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Research Article
Green Synthesis of Manganese Oxide Nanoparticles from *Cassia tora* Leaves and its Toxicological Evaluation

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Abstract

Background and Objective: Green synthesis of nanoparticles is gaining wide acceptability because of their acclaimed lesser toxicity. This study was aimed at synthesizing manganese oxide nanoparticle from *Cassia tora* aqueous leave extract in order to investigate the toxicological effect of the biologically synthesized manganese oxide nanoparticles (MnO_2 NPs). **Materials and Methods:** The biosynthesized MnO_2 NPs was characterized using UV-visible spectrometry, Fourier Transform Infrared (FTIR) spectroscopy and scanning Electron Microscope (SEM). Twenty adult albino rats were randomly divided into four groups, group 1 served as the control while groups 2, 3 and 4 received orally 100, 200 and 400 mg kg^{-1} b.wt., *Cassia tora* leaves biosynthesized MnO_2 NPs, respectively for 21 days. Haematological, liver function and kidney function parameters were carried out. **Results:** X-ray diffraction revealed that the biosynthesized MnO_2 NPs was spherically agglomerated in nature while the particle size was 75 nm. There was no significant difference in Red Blood Cell (RBC), Hemoglobin (Hb), Packed Cell Volume (PCV), White Blood Cell (WBC), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), bilirubin, urea, creatinine, Na^+ and HCO_3^- values in rats orally administered 100, 200 and 400 mg kg^{-1} b.wt., biosynthesized MnO_2 NPs when compared with the control group. However, total protein significantly decreased while K^+ concentration significantly increase. **Conclusion:** This indicates that biosynthesized MnO_2 NPs from *Cassia tora* leaves is not completely safe at these doses tested.

Key words: Nanoparticle, nanotechnology, manganese oxide, green synthesis, *Cassia tora*

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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

Nanoparticles are particles or substances whose diameter is lower[1-3] than 100 nm (10^{-9} m) i.e., ranging from 1-100 nm. Several chemicals can be classified as nanomaterials, most common are the heavy metals and their metal oxides[4]. Synthesized nanoparticles exhibit unique properties lacking in the bulk materials which makes them useful for a wide array of applications[4,5]. Manganese oxide due to their variable oxidation state, magnetic, chemical and electrical properties has a great potential as a new environmentally friendly nanoparticle[5]. Drinking water can be contaminated with incidental nano-manganese oxide. Besides, manganese oxide nanoparticles had been used in waste water treatment.

There are several concerns about the safety of nanoparticles on human health. Nanoparticles, enter human body after oral exposure or inhalation and accumulate in vital organs and tissues such as liver, spleen, brain and gastrointestinal tract[6-8]. The toxicity potential of nanoparticles is dependent on their physicochemical properties such as shape, size, surface area, charge, solubility, geometrical and chemical properties[4,9] for instance, the smaller the size, the easier they are able to enter the human body[10]. Also, the methods used in the preparation of chemically synthesized nanoparticles produces toxic by products and remnants toxic organic solvents are present in the nanoparticle[11,12]. Though, this limitation of conventional methods have been overcome using green synthesis[13]. Since then, various nanoparticles have been synthesized using yeast, fungi, bacteria, algae and plant extract using the green nanotechnology[14]. The use of plant extract in the biosynthesis of ecofriendly nanoparticles is being exploited to a vast extent because plants are widely distributed, safe to handle and has a range of secondary metabolites[15]. This research therefore carried out safety evaluation of biosynthesized manganese oxide nanoparticle from *Cassia tora* in albino rats.

MATERIALS AND METHODS

Collection and preparation of plant materials: The fresh leaves of *Cassia tora* was collected at Adamawa State University, Mubi, Nigeria in August, 2018. It was authenticated by a botanist at the Department of Botany, Adamawa State University, Mubi , Nigeria and specimen was deposited at the herbarium. The leaves were washed with clean water, rinsed with distilled water and air dried for 15 days at room temperature to prevent the destruction of thermo labile constituent of the plant by direct sun rays. The leaves were de-stalked and milled into coarse powder. Ten gram of the powdered leaves of *C. tora* was weighed and put into 500 mL of conical flask containing 150 mL distilled water, it was mixed properly and boiled for 2 min. The aqueous extract was filtered using a muslin cloth and then through a filter paper (Whatman No. 1). The filtrate was used immediately for the biosynthesis of manganese oxide nanoparticle as described by Paul *et al.*[5].

