Green synthesis of Titanium nanoparticles (Ti NPs) using aqueous fruits extract of *Tribulus terrestris* (Dirassa): Synthesis, characterization and Toxicity on Wister Rats

Enas.A.Eisa[1*] and Shama. I. Y.Adam[1] and Nashwa A. A. Eassa[2]

[1]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, Al Neelain, University, Khartoum, Sudan

[2]Department of physics and material Science, Faculty of Science and Technology, Al Neelain, Khartoum, Sudan.

*Corresponding author email: enaseisa90@gmail.com

Abstract

Background: Recently, the green synthesis of metal nanoparticles has gained much interest and there is an increasing demand for various nanoparticles due to their extensive applicability in various areas such as clinical and medical applications, consumer products, electronics and equipment, catalysis, chemistry, energy and gaining more importance due to its simplicity, non-toxic an environmental friendly, cost effective, easy process.

Objective: This study investigate an efficient, sustainable route and eco-friendly technique for green synthesis of titanium nanoparticles using fruits of *Tribulus terrestris*, for their wide availability and medicinal property, and to evaluate the toxicity of different doses Ti-NPs on Wister Rats.

Methods: TiNPs were prepared by the reaction of 0.5M titanium tetrachloride and 20% fruits extract of *Tribulus terrestris* (Dirassa). The TiNPs were characterized by x-ray diffraction (XRD),scanning electron microscopy (SEM), Energy dispersive X-Ray Spectroscopy (EDS), UV-Visible spectroscopy (UV) and tested for their toxicity on Wister rats .The extracts was prepared and given to rats orally once a day for 8 weeks. Body weight was measured weekly. Biochemical effects (serology and hematology) and Histopathological studies of liver, kidney, intestine, brain and heart were performed.

Results: Nanoparticles were characterized. The size of the obtained TiNPs with size 6 nm and the shapes is homogenous under the preparation conditions. Body weight after 8weeks of treatment of rats with Ti nanoparticles was significantly higher in treated groups when compared with control animals ($p < 0.05$), the hematology show significant ($P < 0.05$) decrease in MCHC, MCH and Lymphocytes and increase in neutrophils. ALP and creatinine was significant ($P < 0.05$) decrease in all treated groups .The results of histopathology examination from various treatment groups revealed that the damage in high dose and showed less damage and low index of changes in medium dose and no any change or damage to the vital organs in lower dose.

Key words: Titanium nanoparticles, Toxicity, Dirassa,Titanium, *Tribulus terrestris*, Green synthesis.

Introduction

Green synthesis the development of this relatively new and mostly untapped field of research on the biosynthesis of nanomaterials has been facilitated by the availability of a variety of methods for the synthesis of nano- and micro-scaled materials in the natural environment (Mohanpuri*et al*., 2008). The demand for green production of nanoparticles has significantly increased due to the high costs and toxicity of physical and chemical approaches. Thus, in an effort to find less expensive options, scientists have begun utilizing biological elements/molecules that act as reducing agents, such as plants and eventually plant extracts. The bottom-up strategy is used in the biosynthesis method, which includes either reduction or oxidation reactions. Microbial enzymes' reducing or antioxidant capabilities (Jang *et al*., 2014) or plant phytochemicals' (Lee *et al*., 2014) are usually in charge of reducing metals into their corresponding nanoparticles. The choice of the solvent medium, environmentally friendly reducing agent, and nontoxic stabilizing material is crucial to the success of green synthesis. Due to the hydrophobicity of capping agents, the bulk

of synthesis processes (physical and chemical) heavily rely on organic solvents (Raveendran *et al*., 2003). But in green synthesis, the bio extracts typically contain reducing and stabilizing agents (Nellorea *et al*., 2012).

Titanium NPs Applications in the biomedical, industrial, and optical fields are gradually expanding (Seeger *et al*., 2009). This is because they are suitable for biological, industrial, and medical applications due to their low production costs, high refractive index, photo-stability in solutions, and anticorrosive qualities (Fartkhooni *et al*., 2016).Titanium nanoparticles (NPs) have a variety of sizes, shapes, chemical compositions, and crystalline structures with unique properties like surface functionalization and higher stability that make them useful in a variety of fields in our daily lives (Li *et al*., 2010). Each year, thousands of tons of Ti NPs are used globally in a variety of commercial applications, including plastics, paints, cements, and other products. Recent studies suggested that the majority of the Ti currently produced will be converted into nano forms by the end of year 2026 (Galletti, 2016).Ti nanoparticles are also among the most widely used nanomaterials in medicine, engineering, agriculture, personal care, cosmetics, sunscreens, toothpaste, electronics, clothing, paints, and covers, as well as in food and imaging (Yang *et al*, 2017). TiNPs are also heavily used in nanomedicine, where they are employed in advanced imaging, nanotherapeutics like photodynamic therapy, antimicrobial drugs, and skin care products, as well as the diagnosis and treatment of diseases (Yuan *et al*., 2010). Additionally, numerous researchers have documented the toxic effects of TiO_2 NPs on a variety of organs (Zhao *et al*., 2009). These NPs could be ingested, inhaled, injected intravenously, absorbed through the skin, and distributed throughout the body's vital organs, including the lymph nodes, brain, lung, liver, and kidney (Shakeel *et al*., 2016).These nanomaterials can enter cells and spread quickly in organs and tissues after injection (Mahdieh *et al.*, 2016). Additionally, in order to access various organs and tissues, TiNPs have the capacity to pass through biological barriers like the blood-brain barrier and the blood-placenta barrier (Song *et al*., 2015). According to several studies, Ti NPs accumulated in various organs of experimental animals, primarily the liver, kidneys, spleen, lymph node, lungs, and heart. The liver and kidneys also took 15 days to clear out of the body after administration (Li and Chen, 2011). Infected female mice treated with TiNPs also showed signs of renal damage, such as swelling of the renal glomerulus, as well as hepatocellular degeneration and spotty necrosis. Recent research by (Chang *et al*., 2013) reviewed the presence of nano-Ti was found in various vital organs, including the liver, kidney, spleen, and brain, according to 347 reports on the toxicity of TiNPs. Moreover, the testicular tissue of mice given treatment. More worries are being voiced about the potential risk of exposure to TiNPs to human health and the environment as a result of the increasing number of applications (Yang *et al*., 2017). In order to provide scientific support for the safe application of nanotechnologies, these concerns must be looked into. The current work aims to investigate the potential changes that may be caused by TiNPs on renal tissues because little, if anything, is known about the toxicity of TiNPs on the renal tissues.

