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Efficacy of Zinc Nanoparticles against Castor Oil Induced Diarrhoea in Rat

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ABSTRACT
Diarrhoea which is characterised by three or more episodes of loose or watery stools during a 24 hour period or a decrease in the consistency of the stool, which is typical in patients. The main aim of this study is to determine the AntiDiarrhoeal Study of Biosynthesized Zinc Nanoparticles Using Cassia occidentalis Leaf.Wistar rats were induced diarrhoea with castor oil, group randomly into five groups and treated with loperamide, 10, 20, and 40 mg/kg body weight of zinc nanoparticles of Cassia occidentalis Leaf. After treatment with the ZnNPs gastrointestinal motility, AST, ALT, Catalase, Malondialdehyde and glutathione peroxidase were determined using standard methods. There is significant decrease in gastrointestinal motility of the charcoal in the intestinal tract of the rats. There is significant (P<005) increase in glutathione peroxidase and malondialdehyde antioxidant activities in the treated group compared to Diarrhoeal control. Their significant improvement in liver enzyme markers compared to Diarrhoeal control. The zinc nanoparticles of Cassia occidentalis Leaf can be used in treatment of Diarrhoea.

Keywords: Cassia occidentalis, zinc nanoparticles, diarrhoea, Loperamide, gastrointestinal motility

I. INTRODUCTION

Diarrhoea which is characterised by three or more episodes of loose or watery stools during a 24 hour period or a decrease in the consistency of the stool, which is typical in patients, is described in Ferris et al. (2023). Despite the fact that most episodes are mild, acute episodes of diarrhoea in children may cause significant loss of fluid and dehydration. The first symptoms of diarrhoea should therefore prompt an action to restore lost fluids, thus preventing potentially fatal dehydration. Pathogens such as viruses, protozoa and bacteria may all play a role in causing diarrhoea (Iftikharet al., 2023). Children under the age of five account for 40 percent of all hospital admissions due to diarrhoea worldwide (WHO, 2022).

According to WHO (2022), diarrhoea account for 40 % of children less than 5 years old hospitalized. Rotavirus is one of the deadliest viruses and bacteria that affect humans in emerging countries (Omatola&Olaniran, 2022). Diarrhoea is a leading killer of children, account for 9 per cent of children under age 5 deaths worldwide in 2021. This translates to over 1,200 young children dying each day, or about 444,000 children a year, despite the availability of a simple treatment solution (Peter, & Umar, 2018). In Nigeria, diarrhoea prevalence rate is 18.8% and is one of the worst in sub-Sahara Africa and above the average of 16%. Annually, it accounts for over 16% of child deaths and an estimated 150,000 deaths mainly amongst children under five years (Joseph et al., 2017).

The biggest risk associated with diarrhoea is dehydration due to lose of fluid and electrolytes such as sodium, chloride, potassium, and bicarbonate. There is need to replenish the fluid and electrolytes lose in other to prevent dehydration but without replenishment dehydration set in a later stage (WHO, 2018). World Health Organization recommend the use of Oral Rehydration Salt (ORT) which content some require constituent of compounds in clean water (Peter & Umar, 2018).

Zinc insufficiency is common in the impoverished world despite the prevalence of protein-rich and other dietary sources (Oyewumiet al., 2023). This is despite the fact that zinc is linked to increased rates of infectious disease, including diarrhoea, and fatalities from these diseases (Maywald& Rink, 2022). Treatment plans that include zinc supplements are especially important for helping youngsters recover from sickness such as diarrhoea and maintain good health in the long run (Gombartet al., 2020).

Zinc (Zn) is an essential nutrient element and is involved in many physiological functionsin the body (El-Seedyet al., 2016). Supplementary Zn has increased growth performance, promotedantioxidant and immune activities, improved intestinal microflora, and reduced the incidenceof Diarrhoea (Grilliet al., 2015 and Hassanet al., 2021). Zinc compounds positively affect the digestive tract through increasing mucosal thickness, villi height, activity of enzymes, and regulation of digestive tract micro-organisms (Lee, 2018).

The main aim of this study is to determine the Antidiarreal Study of Biosynthesized Zinc Nanoparticles Using Cassia occidentalis Leaf.

II. METHODOLOGY

Experimental Animals

Twenty (20) healthy rats were obtained from the University of Jos, Plateau state. The rats were allowed to acclimatize with the laboratory condition for one week. The rats were housed in animal cages and have access to food and water with 12 hours day and night cycle. All experiment was carried out according to the laws and principles guiding the use of animals in scientific research.

