Oxidative stress mediated hepatotoxicity induced by ZNP and modulatory role of fruit extract on male Wistar rat

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A R T I C L E   I N F O

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A B S T R A C T

Zinc oxide nanoparticles (ZNP) are being used in various fields viz cosmetics industry as UV protectants, in the food packaging industry due to their anti-bacterial properties, in agriculture as micronutrients, etc. Increased applications of ZNPs in our day to day life, leading to the contamination of the surrounding environment posing a direct or indirect health risk. Various reports suggest that fruits and vegetables are a rich source of phytochemicals having antioxidant properties which help in neutralizing ROS generated on metal toxicity of the body. The present study focuses to study the ameliorative effect of apple (Pyrus malus) extract (E) on ZNP induced toxicity. Therefore, animals were grouped, six in each, exposed to various doses of ZNP (50 and 250 mg/kg), ZNP (50 and 250 mg/kg) + E. The studied parameters were: food intake, water intake, antioxidants assay, zinc accumulation, and histological alterations and was compared to control. Investigation revealed that ZNP induces toxicity as revealed by the alteration in the studied parameter, whereas those exposed to ZNP along with Pyrus malus fruit extract try to reduce the toxicity induced by nanoparticles but at low doses only. This ameliorative effect of fruit extract might be due to the presence of antioxidants scavenging the free radicals generated by ZNPs suggesting that antioxidant-rich fruit may have a protective role and have the potential to reduce the nanoparticles mediated oxidative stress.

1. Introduction

Zinc oxide nanoparticles generally regarded as safe (GRAS), is one such nanoparticle which are being used widely in various fields for the betterment of human needs like, in sunscreens as UV protectant [1], in fertilizers as micronutrient [2], exhibit photoconductivity thus can be used for the device of nanoscale dimensions [3]. Therefore, used variously in a different arena, thereby increases the exposure rate towards humans and there are majorly four routes inhalation, ingestion, injection and dermal through which humans are exposed [4].

Several studies suggested that exposure of nanoparticles (NP) induces reactive oxygen species (ROS) generation, which results in wide spectrum damage to cellular biomolecules including DNA, RNA, protein, cholesterol, lipid, etc. [5]. Several in-vitro studies reported that there is a dose-dependent increase in superoxide dismutase (SOD) and ROS whereas a decrease in reduced glutathione (GSH) and cell viability in human keratinocyte HaCaT [6], spermatocyte cell line (GC2-spd) and Sertoli cell line (TM-4) of the mouse was reported [7], also increased lactate dehydrogenase (LDH) and downregulation of stress-related genes found on exposure to zinc oxide nanoparticles (ZNP) [6,8]. In-vivo model D. melanogaster showed a decrease in an egg to adult viability on exposure to ZNP [8]. The acute test was conducted on five marine organisms that showed alteration in SOD, metallothionein (MT) and heat shock protein (HSP70) level [9]. However, Xiao et al. in his study found a decrease in SOD and CAT activity in ZNP exposed rats [10]. Also, ZNP toxicity found to be associated with the dissolution of Zn to release Zn ion, resulting in a cellular ionic and metabolic imbalance. An experiment conducted on embryonic zebrafish revealed that exposure to zinc oxide nanoparticles induces toxicity, for which an elevated level of zinc ion might be responsible [11]. Thus, increasing nanoparticle exposure found to generate ROS, resulting in the induction of oxidative stress marked by the altered antioxidant level and associated parameters, further their interactions with biomolecules impaired their functionality, and release of certain key marker indicating damages to the particular tissues or organs. Therefore, the usage of antioxidants could be a better strategy to fight against free radical-mediated damage. Although endogenous antioxidants are quite sufficient to maintain the balance between oxidants and antioxidants, however, under stress condition, it is insufficient so to further balance external dietary supplement may be required [12]. Natural antioxidants are found to

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present in all parts of the plant including fruits and vegetables [13] and including them in the regular diet will protect the cellular damage against free radical-mediated oxidative stress [14].