Tribulus terrestris also known as dirssa in Sudan, has medicinal and pharmaceutical value due to the presence of several steroidal saponins, which may explain its use in the development of muscles, physical conditioning, and the treatment of certain diseases (Dinchev *et al*., 2008). The extract has been shown to have antihypertensive and vasodilatory properties (Phillips *et al*., 2006) and also used for urinary dysfunction, asthma, and other conditions (Qureshi *et al*., 2010).

Studies have revealed that the saponins in the extracts of *Tribulus* species had effects on rats with diabetes that were hypoglycemic and hypolipidemic (El-Tantaway and Hassanin, 2007).

2. Experimental

2.1. Materials

Fresh green and mature fruit of *Tribulus terrestris*were collected from garden plants (Khartoum, Sudan) and used for preparation of extract. Titanium (IV) chloride, $TiCl_4$ was purchased from Navi Mumbai, INDIA.

2.2. Animals

Twenty four 3-months old, both sexes Wistar rats, with average body weight ranged from (120-160) g was used in this study. They were housed in propylene cages and were provided bedding with sawdust. The rats were clinically healthy and housed within the premises of Faculty of Science and Technology-Al-Neelain University animal house under standard husbandry conditions, and drinking water provided ad libitum. Animals were acclimatized to the experimental conditions for a period of one week prior to the commencement of the experiment. Animal experiments were designed and conducted in accordance with the guidelines of the Institutional Animal Ethical Committee.

2.3. Characterization techniques of Titanium nanoparticles:

Characterization of nanoparticles is the most important factor to understand and control of nanoparticles synthesis and applications. The characterization is performed using a variety of different techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), UV–Visible spectroscopy and Energy dispersive X-Ray Spectroscopy (EDS). These techniques are proper to determine of different parameters such as particle size, shape, crystallinity, fractal dimensions, pore size and surface area. Moreover, orientation, intercalation, and dispersion of nanoparticles could be determined by these techniques. For instance, the morphology and particle size could be detected by SEM.UV–Vis spectroscopy is used to confirm sample formation by showing the Plasmon resonance.

3. Methods

3.1. Preparation of fruits extract:

The plant's fruits was washed, dried and extracted with sterile distilled water. The extract was prepared by taking 20 g of the plant part material with 100 mL of deionized water, the mixture was boiling for 1hour in $80c^{o}$. The mixture was cooled and this extract is filtered by whatman no. (1) Filter paper this filtrate was used as the extract for the preparation of titanium nanoparticles (TiNPs).

3.2. Preparation of Titanium nanoparticles (Ti NPs)

Eighty ml of *Tribulus terrestris* fruits extract was added to 80 ml of 0.5 M aqueous $TiCL_4$ solution, with stirring magnetically at room temperature. The mixture of titanium tetra chloride and leaf extract at the end time was light yellow, after 1 hour the color was changed to brown.

The mixture was subjected to shaking on the stirrer for 4 hrs. At the end of reaction time, the mixture color was creamy. In this process nanoparticles were formed, after those add ammonia drop to drop to achieve pH of solution became 7. The nanoparticles were dried at 100°Cfor overnight and calcined at 450°Cfor 3 hours.

3.3. Experimental design

Twenty four adult Wistar rats were divided randomly to 4 groups, each of 6 rats. Group 1 continued to be fed the normal diet and served as control. Groups 2,3 and 4 were given Titanium *T. terrestris* fruits nanoparticles at 50,150 and 300 mg/kg /day orally, respectively. All rats received their designated experimental oral doses for 4 weeks. Initial and final body weight and body weight gain for each group were recorded at the first day of experimental dosing every weeks until the end of experiment. Blood samples were collected at slaughter. At necropsy, all rats were examined to identify gross lesions and specimens of the live, kidney and intestinewere fixed in 10% neutral buffered formalin and processed for histopathology

3.4. Hematological analysis

Blood samples were collected in dry test tubes containing anticoagulant EDTA (Ethylene diamine

tetra acetic acid) for determination of hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBC) counts, white blood cell (WBC), and differential WBC counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), and the measuring techniques were performed according to an automated heamatology analyzer.

3.5. Serobiochemical analysis

Blood sample were collectedat slaughter in plain container and allowed to clot and sera were separated by centrifugation at 3000 r.p.m for 5 min and stored at -20C until analyzedfor the activities of serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase (ALP) and for concentration of total protein, albumin, globulin, bilirubin, urea and creatinine. The following methods for enzyme activity of control and tested rats were performed according to the instructions in the manual of Roche Diagnosis Hitachi 902 Analyzer.

3.6. Histological examination

For histological analysis, Necropsy was conducted and all rats were examined to identify gross lesion. Specimens of the liver, Kidney, and small intestines were collected, immediately fixed in 10% formalin, embedded in paraffin wax. Subsequently, sectioned at 5µm thick with microtome and stained with haematoxylin and eosin (H&E) using Harris's hemalum. Staining was performed at room temperature and tissues were observed under a light microscope.

3.7. Statistical analysis

The results were examined by independent Sample`s t-test using SPSS software (Statistical Package for the Social Sciences, version 20 , SPSS Inc, Chicago, Illinois, USA). All results were shown as means ± standard deviation (SD) and a $p<0.05$ was determined as statistically significant (Snedecor, 1989).

4. Result

4.1. Characterization of the Ti- Dirassa NPs under different conditions

UV-Vis Spectroscopy

UV-Vis min 1240 spectrometer was used to record the absorption spectrum of TiO_2 in range from 280nm to 350nm as shown in fig (1), the maximum value of absorption of pure TiO_2 1.134 (a.u) wile for TiO_2 with plant equal 1.008 (a.u) at 300 nm for both samples corresponding to photon energy 4.133 eV, the absorption edge was also calculated using Urbach's equation at wavelength 324 nm with energy band gap 3.8 eV, it was observed that TiO_2 increase the absorption value of plant extraction.

