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Stannous Oxide Nanoparticles Health Hazardous on the Animal Laboratory, Alexandria, Egypt

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ABSTRACT

The present study was carried out at the Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University and Chemistry Laboratory, Department of Chemistry, Faculty of Science, Alexandria University. The aim of this study was to focus on the synthesis and characterization of tin oxide (SnO2) nanoparticles and study the effect of different concentrations of tin oxide nanoparticles on lipid peroxidation, antioxidant defense system, biochemical parameters, in addition to histology and immune histochemistry in liver and kidney of male rats. Fifty male rats (150-170g) were divided into four groups of 10 rats for each. The first group was used as control, while group II, group III, group IV and group V were treated orally with Tin oxide NPs at a dose of 1/150 LD50, 1/100 LD50, 1/50 LD50, and 1/25 LD50 mg/kg BW/day for a period of three weeks; at the end of the experimental period, blood samples, liver and kidney were collected for the investigation of different parameters. Tin oxide (SnO2) nanoparticles was prepared and characterized using different instruments such as Thermal Gravimetric Analysis (TGA), High Resolution- Transmission, Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Fourier Transform Infrared spectroscopy (FT-IR), X-Ray Diffraction (XRD). Treatment with tin oxide (SnO2) nanoparticles different concentrations increased liver and kidney thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (H2O2) and decreased the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and reduced glutathione (GSH) content as compared to control. Protein contents were significantly decreased when rats treated with tin oxide (SnO2) nanoparticles different concentrations in comparison to control group. Treatment with tin oxide (SnO2) nanoparticles different concentrations caused significant increase in urea and creatinine. The increase in urea and creatinine concentration is considered as significant markers of renal dysfunction. Treatment with tin oxide (SnO2) nanoparticles different concentrations caused significant decrease in the activities of aspartate aminotrasferase (AST), alanine aminotrasferasehe (ALT), alkaline phosphatase (ALP) and significant increase in lactate dehydrogenase (LDH) in rat liver and kidney homogenates. Histopathological and immunohistochemical changes were observed and this confirmed the biochemical perturbations occurred due to tin oxide (SnO2) nanoparticles different concentrations in rat liver and kidney.

Keywords: *Nanoparticles, Health hazardous, stannous oxide, rates*

INTRODUCTION

Particles in the nano-sized range have been present on earth for millions of years and have been used by mankind for thousands of years. Soot for instance, as part of the Black Carbon continuum, is a product of the incomplete combustion of fossil fuels and vegetation; it has a particle size in the nanometere micrometer range and therefore falls partially within the ‘‘nanoparticle’’ domain. Recently, however, nanoparticles (NP) have attracted a lot of attention because of our increasing ability to synthesize and manipulate such materials. Today, nanoscale materials find use in a variety of different areas such as electronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic and material applications. Because of the potential of this technology there has been a worldwide increase in investment in nanotechnology research and development (Guzman et al., 2006). Data on the current use and production of NP are sparse and often conflicting.

One estimate for the production of engineered nanomaterials was 2000 tons in 2004, expected to increase to 58,000 tons in 2011-2020

(Maynard, 2006). The forecasted huge increase in the manufacture and use of NP makes it likely that increasing human and environmental exposure to NP will occur. As a result NP are beginning to come under scrutiny and the discussion about the potential adverse effects of NP has increased steadily in recent years; in fact it has become a top priority in governments, the private sector and the public all over the world (Roco, 2005; Helland et al., 2006; Siegrist et al., 2007). Several reviews have summarized the recent developments in this field. Most attention has thus far been devoted to the toxicology and health implications of NP (Kreyling et al., 2006; Lam et al., 2006; Nel et al., 2006; Helland et al., 2006), while the behavior of NP in the environment (Biswas and Wu, 2005; Wiesner et al., 2006) and their ecotoxicology (Colvin, 2003; Moore, 2006) have been less often reviewed. However, no systematic description of natural and anthropogenic NP and their occurrence, fate and effects on the environment is yet available. This review therefore gives an overview on the behavior of NP in the environment; both manufactured and unintentionally produced, and also make comparisons between them and natural NP. The knowledge gained with natural NP will be of invaluable help for assessing the behavior and effects of engineered NP in the environment.

Nanoparticle types

Nanotechnology is defined as the understanding and control of matter at dimensions of roughly 1-100 nm, where unique physical properties make novel applications possible (EPA, 2007). NPs are therefore considered substances that are less than 100 nm in size in more than one dimension. They can be spherical, tubular, or irregularly shaped and can exist in fused, aggregated or agglomerated forms. Fig. 1 shows how the NP fit into other size-dependent categories that have been used for many decades. The commonly used definition of ‘‘dissolved’’ is in most cases operationally defined by all compounds passing through a filter, in many cases with a cutoff at 0.45 mm. The colloidal fraction is defined as having a size between 1 nm and 1 mm (Buffle, 2006), therefore overlapping with the NP. Separation between dissolved, colloidal and particulate matter in natural waters is normally given by the availability of analytical methods that can distinguish among these fractions without introducing artifacts during the measurement process (Lead and Wilkinson, 2006). Alternatively, Gustafsson and Gschwend (1997) have also suggested a ‘‘chemcentric’’ definition of colloids and proposed that a colloid is any constituent that provides a molecular milieu into and onto which chemicals can escape from the aqueous solution and whose environmental fate is predominantly affected by coagulation breakup mechanisms, as opposed to removal by settling. Particles in the atmosphere have normally been classified according to their size, e.g. PM10 or PM0.1, the latter corresponding to the definition of NP.