Therefore, the objective of the present study is to investigate the effect of ZNP and modulatory effect of fruit juice on oxidative stress marker likesuperoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), lactate dehydrogenase (LDH) and histopathological changes in hepatic tissue.

2. Material and methods

2.1. Characterization of ZNPs

Zinc oxide nanoparticles purchased from Sigma Aldrich (USA) were characterized by different techniques. Optical properties by UV–vis spectrophotometer (Shimadzu), to study the crystalline nature of nanoparticles X-ray diffractometer (XRD, PANalytical X’pert PRO 2200) was used with CuKa radiation (at λ =1.5406 Å). Shape and surface morphology was studied by scanning electron microscopy (SEM) along with elemental composition through energy dispersive X-ray fluorescence (EDXRF) spectrophotometer (PAN analytical Epsilon 5) was performed. The ultrastructural study was performed through TEM, where 2–3 µl of the ultrasonicated samples were drop cast over a copper mesh, air-dried and were examined by high-resolution transmission electron microscopy (JEOL 2100 F). Further to quantify the surface charge, the zeta potential was measured through the electrophoretic light scattering (ZC-2000, Microtec, Japan) where the surface charge of the metal oxide was evaluated under an applied electric field.

2.2. Experimental methods

2.2.1. Preparation and dose selection of ZNP

Experimental dose, precisely 50 and 250 mg/kg body weight were selected based on the lethality test. Doses of ZNP were prepared in distilled water and were ultrasonicated for 15 min before its administration to the rats. For oral gavage, fruit juice was extracted simply by macerating the fruit with the help of mortar pestle and was later filtered with muslin cloth for further use and it is coded as ‘E’ (Apple Extract Juice).

2.2.2. Animal treatment

Male Wistar rats of 150–200 g were obtained from central laboratory animal resource (CLAR) Jawaharl Nehru University (JNU) all the protocols were done per Institutional animal ethical committee (IAEC), Jawaharlal Nehru University, New Delhi. Before the experiment animals were housed in polypropylene cages under standard environmental conditions (22 ± 3 °C, 55% ± 15% RH and 12 h light/dark cycle) with access to normal basic diet and water ad libitum for acclimatization. Later animals were divided into six(6) groups, and six (6) animals in each group were divided as mentioned in Fig. 1. Animals exposed to ZNP once a week for four weeks along with fruit extract on every alternative day, during this periodwater intake, food intake and feces excretion were monitored regularly all animals were sacrificed to terminate the experiment. Blood sample was collected for the blood profile analysis, organs like liver washed with normal saline solution, sucked dried and weighted. The organ coefficient of the liver was expressed as (wt. weight of the individual tissues)/(whole body-weight of individual rat in g).

2.2.3. Zinc content in organ

Metal content analysis was done as per Singh et al. (2013) with a slight modification, Tissue samples were dried initially in an oven at 50 °C, further powdered with the help of mortar and pestle, Later the pre-digested powdered samples in nitric acid was heated at 80 °C than in 70 % perchloric acid and digested till dried completely. Then the required volume is maintained in Milli-Q and filtered through nitrocellulose filter paper. Samples were run in AAS (Thermofisher scientific-651187) to determine the zinc content in the Liver.

2.3. Biochemical analysis

2.3.1. Oxidative stress marker

Various biochemical markers were measured to address the oxidative stress state of the cellular environment assessed by various methods such as the specific activity of phase I enzyme such as NADPH-cytochrome. P450 reductase was assessed according to the method of Omura and Takesue [15] whereas NADH-cytochrome b5 reductase was assessed by the method of Mihara and Sato [16]. Superoxide dismutase (SOD) activity was measured by the procedure of Marklund and Marklund [17], Catalase (CAT) activity in liver was assayed following the procedure of Aebi [18], Glutathione peroxidase (GPX) activity was measured by the coupled assay method as described by Paglia and Valentine [19], whereas the activity of glutathione reductase (GR) was determined by the procedure as described by Carlberg and Mannervik [20]. Lactate dehydrogenase activity (LDH) was measured by Bergmeyer and Bernt [21].