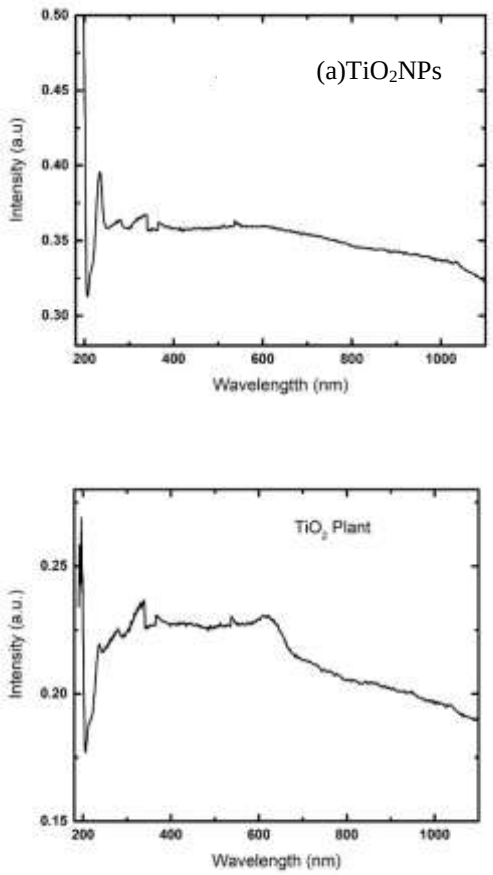

Fig (1): UV-Vis Spectroscopy of synthesized Ti nanoparticles (a) Titanium dioxide nanoparticles (b) Titanium *Tribulus terrestris* fruit nanoparticles

XRD Characterization

The XRD analysis was done to confirm the crystalline nature and particle size of the biologically synthesized TiO_2 NPS using *T. terrestris* fruit extract plant, (Fig.2) represent the XRD pattern of TiO_2. The formation of titanium dioxide nanoparticles. The formation of TiO_2 was confirm with tetragonal body-centered structure with diffraction angels 25.27º, 37.96º 48.07º, 53.7º, 55 º, 62.7 º,75.3 º at planes (101, 004, 200, 105, 211, 204, 215) respectively, the particle size was calculated using scherrer's equation as follow

$$D = \frac{0.9\lambda}{\beta cos\theta}$$

Where D is particle size, λ is wavelength of X-ray (1.546 Å), β is full width at half maximum and θ is diffraction angle, the particle size of properad TiO_2 equal 6.6 nm and this size is suitable for bio applications.

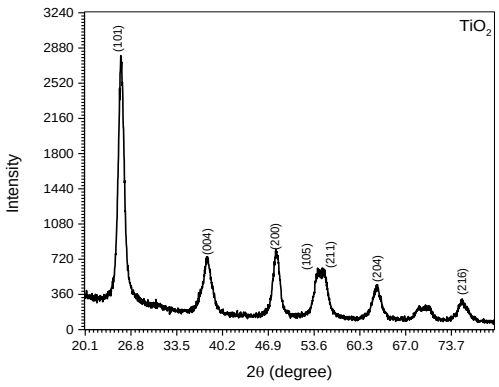

Fig (2): X-ray diffraction pattern of green synthesized Anatase TiO_2 NPs.

The SEM and EDX Characterization

The SEM image of TiO_2 NPs was shown in (fig3). It clearly that the samples particles have un uniform shapes with un homogenized surface, EDX result show that the sample consist of Titanium and oxygen.

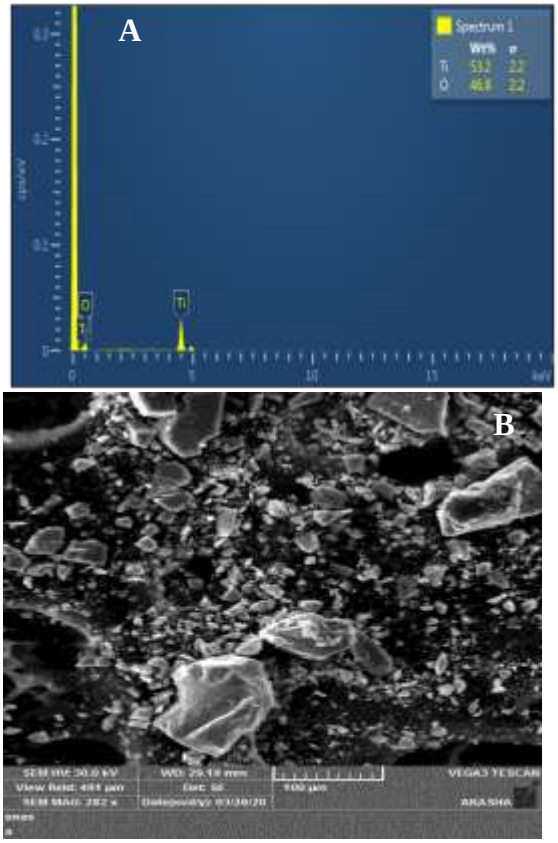

Fig (3): (a) EDS spectrum of green synthesized. (b) SEM images of green synthesized Ti NPs

4.2. Change in body weight

Body weight and body weight gain of rats given oral doses of Titanium nanoparticles with Dirassa aqueous extract at different doses for 8 weeks were presented in Table 1. After 4 weeks of the experiment there was a significant increase ($P<0.05$) and after 8 weeks was a significant decrease on the body weight of treated groups (2, 4 and 4) when compared to normal control (group 1). No death among the rats recorded along the treatment.

A

Table.1. The effect of ***T. terrestris*** fruits Titanium nanoparticles (Ti-NPs and aqueous extract of ***T. terrestris*** fruits on **body weight (g)** in rats for 8 weeks

Treatment groups		**Body weights gain (g) of rats in grams**	
	Pretreatment Body weight (g) 0 week	**Post Pretreatment**	
		Body weight gain (g) After 4 weeks	**Body weight gain (g) After 8 weeks**
1. Normal Control	141.8±17.4	7.5±9.5	50.7±25.5
2. Nanoparticles 50mg/kg (Low Dose)	139.6±6.9	21.0±7.8*	20.0±10.6*
3. Nanoparticles 100mg/kg (Medium Dose)	143.0±11.2	37.0±7.1*	0.7±11.4*
4. Nanoparticles 300mg/kg (High Dose)	140.6±5.7	26.0±7.7*	22.6±7.0*

Values are expressed as mean body weight (g) ± S.E. (*Standard Error*); n=6 rats in each group.*Significant = (*P<0.05*) as compared to normal control. (Student's t test); NS = not significant.

4.3. Hematological analysis

The hematological data after 8 weeks of treatment with Titanium nanoparticles are presented in Table 2. Hematological changes of treated rats with different doses showed significant decrease in lymphocytes and increase of neutrophils with (p-value <0.05) in comparison with untreated group. Also, there was significant decrease in mean concentration of WBCs and Hb in group 4 and significant increase in RBCs in group 3(p-value <0.05), when compared with untreated group with while other hematological parameters showed no significant change

Table.2. Hematological changes in rats given **Titanium*T. terrestris*** fruits nanoparticles (**Ti*Tt* NPs**) orally for 8 weeks.

