

Antidiarrheal Activity of Biosynthesized Zinc Oxide Nanoparticle Using *Cassia occidentalis* in Castor Oil Induced Diarrhea Rats

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Abstract - Diarrhea is among the leading cause of death especially among children aged under 5 years. Zinc has been included in the management and treatment of diarrhea. Nanoparticle is gaining importance in medicine. The aim of this study is to evaluate the antidiarrheal activity of biosynthesized zinc oxide nanoparticle in castor oil-induced diarrheal rats. Zinc oxide nanoparticle (ZnONP) was biosynthesized using *Cassia occidentalis* leaf. The biosynthesized ZnONP was characterized using ultraviolet (UV)-spectroscopy and Fourier transform infrared spectroscopy (FTIR). Twenty five albino rats were randomly grouped into five groups. All rats, except the control, were induced with diarrhea. Group1 received normal saline, group 2 received 3 mg/kg b.wt. loperamide while groups 3, 4 and 5 received 10, 20, and 50 mg/kg b.wt. biosynthesized ZnONP. UV spectroscopy showed an absorption peak at 320 nm. The SEM micrographs reveal the nanoparticles as flake like which are well agglomerated.. The SEM micrographs reveal the nanoparticles as flake like which are well agglomerated. The biosynthesized zinc oxide nanoparticle significantly delayed the onset of diarrhea, decreased the number of wet feces, increased the percentage gastrointestinal tract (GIT) inhibition and decreased the volume of intestinal fluid. Biosynthesized zinc oxide nanoparticle from *Cassia occidentalis* leaf can be used in the treatment of diarrhea.

Keywords: diarrhea, biosynthesized, nanoparticle, zinc, *Cassia occidentalis*.

I. INTRODUCTION

Nanoparticles are particles with their size ranging between 10 – 1000 nm [1]. Nanoparticles have unique properties which the bulk material lack and this makes them useful for a lot of applications [2].[3]. Nanoparticle is gaining importance in medicine. The importance includes prevention, early detection, enhanced diagnosis and follow up of diseases [4]. There is increasing optimism that nanomedicine will bring

important advances in the diagnosis, treatment and prevention of diseases [5].

Metal nanoparticles are being used in medical, cosmetic, pharmaceutical and nutritional industries [6]. These nanoparticles are gaining much attention. For examples, Zinc oxide nanoparticles are used in cosmeceutical and medical application for ultraviolet ray protection in semi-solid forms such as skin cream [7]. Selenium nanoparticles is been applied in agricultural industry for better plant development and growth [8]. It has antimicrobial, anticancer, antioxidant properties and has lesser toxicity when compared to the selenite counterpart [9] – [11]. Bismuth oxide nanoparticle can be used as an x-ray absorbent [12]. Gold nanoparticles are being used for bioassays and biosensor system and in the hyperthermia of cancerous cells [13][14] Antibody conjugated gold nanoparticles can be used for detection of small amounts of antigen based on surface plasmon resonance (SPR) spectra [15].

Nanoparticles are synthesized either chemically, physically or biologically. Chemically synthesized nanoparticles are associated with many harmful effects [4]. Chemically synthesized nanoparticles produce toxic products and organic solvents that maybe toxic still remains in the nanoparticle after synthesis [16] [17]. Physically synthesized nanoparticles do not have the desirable characteristics such as morphology, size and shape distribution as well as crystallinity composition [18]. In synthesizing metal nanoparticles using green chemistry, various biological materials which include algae and plant extracts and different micro-organisms such as fungi, bacteria and yeast have been utilized [18] [19]. The use of plant extracts for synthesis of nanoparticles may be better than other biological methods because it is more affordable, eliminates the elaborate preservation of cell cultures and is suitable for large scale synthesis [20]. Plants have a wide collection of phytochemicals that are used to overcome defence and stress conditions [21]. These phytochemicals serves as either reducers or stabilizers and they are a safe approach, producing desirable metal nanoparticles with

acceptable structural properties [14]. Among these phytochemicals, flavonoids and phenolic acids have been extensively studied for their applications in nanomedicine [14]. Phenolic acids such as gallic acid have been widely used to synthesize different metal or metal oxide nanoparticles which include gold, selenium and silver [14]. Metal nanoparticles synthesized by phenolic compounds have higher stability than those synthesized by other organic or inorganic reducing agents such as sodium borohydride or citrate [22]. Therefore, the use of plant extracts in green chemistry for the synthesis of nanoparticles is gaining a lot of attention now.