Biosynthesis of manganese oxide nanoparticles: The method according to Paul *et al.*[5] was adopted in the synthesis of manganese oxide nanoparticles. Five milliliter of aqueous leaves extract of *C. tora* was added to 50 mL of aqueous solution of 0.2 M potassium permanganate ($KMnO_4$) while, heating and stirring at 70°C and pH 7 for 60 min. The $KMnO_4$ solution changed from colourless to brown with formation of precipitate. The precipitate was centrifuged at 3000 rpm for 15 min and washed with distilled water 3 times.

Characterization of MnO_2 nanoparticles: The biosynthesized MnO_2 NPs was characterized according to the method described by Chatterjee *et al.*[16] using UV-visible spectroscopy, FT-IR, SEM and XRD.

UV-visible spectroscopy: The nanoparticle was dissolved in distilled water sonicated and transfer into curvette inserted into the UV-visible spectrophotometer. The absorbance was measured at 250-800 nm.

Fourier Transform Infrared (FT-IR) spectroscopy: The biomolecule responsible for the reduction of MnO_2 NPs was determined with a Perkin Elmer Fourier Transform Infrared (FT-IR) spectrophotometer. Dried samples of the plant extract and MnO_2 NPs were separately loaded on the FT-IR sample cell and scanned at 4000-400 cm^{-1}.

Scanning Electron Microscopy (SEM): Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry for analysis[17].

X-ray Diffraction measurements (XRD): About 1 g of the powdered sample was prepared using the sample preparation block and compressed in the flat sample holder to create a flat, smooth surface that was later mounted on the sample stage in the XRD cabinet. The sample was analyzed using the reflection transmission spinner stage using the Theta-Theta settings. The 2θ starting position was 0.00483 and ends at

75.000 with a 2θ step of 0.026 at 3.57 sec/step. Tube current was 40 Ma and the tension was 45 VA. Fixed divergent slit size of 1° was used and the goniometer radius was 240 mm. The average particle size of the biosynthesized nanoparticles was calculated using Debye-Scherrer equation:

$$D = \frac{K\lambda}{\beta}\cos\theta$$

Where:

D = Crystalline size of MnO_2 NPs
λ = Wavelength of x-ray source (0.1541 nm) used in XRD
β = Full width at half maximum of the diffraction peak
K = Scherrer constant with value from 0.9-1
θ = Bragg angle

Experimental animals: Total 20 healthy adult albino rats weighing between 120-150 g, were used as the mammalian experimental model. The rats were purchased from National Veterinary Research Institute, Vom, Plateau state Nigeria. The rats were housed in animal cages with access to food and water *ad libitum* and were left to acclimatize for 1 week before commencement of experiment. All the experimental procedures were conducted in accordance with the standard guideline principles on the use of living laboratory animals in scientific research.

Experimental design: The rats were randomized into 4 groups containing 5 animals each. Groups 1, received normal saline while groups 2, 3 and 4 received100, 200 and 400 mg kg^{-1} b.wt., of biosynthesized manganese oxide nanoparticle, respectively for 21 days.

Biochemical parameters analysis: On the 22nd day, all rats were euthanized with 100% chloroform. Blood samples from each rat was collected in 2 sample containers, ethylenediaminetetraacetic acid containing sample bottle for estimation of haematological parameters and plain sample bottles which was centrifuged and supernatant (serum) used for biochemical parameters analysis. Alanine aminotransferase and aspartate aminotransferase was done using the method described by Reitman and Frankel[18]. Alkaline phosphatase, bilirubin, albumin, total protein, urea, creatinine and electrolytes was done using the methods described by Wright *et al.*[19], Jendrassik and Groff[20], Doumas *et al.*[21], Kingsley[22], Fawcett and Scoth[23], Taussky and Brahen[24] and Kulpmann[25].

Statistical analysis: Data are expressed as Mean±Standard Error of Mean (SEM). The statistical analysis was done using one-way Analysis of Variance (ANOVA) followed by the Duncan multiple test. The $p<0.05$ was considered significant.