Parameters	**1.Control(normal diet)**	**2.Ti*Tt*NPs(50mg/kg/day)**	**3.Ti*Tt*NPs(150/kg/day)**	**4.Ti*Tt*NPs(300mg/kg/day)**
Hb (g/dl)	12.2±0.93	11.5±1.62NS	12.3±0.93NS	10.9±1.86*
RBC ($x10^6mm^3$)	7.08±0.48	8.2±0.83NS	11.1±2.30*	6.10±1.05NS
MCV (m^3)	56.3±0.91	55.7±0.62NS	59.0±1.98*	55.7±0.80NS
MCH (pg)	17.2±4.0	15.2±2.46*	14.1±3.05*	17.8±0.20NS
MCHC (g/L)	30.6±1.06	27.5±4.50*	24.9±5.80*	31.9±0.46NS
WBC ($X10^6mm^3$)	4.56±1.02	4.74±1.03NS	5.33±0.82*	3.04±0.38*
Lymphocytes (%)	67.7±4.67	25.15±10.3*	52.0±2.04*	54.4±2.49*
Neutrophils (%)	32.3±4.66	67.0±10.3*	47.9±2.04*	45.6±2.49*

Values are expressed as means ± S.E. *(Standard Error).* (n = 6 rats in each group); NS = not significant;* Significant= (*P*<0.05).

4.5. Serobiochemical analysis

Serobiochemical changes for rats given Titanium nanoparticles for 8 weeks are presented in table 3. After 8 weeks of treatment showed significant decrease in activity of AST and increase in group 4 than control. ALT increase in group 2 and decrease in group 4. ALP increase in group 3 and decrease in group 4.The concentrations of total protein, Albumin were significant increase in group 4 and creatinine in all groups were significant decrease than control in group (1). While the concentration of Glucose showed significant decrease in groups 3 and 4 when compared with control.

4.4. Histopathological changes

Microscopic examinations after 8 weeks of treatment of the daily oral doses of TiNPs included the changes in the liver, kidneys and intestines in group 3. On microscopy, there was fatty cytoplasmic vaculation of the hepatocytes (figs 4 and 5). Glomerularchangs, fatty change, packing, dilation, epithelial cell degeneration or necrosis of renal tubules in cortex were detected in rats orally given 300 mg/kg/day of TiNPs Figs.6 and 7. Showed low index of changes in treated groups included mild catarrhal enteritis with infiltration of lymphocytes in the lamina propria fig 8 . These changes were less marked in group 1and 2. No lesions were observed in the heart. These changes were less marked

in group 2. Control rats (Group1) showed no significant lesions. No death among the rats recorded along the treatment.

Table.3. Changes in serum constituents of rats given Ti*Tt*NPs (Titanium *T. terrestris* fruits nanoparticles) orally for 8 weeks.

	Groups			
Parameters	**1.Control (normal diet)**	**2.Ti*Tt*NPs (50mg/kg/d)**	**3.Ti*Tt*NPs (150/kg/day)**	**4.Ti*Tt*NPs (300mg/kg/day)**
ALP (IU)	198.05±35.49	191.8±41.67*	204.2±36.97*	117.8±25.88*
ALT (IU)	36.21±6.77	44.28±8.05*	35.80±8.02NS	23.66±5.14*
AST(IU)	163.18±10.04	164.4±17.77NS	161.4±10.82NS	123.8±28.41*
Total protein (g/dl)	11.61±0.50	12.13±1.29NS	14.12±0.5NS	3.44±0.70*
Albumin(g/dl)	4.24±0.60	4.14±0.20NS	4.39±0.29*	11.08±2.27*
Globulin(g/dl)	7.37±0.50	7.99±1.26NS	9.73±1.13*	9.06±0.57*
Glucose (mg/dl)	74.69±12.28	58.06±11.38NS	64.82±7.07*	49.54±13.76*
Creatinine(mg/dl)	1.08±0.16	0.69±0.03*	0.78±0.05*	0.67±0.14*

Values are expressed as means ± S.E. *(Standard Error).* (n = 6 rats in each group); NS = not significant;* Significant= (*P*<0.05) AST; aspartate transaminase, ALT; alanine transaminase, ALP; alkaline phosphatase.

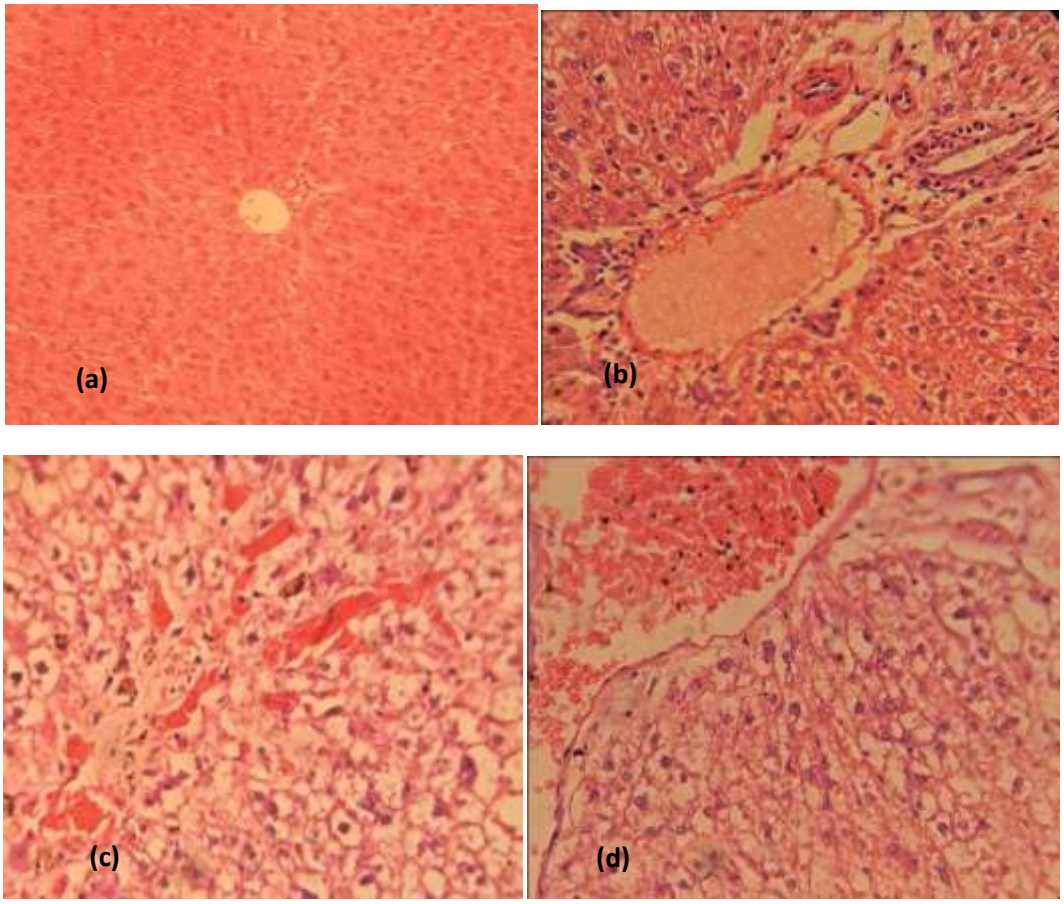

Fig .4. : Liver in a rat receiving oral dose ofTitanium nanoparticlesat 300 mg/kg/day for 8 weeks. Showing no lesions andnormal liver histology, was observed in control (a) Sever cytoplasmic fatty changes of Cytoplasmic hepatocytes(b,c) showing necrosis of centerlobularhepatocytes. (d) .H&E staining 100 X

.