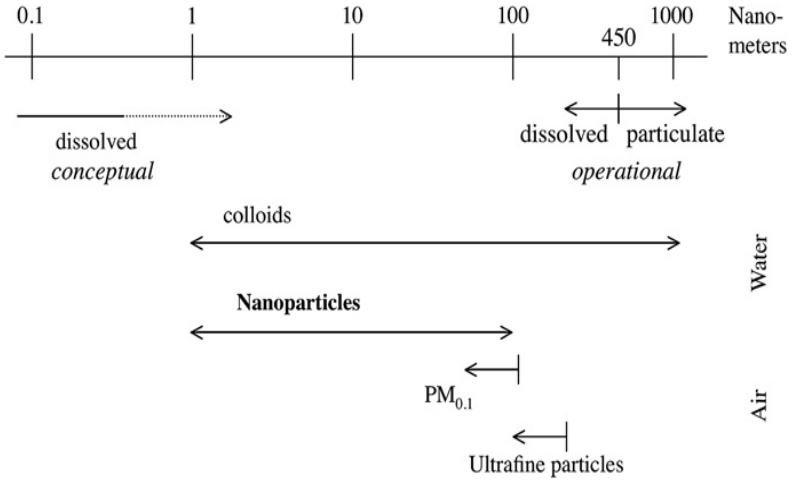

Figure1. *Definitions of different size classes relevant for nanoparticles.(Nowack and Bucheli, 2007)*

NP can be divided into natural and anthropogenic particles. The particles can be further separated based on their chemical composition into carbon-containing and inorganic NP. The C-containing natural NPs are divided into biogenic, geogenic, atmospheric and pyrogenic NP. Examples of natural NPs are fullerenes and CNT of geogenic or pyrogenic origin, biogenic magnetite or atmospheric aerosols (both organic such as organic acids and inorganic such as sea salt). Anthropogenic NP can be either inadvertently formed as a by-product, mostly during combustion, or produced intentionally due to their particular

characteristics. In the latter case, they are often referred to as engineered or manufactured NP. Examples of engineered NP are fullerenes and CNT, both pristine and functionalized and metals and metal oxides such as TiO2 and Ag. Engineered NP are the main focus of the current research on NP in the environment, but some of them occur also naturally, e.g. as inorganic oxides or fullerenes.

Nanoparticles are of great concern to the environment because of their small size and high catalytic properties. Attention must be given to the potential of particles to interact with organisms and ecosystems in unexpected ways. Nanoparticles may get released into the environment in high amounts in all stages of production, recycling and disposal. Nano-forms of particles are produced from various natural as well as anthropogenic sources such as volcanic eruptions, physical and chemical weathering of rocks, glacial ice cores, oceans, surface waters, groundwater, atmospheric water, treated drinking water, diesel exhaust, electroplating and welding etc. (Cyrys et al., 2003; Siegmann et al., 2008). These particles may aggregate to form higher size particles or may persist in air, water, soil and biological systems (Beduneau et al., 2009). Moreover, these materials can enter the environment on a continual basis from washing off of consumer products. Currently very little is known about the behavior of nanoparticles in the environment and their interaction with the biological systems (Oberdörster et al., 2005).

Nanoparticles can enter inside of an organism through various routes such as dermal, oral and respiratory tract to other parts of the body. Any combustion process produces ultrafine particles such as diesel exhaust particles, carbon black and fly ashes in vast numbers (Evelyn et al., 2003; Roy et al., 2014). The human population is routinely exposed to these particles in the ambient atmosphere, primarily from diesel fumes. The inhalation could be the most frequent route of exposure to nanoparticles present in the environment. Aerosols containing nanometer size metallic particles (e.g., Zinc oxide, tin) caused an acute adverse response in welders known as metal fume fever (Antonini, 2003). The inhaled nanoparticles can enter into the bloodstream after passing through the respiratory system. Recent studies have shown that nanoparticles can cross the blood-brain barrier and exhibit effects on the central nervous system (Long et al., 2006). These ultrafine particles can cause major pulmonary and cardiac diseases (Adar et al., 2007).

Nanoscience and nanotechnology

Nanotechnology play an important role in our society especially in solving many different problems. Table 1 gives an idea aboutapplication of nanotechnology in different fields and the most important one of this problem is water pollution (Fleischer and Wald, 2008). Nanotechnology is related to the different structures of matter with dimensions in the order of a billionth of a meter and concern with study of material on nano scale (1 nm = 10-9 m) and it plays a great role with nano science in production of nanoparticles (NPs) (smaller than 100 nm), generally (> 1 nm and < 100 nm). Nanoparticles can exist in different shapes such as spheres, rodes, wires and tubes (Liu, 2006). Nanoparticles with 10-6 atoms or less are generally characterized by some properties that are different from those of the same atoms bonded together to form bulk materials because size-dependent properties are often exhibited (Braun et al., 1997; Cohen, 2001). Nanoparticles are chemically reactive and conduct electricity more effectively than other materials (Poole and Owens, 2003).

The physical and chemical characteristics of nanosized materials can differ substantially from those of bulk materials. Nanoparticles have higher density of active sites per unit mass due to their larger specific surface area. , small size and large surface area so that affect on their electronic properties and make them have many application such as catalyst in hydrogenation reaction of monoaromatics, synthesis of diesel fuel and removal of organic pollutants from water (Gutpta et al., 2013; Cantarella et al., 2016).

Nanomaterials can give an efficient approach for water purification. Indeed, recent studies suggested that many of the issues involving water quality could be solved using nanosorbents, nanocatalysts, bioactive nanoparticles, nanostructured catalytic membranes and other products and processes resulting from the development of nanotechnology.

In addition, the properties of nanostructures can be tuned toward specific environmental remediation applications through surface modification, by modifying the nanomaterial surface with a second material to optimize the

desired remediation process (Zhang et al., 2016).

Table1. *Application of nanotechnology in the different field* ***(Pal et al., 2011)***

Applied field	Application
Nanomedicines	Nano drugs, Medical devices, Tissue engineering
Chemical and Cosmetics	Nanoscale chemicals and compounds, paints, coatings etc
Materials	Nanoparticles, carbon nanotubes, biopolymers, points, coatings
Food Sciences Environment and Energy	Processing, nutracetical food, nanocapsules. Water and air purification filters, fuel cells, photovoltaic
Military and Energy	Biosensors, weapons, sensory enhancement
Electronics	Semiconductors chips, memory storage, photonica, optoelectronics
Scientific Tools	Atomic force, microscopic and scanning tunnelling microscope
Agriculture	Atomic force, microscopic and scanning tunnelling microscope.

Preparation of Nanoparticles

Several methods can be used for nanoparticles synthesis. The selection of appropriate method for the preparation of nanoparticles depends on the physicochemical character of the polymer and the drug to be loaded (Pal et al., 2011). In the past few years, the solid-state synthesis of oxides and nanomaterials has been developed to the highest theoretical and technological levels. The solid-state synthesis is still the best method for many preparations of ceramics and other large-scale industrial productions. New solution-based methods have been developed for this purpose over the past decades and their innovative potential for accessing a wide spectrum of nanoscale oxides.