Collection of Plant Sample

Cassia occidentalis leaf was collected at Mugulvu, Mubi South Local Government, Nigeria in the month of March. The plant was authenticated at Forestry Department, Federal Polytechnic, Mubi. The leaf was shade dried and grounded into powder using pestle and motar.

Preparation of Plant Extract.

Aqueous leaf extract of Cassia occidentalis was prepared according to Rajeshkumaret al. (2018) with slight modifications. The powdered sample weighing 20 g was dissolved in 200 mL of water and boiled for 10 minutes. The extract was cooled at room temperature, filtered through Whatman filter paper no 1 and the extract was stored at 4^0 C.

Green synthesis of zinc oxide nanoparticles. Zinc acetate dihydrate salt [Zn (O_2CCH_3) $2(H_20)_2$] (0.25 M) solution was prepared by adding 22.92 g zinc acetate dihydrate salt to 500 ml of distilled water in a flask. Further, 10 ml of Aqueous leaf extract of Cassiatora added dropwise into the zinc acetate solution under continuous stirring at 60 ℃ for 2 hours on a magnetic stirrer. While stirring, NaOH was added dropwise in the above solution to maintain the pH at 12. Plant extract acted as a reducing agent. The formation of light yellow color suspended particles and visible color change of the solution from brown to yellow indicated the formation of zinc oxide nanoparticles. This mixture was then centrifuged at 6000 × g for 15 min and supernatant was discarded to collect the pellet in a Petri plate. Afterwards, it was placed overnight in a hot air oven for drying at 60 ℃. Calcination of yellow-coloredZnONPs was done in a muffle furnace at 400 ℃ for 4 hours to remove impurities (Rafiqueet al., 2022). Influence of the plant extract on production of ZnONPs was investigated by varying the ratio of plant extract to salt (v/w) and yield of prepared ZnONPs was calculated by following formula.

$$\text{Yiled } (\%) = \frac{\text{Experimental ZnO weight}}{\text{Theoretical ZnO Weight}} \text{ x } 100$$

Castor oil induced diarrhoea

The method followed by Umeret al. (2013) was used for this study (Sisayet al., 2017). Male wistar rats was fasted for 18 h, randomly allocated to five groups of four animals each. One hour after administration of the respective doses of castor oil, they were individually placed in cages where the floor was lined with white paper. During an observation period of 2 hour, the onset of diarrhoea and the number of faecal output (wet faeces was recorded for individual rat. The number of faecal output was determined according to the formulae (Araet al., 2013; Tadesseet al., 2014).

Experimental Design

All the rats where induced with Diarrhoea using 1 ml castor oil except the normal control group. In all models, the normal control group was admin+istered with 1 ml distilled water, group 2 serve as Diarrhoeal control but no treatment and group 3 was treated with loperamide as standard drug. The other groups (i.e. group 3, 4, 5, 6), were administered 10mg/kg, 20 mg/kg and 40 mg/kg body weight dose of the zinc nanoparticle sample.

S/N	Group	Treatment
1	Group 1	Normal
2	Group 2	Diarrhoea without Treatment
3	Group 3	Diarrhoea + Loperamide
4	Group 4	Diarrhoea + 10 mg/kg bw of ZnNp

5	Group 5	Diarrhoea + 20 mg/kg bw of ZnNp
6	Group 6	Diarrhoea + 40 mg/kg bw of ZnNp

Gastrointestinal Motility Test

From the above, after 1 h each animal will be given 1 ml of charcoal meal (10%) activated charcoal in 5% gum acacia) via the oral route. All animals will be sacrificed 30 min thereafter, and the distance covered by the charcoal meal in the small intestine, from the pylorus to the caecum will be measured and expressed as percentage of distance moved (Pazhaniet al., 2001).

Blood sampling and serum biochemical analysis. Blood samples (2 ml) were collected on 35th day from the wing vein of all the birds in plain tubes, allowed to clot and then centrifuged at 4000 rpm for 10 min. The obtained serum was stored at − 20 °C for biochemical analysis (Grądzkiet al., 2020). Serum biochemical profile including aspartate transferase (AST), and alanine transferase (ALT) was evaluated by using a randox kits following the manufacturer's guidelines (Sokołet al., 2015).