RESULTS

Figure 1 shows the UV visible absorption spectrum of MnO_2 NPs biosynthesize from *Cassia tora*.

The UV visible spectroscopy gave a sharp narrow absorption maxima at 290 nm, which can be attributed to the formation of MnO_2 nanoparticles. Figure 2 shows the FTIR for *Cassia tora* leaves extract and MnO_2 nanoparticles. The result observed from *Cassia tora* leave gave the IR band (Table 1) while manganese oxide nanoparticles gave IR band as shown in Table 2. Figure 3 shows SEM image of the MnO_2 NPs. It revealed the morphology of the MnO_2 NPs to be

Table 1: Fourier Transform Infrared (FT-IR) of *Cassia tora* leaves extract

Frequency	Functional group	Type of vibration	Characteristic absorption	Intensity
3280.20/68.69	OH	Stretched (H Bonded)	3200-3600	Strong, broad
2919.03/74.90	OH	Stretching Carboxylic acid		Strong
2850.30/80.53	C-H	Stretch of aliphatic alkane		Strong
1587.75/67.52	C-O	Stretched	1550-1640	Strong
1247.11/71.24	C-O	Stretched	1050-1600	Strong
1012.37/39.66	C-N	Stretched aliphatic amine	1000-1360	

Table 2: Fourier Transform Infrared (FT-IR) analysis of biosynthesized MnO_2 NP from *Cassia tora* leaves

Frequency	Functional group	Type of vibration	Characteristic absorption	Intensity
3323.98/52.65	O-H	Stretched (H Bonded)	3200-3600	Strong, broad
1614.44/47.32	C-O	Bend	1550-1640	Strong
1359.89/57.61	C-H	Stretch, bending	1350-1480	Strong
1308.98/38.56	C-O	Stretched	1210-1320	Strong
809.59/42.92	Mn-O		1000-400	Strong
707.45/47.16	Mn-O		1000-400	Strong

Table 3: X-ray Diffraction (XRD) of *Cassia tora* biosynthesized MnO_2 nanoparticle

H	K	L	FWMH	d (A^0)	2θ
2	1	1	0.2070	3.91978	22.6859
1	0	1	0.1812	3.00211	29.76084
2	2	2	0.1294	2.69187	33.2845
4	0	0	0.2070	2.29075	39.3328
2	1	0	0.4141	2.04426	44.3111
1	3	4	0.1553	1.84537	49.3150
2	2	0	0.3106	1.49633	62.0239
0	0	2	0.2070	1.39013	67.3674

H, K, L: Lattice planes, FWMH: Full width at half maximum

Table 4: Effect of biosynthesized MnO_2 Nps from *Cassia tora* leaves on some haematological parameters

Groups	RBC (10^{12} L^{-1})	WBC ($\times 10^9$ L^{-1})	PCV (%)	HB (g dL^{-1})
1	3.55±0.24^a	9.75±0.51^a	31.25±1.03^a	9.97±0.91^a
2	3.87±0.13^a	9.97±0.40^a	31.37±0.47^a	9.97±0.40^a
3	3.75±0.18^a	9.65±0.46^a	34.50±3.17^a	10.62±0.23^a
4	3.85±0.06^a	9.77±0.46^a	32.72±0.49^a	11.02±0.34^a

Values are expressed as Mean±SEM, (n = 5), values along the same column with same superscript are not significantly different (p<0.005), RBC: Red blood cells, PCV: Packed cell volume and HB: Hemoglobin

Table 5: Effect of *Cassia tora* biosynthesized MnO_2 NPs on some liver function parameters

Groups	Total protein (g dL^{-1})	Serum albumin(g dL^{-1})	ALP (IU L^{-1})	ALT (IU L^{-1})	AST (IU L^{-1})	Total bilirubin (mmol L^{-1})	Con. bilirubin (mmol L^{-1})
1	77.50±0.64^b	36.25±0.64^a	21.25±0.479^a	4.25±0.25^a	6.50±0.28^a	11.00±0.40^a	8.75±0.25^a
2	73.25±1.75^{ab}	37.00±0.91^a	18.50±0.65^a	5.00±0.40^a	7.75±0.85^a	12.00±0.81^a	7.75±0.85^a
3	72.00±2.38^{ab}	38.25±1.10^a	20.50±0.64^a	5.75±1.03^a	6.50±0.64^a	12.25±1.10^a	8.00±0.91^a
4	67.57±2.65^a	35.50±2.21^a	23.00±2.64^a	5.75±0.85^a	8.12±0.87^a	11.75±0.85^a	7.50±0.64^a