Several authors focus on low-temperature methods in solution with special emphasis on their most recent developments that have a high potential for time- and energy-saving technical processes. There are other interesting approaches to nanomaterials synthesis in high-boiling, coordinating solvents or through flame synthesis. The efficiency of high-temperature and gas-phase pathways to complex oxide nanoparticles has also been reviewed (Titirici et al., 2006; Bowen and Carry, 2002; Byrappa and Yoshimura, 2008). Pulsed laser deposition techniques furthermore plays an important role in the fine-tuning of complex oxide thin films.

Interactions of Nanoparticles with Biological Systems

To understand the mechanisms for nanoparticle toxicity, information is needed of the response of living systems to the presence of nanoparticles of varying size, shape, surface and chemical composition. Several possible mechanisms of action in the toxicity of particles in general have been postulated (Donaldson et al., 2004; Dybing et al., 2004).

It is known that nanoparticles can cross the cell membrane because of their small size, but the mechanism of internalization is currently under debate (Rothen-Rutishauser et al., 2007; Heinlaan et al., 2008). Even non-phagocytic cells such as red blood cells have been shown to internalize nanoparticles. Ultrafine particles appear to pass the pulmonary epithelial barrier and reach into the bloodstream, raising the possibility of direct contact with the vascular endothelium. Even nanoparticles have been known to localize to regional lymph nodes when administered dermally (Cross et al., 2007). Therefore, when nanoparticles enter the systemic circulation, they encounter a complex web of immune cells and plasma proteins. Nanoparticles physicochemical properties such as size, surface area, surface energy and charges are important for binding with biomolecules and may determine the fate of nanoparticles in cells (Aggarwal et al., 2009). Once inside the cell,

nanoparticles may induce adverse effects such as enhanced expression of pro-inflammatory cytokines, generation of reactive oxygen species (ROS) and DNA strand breaks (Rothen-Rutishauser et al., 2007). Direct exposure of human alveolar endothelial cells (HAECs) to ultrafine particles (Fe2O3, Y2O3 and ZnO) for 1–8 h with different concentrations (0.001–50 μg/ml) has been shown to induce an inflammatory response (Gojova et al., 2007).

Nanoparticles can bind with several bio-molecules including DNA, small peptides and proteins (Niemeyer, 2001). Protein binding is one of the key elements that affect the distribution of the nanoparticles throughout the body. ZnO NPs (30 nm) are able to bind proteins with important biological functions, including immunoglobulins, lipoproteins, albumin, α-1- antichymotrypsin, α-2-macroglobulin, and transferring (Deng et al., 2009). It has been suggested that adsorption to plasma proteins depends primarily on nanoparticle surface hydrophobicity or charge. Protein adsorption on various materials has been widely studied and found that various factors such as electrostatic interaction, hydrophobic interaction and specific chemical interactions between protein and the adsorbent play important roles.

In several reports, the mechanisms of selective adsorption have been attributed to the electrostatic interaction of many proteins (Zhang et al., 2006; Lynch et al., 2007). A study by Patil et al. has shown that microemulsions of nanoceria particles (3–5 nm) having negative zeta potential did not significantly adsorb protein (Patil et al., 2007). On the contrary, the particles of positive zeta potential of hydrothermal nanoceria particles of 8–10 nm size were found to favour the protein adsorption. Further, it can be clearly said that the surface charge properties of materials influence the protein adsorption process under physiological conditions. By varying the surface charges, the electrostatic interaction between the protein and the adsorbent for selective adsorption of a particular protein can be varied.

The aim of this study was to focus on the synthesis and characterization of tin oxide (SnO_2) nanoparticles and study the effect of different concentrations of tin oxide nanoparticles on lipid peroxidation, antioxidant defense system, biochemical parameters, in addition to histology and immunohistochemistry in liver and kidney of male rats.

MATERIAL AND METHODS

The present study was carried out at Environmental Toxicology Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, in collaboration with Chemistry Laboratory, Faculty of Science, Alexandria University, Alexandria, Egypt.

Chemicals

All chemicals used in this study are of analytical reagent grade and used as received. Tin(II)chloride dehydrate ($SnCl_2$ H_2O, Formula weight 225.65 g/molandassay 99 % purity) was purchased from ACCMA company, Egypt. Ammonia solution ($NH_3.H_2O$, Formula weight 17.03 andassay 28 %) was purchased from ADWIC Company, Egypt. Ethanol (Formula weight 46.064 g/molandassay 95%) was from ADWIC Company.

Synthesis of SnO2 nanoparticles (Nano-SnO2)

The preparation of Nano-SnO_2 material was performed according to the following procedures (Krishna and Komarneni, 2009; Yang et al., 2004). 2.0 g of tin (II) chloride monohydrate (SnCl2.H2O) was dissolved in in 100 mL distilled water.

To this solution ammonia solution was added drop by drop under stirring. The resulting gel is composed of tin (II) hydroxide ($Sn(OH)_2$) was then filtered, washed several times with distilled water and dried at 80 ºC for 24 hours to remove adsorbed water molecules. Finally, tin Oxide nanoparticles (Nano-SnO_2) were produced by heating in a muffle furnace at 550 ºC for 2 hours.

Characterization of Nano-SnO2

Several instrumental techniques were used to characterize nanoparticle Nano-SnO_2. These include thermal gravimetric analysis (TGA), Fourier transform infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), high resolution-transmission electron microscopy (HR-TEM), X-Ray Diffraction.

Thermal Gravimetric Analysis (TGA)

Thermal gravimetric analysis (TGA) and thermo analytical curves of Nano-SnO_2 was acquired using Perkin Elmer TGA7 Thermo balance. The selected operating conditions were temperature heating range = 20-700 ºC, heating rate = 10 ºC min^{-1}, flow rate = 20 ml min^{-1} pure nitrogen atmosphere and the sample mass was taken in the range of 5.0-6.0 mg.

High Resolution- Transmission Electron Microscopy (TEM)

High resolution - transmission electron microscope (HR-TEM) model JEM-2100 was used to image the Nano-SnO_2 material. The HR-TEM technique includes scanning image observation device to give bright and dark-field STEM images at 200 kV. Also, the unit comprises energy dispersive X-ray analyzer model JED-2300T to examine the images and particle size of nanosilica and other functionalized nanosorbents.