Diarrhea is the passing out of watery or loose stools more than three times a day [23]. In developing countries, diarrhea is one of the major diseases especially in children [24]. Severe diarrhea can cause dehydration which is life threatening [25]. The first treatment usually administered in diarrhea cases is oral rehydration therapy (ORT) [26]. Oral rehydration therapy does not decrease the frequency, duration and volume of diarrhea [27]. Numerous studies have shown that taking zinc supplement significantly decrease the duration and severity of diarrhea [28] [29]. United Nations children's fund (UNICEF) and World health organization (WHO) recommended zinc supplementation in the management of diarrhea in children [30]. This study therefore evaluated the antidiarrheal activity of biosynthesized ZnO nanoparticle using *Cassia occidentalis* in castor oil-induced diarrhea rats.

II. METHODOLOGY

2.1 Experimental Animals

Twenty five albino rats weighing between 120 g – 150 g were obtained from National Veterinary Research Institute (NVRI), Vom, Plateau State. The rats were kept in a ventilated cage and acclimatized for seven days. The rats were given standard feed and water ad-libitum. They were handled according to the International Guiding Principles for Biomedical Research involving Animals (CIOMS/ICLAS, 2012) and protocol approved by the Adamawa State University, Mubi ethical committee with approval number ADSU/IACEC/ANP-A045/2024.

2.2 Plant Preparation

Cassia occidentalis leaves were collected at Adamawa State University, Mubi, Mubi north local government area and was identified by a soft ware plantnet. *Cassia occidentalis* fresh leaves were collected, washed and rinsed with distilled water. The leaves were used immediately. The aqueous extract was prepared by mixing 10 g of fresh leaves with 100 mL of water and boiled for 10 minutes with constant stirring on a magnetic stirrer. The leaves in water were allowed to cool for hours and

were filtered through Whatman no. 1 filter paper. The filtrate was used immediately [31].

2.3 Synthesis of Zinc Oxide Nanoparticles

The method described by Sutradhar and Saha [32] with slight modifications was used in the synthesis of zinc oxide nanoparticles. An aliquot amount (10 mL) of aqueous leaves extract of *C. occidentalis* was added to 50 mL of 0.2 M zinc nitrate ($Zn(NO_3)_2$) with continuous stirring at 25°C and pH 7 for 60 min. The formation of the nanoparticle was ascertained by the formation of precipitate. The precipitate was centrifuged for 15 min at 3000 rpm and washed 3 times with acetone. The precipitate was subjected to characterization as follows;

2.4 UV-Visible Spectroscopy

About 0.01 g of the zinc oxide nanoparticles was dissolved in 20 mL of distilled water. This was transferred into the cuvette and inserted into the spectrophotometer. The absorbance was scanned between 200 nm to 600 nm. The result was transferred to Microsoft excel where the data was analyzed.

2.5 FT-IR Spectroscopy

Dried *C. occidentalis* leaf extract and zinc oxide nanoparticle separately were loaded on the FT-IR sample cell and scanned at 4000 cm^{-1} to 400 cm^{-1} .

2.6 XRD analysis

Using the sample preparation block, 1 g of the powdered sample was weighed and compressed in the flat sample holder, creating a smooth, flat surface that was mounted on the sample stage in the XRD cabinet. The reflection transmission spinner stage using the Theta-Theta settings was used to analyze the sample. 2 θ starting position was 0.00483 and ends at 75.000 with a 2 θ step of 0.026 at 3.57 seconds per step. Tube current was 40 Ma and the tension was 45 VA. The goniometer radius was 240 mm. Fixed Divergent Slit size of 1° was used

2.7 Scanning Electron Microscopy (SEM)

The pellet was subjected to scanning electron microscopy analysis. A very small amount of the sample was dropped on the grid to produce a thin film of the sample. A blotting paper was used to remove the extra solution and then the film on the SEM grid was allowed to dry for analysis.