Details of the methods were incorporated in (S1).

2.3.2. Total protein estimation

Total protein content was estimated by using the Bradford [22] method. The supernatant was diluted 50 times and 10 µl of this diluted protein was taken and to this 200 µl of Bradford reagent and kept in dark for 10 min. After 10 min of incubation, OD was measured at 595 nm.

2.3.3. Hematological study

Hematological and immunological parameter like red blood corpuscle (RBC), hemoglobin (Hb), mean corpuscular value (MCV), eosinophils, neutrophils and lymphocytes through the automated hematological analyzer (KX-21, Sysmex, Transasia, India).

2.3.4. Histopathological examination

After 28 days animals were sacrificed and dissected for histological study. Briefly, liver tissue was fixed in 10 % formalin and implanted in a paraffin block, finely sliced into 5 µm thick and then placed on glass slides further stained with hematoxylin-eosin (HE) and observed under the light microscope for histopathological analysis.

2.4. Statistical analysis

The data were analyzed by Graphpad prism 5, a statistical software system used to perform a post hoc multiple comparison test (Bonferroni’s multiple comparison test) after ANOVA and presented as mean ± SEM (n = 6). A p-value below 0.05 was considered as statistically significant level.
3. Result

3.1. Characterization of ZNPs

Fig. 2 showed various techniques used for the characterization of ZNPs. UV–vis spectrum reveals the strong absorption peak observed at 362.5 nm. The crystalline nature of ZnO NPs was further confirmed by XRD (PANalytical X’Pert Pro X-ray diffractometer) with Cu Kα radiation (λ = 0.154 nm) diffraction peak observed at 2θ = 36.48° which is found to originate from (101) planes of ZnO indicating that the ZNPs, UV–vis spectrum reveals the strong absorption peak observed at 362.5 nm. The crystalline nature of ZnO NPs was further confirmed by XRD (PANalytical X’Pert Pro X-ray diffractometer) with Cu Kα radiation (λ = 0.154 nm) diffraction peak observed at 2θ = 36.48° which is found to originate from (101) planes of ZnO indicating that the
samples were polycrystalline wurtzite structure (Zincite, JCPDS 5-0664) based on Scherrer equation as follows:

\[ D = \frac{0.9\lambda}{\beta \cos \theta} \]

Where, ‘D’ is the average particle size  
\( \lambda \) is the wavelength of X-Ray (0.154 nm)  
\( \beta \) is FWHM (Full Width at Half Maximum)  
\( \theta \) is the diffraction angle

And the calculated average crystallite particle size is 16.81 nm. Further, SEM image revealed the smooth surface of nanoparticles and found out to be average 98.25 nm; SEM-EDX conducted to know the elemental composition of nanoparticles and confirms the presence of three elements, namely zinc, oxygen, and gold. Whereas, the detailed study about the morphology of the nanoparticles was conducted through TEM. The TEM micrographs revealed that ZNP of various shapes viz. from spherical to rod shape, further prepared histogram reveals that highest no. of particles falls in the range 10–20 nm, however average particle size is 28.5 nm. HR-TEM shows a single crystal with a lattice constant of 0.29 nm. Further Zeta potential graph reveals that the purchased zinc oxide nanoparticles had a positive surface charge is approximately +26 mV.

3.2. Effect of ZNP on relative body weight

Intra-peritoneal exposure of ZNP in the present study revealed insignificant changes in relative body weight in all the exposed group as compared to control (Fig. 3).