Scanning Electron Microscopy (SEM)

Scanning electron microscope (SEM) (JSM-6360LA, JEOL Ltd.), (JSM-5300, JEOL Ltd.) and an ion sputtering coating device (JEOL-JFC-1100E) were used to examine the images and characterize the surface morphology and particle size of Nano-SnO_2.

Fourier Transform Infrared spectroscopy (FT-IR)

The FT-IR spectrum of Nano-SnO_2 was recorded by using a BRUKER optics vertex 70 Fourier transform infrared spectrophotometer in the range of 400-4500 cm^{-1}.

X-Ray Diffraction (XRD)

The XRD analysis of Nano-SnO2 was acquired using CHMIEYZ mad in Japan, model number 7000 with x-ray tube of target Cu, voltage 30.0 kV, 30.0 mA and wave length 1.5 oA.

pH measurements

A calibrated ORILON RESEARCH pH-meter by using three standard buffers solutions, 4.01, 7.00 and 9.21 was used to perform the PH-measurements.

Animals and Experimental Design

Fifty male Albino Wistar rats weighing 150-170g were obtained from the animal house of the Faculty of Medicine, Alexandria University. Animals were handled in accordance with the principles of laboratory animal care as contained in NIH guide for laboratory animal welfare and the experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were housed in stainless steel bottomed wire cages and maintained at a temperature of 22 ± 2°C, relative humidity of 40-60%, with a 12 h/12 h light/dark cycle and free access to pellet diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch; Egyptian Company of Oils and Soap Kafr-Elzayat- Egypt) and water *ad libitum*. After two weeks of acclimatization, rats were randomly assigned to four groups with ten animals each.

The test substances were administered to the animals according to the following experimental protocol:

- **Group I (control)**: Control rats were orally administered distilled water via ball-tipped curved intubation needle on a daily basis for a period of 3 weeks.
- **Group II (Tin oxide NP)**: Rats were treated orally with Tin oxide NP at a dose of 1/150 LD_{50} mg/kg BW/day for a period of three weeks.
- **Group III (Tin oxide NP)**: Rats were treated orally with Tin oxide NP at a dose of 1/100 LD_{50} mg/kg BW/day for a period of three weeks.
- **Group IV (Tin oxide NP)**: Rats were treated orally with Tin oxide NP at a dose of 1/50 LD_{50} mg/kg BW/day for a period of three weeks.
- **Group V (Tin oxide NP)**: Rats were treated orally with Tin oxide NP at a dose of 1/25 LD_{50} mg/kg BW/day for a period of three weeks.

At the end of the experimental period, rats were starved overnight, euthanized then dissected. Blood samples were taken from the aorta and collected into glass tubes with no anticoagulant and used for biochemical analyses.

The abdominal cavity of each rat was opened where the liver and kidney were excised. These tissues were further used for biochemical analysis, histological and immunohistochemistry analysis.

Blood and Serum Samples

Blood samples were individually collected from the aorta of each rat in non-heparinized glass tubes. Serum was separated by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at -18 °C until analysis.

Tissue Samples

After scarification of rats, liver and kidney were immediately removed and washed by cold saline; weighed and washed using chilled saline solution 0.9%. Tissues were minced and homogenized (10% w/v) in ice-cold sodium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a Potter–Elvehjem type homogenizer.

The homogenates were centrifuged at 10,000 xg for 20 min at 4°C. The resultant supernatants were used for the analysis of the different enzyme activities, free radicals and biochemical parameters.

Determination of Urea Concentration

Urea level in serum was assayed by using commercial kit that was supplied by Diamond, Egypt. Urea was estimated according to the method of Patton and Crouch (1977).

Determination of Creatinine Concentration

Creatinine concentration in serum was assayed by using commercial kit that was supplied by Diamond, Egypt. Creatinine was estimated according to the method of Henry et al. (1974).

Determination of Alanine Aminotransferase (ALT) Activity

The alanine aminotransferase (ALT; EC 2.6.1.2) activity in serum and liver was assayed according to the method of Reitman and Frankel (1957).

Determination of Aspartate Aminotransferase (AST) Activity

The aspartate aminotransferase (AST; EC 2.6.1.1) activity in liver was assayed according to the method of Reitman and Frankel (1957).

Determination of Lactate Dehydrogenase Activity

Lactate dehydrogenase activity (LDH; EC 1.1.1.27) was determined by the method of Cabaud and Wroblewski (1958) by kinetic evaluation using commercial kits (Bio Systems S.A Costa Brava30, Barcelona, Spain).

Determination of Serum Alkaline Phosphatase Activity

Alkaline phosphatase (ALP; EC 3.1.3.1) activity in serum was assayed by using commercial kit that was supplied by BioMérieux Co, from France (Principato et al., 1985).

Determination of Protein Concentration

The protein concentration was determined by method of Lowry et al. (1951) using bovine serum albumin as a standard.

Results & Discussion

Thermal Gravimetric Analysis (TGA) of Nano-Sno2

Figure (2) shows the TGA thermogram of Nano-SnO_2 and refers to the presence of four successive thermal degradation steps. The first decomposition stepwas characterized at temperature range 33.3 – 112.8 ºC due to a loss of 0.37 mg of the starting mass to represent a 2.52% loss from Nano-SnO_2 due to loss of adsorbed water molecules from the surface. The weight loss in the second degradation step of this nanomaterial corresponds to 0.51 mg (3.47%) at temperature range of 112.8 – 231.1 ºC due to thermal decomposition and partial loss of oxygen atom from Nano-SnO_2.

