as one of the most important health problems and leading cause of mortality and morbidity in the developing countries (Rajamanickam *et al*., 2010) and produces more illness and causes death of more infants and children below 5 years old than all other diseases combined (Dalal *et al*., 2011). Diarrhea is considered as one of the leading causes of growth retardation and death in infants (Petri *et al*., 2008).

Acute diarrhea remains a leading cause of childhood deaths despite the undeniable success of Oral Rehydration Therapy (ORT). Oral rehydration solution (ORS) saves children's lives, but does not seem to have any effect on the length of time the children suffer with diarrhea. Hence, new revised recommendations have been formulated by the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF), in collaboration with the United States Agency for International Development (USAID) and other experts. It recommends zinc salt along with low osmolarity ORS, with reduced levels of glucose and salt, during acute diarrhea, which reduced the duration and severity of the episode; and zinc supplementation given for 10-14 days lowers the incidence of diarrhea in the following two to three months (WHO / UNICEF Joint Statement, 2004).

Zinc is an essential micronutrient for human growth, development, and maintenance of the immune system. Zinc supplementation has been found to reduce the duration and severity of diarrheal episodes and likelihood of subsequent infections for 2–3 months (Bhutta *et al*., 2000). Zinc supplements are generally accepted by both children and caregivers and are effective regardless of the type of common zinc salt used (zinc sulphate, zinc acetate or zinc gluconate). The positive action of zinc in acute diarrhea are due to the ability of zinc to improve absorption of fluid and electrolyte, improve regeneration of the intestinal epithelium, increase the levels of brush border enzymes, increase protein synthesis, cell growth and differentiation, improve immune function, and regulate oxidative stress (Berni *et al*., 2011; Patel *et al*., 2005). Zinc inhibits toxin-

induced cholera, but not Escherichia coli heat-stable, enterotoxin-induced, ion secretion in cultured Caco-2 cells (Berni *et al*., 2005).

Literature review however revealed dearth of information about the effect of zinc on intestinal motility during diarrhea. Alterations in intestinal motility (usually increased propulsion) are observed in many types of diarrhea. This research was therefore carried out to investigate the effect of zinc on intestinal motility during diarrhea and the mechanism by which zinc might affects motility.

MATERIALS AND METHODS

Male albino Wistar rats (80-100g) were used for the experiments. The animals were housed under standard controlled environmental conditions with a 12 hour light/dark cycle, with food (Pfizer Feed Plc, Nigeria) and water provided *ad libitum*. The animals were allowed to acclimatize for one week before the experiments.

The rats were then kept in plastic cages whose floors were lined with white blotting paper for two hour daily in order to allow them to be familiar with the environment two weeks. Wire gauze was placed about 2 cm above the papers so as to prevent the rats from eating up the papers. For all the experiments, the animals were fasted for 18 hours before treatment.

Effects of zinc on fecal pellet output in castor oil induced diarrhea

The antidiarrheal activity of zinc sulphate was evaluated according to the method described by Teke *et al*. (2007). The animals were fasted for 18 hours and divided into five groups of five animals each. All rats were put in a separate cage. Each animal was given 1ml castor oil. After thirty minutes, each animal in the different groups were treated as follows: group 1 was administered 10ml/Kg normal saline (p.o); group 2, loperamide (3mg/Kg, p.o); group 3, zinc sulphate (25mg/Kg, p.o); group 4, zinc sulphate (50mg/Kg, p.o); group 5, zinc sulphate (100mg/Kg, p.o) and group 6, zinc sulphate (150 mg/Kg, p.o). The fecal pellets of the rat was counted every 2 hours from the time of zinc treatment for the first 8 hours and weight of faeces overnight (24 hours) after diarrhea induction were obtained. The presence of wet faeces was noted for each animal. The pellets were air dried for another 24 hours before weighing. The average weight of the pellets was taken as the output for each rat in the group.

Effects of blockers on fecal pellet output in castor oil treated animals given zinc.

The dose that produced the greatest effect on fecal pellet output was used as the working dose for this study. Twenty-five rats were divided into five groups of 5 rats each. Each rat in group 1 was pre-treated with 10ml/Kg normal saline (i.p.), while each rat in group 2 was given 10 mg/kg propranolol (i.p), group 3, 1mg/Kg prazosine (i.p), group 4 was given 2.5mg/Kg nifedipine (i.p) and group 5 was given 0.1mg/Kg atropine (i.p). Thirty minutes later, each animal was given 1ml of castor oil and after 30 minutes, each rat was given the working dose of zinc. Fecal pellet output was recorded every hour for 4 hours.