2.8 Induction of Diarrhea

Castor oil (3 mg/kg b.wt) was administered to the rats orally for the induction of diarrhea.

Experimental Design

Twenty five albino rats were randomly grouped into five groups. All rats were induced with diarrhea. Group 1 received normal saline, group 2 received 3 mg/kg b.wt. loperamide while groups 3, 4 and 5 received 10, 20, and 50 mg/kg b.wt. biosynthesized ZnONP.

2.9 Inhibition of Wet Faeces

Albino rats of both sexes fasted for eighteen hours. They were grouped and treated as described above. One hour after administration, they were placed individually in cages lined with white cardboard paper. They were observed for four hours. The onset of diarrhea and the number of wet feces was recorded for individual rat.

2.10 Gastrointestinal Motility Test

Albino rats of both sexes fasted for eighteen hours. They were grouped and treated as described above. After 30 mins of administration, 1 mL of charcoal meal (10 % activated charcoal in 5 % gum acacia) was administered. After 30 minutes of administration, all the animals were sacrificed. The distance travelled by the charcoal meal in the small intestine from the pylorus to the caecum was measured and expressed as percentage inhibition of gastrointestinal motility [33].

% inhibition of gastrointestinal motility = $\frac{\text{length of small intestine} - \text{distance travelled by charcoal}}{\text{length of small intestine}} \times 100$

2.11 Castor Oil - induced Enteropooling Test

Castor oil-induced enteropooling test was done by the method of Meite et al.[34]. Albino rats of both sexes were fasted for 18 hrs. They were grouped and treated as described above. The rats were sacrificed after an hour of administration. The small intestine from the pylorus to the caecum was removed. The intestinal contents were milked into a measuring cylinder. The weight and volume of the intestinal fluid was measured.

2.12 Statistical analysis

Results were expressed as mean \pm S.E.M. One way analysis of variance (ANNOVA) was used followed by Duncan multiple test. $P < 0.05$ was considered statistically significant. Statistical Package for Social Sciences (SPSS) version 21.0 was used for the statistical analysis.

III. RESULTS AND DISCUSSIONS

The UV visible spectroscopy analysis of the zinc oxide nanoparticle gave an absorption maximum at 360 nm.

Muhammad *et al.*[35] also reported the absorption peak of zinc oxide nanoparticle to be 360 nm. The absorption peak for ZnO nanoparticle has been found to be between 360 and 365 nm [36][37]. This indicates that the nanoparticle synthesized is ZnO. The narrow nature of the band and its singleness is an indication that the particles are same size, monodisperse and well aggregated.

FTIR are employed in determining organic functional group present in organic compound or other substance. Eight different absorption peaks were observed in the plant extract as shown in Figure 2 at wave numbers which corresponds to a particular organic functional group. The broad band observed at 3287.84 cm^{-1} corresponds to H-bonded O-H stretch alcohols. The peak at 2918.37 cm^{-1} is assigned to C-H stretch of alkane. The absorption band at 2850.07 cm^{-1} corresponding to C-H stretch of alkane. The band at 1730.93 cm^{-1} was given CHO of saturated amide. The strong band at 1592.31 cm^{-1} reveals the presence of N-H bending of amide groups. Another absorption band was observed at 1406.97 cm^{-1} it was assigned to C-H bending of saturated aliphatic. The stretched absorption band at 1239.96 cm^{-1} indicates the C-O of ether while the absorption peak of 1024.02 cm^{-1} correspond to C-O stretched alcohol. This is an indication that the aqueous leaf extract of *Cassia occidentalis* may contain bioactive molecules like carboxylic acids, proteins, alcohol, alkaloid, flavonoid, etc. There is a shift in these absorption band when compared to the FTIR of the nanoparticle as seen in Figure 2. The band at 3287.84 cm^{-1} shifted to 3316.04 cm^{-1} , 1592.31 cm^{-1} shifted to 1575.13 cm^{-1} , 1406.97 cm^{-1} to 1416.05 cm^{-1} , 1024.02 cm^{-1} to 999.36 cm^{-1} while the peaks at 2918.37 cm^{-1} , 2850.07 cm^{-1} , 1730.93 cm^{-1} , 1239.96 cm^{-1} were absent in the nanoparticle. The peak at 928.96 cm^{-1} presents in nanoparticle but absent in the plant extract was assigned to ZnO. Mohammad and Aneelida [38] reported that during the biosynthesis of nanoparticle there is a shift in the peak position.