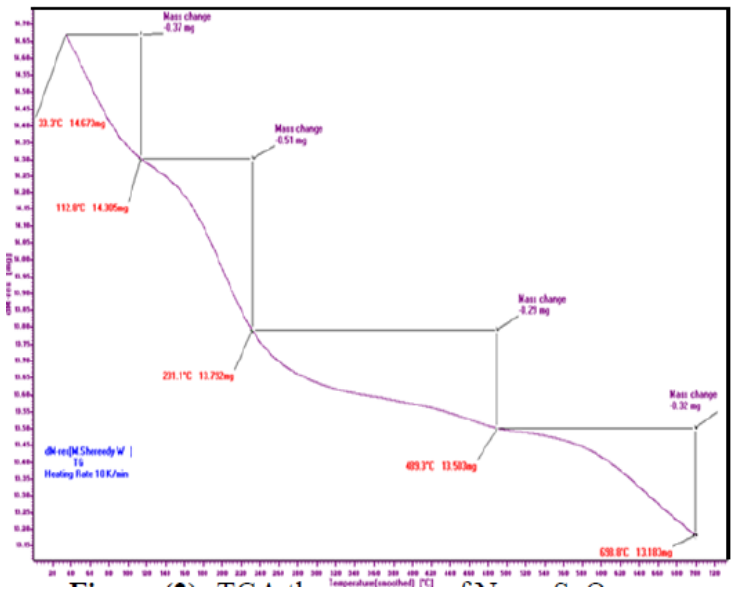

Figure2. *TGA thermogram of Nano-SnO_2*

The weight loss in the third and fourth degradation steps of this nanomaterial corresponds to 0.29 mg (1.97%) and 0.32 mg (2.18%) at temperature ranges of 231.1 - 489.3 ºC and 489.3 – 698.8 ºC, respectively which are mainly related to the loss of partial oxygen atom

loss. The total percentage losses from Nano-SnO_2 can be determined to correspond to 10.14%.

High Resolution-Transmission Electron Microscopy (HR-TEM) of Nano-Sno2

Transmission electron microscopy is a microscopic technique in which abeam of electron passes through an Ultra-thin specimen, interacting with the specimen as it passes. Therefore, an image is formed from the interaction of electrons transmitted through the specimen. The image is then magnified and focused onto the imaging device such as afluorescent screen on a layer of photograph film or to be detected by sensor such as charge-coupled device (Lu et al., 2009; Dai et al., 2002).

Several images of theHR-TEM image of SnO_2 nano materials are shown in Figs. 18-22 with different magnification orders. As shown in these figures homogeneous nano crystalline grainswith a uniform particle. The HR-TEM micrograph can also give us the chance to compute the statistical grain size and it reveals and the presence of non-agglomerated tin oxide nanoparticle with uniform particle size as 18.44 nm as represented in Fig. 23. The results of this study confirm the presence of single-crystal SnO_2nanostructure.

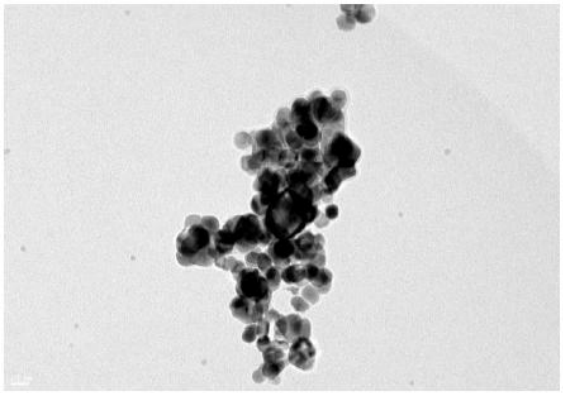

Figure3. *HR-TEM image # 1 of Nano-SnO2at magnification order*

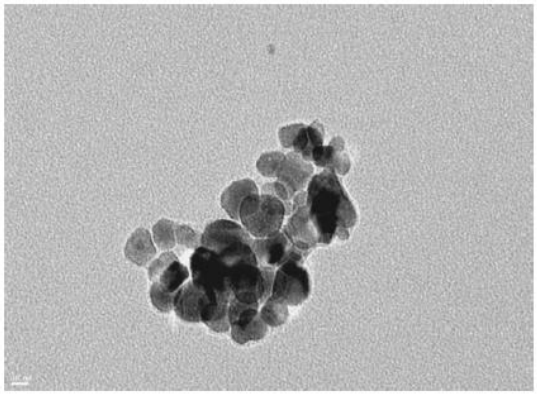

Figure4. *HR-TEM image # 2 of Nano-SnO2 at magnification order*

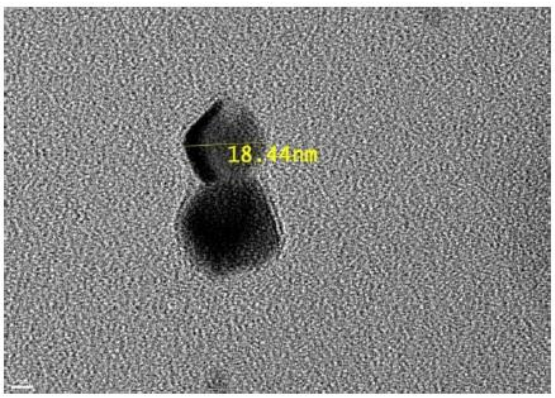

Figure5. *Determination of particle size of Nano-SnO2 by HR-TEM*

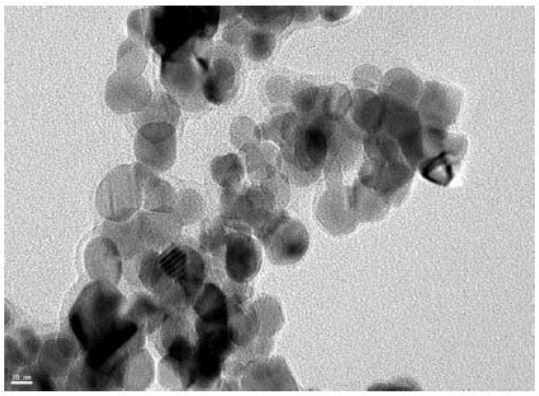

Figure6. *HR-TEM image # 3 of Nano-SnO2 at magnification order*

Scanning Electron Microscopy (SEM) of Nano-Sno2

Scanning electron microscope (SEM) uses focused beam of high- energytic electrons to generate a variety of signals at the surface of solid specimen. The driven signals from electron sample interactions reveal information about the sample including external morphology (texture), chemical composition, crystalline structure and orientation of material making up the sample.Three-dimensional image is generated that displays spatial variation in these properties. Areas approximately 1 cm to 5 microns in width can be imaged in scanning mode using congenital SEM techniques (magnification ranging from 20x to approximately 50,000x and spatial resolution of 50 to 100 nm)(He and Zhou 2013; Ayeshamariam 2013). Scanning electron microscope (JSM-6360LA, JEOL Ltd.), (JSM-5300, JOEL Ltd.) and an ion sputtering coating device (JOEL-JFC-1100E) were used to examine the images and particle size of Nano-SnO2. The surface and textural morphology of Nano-SnO2 were characterized by the SEM

imaging as represented in Figs. 24- 25 at two different magnification order 35,000x and 50,000x, respectively. The SEM of Nano-SnO2shows the existence of particles in aggregate structures with the particle size range of 34.09-56.82 nm.