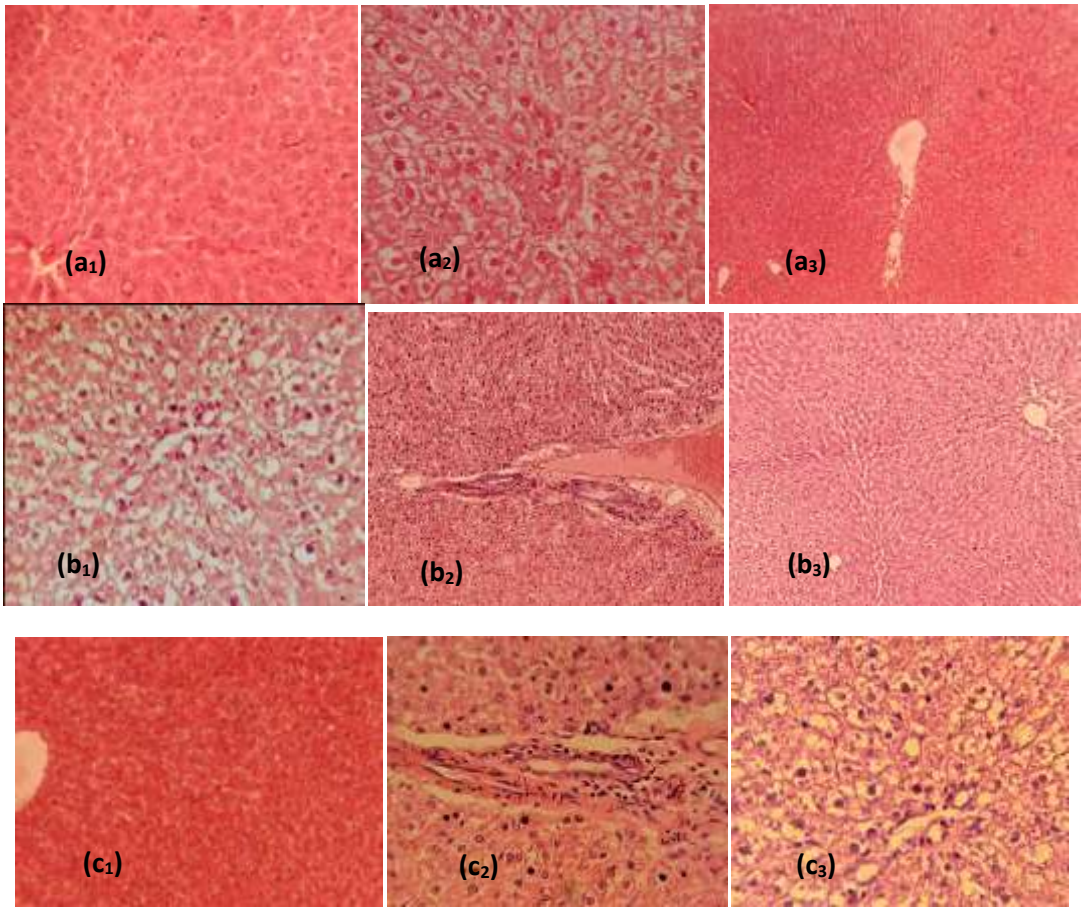

Figure .5.: Shows a comparison of liver damages ($a_{1,2,3}$) represents liver of normal rat- No changes observed in animals of the normal control group showing normal hepatic cell. ($b_{1,2,3}$) represents 50 mg/kg/day *T. terrestris* fruit Titanium nanoparticles show Slight changes and ($c_{1,2,3}$)rat treated with 150 mg/kg/day *T. terrestris* fruit Titanium nanoparticles (G3) show - Cytoplasmic fatty change and Necrosis in central vine for 8 weeks. (H & E) ×100