Effects of zinc on intestinal transit

The method of Gamaliel *et al*., (1996) was used. Fifteen rats were divided into three groups of 5 rats each. Each animal was given 1ml of castor oil. Thirty minutes after castor oil administration, animals in group 1 were given 3ml/Kg normal saline (p.o), animals in group 2 were administered 0.8ml of working dose of zinc and animals in group 3 were given 0.1mg/kg standard drug atropine (i.p). Thirty minutes after the last dose of drug was given 10% activated charcoal in physiological saline in a volume of 3ml/Kg was administered to each animal. One hour after charcoal meal, the rats were sacrificed and the abdomen immediately cut open to dissect out the whole small intestine (pylorus region to caecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured for obtaining the percentage of the entire small intestine travelled by the activated charcoal as described below:

$$\%\ \text{distance travelled} = \frac{\text{distance travelled}}{\text{Total length of the intestine}} \times 100$$

Statistical analysis:

Results were presented as mean ± standard error of mean (SEM). The student t-test was used to determine the significant difference between two groups. Confidence interval of 95% was taken as statistically significant. Data was analysed using SPSS version 17 software.

RESULTS

Effects of zinc on wet fecal pellet output

Result showed that castor oil administration induced diarrhea (wet faeces) in the animals. Zinc sulphate administration reduced the number of faecal pellet output. This inhibition was not effective at 25mg/Kg Zinc sulphate administration ($p>0.05$). However, zinc sulphate at a dose of 50mg/Kg, 100mg/Kg and 150mg/Kg significantly ($p<0.05$) reduced the number of wet feacal pellets compared with control. Table 1 showed that 8 hours after rats were given castor oil, the mean number of wet faeces in control rats was 6.8 ± 0.25. Zinc sulphate (50mg/Kg) significantly ($p< 0.05$) reduced the number of wet faeces (5.0 ± 0.41), a dose of 150mg/Kg significantly ($p< 0.001$) reduced wet fecal pellet output (3.3 ± 0.25) and the most effective dose in this study was 100mg/Kg which significantly ($p< 0.001$) reduced the number of wet faeces to 3.0 ± 0.0. The 100mg/Kg dose was therefore

taken as the Working Dose. The standard antidiarrhea drug loperamide (3mg/Kg) produced a significant reduction ($p< 0.001$) in number of wet faeces output all through the 8 hours of study. The reduction in number of wet output in loperamide treated animals was not significantly different from that in zinc treated (100mg/Kg)

Effects on zinc on mean weight of faeces after 24 hours castor oil induced diarrhea

Figure 1 showed that all doses of zinc sulphate used in this study (25, 50, 100, 150mg/Kg) produced significant ($p< 0.05$) decrease in the mean weight of faeces after 24 hours of diarrhea induction and drug treatment. Loperamide (3mg/Kg) also produced a decrease in faecal weight after 24 hours of treatment as shown in Figure 1.

Effects of blockers on wet faecal pellet output in castor oil treated animals given zinc

Result showed that by the 4th hour after diarrhea was induced, propranolol and nifedipine significantly ($p< 0.001$, 0.001 respectively) abolished the antidiarrhea effect of zinc sulphate. There was no significant difference ($p> 0.05$) in number of wet faeces between prazosine treated animals and animals given normal saline, while the antidiarrheal effect of zinc sulphate was further potentiated by atropine. There was no wet faeces in animals treated with atropine. (Table 2)

Effects of blockers on mean weight of faeces after 24 hours

Figure 2 showed that propranolol and nifedipine increased the mean faecal weight when administered to rats before zinc sulphate treatment after diarrhea has been induced in rats; however this increase were not significant ($p> 0.05$). Prazosine decreased the mean weight of faeces of rats; however this decrease was not significant ($p> 0.05$). The mean weight of faeces was significantly reduced by atropine ($p< 0.05$).