Values are expressed as Mean±SEM, (n = 5), values along the same column with same superscript are not significantly different, values with different superscript are significantly different (p<0.05), ALT: Alanine aminotransferase, ALP: Alkaline phosphate, AST: Aspartate aminotransferase

Table 6: Effect of *Cassia tora* leaves biosynthesized MnO_2NPs on serum kidney function parameters

Groups	Na^+ (mmol L^{-1})	K^+ (mmol L^{-1})	Cl^- (mmol L^{-1})	HCO_3^- (mmol L^{-1})	Urea (mmol L^{-1})	Creatinine (mmol L^{-1})
1	135.50±3.87^a	2.88±0.25^a	99.29±1.71^a	25.00±3.16^a	2.93±0.09^a	118.50±15.02^a
2	138.00±2.16^a	3.13±0.35^{ab}	99.50±1.29^a	25.00±1.83^a	2.88±0.25^a	125.75±5.56^a
3	139.50±2.08^a	3.23±0.46^{ab}	100.50±1.11^a	22.75±1.71^a	3.05±0.13^a	124.00±9.38^a
4	138.25±1.71^a	3.60±0.18^b	100.00±2.58^a	24.25±2.58^a	3.10±0.24^a	125.25±9.54^a

Values are expressed as Mean±SEM, (n = 5), values along the same column with same superscript are not significantly different, values with different superscript are significantly different (p<0.05), Na+: Sodium ion, K^+: Potassium ion: Cl: Chloride ion and HCO_3: Bicarbonate ion

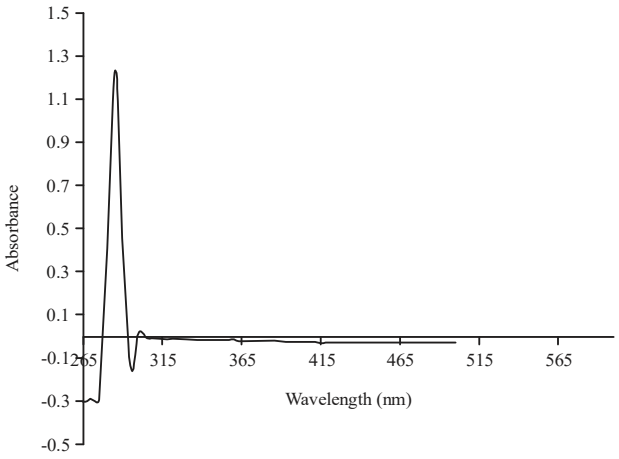

Fig. 1: UV-visible absorption spectrum of manganese oxide nanoparticles biosynthesized from *Cassia tora* leaves extract

an agglomerated polymorphic material. The XRD pattern of the biogenic MnO_2 NPs shows eight intense Bragg reflections as shown in Fig. 4. Table 3 present various data obtained from XRD analysis which was used in calculation of the particles size.

The effect of 21 days oral administration of biosynthesized MnO_2 NPs on haematological parameters of male and female albino rats is shown in Table 4. The administration of the biosynthesized NPs at all doses did not cause any significant effect on the RBC, WBC, PCV and HB as compared to the control. There was no significant difference (p<0.05) in all the haematological parameters evaluated when compared with the control at all doses of biosynthesized MnO_2 NPs.