Determination of antioxidant enzymes activity. Antioxidants like catalase (CAT), Malondialdehyde and Glutathione peroxidase (GPx) activities were evaluated by using method of Zhang et al. (2008).

Statistical Analysis

All data are expressed as means ± SD (standard deviation). The results, calculated as the means for each pen, were analysed by one-way ANOVA using the SPSS version 26 statistical package. Differences between means were determined by Duncan's post hoc test, and p <0.05 were taken to indicate various levels of statistical significance.

III. RESULTS

Table 1: Effect of biosynthesized zinc nanoparticles on onset Diarrhoea and number of wet faeces

	Onset Diarrhoea (Min)	Number of wet faeces
Diarrhoea Control	10.30 ± 6.01	3.00 ± 1.00^{c}
Diarrhoea + Loperamide	75.80 ± 1.12	2.70 ± 0.57^{bc}
Diarrhoea + 10 mg/kg BW of Zn Nanoparticles	28.20 ± 1.12	2.30 ± 0.58^{b}
Diarrhoea + 20 mg/kg BW of Zn Nanoparticles	30.90 ± 0.97	2.00 ± 1.00^{b}
Diarrhoea + 40 mg/kg BW of Zn Nanoparticles	42.80 ± 5.71	1.00 ± 0.91^{a}

Values are express as mean ± SEM of four determinants. Values along same column with same superscripts are not significantly different at p<0.05.

Table 2: Effect of Biosynthesized Zinc Nanoparticles on Gastrointestinal Tract Inhibition

	Length of Intestine (cm)	Distance travel by charcoal (cm)	Inhibition (%)
Normal Control	112.00 ± 2.86^{a}	70.70 ± 2.71^{d}	36.88
Diarrhoea Control	125.00 ± 2.29^{b}	41.70 ± 1.04^{b}	66.64
Diarrhoea + 10 mg/kg BW of Zn Nanoparticles	111.70 ± 1.51^{a}	43.30 ± 3.64^{c}	61.24
Diarrhoea + 20 mg/kg BW of Zn Nanoparticles	102.30 ± 1.32^{b}	29.70 ± 3.21^{a}	70.96
Diarrhoea + 40 mg/kg BW of Zn Nanoparticles	136.70 ± 1.52^{c}	36.70 ± 2.89^{b}	75.76

Values are express as mean ± SEM of four determinants. Values along same column with same superscripts are not significantly different at p<0.05.

Table3: Effect of biosynthesised zinc nanoparticles on serum antioxidant activity

	GPX (X 10^{-4}) (µ/L)	Catalase (X 10^{-3}) (µ/L)	MDA (X 10^{-4}) (mg/dL)
Normal Control	21.00 ± 4.51^{a}	16.00 ± 1.00^{d}	34.83 ± 2.30^{b}
Diarrhoea Control	23.59 ± 0.85^{c}	1.33 ± 1.08^{a}	68.22 ± 2.98^{c}