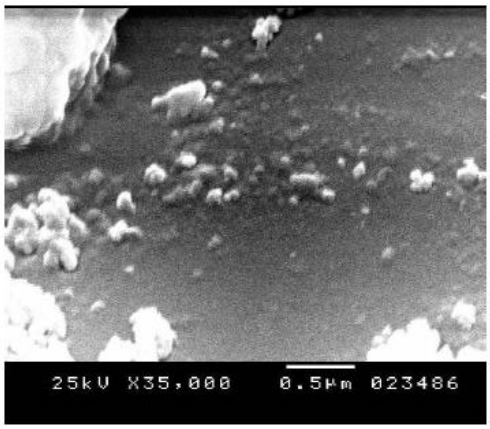

Figure7. *SEM image of Nano-SnO2at magnification order = 35,000x*

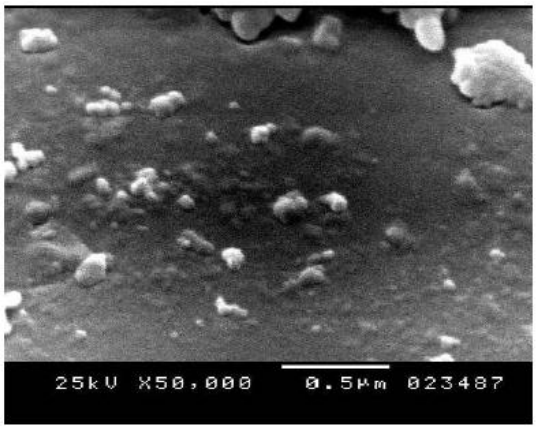

Figure8. *SEM image of Nano-SnO2 at magnification order = 50,000x*

Effect of Sno2 Nano Particles Different Concentrations on Oxidative Stress Markers in Rat Liver

None of the rats treated with chlorpyrifos, abamectin or their combination, showed signs of morbidity or mortality during the study. Regarding SnO2 NPs, few studies have assessed their toxic effect. Therefore, the present results cannot compared with others.

Based on the data in Table (3) and Figures 3-8, rats treated with different concentrations of Tin oxide NPs showed a significant increased peroxidation index of lipids, TBARS and H2O2 in comparison with the control and other Tin oxide NPs groups in concentration dependent manner. On the other hand GSH content was significantly decreased as compared to control. The effect of tin oxide NPs was concentration dependent. This results are in agreement with Abbasalipourkabir et al. (2015) who found that rats received 50, 100, 150 and 200 mg/kg nZnO, respectively showed oxidative stress. Also, a significant increase in MDA levels were observed in the liver of rats treated with gold nanoparticles on both 3 and 7 days post-dosing. This finding suggests that gold NPs of 10 nm diameter produce significant lipid peroxidation in rat liver however lungs and heart do not show any oxidative stress (Khanet al., 2012). It has been found that gold nanoparticles with a long blood circulation time can accumulate in the liver and spleen, and significantly affect the gene expression (Balasubramanian et al., 2010; Cho et al., 2009). Thus the hepatotoxicity of gold nanoparticles may be attributed to accumulation of nanoparticles in liver. In addition, Ebabe Elle et al. (2013) indicate that exposure to 500 mg/d/kg BW of AgNPs results in liver damage by a dyesregulation of lipid metabolism, highlighting liver and heart as the most sensitive organs to the deleterious effects. Moreover, SnO2 NPs showed a higher toxicity to A549 than the HCT116 cancer cells and the proliferation of both cancer cell lines was size and dose dependent due to ROS generation (Tammina et al., 2017).

Table3. *Effect of different concentrations of tin oxide nanoparticles on the level of thiobarbituric acid reactive substances, hydrogen peroxide and reduced glutathione content in rat liver*

Parameters	Experimental groups				
	Cont.	C1	C2	C3	C4
TBARS (nmol/g tissue)	33.21 ± 1.23^{d}	39.78 ± 1.35^{c}	41.98 ± 1.35^{bc}	44.55 ± 1.57^{ab}	48.36 ± 1.59^{a}
H_2O_2(µmol/g tissue)	79 ± 2.68^{d}	91 ± 2.86^{c}	100 ± 3.33^{b}	105 ± 2.73^{b}	116 ± 3.95^{a}
GSH (mmol/mg protein)	2.04 ± 0.068^{a}	1.73 ± 0.042^{b}	1.49 ± 0.039^{c}	1.32 ± 0.054^{d}	1.11 ± 0.047^{e}

Values are expressed as means ± SE; n=7 for each treatment group. ^{abcd}Mean values within a row not sharing a common superscript letters were significantly different, $p< 0.05$.

Oxidative stress has been marked a possible mechanism of nanoparticles toxicity (Ahamed et al., 2011). Zinc oxide nanoparticles have been proposed to increase lipid peroxidation and induce oxidative stress, it's possible mechanism is involved in the toxicity of nanoparticles

(Sharma et al., 2011). In the current study, the potential of SnO2 nanoparticles to induce oxidative stress was studied by measuring TBARS, H2O2, GSH, SOD, CAT, GPX, GR and GST in adult male rats. A significantly increased level of MDA was observed in the liver of the experimental groups treated with SnO2nanoparticles at different concentrations (1/150, 1/100, 1/50 and 1/25 LD50) compared to the control. The results of this study are comparable to Sharma et al. (2011), who reported that DNA damagein human epidermal cells, is possibly because of lipid peroxidation and oxidative stress. It is suggested that the small size of nanoparticles makes them able to interact with DNA directly (Sharma et al., 2009). According to the study of Hackenberg et al. (2011), DNA damage and inflammation in human nasal mucosa cells in vitro due to ZnO nanoparticles even at low concentrations has already been reported. Also, Rizk et al. (2017) reported that different doses of TiO2NPs (50, 250 and 500 mg/ kg body weight) intrapertioneally injected to mice for 7, 14 and 45 days induced oxidative stress and biochemical disturbance in dose and time dependent manner and this is similar to our results.