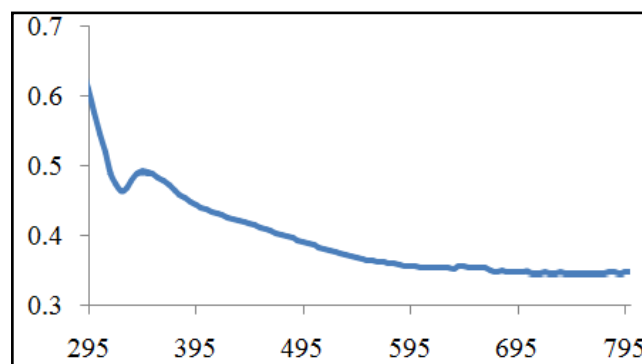


Figure 1: UV Visible spectra of biosynthesized zinc oxide nanoparticle with an absorption maximum at 355 nm

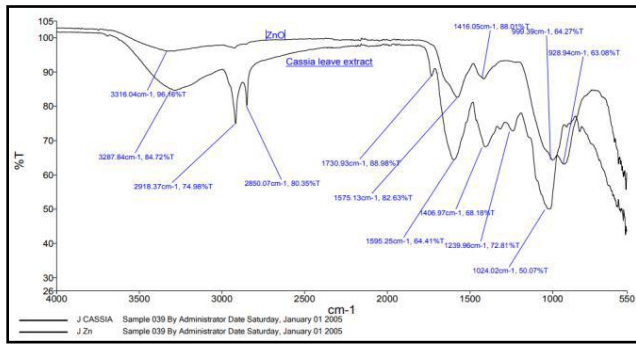


Figure 2: Fourier Transform Infra-red (FT-IR) Spectroscopy Analysis of biosynthesized zinc oxide nanoparticle

The EDX analysis of the zinc oxide nanoparticle was carried out in order to determine the elemental composition of the biosynthesized zinc oxide nanoparticle. The result revealed the presence of elements like zinc, carbon, oxygen, potassium, silicon, calcium, magnesium and sodium at a very low concentration. The presence of carbon may be due to the plant used in the bioreduction. The zinc and oxygen is present at very high concentration as seen in Figure 2, indicating that the nanoparticle contains mainly zinc oxide. The result of this study is similar with that of Azhar *et al.* [39].

The morphological and structural studies for Zinc oxide nanoparticles synthesized by biological method were investigated using scanning electron microscope and displayed in Figure 5. The SEM micrographs reveal the nanoparticles as flake like which are well agglomerated. The result is similar to the one reported by Shafqat *et al.* [40].

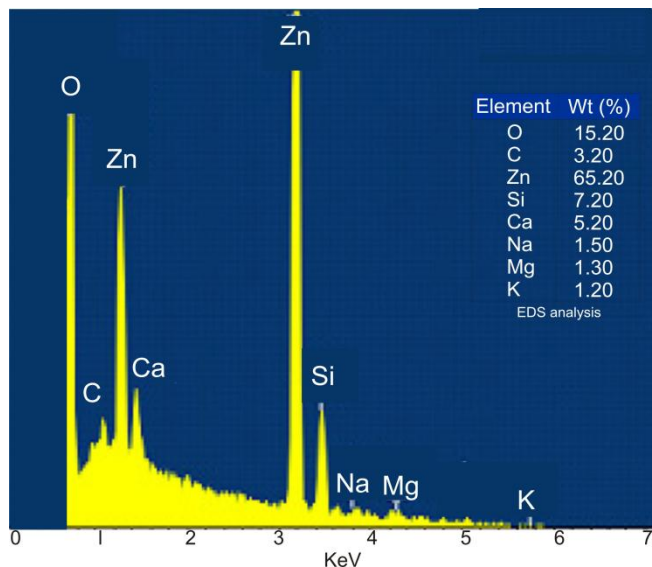


Figure 3: Energy Dispersive Spectroscopy Analysis of biosynthesized zinc oxide nanoparticle