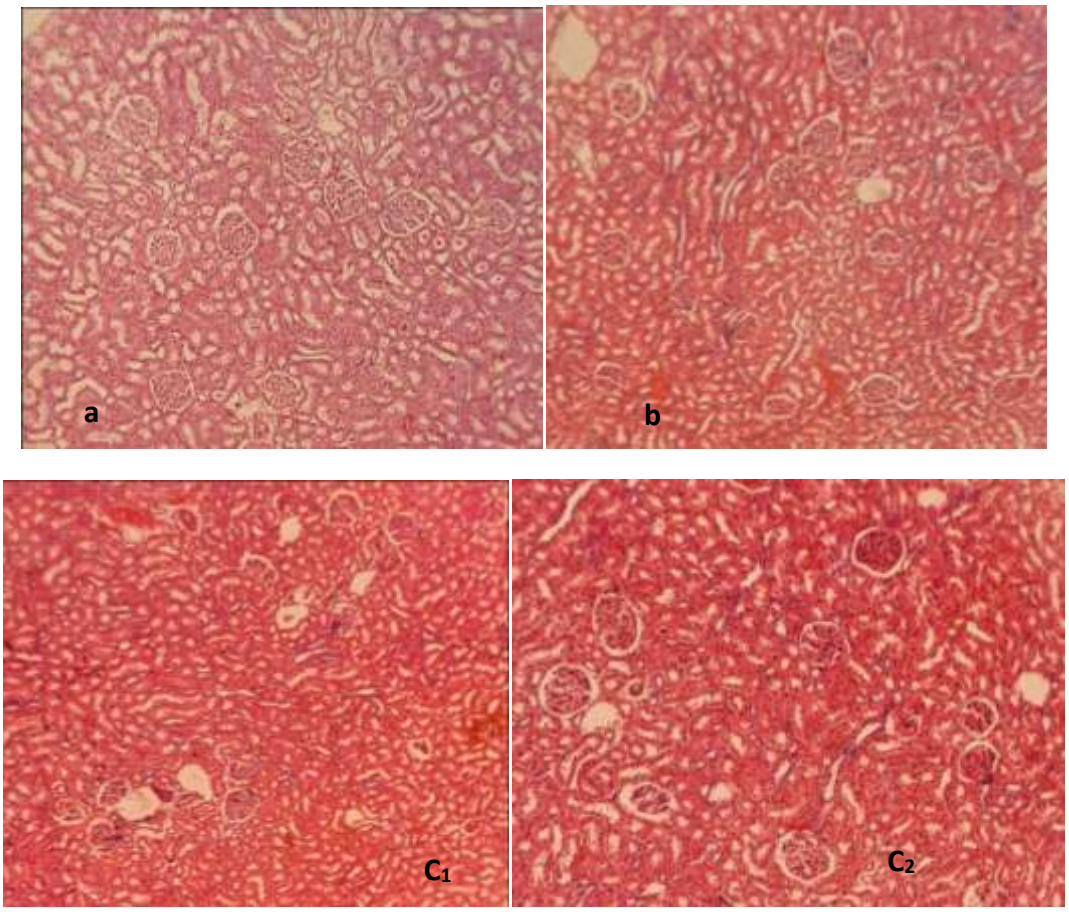

Fig.6.: Shows a comparison of kidneys of rats received daily oral doses of *T. **terrestris*** fruit Titanium nanoparticles for 8 weeks showing, (a) control ,(b) represents 50 mg/kg per day , degeneration and dilatation of renal tubules in cortex.($c^{1,2}$) represents 150mg/kg per day – Necrosis, packing and fatty change , (H & E) ×100.

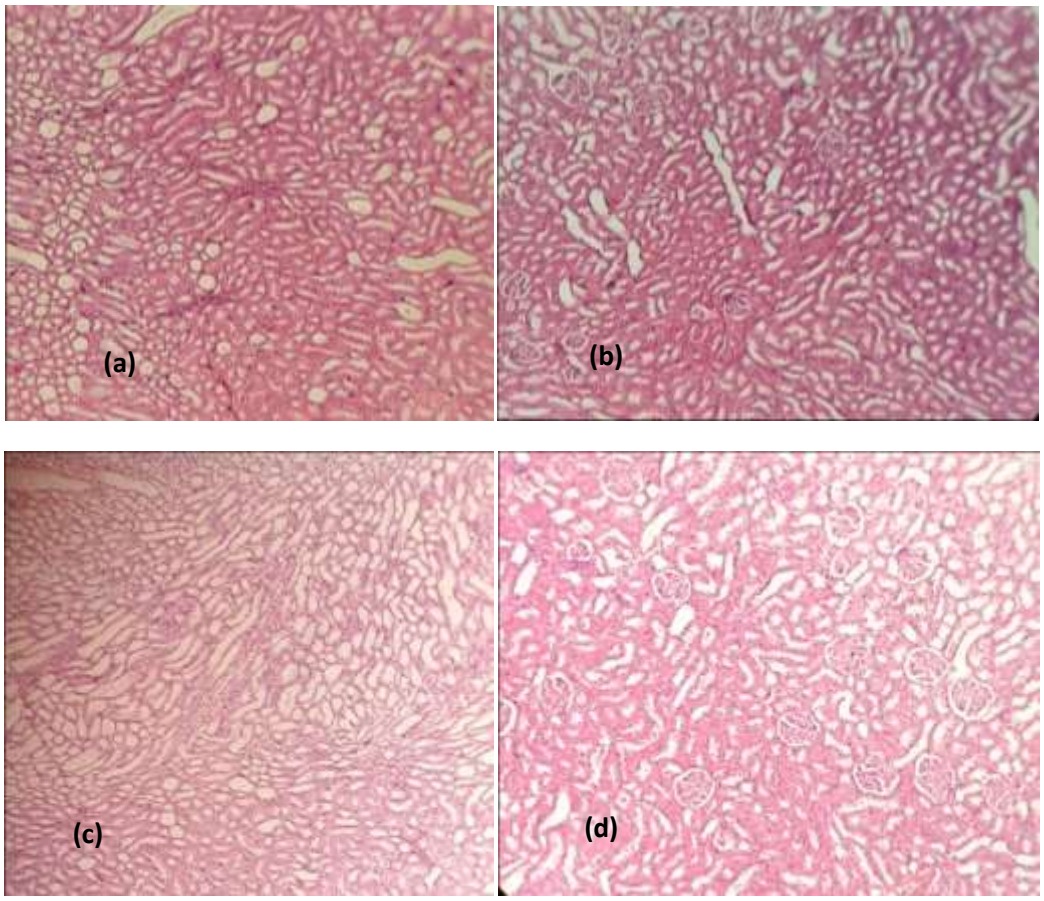

Fig .7. :kidney in a rat receiving oral Titanium nanoparticles300 mg/g/day for 8 weeks. Showing no lesions was observed in control (a) Sever cytoplasmic fatty vaculation of renal tubules in cortex (b)degeneration(c)dilatation(d)Change in the glomerular in kidney. H&E staining 100 X.