In the present study, a significant decrease in GSH levels in rats treated with different concentrations of tin oxide was observed. Nasr et al. (2016) also observed a similar depletion of GSH and suggested that this may be responsible for enhanced LPO. GSH is well known for its antioxidant properties and its role as redox modulator in related enzymes. SnO2 nanoparticles treatment resulted in a significant decrease in the levels of reduced glutathione (GSH) in liver homogenate. It appears that elevation in lipid peroxidation is a consequence of depleted GSH stores, which are otherwise capable of moderating the levels of LPO (El-Demerdash et al, 2012). Therefore, reduced level of GSH enhances the toxic effect, because GSH plays an important role in detoxification of ROS. GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including the GSH peroxidases (Hayes et al., 2005). Under oxidative stress, glutathione is consumed by the glutathione related enzymes to detoxify peroxides produced due to increased lipid peroxidation (Cathcart, 1985). In this study, the observed increase in lipid peroxidation and a concomitant depletion in GSH activity, suggests that the increased peroxidation may be a consequence of depleted GSH stores.

Effect of Sno2 Nanoparticles Different Concentrations on Antioxidant Enzymes Activity in Rat Liver

As shown in Table 4; SOD, CAT, GPx, GR and GST activities were significantly decreased in rats treated with different concentrations of SnO_2 nanoparticles as compared to control. The cellular antioxidant system comprises of integral antioxidants like GSH, and also different free radical scavenging antioxidant enzymes against oxidative injury. Among the antioxidant enzymes, SOD, CAT, GPx, GR and GST are the first line of defense against oxidative injury. The inhibition of antioxidant system may cause the accumulation of H2O2 or products of its decomposition (Halliwell, 1994). SOD quenches O-2 into the corresponding H2O2 and H2O (Fridovich, 1972). The decrease of GPx activity induced by SnO2 nanoparticles in the present investigation may be attributable to a direct inhibitory oxidative effect on the enzyme.

Table4. *Effect of different concentrations of tin oxide nanoparticles on the activities of antioxidant enzymes in rat liver*

Parameters	Experimental groups				
	Cont.	C1	C2	C3	C4
SOD (U/mg protein)	77.03 ± 2.52^{a}	65.79 ± 1.73^{b}	61.74 ± 1.68^{b}	55.19 ± 1.59^{c}	46.01 ± 1.82^{d}
CAT (µmol/hr/mg protein)	46.09 ± 1.86^{a}	38.24 ± 1.13^{b}	$34.93\pm1.04b^{c}$	31.67 ± 1.02^{c}	27.01 ± 1.02^{d}
GPx(U/mg protein)	0.949 ± 0.028^{a}	0.807 ± 0.030^{b}	0.663 ± 0.024^{c}	0.575 ± 0.021^{d}	0.527 ± 0.020^{d}
GR (U/mg protein)	1.35 ± 0.034^{a}	1.10 ± 0.042^{b}	1.02 ± 0.038^{b}	0.93 ± 0.024^{c}	0.78 ± 0.023^{d}
GST(µmol/hr/mg protein)	1.13 ± 0.038^{a}	0.96 ± 0.024^{b}	0.89 ± 0.033^{b}	0.80 ± 0.021^{c}	0.72 ± 0.021^{c}

Values are expressed as means ± SE; n=7 for each treatment group. ^{abcd}*Mean values within a row not sharing a common superscript letters were significantly different, p< 0.05.*

The endogenous GSH, synthesized mainly in the liver, plays an important role in the system of cell defense. It is involved in detoxification of many xenobiotics through conjugation of toxic metabolites in the second phase of biotransformation (**Jurczuk *et al.*, 2006**). In the current study, the levels of GSH and the activities of SOD and GST were significantly reduced in the studied tissues of SnO_2 nanoparticles -treated rats in comparison with

controls. The substrate of SOD is the superoxide radical anion which is generated by the transfer of one electron to molecular oxygen. This is responsible both for the direct damage of biological macromolecules and for generating other reactive oxygen species. SOD keeps the concentration of superoxide radicals at low levels and therefore plays an important role in the defense against oxidative stress (Fridovich, 1997).The present results are coincident with Abbasalipourkabir et al. (2015) who found that treatment groups with ZnO nanoparticles induced significant decrease in SOD activity by increasing the concentration of ZnO nanoparticles, thus indicates oxidative stress. However, Sharma et al. (2011) reported significantly ($p < 0.05$) reduced SOD activity in cells after 24 h treatment with 0.008, 0.08 and 0.8 mg/ml ZnONPs, compared to unexposed cells (Sharma et al., 2009). The decrease in the SOD and GPx activity and increase in LPO could explain the induction of free radicals in SnO2 nanoparticles -treated rats. Among the endogenous detoxifying systems, GST plays a critical role in protection against electrophiles and products of oxidative stress by catalyzing the conjugation of GSH to a variety of electrophilic compounds (Hayes et al., 2005).

Our results coincident with the study of Ranjbar et al. (2005) who reported that GST is one of enzyme system involved in the detoxification of organophosphorus insecticides to non-toxic products or by rapidly binding and very slowly turning over the insecticide. GST is a part of adaptive response of rat hepatocytes to stress after SnO2 nanoparticles treatment.

This can be understood in view of the fact that organophosphorus insecticides consume GSH through a detoxification reaction and/or that GST catalyzes this reaction between GSH and xenobiotics, regulating possible harm. Moreover, several authors reported that organophosphrous insecticides were in vitro GSH-conjugated by GST. GPx and GST contribute to detoxify the products of oxidative stress; through GST relative contribution is more significant than GPx contribution (Kondala Rao and Shaha, 2000). Also, Abbasalipourkabir et al. (2015) found that glutathione peroxidase, the other antioxidant enzyme, was reduced in the group treated with higher concentration of ZnO nanoparticles compared to the control group showing oxidative stress in the treatment groups. The second line of defense includes the non-enzymatic radical scavenger GSH, which scavenges residual free radicals resulting from oxidative metabolism and escaping decomposition by the antioxidant enzymes. In the present work, the GSH concentration, a key cellular non-enzymatic antioxidant, was also determined. During the metabolic action of GSH, its sulfhydryl group becomes oxidized resulting with the formation of the corresponding disulphide compound, GSSG (oxidized form).In the present study, GSH concentration and GPx activity were positively correlated. It is known GPx is involved together with GSH in the protection against ROS. Some oxidants directly react with GSH and generate free radicals by reducing the internal redox status.