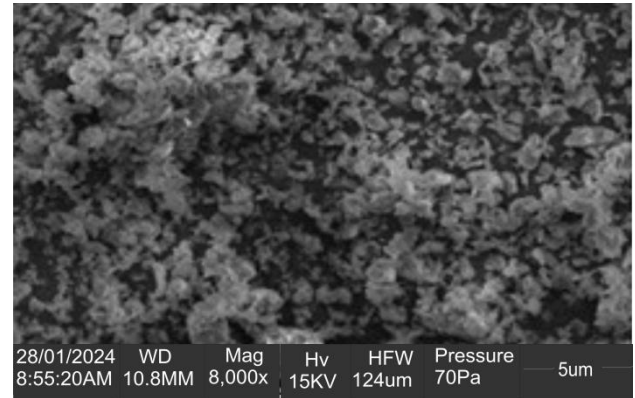


Figure 4: Scanning Electron Micrograph of biosynthesized zinc oxide nanoparticle

The crystalline structures of the prepared powders were analyzed by X-ray diffractometry (XRD) in the angular region of 2θ between 20° - 70°. The instrument was operated at 40 kV and the spectra were recorded at scanning speed of 8°/min. For determining the crystallite size and the corresponding lattice strain, the analysis of the XRD pattern lines is necessary and effective [41].

X-ray diffraction patterns of the ZnO nanoparticles synthesized by biological method is shown in Fig. 5. XRD patterns applied according to Bragg's law ($\lambda = 2d \sin \theta$), where λ is the wavelength of Cu radiation, d is the interplanar spacing and θ is the diffraction angle [36]. The XRD spectra indicate that the ZnO crystal has Body Center Cubic (BCC) structure with average size of 50.39 nm in standard DB card number as given in Table 1.

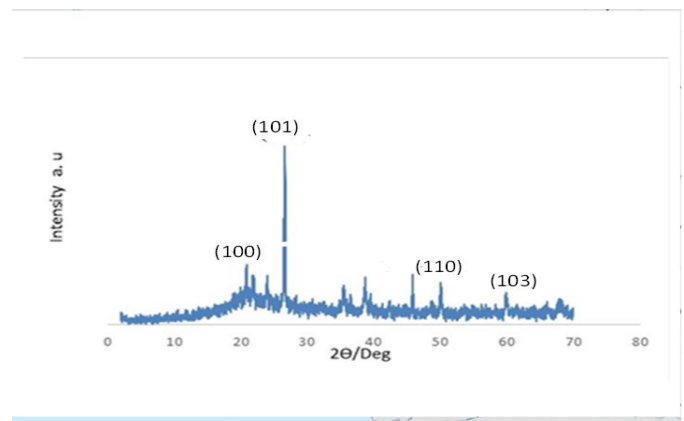


Figure 5: XRD analysis of zinc oxide nanoparticle

Table 1: XRD analysis of zinc oxide nanoparticle

Peak	2θ (degree)	FWHM	Size (nm)	DB Card No.
1	20.88	0.19	48.24	01-071-4916
2	26.61	0.18	51.26	00-003-0620
3	45.83	0.16	53.52	00-001-1076
4	59.81	0.19	49.50	00-001-1136

The effect of biosynthesized ZnONP on onset and number of wet feces in castor oil-induced diarrheal rats is shown in (Table 2). Biosynthesized ZnONP significantly increased the time for the onset of diarrhea when compared with the untreated diarrhea group. It also significantly decreased the number of wet feces when compared with the negative control. 50 mg/kg b.wt. ZnONP significantly decreased number of wet feces when compared with loperamide. The observed significant increase in the onset of diarrhea indicates that the biosynthesized zinc oxide nanoparticle delayed the onset of diarrhea. Also, decrease in the number of wet feces by biosynthesized ZnONP observed indicates the antidiarrheal property of biosynthesized ZnONP. This activity might be brought about by stimulation of the re-absorption of water from the intestinal lumen.