The concentration of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Bilirubin, total protein and conjugated bilirubin in the serum of the albino rats estimated during the 21 days oral

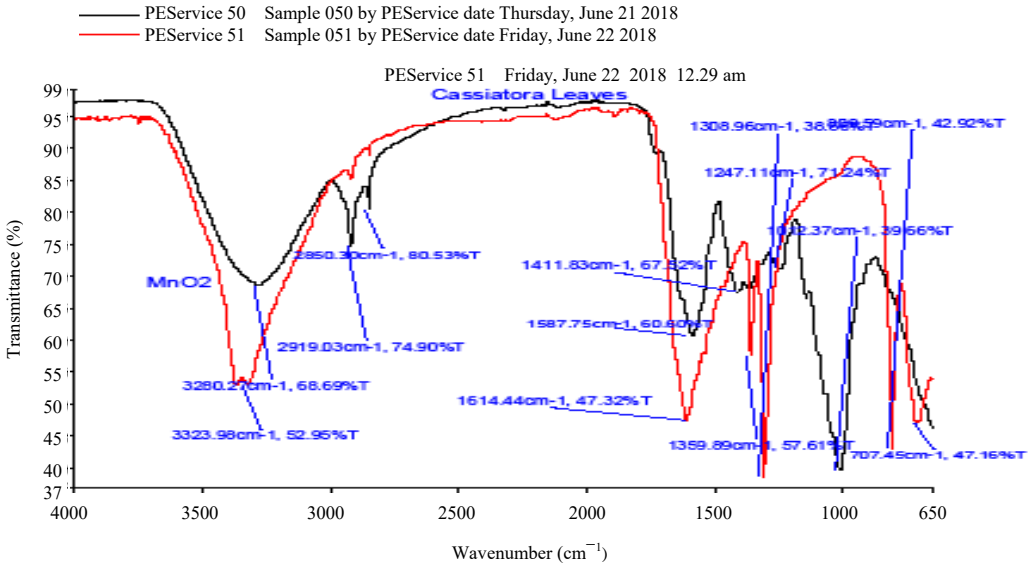

Fig. 2: Fourier Transform Infrared (FTIR) spectra of MnO_2 nanoparticles synthesized using *Cassia tora* leaves extract

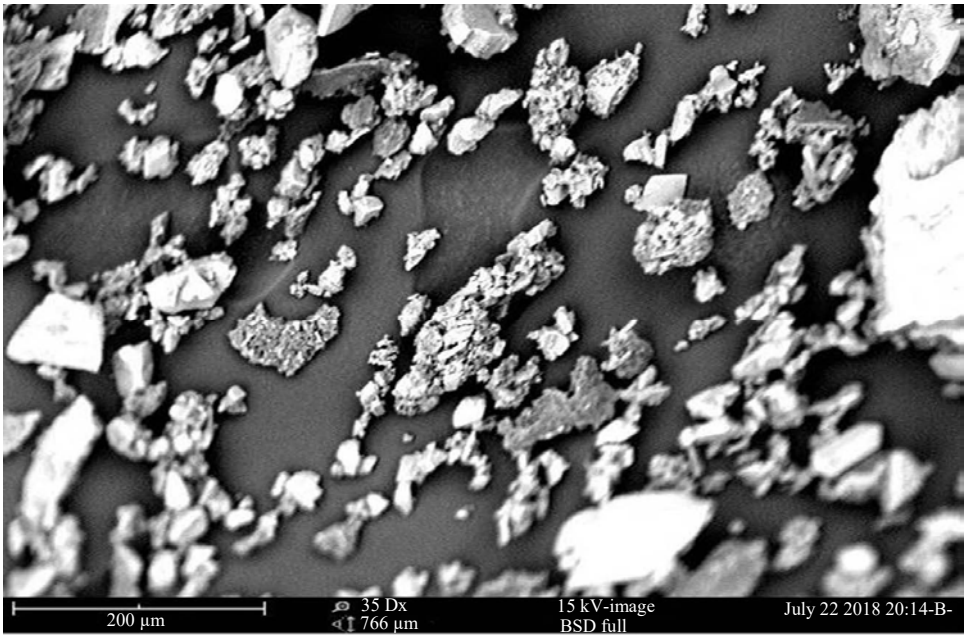

Fig. 3: SEM image of cassia tora biosynthesized MnO_2 NP

administration is shown in Table 5. The result revealed that the oral administration of the biosynthesize NPs did not cause any significant increase in the activity and concentration of the enzyme biomarkers and bilirubin in the serum but there was a dose dependent significant decrease ($p>0.05$) in the concentration of total protein. The result of the effect of *Cassia tora* leaves biosynthesized manganese oxide nanoparticles on kidney function parameters is shown in Table 6. There was no significant difference in all the kidney function parameters tested for except the K^+ concentration that significantly increased ($p<0.05$) in a dose dependent manner.