On the other hand, some oxidants initially are metabolized, and their metabolites generate free radicals that react with GSH. The decrease in GSH levels could be due to the presence of free radicals produced by insecticides. These effects have been previously observed by other authors in vitro and in vivo.

Effect of Sno2 Nanoparticles Different Concentrations on Enzymatic Activities and Protein Content in Rat Liver

Liver is the detoxifying center for any foreign compounds that enter the body. It plays an important role in the biotransformation process for these compounds and also it performs different kinds of biochemical, synthetic and excretory functions. So, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present everywhere and human can be exposed to them (Evans, 2009).In the present study, rats treated with SnO2 nanoparticles different concentrations for three weeks showed significant decreases in ALT, AST and ALP activities and protein content in rat liver while LDH activity was significantly increased (Table 5 and Figures 36-40).

These enzymes are secreted into the blood after liver and kidney injury, resulting in an increase of their activities in serum samples (Gokcimen et al., 2007). Lipid peroxidation is known to disturb the integrity of cellular membranes, leading to the leakage of cytoplasmic enzymes (Bagchiet al., 1995). Therefore, the perturbations observed in enzyme activities in liver in the present study could be due to the necrosis of liver, kidney and lung (El-Shenawy et al., 2009). Protein levels in rat liver were decreased after administration of SnO2 nanoparticles different concentrations. The

decrease in the levels of protein might be due to changes in protein synthesis and/or metabolism.

Table5. *Effect of different concentrations of tin oxide nanoparticles on the enzyme activities and protein content in rat liver*

Parameters	Groups				
	Cont.	C1	C2	C3	C4
AST (U/mg protein)	114±3.88^{a}	99±3.52^{b}	90±2.97^{b}	72±2.60^{c}	64±1.69^{c}
ALT (U/mg protein)	156±3.76^{a}	134±4.86^{b}	125±2.13^{b}	102±3.51^{c}	92±3.12^{c}
LDH(U/mg protein)	1190±39.92^{d}	1355±37.10^{c}	1439±47.89^{bc}	1536±42.33^{ab}	1647±46.22^{a}
ALP(U/mg protein)	388±14.51^{a}	336±11.02^{b}	295±10.61^{c}	264±9.56^{cd}	238±7.96^{d}
Protein (mg/g tissue)	187±5.89^{a}	165±5.83^{b}	143±4.27^{c}	127±2.18^{d}	112±4.16^{e}

Values are expressed as means ± SE; n=7 for each treatment group. ^{abcd}Mean values within a row not sharing a common superscript letters were significantly different, p< 0.05.

Liver disorders can be determined by serological markers such as alanine transaminase, ALT (Wang et al., 2006). The abnormal increase of serum ALT concentrations, may suggest liver hepatotoxicity (Lynch et al., 2005). The results of this study showed that SnO2 nanoparticles decreased the activities of AST, ALT and ALP and this is in agreement with Abbasalipourkabir et al. (2015) who reported that the activity of ALT increased significantly in all groups treated with ZnO nanoparticles compared to the control group. Also, they suggested that treatment with ZnO nanoparticles at 50 mg/kg body weight had no toxic effect on the liver while its concentration at 100 mg/kg body weight and above produced a dose dependent toxicity in the liver and this is in a good accordance with the present results where SnO2 nanoparticles showed dose-dependent effect. Also, Sharma et al. (2011) reported significantly ($p < 0.05$) higherlevels of ALT compared to the control mice followed by treatment with 300 mg/kg Zinc oxide nanoparticles. It was suggested that to find liver damage, ALT activity is usually determined with AST (Wang et al., 2006).

The enzyme LDH can be used as an indicator for cellular damage, clinical practice, and cytotoxicity of pollutants. LDH activity indicates the switching over of anaerobic glycolysis to aerobic respiration. The changes in the dehydrogenase activity in acetamiprid and emamectine benzoate - treated rats (Table 5) may be due to severe cellular damage, leading to increased release of dehydrogenase that impaired carbohydrate and protein metabolism (Sivakumari et al., 1997). The elevation of lactate also indicated metabolic disorders and a clear response against energy depletion. Sancho et al. (1998) observed the same response when European eels were exposed to fenitrothion.

The alterations in ALP activity in liver (Table 5) caused by SnO2 nanoparticles are in accordance with the findings of Szilagyi et al. (1994), and Ochmanski and Barabasz (2000).Szilagyi et al. (1994) referred the high levels of plasma ALP to an increased osteoblastic activity, provoked by the disturbance of bone formation, caused in turn by aluminum. Ochmanski and Barabasz (2000) reported that Aluminum may bind to DNA, RNA and inhibit the activity of ALP. However, Rahman et al. (2000) suggested that the decrease in the activities of ALP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, this showing the stress condition of the treated animals. In addition, they reported that the increase in the activity of ALP in blood might be due to the necrosis of liver, kidney and lung.

Protein content in liver of rat treated with SnO_2 nanoparticles different concentrations showed a significant ($p<0.05$) decrease as compared to control (Table 5). Protein is one of the main cellular components susceptible to damage by free radicals. The reduction in liver proteins caused by SnO_2 nanoparticles could be attributed to the damaging effect of nanoparticles on liver cells or due to degradation of protein caused by ROS generated by SnO_2 nanoparticles. Thus, SnO_2 nanoparticles toxicity might be due to the release of chemically unstable metabolites. These results are very similar with the findings of several authors who found decrease in protein liver in living organisms exposed to xenobiotics (El-Demerdash 2011;El-Demerdash and Nasr, 2014; El-Demerdash et al., 2016). The decrease in protein content is probably a physiological adaptation of living organism to combate stress produced by the pesticide to overcome the stress (Korkmaz et al., 2009).

CONCLUSION & RECOMMENDATION

Conclusively, it is clear that tin oxide (SnO_2) nanoparticles induced pronounced hazardous

effects in rat liver and kidney in a dose dependent manner. Estimation of lipid peroxidation, enzymatic, non-enzymatic antioxidants in addition to biochemical parameters could be used as biomarkers for the harmful effect of tin oxide (SnO_2) nanoparticles.

Finally, It Can be Recommended that

- Data related to harmful effect in mammals is not sufficient so, further research is needed to study the impact of SnO_2 nanoparticles on human health and safety.
- Also, the use of well-characterized NPs and a representative type of cells are important in NPs toxicity assays to better understand their mode of interaction.

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