The effect of biosynthesized ZnONP on gastrointestinal tract inhibition is shown in (Table 3). Biosynthesized ZnONP significantly increased percentage inhibition of gastrointestinal tract (GIT) motility. The percentage inhibition of the group treated with 50 mg/kg b.wt. biosynthesized ZnONP was greater (73.15%) than the loperamide treated group (68.96%). The increase in percentage inhibition of GIT motility indicates that the biosynthesized ZnONP inhibits peristaltic activity by activation of the sympathetic innervations [42].

The effect of the biosynthesized ZnONP on enteropooling in castor oil-induced diarrheal rats is shown in (Table 4). The volume of the intestinal fluid of all the groups treated with biosynthesized ZnONP significantly decreased when compared with the diarrheal control. 50 mg/kg b.wt. biosynthesized ZnONP significantly decreased the volume (1.1 ± 0.03) when compared with the loperamide treated group (1.8 ± 0.01). Inhibition of peristaltic activity improves water and nutrient reabsorption from the GIT. This is the reason for the decreased volume of intestinal fluid observed in this study. Decrease in the number of wet feces as well as in the volume of intestinal fluid leads to a decrease in the severity of diarrhea. Zinc has been reported to reduce the severity of diarrhea [28] [29].

The mechanism of zinc antidiarrheal action is not well understood but in the intestine zinc plays different roles such as the regulation of fluid transport in the intestine, mucosal integrity and modulation of expressing of genes coding important zinc dependent enzymes such as cytokine which have an important role in modulation of oxidative stress and in the immune system [43][44]. Zinc also acts as a K⁺ channel blocker of cAMP mediated chlorine secretion [24].

Table 2: Effect of biosynthesized ZnONP on onset and number of wet feces in castor oil-induced diarrheal rats

Group	Onset of diarrhea (min)	Number of wet feces
1	14.30 ± 0.61 ^a	3.0 ± 1.00 ^c
2	82.80 ± 1.21 ^d	2.7 ± 0.57 ^{bc}
3	31.20 ± 1.12 ^b	2.3 ± 0.58 ^b
4	34.90 ± 0.97 ^b	2.0 ± 0.10 ^b
5	50.80 ± 5.71 ^c	1.0 ± 0.91 ^a

Values are expressed as means ± SEM n=5. Values with the same superscript are not significantly different (p < 0.050).

Table 3: Effect of biosynthesized ZnONP on gastrointestinal tract inhibition in castor oil-induced diarrheal rats

Groups	Percentage GIT inhibition
1	41.23
2	68.96
3	62.34
4	69.99
5	73.15

Table 4: Effect of biosynthesized ZnONP on enteropooling in castor oil-induced diarrheal rats

Group	Weight of intestinal fluid (g)	Volume of intestinal fluid (mL)
1	3.6 ± 0.59 ^a	3.4 ± 0.02 ^a
2	1.6 ± 1.10 ^a	1.8 ± 0.01 ^c
3	1.8 ± 1.00 ^a	2.8 ± 0.12 ^b
4	1.6 ± 1.50 ^a	2.1 ± 0.20 ^b
5	1.9 ± 1.34 ^a	1.1 ± 0.03 ^d

Values are expressed as means ± SEM. n=5. Values with the same superscript are not significantly different (p < 0.05)

IV. CONCLUSION

Biosynthesized zinc oxide nanoparticle from *Cassia occidentalis* leaf delayed the onset and severity of diarrhea. It can be used in the treatment of diarrhea.

Conflict of interest

Authors declare that there is no conflict of interest.

Funding

This study was funded by Nigeria Tertiary Education Trust Fund (TETFund) through Institution Based Research (IBR) grant (TEFT/ DR&D/UNI/MUBI/RG/2023/VOL.1.

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Citation of this Article:

Maryam Usman Ahmed, Ismaila Yada Sudi, & Hassan Wafi Garba. (2024). Antidiarrheal Activity of Biosynthesized Zinc Oxide Nanoparticle Using *Cassia occidentalis* in Castor Oil Induced Diarrhea Rats. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 8(8), 10-16. Article DOI <https://doi.org/10.47001/IRJIET/2024.808002>