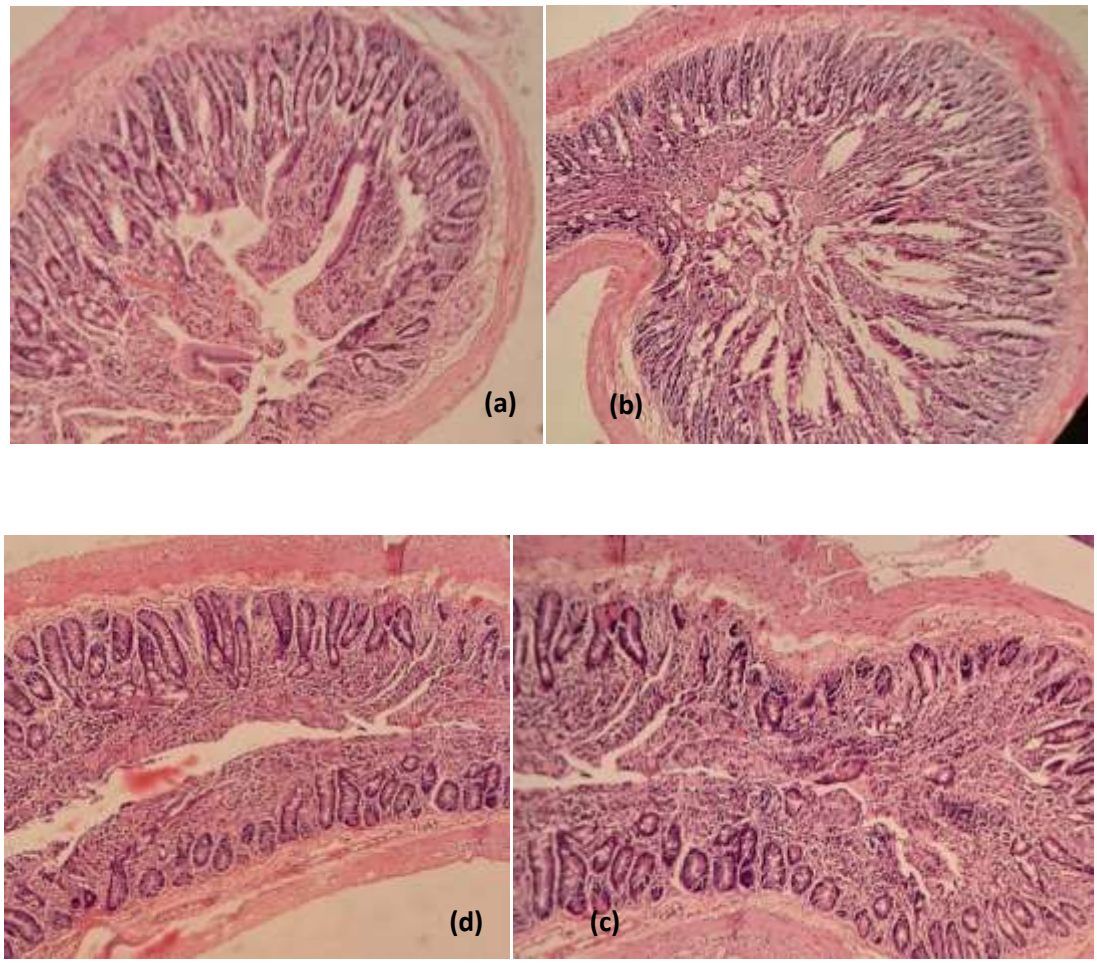

Fig.8.: Catarrhal enteritis and Desquamation of the intestinal epithelium and degeneration of the intestinal villi in a rat receiving oral *T. terrestris* fruit Titanium nanoparticles at 50(a),150 (b) and 300mg/kg/day, showing mild catarrhal enteritis and lymphocytic infiltration in intestinal lamina Propria.(c).Control(d) for 8 weeksH&E X100.

5. Discussion

Titanium nanoparticles have been widely used in industry and medicine. Green Ti nanoparticles characterization white colored titanium dioxide nanoparticles were prepared using the extract of *T. terrestris* and characterized using UV, XRD, SEM and EDS.

UV-vis spectral the present study has described the synthesis of TiO_2 NPs by the *T. terrestris* fruit extract mediated reduction of the aqueous titanium ions. Formation of TiO_2 NPs in aqueous solution was confirmed by using UV–vis spectral analysis. Results showed that the reduction of titanium ions and the generation of TiO_2 NPs were completed after overnight incubation at room temperature. The formation of creamy color indicated the reduction of titanium ions.

The absorption spectra of the TiO_2 NPs formed in the solution had absorbance peaks at around (280-350) nm for the plant extract solution exposed to TiO_2 (Ambika and Sundrarajan, 2016) Fig (1).

The XRD analysis was done to confirm the crystalline nature and particle size of the biologically synthesized Tio_2 NPS.XRD patterns of green synthesized TiO_2 nanoparticles were observed at 25.27°, 37.96°, 48.07°, 53.88°, 55°, 62.7°and 75.3° can be attributed to the 101, 004, 200, 105, 211, 204, and 215 crystalline Anatase structures of synthesized titanium dioxide nanoparticles (Fig. 2). These results were confirmed using Joint Committee on Power Diffraction Standards (JCPDS) No. 21 1272.

The calculated crystallite was found to be 6.6 nm for TiO_2 nanoparticles. The diffraction peak of the green synthesized TiO_2 nanoparticles is comparatively sharp (Varahalarao and Mohan, 2014). This phytochemical coating may enhance the stability and the dispersibility of the nanoparticles, which in turn may enhance their bioavailability, making them suitable for biological applications (Hariharan *et al*., 2017).

SEM and EDS the image was observed with magnification of 20 μm. The TiO_2 nanoparticles were observed with irregular particle structure. The size was 6.6 nm nanoparticles. The nanoparticles were dispersed evenly on the surface with development of aggregate nanoparticles which revealed that powder particles are marginally agglomerated showing the view of spherical nanoparticles (Zahir *et al*., 2015).

The SEM image of Tio_2 NPs is shown in (Fig.3b). It clearly shows that the particles consist of agglomerated and nearly spherical in shape. Therefore, the previous researcher reported that this kind of results only comes in metal oxides (Suresha *et al.*, 2015)

The EDS spectrum image shows the elemental composition, which is present in the Tio_2 NPs and is showed in (Fig.3 a). It displays three strong peaks which are identified as titanium and oxide molecules (Dhaneswar *et al*., 2013).

However, the safety of TiNPs exposure remains unclear,but it has serious adverse effects including hematotoxicity and hepatotoxicity in both animals and humans, the results of the present study indicated that, titanium is toxic to rats at dosed 300mg/kg/day for 60 days orally. This observed that pathological ,hematological and biochemical change were indicative of TiNPs affecting the intestines,kidneys and small changein brain and these could explain the change in creatinine and liver enzyme.

Titanium nanomaterials were observed to translocate into the blood, following oral or intraperitoneal exposure, and thereafter distribute to secondary targets, including the liver, spleen, lungs, and kidneys (Johnston *et al*., 2009)

Treating rats with TiNPs induced a noticeable decrease in Lymphocytes,neutrophils, MCHC and increase in WBC and Neutrophils in group 3.

This study demonstrated that liver enzymes ALP, ALT and AST measured in the serum increased in group 1 and group 2 .An increase in the levels of transaminases is related to damage of the liver, kidney, heart, and other tissues in the state of stress influenced by xenobiotic (Li et al., 2012). Therefore, data presented here suggest that the liver of rats that treated with TiNPs, may be damaged, as the liver is a major target of nanoparticle accumulation. Previous studies also have indicated that TiNPs administration leads to aggregation in the vital organs such as, liver, brain, lung, spleen, and kidney (Gui *et al*., 2011).

The maintenance of blood glucose homeostasis is always of primary importance and is required for the optimum function of the brain and nervous system (Suh*et al*., 2007). There is some evidence indicating that NPs can disrupt glucose metabolism. Shin *et al*. (2019) reported that NPs increased reactive oxygen species (ROS) and caused reduced glucose uptake and alteration in the glucose metabolic function. Thesis study agreement with result show in all groups.