Table 1. Effects of Zinc treatment on number wet feacal output in castor oil treated rats

Treatment	2 hours	4 hours	6 hours	8 hours
Control	3.8 ± 0.25	5.5 ± 0.29	6.5 ± 0.29	6.8 ± 0.25
3mg/Kg Loperamide	2.3 ± 0.25**	2.3 ± 0.25**	2.5 ± 0.29***	2.5 ± 0.29***
25mg/Kg Zinc sulphate	4.0 ± 0.41	5.3 ± 0.25	6.3 ± 0.26	6.5 ± 0.29
50mg/Kg Zinc sulphate	3.5 ± 0.29	4.3 ± 0.25*	5.0 ± 0.41*	5.0 ± 0.41*
100mg/Kg Zinc sulphate	3.0 ± 0.0*	3.0 ± 0.0***	3.0 ± 0.0***	3.0 ± 0.0***
150 mg/Kg Zinc sulphate	2.5 ± 0.29*	3.3 ± 0.25**	3.3 ± 0.25***	3.3 ± 0.25***

N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$, **= significant compared with control at $p< 0.01$, *** = significant compared with control at $p< 0.001$

Table 2: Effect of blockers on number of wet faeces in castor oil treated animals given zinc.

Treatment	1 hours	2 hours	3 hours	4 hours
Control	0.5 ± 0.29	1.8 ± 0.25	2.5 ± 0.29	3.0 ± 0.41
Propranolol	3.3 ± 0.25***	4.8 ± 0.25***	5.5 ± 0.29***	5.8 ± 0.25***
Prazosine	2.0 ± 0.0**	3.0 ± 0.0**	3.8 ± 0.25*	3.8 ± 0.25
Nifedipine	2.8 ± 0.25**	4.8 ± 0.25***	5.5 ± 0.29***	6.0 ± 0.0***
Atropine	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$, **= significant compared with control at $p< 0.01$, ***= significant compared with control at $p< 0.001$

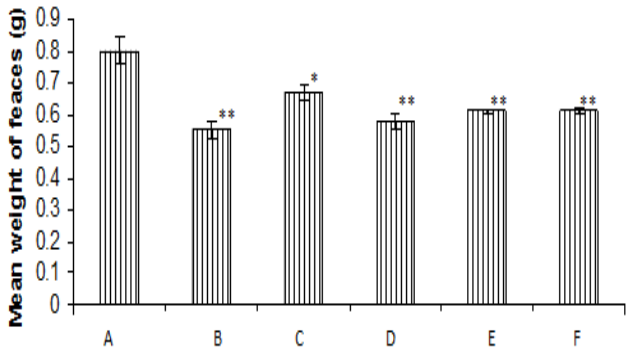

Figure 1: Mean weight of faecal output after 24 hours of castor oil induced diarrhea. N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$, **= significant compared with control at $p< 0.01$, *** = significant compared with control at $p< 0.001$. A=control, B=3mg/Kg Loperamide, C=25mg/Kg $ZnSO_4$, D=50mg/Kg $ZnSO_4$, E=100mg/Kg $ZnSO_4$, F=150 mg/Kg $ZnSO_4$

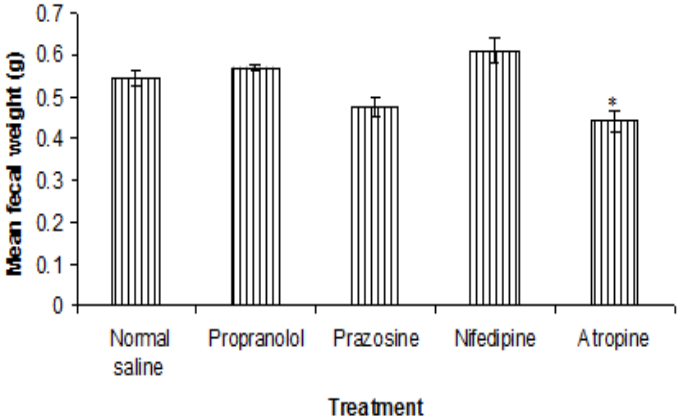

Figure 2: Effect of blockers on mean faecal weight after 24 hours. N= 5, values are presented as mean ± SEM. *= significant compared with control at $p< 0.05$.