DISCUSSION

The biosynthesis of manganese oxide nanoparticles from potassium per manganese using *Cassia tora* leaves extract as reducing agent was confirmed by the use of optical

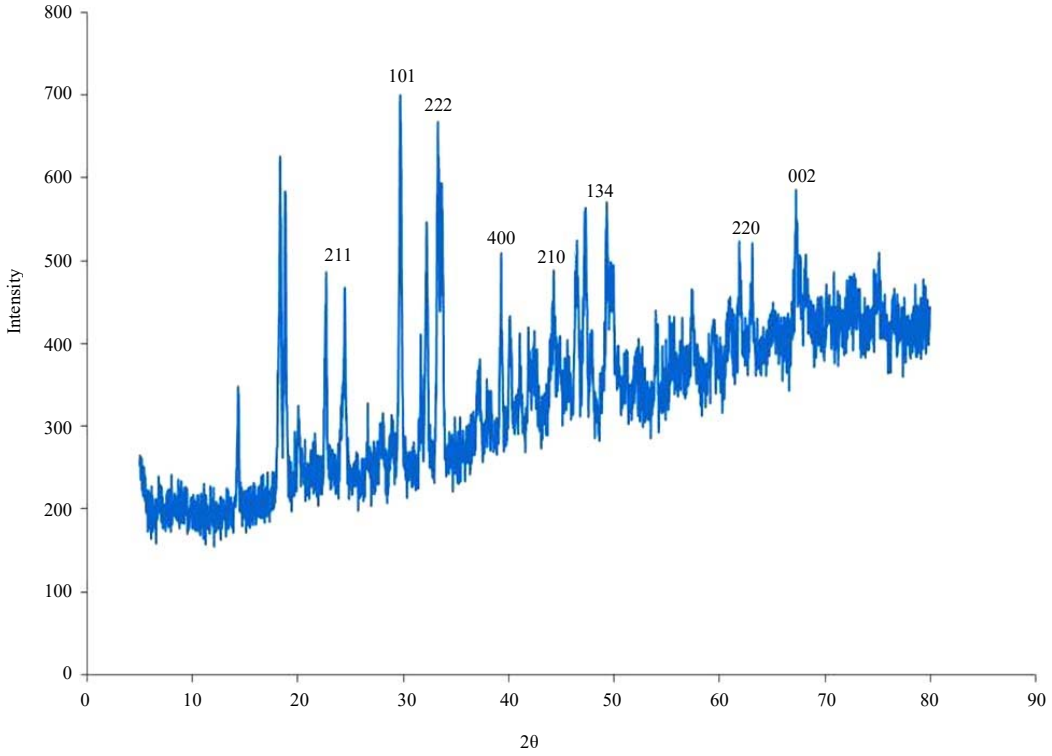

Fig. 4: X-ray diffraction pattern obtained for *Cassia tora* biosynthesized MnO_2 nanoparticle

property, UV-visible spectroscopy, FTIR spectroscopy, SEM and XRD. UV-visible spectroscopy is a useful technique in confirming the formation of metal nanoparticles in aqueous solution[26]. Metallic nanoparticles display characteristic optical absorption spectra in the UV-visible region called Surface Plasmon Resonance (SPR) which is due to the physical absorption of light by metallic nanoparticles and this leads to a coherent oscillation of the conduction electron[27]. This is a small particle effect since it is absent in individual atoms as well as in their bulk structures. UV-visible spectra of MnO_2 nanoparticles show the characteristic fingerprint of the Surface Plasmon Resonance (SPR) spectra with absorbance at 285-305 nm with peak maxima at 290 nm, which can be attributed to the formation of MnO_2 nanoparticles.