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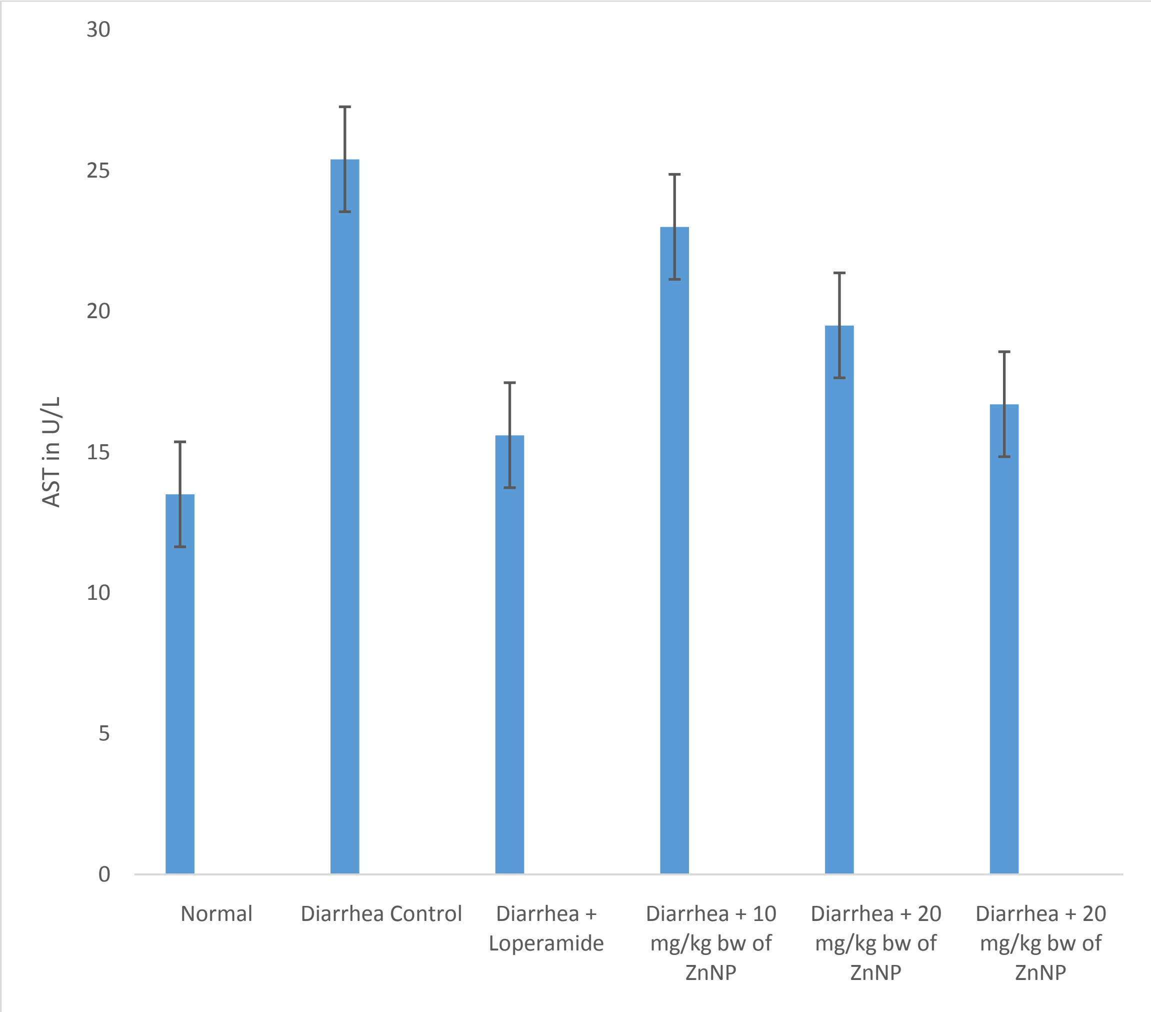
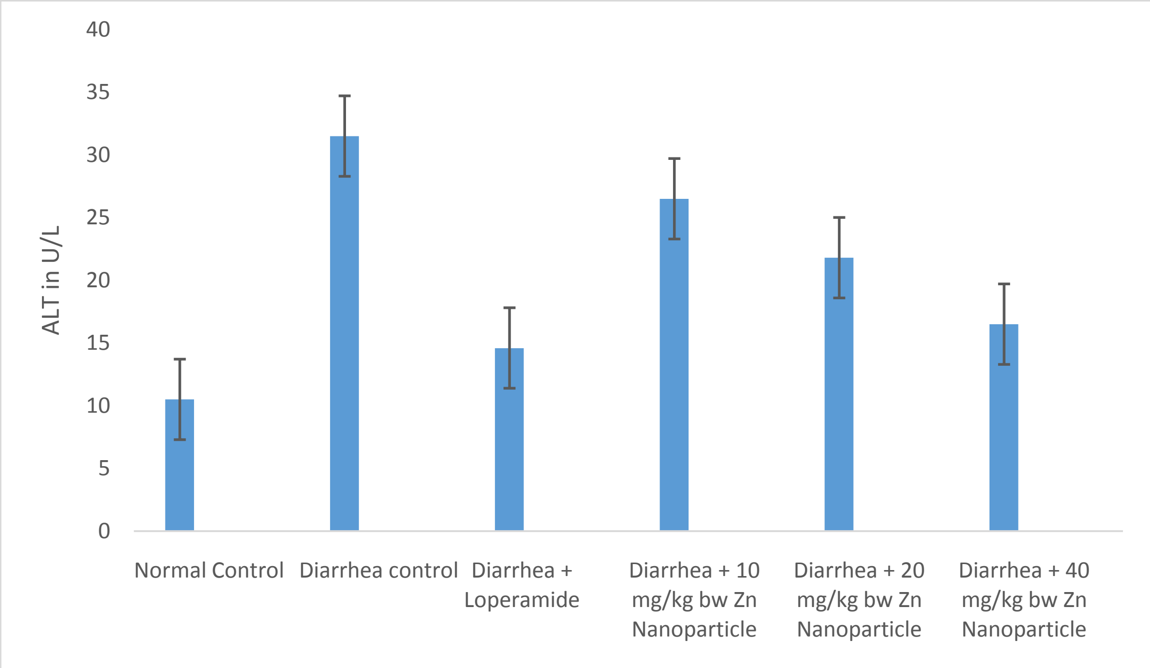
Diarrhoea + 10 mg/kg BW of Zn Nanoparticles	23.00 ± 0.96^{c}	1.36 ± 0.10^{a}	20.07 ± 1.37^{a}
Diarrhoea + 20 mg/kg BW of Zn Nanoparticles	21.94 ± 0.47^{b}	2.34 ± 0.12^{b}	16.09 ± 1.37^{a}
Diarrhoea + 40 mg/kg BW of Zn Nanoparticles	20.33 ± 0.80^{a}	3.23 ± 0.13^{c}	16.00 ± 0.25^{a}