The decrease in the number of red blood cells after exposure to Tio_2NPs in group3 may be due to the damage of those red cells and the reduced rate of red blood cell formation, caused by the deterioration of the hematopoietic system, resulting in the development of anemia, followed by hypoxia (Mortensen *et al*., 2010). Also, results are disagreement with Abdou *et al.* (2018) who reported that, administration of TiNPs (500 mg/kg bw) orally every other day for 60 days to rats produced significant elevation in serum urea and creatinine. They was decrease in creatinine in all groups.

Conclusion:

In totality, the Ti is a toxic substance at high dose and in lower dose is safe. Due to their benign and stable nature, these Ti NPs may be well utilized in industrial and remedial purposes and processes of water pollution remediation.

Reference

Abdou K.H., Moselhy W.A., Mohamed H.M., ElNahass E. and Khalifa A.G.(2018).Moringaoleifera Leaves Extract Protects Titanium Dioxide Nanoparticles-Induced Nephrotoxicity via Nrf2/HO-1 Signaling and Amelioration of Oxidative Stress. Biological Trace Element Research, 187(1): 181-191.

Chang X., Zhang Y., TangM. and Wang B. (2013).Health effects of exposure to nano-TiO2: a meta-analysis of experimental studies. Nanoscale Res. Lett., 8(1):51.

Dinchev D., Janda B., Evstatieva L., Oleszek W., Aslani M.R. and Kostova I. (2008). Distribution of steroidal saponins in *Tribulus terrestris* from different geographical regions. Phytochem. 69: 176-186.

El-Tantawy W.H., Temraz A., El-Gindi O.D. (2007). Free serum testosterone level in male rats treated with *Tribulus Alatus* extracts. Int. Br. J. Urol. 33(4): 554-559.

Fartkhooni F. M., Noori A. and Mohammadi, A. (2016). Effects of titanium dioxide nanoparticles toxicity on the kidney of male rats. Int. J. Life Sci., 10(1):65-9.

Galletti A. (2016).Toxicity Evaluation of TiO2 Nanoparticles Embedded in Consumer Products. Thesis. Miami, University of Miami.

Jang E., Ryu B.H., Shim H.W., Ju H., Kim D.W. and Kim T.D. (2014).Adsorption of microbial esterases on Bacillus subtilis-templated cobalt oxide nanoparticles. Int. J. Biol. Macromol., 65, 188–192.

Johnston H. J., Hutchison G. R., Christensen F. M., Peters S., Hankin S. (2009).and Stone V. Identification of the mechanisms that drive the toxicity of TiO2 particulates: the contribution of physicochemical characteristics. Part FibreToxicol., 6:33.

Lee J., Park E.Y. and Lee J. (2014). Non-toxic nanoparticles from phytochemicals, preparation and biomedical application. Bioproc. Biosyst. Eng., 376, 983–989.

Li J. J., Muralikrishnan S., Ng C. T., Yung L. Y. and Bay B. H. (2010).Nanoparticle induced pulmonary toxicity. Exp. Biol. Med. (Maywood), 235(9):1025- 33.

Li Y. F. and Chen C. (2011).Fate and toxicity of metallic and metal-containing nanoparticles for biomedical applications. Small, 7(21):2965-80.

Mahdieh Y., Sajad S., Mahmoudreza G., Ali B., Hossein D., Mohammad A. and Mehrdad M. (2016).The effects of titanium dioxide nanoparticles on liver histology in mice. J. Chem. Pharm. Res., 8(4):1313-6.

Mohanpuri P., Rana N.K., and Yadav S.K. (2008).Biosynthesis of nanoparticles, technological concepts and future applications. J. Nanopart. Res. 10, 507–517.

Mortensen M., Ferguson D. J. P., Edelmann M., Kessler B., Morten K. J., Komatsu M., and Simon A. K. (2010). Loss of autophagy in erythroid cells leads to defective removal of mitochondria and severe anemia in vivo. Proceedings of the National Academy of Sciences, 107(2), 832-837

Nellorea J., Paulineb P.C. and Amarnath K. (2012).Biogenic synthesis by Sphearanthusamaranthoids towards the efficient production of the biocompatible gold nanoparticles. Dig. J. Nanomater. Bios. 7, 123–133.

Phillips S.J., Anderson R.P. and SchapireR.E. (2006). Maximum entropy modeling of species geographic distributions. Ecol. Model., 190: 231-259.

Qureshi R., Bhatti G.R. and MemonR.A. (2010). Ethnomedicinal uses of herbs from northern

part of Nara desert, Pakistan. Pak. J. Bot., 42(2): 839-851.

Raveendran P., Fu J., Wallen S.L. (2003).Completely green synthesis and stabilization of metal nanoparticles. J. Am. Chem. Soc., 12546, 13940–13941.

Seeger E. M., Baun A., Kästner M. and Trapp S. (2009).Insignificant acute toxicity of TiO2 nanoparticles to willow trees. J. Soils Sedim., 9(1):46-53.

Shakeel M., Jabeen F., Shabbir S., Asghar M. S., Khan M. S. and Chaudhry A. S.(2016). Toxicity of nano-titanium dioxide (TiO2 - NP) through various routes of exposure: a review. Biol. Trace Elem. Res. 172(1):1-36.

Shin T. H., Seo C., Lee D. Y., Ji M., Manavalan B., Basith S., Chakkarapani S. K., Kang S. H., Lee G., Paik M. J., andPark C. B. (2019). Silica-coated magnetic nanoparticles induce glucose metabolic dysfunction in vitro via the generation of reactive oxygen species. Archives of Toxicology, 93(5), 1201–1212.

Snedecor, G. W. and Cochran, W. C. (1989). Statistical Methods, 8th Edn, Iowa State University Press, Ames, Iowa.

Song B., Liu J., Feng X., Wei L. and Shao L. A.(2015).review on potential neurotoxicity of titanium dioxide nanoparticles. Nanoscale Res. Lett., 10(1):1042.

Suh S. H., Paik I. Y., and Jacobs K. (2007). Regulation of blood glucose homeostasis during prolonged. Molecules and Cells, 23(3), 272–279.

Suresh U., Murugan K., Benelli G., Nicoletti M., Barnard D.R., Panneerselvam C., Mahesh Kumar P., Subramaniam J., Dinesh D., Chandramohan B. (2015). Tackling the growing threat of dengue: *Phyllanthusniruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedesaegypti* (Diptera: Culicidae). Parasitol Res 114:1551–1562

Yang Y., Qin Z., Zeng W., Yang T., Cao Y., Mei C. and Kuang Y. (2017).Toxicity assessment of nanoparticles in various systems and organs.Nanotechnol. Rev., 6(3):279-89.

Zhao J., Ding W. and Zhang F. (2009).Effect of nano-sized TiO2 particles on rat kidney function by metabonomicapproach. J. Toxicol., 23:201-4.