The Fourier Transform Infrared (FTIR) analysis was performed to identify the possible biomolecules responsible for the reduction of manganese ions and capping of the reduced manganese oxide nanoparticles biosynthesized using *Cassia tora* leaves extract. The bands at 809.59 and 707.45 cm^{-1} found in the metal oxide correspond to the Mn-O bond. This indicates that the synthesized nanomaterial is manganese oxide. Absorption peak observed at 3323.89 and 3280.20 cm^{-1} present in the metal oxide and *Cassia tora* leaf extract respectively may be due to OH stretching vibrations of water. The absorption peaks at 2919.03 cm^{-1} observed in the *Cassia tora* leaf corresponds to C-H bending while the absorption band at 2850.75 cm^{-1} was ascribe to C-H stretch of alkane. The absorption band at 1614.44 cm^{-1} in the metal oxide and 1587.75 cm^{-1} in the *Cassia tora* leaf are assigned to C=O of amide. The peaks at 1359.89, 1308.96 cm^{-1} of the metal oxide and 1247.83 cm^{-1} of the plant may be due to C-O stretching vibrations, while the absorption band at 1012.37 cm^{-1} in the plant is for C-N stretching amine. The presence of some of the peaks (2919.03, 2850.75 and 1012.37 cm^{-1}) in the plant and its absence in the metal oxide nanoparticle indicates those of the biochemical molecules responsible for the bioreduction. Their absent may be due to their participation in the bio-reduction which led to their oxidation.

Scanning Electron Microscope (SEM) analysis of the *Cassia tora* biosynthesized nanoparticle revealed that the manganese oxide nanoparticles exhibit agglomeration which occurred during the synthesis process. The polymorphic morphology of material observed from the SEM image was very alike to that synthesized by Jayandran *et al.*[14].

X-ray diffraction analysis was carried out to confirm the crystalline size and nature of the manganese oxide nanoparticles. The eight intense Bragg reflections observed may be indexed to Face Centered Cubic (FCC) structure of manganese oxide. A comparison of obtained XRD spectrum with the standard, confirmed that the manganese oxide nanoparticles formed were in the form of crystals as evidenced by the peak at the 2θ values. The un-assigned reflections could be due to crystallization of bio-organic molecules that may occur on the surface of the nanoparticles. The broadening of XRD peaks around the bases indicated that the manganese particles were in nan-orange[28]. The average particle size was found to be 75 nm. The XRD patterns displayed in this study are in good agreement with the earlier research reported for green synthesis of silver nanoparticles by Jayandran *et al.*[14].

Evaluation of haematological indices provide useful information on the adverse effects of foreign components on the blood and also explain blood related functions of chemical compounds[29]. The non-significant effect of the biosynthesized MnO_2 NPs on RBC, HB, WBC and PCV throughout the experimental period is an indication that there were no destructions of matured RBC and no change in the rate of production of RBC's (erythropoiesis). White blood cells defend the body against infections or any foreign bodies[30]. Therefore, the non- significant different in WBC indicate that the MnO_2 NPs did not produce any toxic effect.

Elevated serum levels of ALT, a cytosolic enzyme predominantly present in the liver, indicates hepatocellular injury. Elevated serum bilirubin level is an indicator of excretory dysfunction of the liver. The non-significant difference in ALT, total and conjugated bilirubin indicates that *Cassia tora* biosynthesized MnO_2 NPs did not affect hepatocellular integrity and excretory function of the liver cells. Total protein is the sum total of plasma proteins, albumin and globulin. Albumin and most globulins are synthesized in the liver[31]. It is therefore a useful index for the synthetic capacity of the liver. The significant decrease in the total protein concentration in rats administered *Cassia tora* leaves biosynthesized MnO_2 NPs indicates dysfunction in the synthetic capacity of the liver. Zaitseva *et al.*[32] also reported that chemically synthesized manganese oxide nanoparticles after per-oral intake for 30 days resulted in a liver protein synthesizing function, in addition to reduced body weight, liver cell membrane damage connected to higher serum AST and ALT. One of the major functions of the kidney is maintenance of electrolyte balance[33]. The significant increase in serum potassium ion concentration reflects a dysfunction in tubular reabsorption by the nephrons of the kidney.

CONCLUSION

This study concludes that the Manganese oxide nanoparticle biosynthesized from the aqueous leaf extract of *Cassia tora* posses slight toxicity on the liver and kidney and have somehow harmful effects.

SIGNIFICANCE STATEMENT

This study synthesized manganese oxide nanoparticle from *Cassia tora* leaf and discovered that consumption of this biosynthesized manganese oxide nanoparticle is not completely safe for consumption at the doses tested. Thus a new theory can be carried out on lesser dose.

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