Values are express as mean ± SEM of four determinants. Values along same column with same superscripts are not significantly different at $p<0.05$.
(GPX = Glutathione Peroxidase, MDA = Malondialdehyde)





IV. DISCUSSION

Diarrhoea is the condition of having three or more loose or liquid stools per day or as having more stool than is normal for that person (Dong & Zeng, 2020). Diarrhoea results from unsteady between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in intestine motility and an excess loss of fluid in the faeces (Babaleet al., 2018).

Castor oil causes Diarrhoea in animals due to the action of its active metabolite, ricinoleic acid derived from hydrolysis of its triglyceride in the duodenum by pancreatic lipase (Sambhakaret al., 2023). The released ricinoleic acid causes irritation, inflammation of the intestinal mucosa it also stimulates intestinal hyper motility and hyper secretion (Ezejaet al., 2012). Treatment with different concentration of ZnNPs caused decrease in the number of wet feces in the rats. This shows that ZnNPs have the potentials to inhibit castor oil induced Diarrhoea in rats. The Diarrhoea control has recorded the lowest percentage inhibition may be no inhibiting agent administered. This study tally with the research carried out by Yacobet al., (2016), Mohammed &Alhassan, (2020).

The observed significant increase in the glutathione peroxidase (GPx) antioxidant enzyme concentration in Diarrhoeal control group may suggest an occurrence of oxidative stress demonstrates that Diarrhoea can be accompanied by an oxidative stress status in the intestinal fluid (Koriem& Farouk, 2023) Administration of zinc nanoparticles to the Diarrhoea induced rats at 10, 20 and 40 mg/kg bw shows a decreased in the elevated level of GPx concentration in a dose dependent manner suggesting an improvement in the antioxidant activity.

Unsalet al (2020) reported that zinc is one of the exogenous antioxidant considered as multipurpose trace element and it is suggested for remedial supplementation of many stress and pathological conditions. The concentration of catalase shows significant ($p<0.005$) in the ZaNPs treated groups when compared with the Diarrhoea control group suggesting an improvement in the activity of catalase enzyme to suppress free radical and oxidative stress (Pradhan et al., 2021)

The observed decreased in MDA concentration is an indication ZnNPs can suppress and inhibit free radicals that leads to oxidative stress. Low concentration of MDA may suggest that zinc can improve antioxidant enzyme activity to prevent lipid membrane damage caused by ROS and thus, decrease in lipid peroxidation (Weisanyet al., 2012). The concentration of zinc can reduce the concentration of malondialdehyde (MDA) and enhance antioxidant activities to suppress the generation of ROS Malondialdehyde (MDA) is an end product of lipid peroxidation (Farouk & Al-Amri, 2019).

Administration of castor oil showed a significant increase level of AST and ALT in the serum of infected rats, similar results reported by Mundet al., (2017). The rise in enzyme activity occurs may be due to cell wall damage, inflammation, and heavy blood loss (Khedret al., 2021). Treatment with different concentration of zinc nanoparticles showed significant decrease in the enzyme activity.

In conclusion, the zinc nanoparticles of Cassia occidentalis have anti-Diarrhoeal properties by reduction in wet droplets, gastrointestinal motility and improved in vivo antioxidant activities.

Recommendation

Further studies should be carried out on characterization and toxicology of zinc nanoparticles of Cassia occidentalis.

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