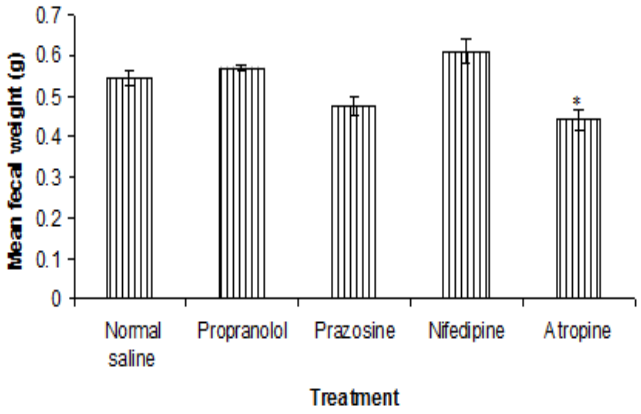

Figure 3: Percentage distance moved by charcoal meal through the small intestine. N= 5, values are presented as mean ± SEM. *= significant compared with control at p< 0.05, **= significant compared with control at p< 0.01

Effects of zinc on intestinal transit

In control animals given normal saline, charcoal meal moved 85.7 ± 2.35% through the length of the small intestine. Zinc sulphate treatment (100mg/Kg) significantly (p< 0.05) reduced the distance moved by charcoal (60.7 ± 7.13%), while the standard drug atropine significantly (p< 0.01) reduced distance moved by charcoal (65.9 ± 4.08%) as shown in Figure 3.

DISCUSSION

The result of this study agrees with previous reports on the effect of zinc on diarrhea and in addition to earlier mechanisms of action elucidated on zinc in its antidiarrhea activities, this study showed that zinc treatment reduced intestinal motility during castor oil induced diarrhea. Zinc sulphate was not very effective at the lower doses of 25mg/Kg and 50mg/Kg. However, zinc was more effective at higher doses and a dose of 100mg/Kg was more effective than 150mg/Kg. The induction of diarrhea with castor oil results from its hydrolytic product, ricinoleic acid, (Iwao and Terada, 1962). The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion, (Pierce *et al.*, 1971) resulting in the generation of giant contractions of the transverse and distal colon (Croci *et al*., 1997). A potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting either gut motility and/or electrolyte out flux (Croci *et al.*, 1997), therefore zinc sulphate is an important antidiarrheal agent.

The result of this study revealed that the antidiarrheal effect of zinc occur by stimulation of β-adrenergic receptors in the intestine. Gati *et al*., (1975) had previously suggested that epinephrine exerts its inhibitory effect on gastric motility via beta adrenergic receptors, thus activation of the sympathetic innervations of the intestines results in the inhibition of peristaltic activity and a reduction in tone. Akomolafe *et al*., (2004) reported that both alpha and beta receptor stimulation are involved in antidiarrhea mechanism. Mohammed *et al*., (2009) also reported that the hydro methanolic portion of *Indigofera pulchra* extract possessed antidiarreal effect and that it acted through β adrenergic receptors. Therefore the adrenergic pathway is important in antidiarrhea mechanism. Many antidiarrhea drugs also exert their effects through α2-adrenoceptors pathway (Hsu, 1982; Ruwart *et al*., 1980). Activation of the pre-junctional α2-adrenoceptors on the parasympathetic terminals plays an important role in the inhibitory action of sympathetic nerve stimulation of gastrointestinal motility by inhibiting acetylcholine release (Berthelsen & Pettinger, 1977). The sympathetic nervous system also controls the balance between absorption and secretion in the ileum through activation of the mucosal α2-adrenoceptors.

This study also revealed that zinc sulphate reduced intestinal motility during diarrhea by a mechanism involving L-type Ca (2+) channels. Nifedipine, a Ca^{2+} blocker abolished the antidiarrheal effect of Zinc. This is in line with previous work done on antidiarrheal drug (Borrelli *et al*., 2006). Contractile activity in smooth muscle is initiated by a Ca^{2+}-calmodulin interaction to stimulate phosphorylation of the light chain of myosin, enabling the molecular interaction of myosin with actin thus causing contraction.

Zinc sulphate, acetate, and gluconate are all acceptable zinc salt formulations, of which zinc sulfate is low-cost, efficacious and safe. Zinc sulphate tablets may be dispersed in breast milk, in oral rehydration solutions, or in water on a small spoon; older children may chew the tablets or swallow them with water (Bajait and Thawani, 2011). Thus oral zinc administration provides substantial benefit in the reduction of stool output, frequency, and duration, combined with safety, efficacy, and affordability in acute diarrhea. This study reveals that zinc sulphate in addition to the initial mechanisms of action elucidated, reduces intestinal motility by activating β-adrenergic receptors and L-type Ca (2+) channel.

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