3.3. Effects of ZNP on zinc distribution in liver and organ coefficient (liver)

And the calculated average crystallite particle size is 16.81 nm. Further, SEM image revealed the smooth surface of nanoparticles and found out to be average 98.25 nm; SEM-EDX conducted to know the elemental composition of nanoparticles and confirms the presence of three elements, namely zinc, oxygen, and gold. Fig. 4a showed the effect of ZNP on zinc distribution in the liver of exposed animals, the present investigation exhibited that, at a higher dose of ZNP alone and ZNP + Extract, showed significant accumulation of zinc in the liver of treated animals as compared to control groups. However, a lower dose of ZNP did not show any significant accumulation in both the abovementioned experimental conditions.

The effect of ZNP on the liver coefficient was presented in (Fig. 4b). Results indicated that there was no significant change was observed organ coefficient in the treated groups as compared to the control.

3.4. Effects of ZNP on LDH level

The LDH (lactate dehydrogenase) is marker enzymes, act as significant indicators of tissue injury. (Fig. 4c) indicated a marked *p < 0.05, ***p < 0.001) upsurge in the levels of LDH in both experimental doses of ZNP treated animals whereas animals treated with ZNP + Extract showed a significant decrease in level of LDH as compared to control. But there was no substantial alteration in marker enzymes found in rats treated with the only extract as compared to control animals.

3.5. Effects of ZNP on water intake, food intake, and fecal excretion profile

Fig. 4d, water intake study showed significant (**p < 0.01) increase in water intake in high dosed ZNP treated groups as compared to control, however significantly decreased in water intake pattern in ZNP + Extract treated animal groups when compared with only ZNP treated rats. Only extract-administered groups of animals did not show any alteration concerning untreated rats.

Further (Fig. 4e) food intake profile of the rats during the exposure, exhibited a significant (**p < 0.01) increase in food intake at high doses (250 mg/kg) of ZNP treated group, however, these changes were normalized in the animal supplemented with ZNP along extract when compared with control.

Fecal excretion profile (Fig. 4f) showed significant (**p < 0.001, *p < 0.05) increase in fecal excretion observed in ZNP(250 mg/kg) exposed groups, ZNP (50 mg/kg) along with fruit extract and only extract-treated rats as compared to control. However, those who received a high dose of ZNP (250 mg/kg) along with fruit juice and extract supplemented group showed significant (**p < 0.001) decrease as compared to ZNP (250 mg/kg) exposed group.

3.6. Effect of fruit extract on phase I enzyme and on biomarker of oxidative stress

The activity of NADPH cytochrome P450 R (Fig. 5a) which belong to the group of Phase 1 enzymes, showed significantly (**p < 0.05) increases in high dose ZNP treated rats as compared to control rats, whereas the group received extract only shows significant (**p < 0.05) decrease as compared to those received a high dose of ZNP only. Also, NADH Cytochrome b5R (Fig. 5b) showed a similar trend but those change remains insignificant as compared to the control.

Further high dosed ZNP exposed as well as ZNP along with extract-treated animal (Fig. 5c) showed significantly (**p < 0.001, **p < 0.01) decrease in SOD activity as compared to control, however, groups received fruit extracts only, exhibited significant (**p < 0.001, *p < 0.05) increase to both high dose ZNP treated group as well as high dose ZNP treated group along with extract respectively. In case of CAT (catalase) activity (Fig. 5d), significant (*p < 0.05, ***p < 0.001) decrease in CAT activity was observed in a dose-dependent manner in ZNP treated groups, similarly, groups received a high dose of ZNP along with extract also exhibited significant (**p < 0.001) decrease, in contrast, to control, on the other hand, those received extract only showed significant (**p < 0.001) increase in CAT activity to both groups received high dose of ZNP as well as a high dose of ZNP along with extract but changes remains insignificant as compared to control.

GPX (glutathione peroxidase) (Fig. 5e) showed a reduction in its activity with an increasing dose of ZNP but found to increase in those received ZNP along with extract, however, these changes remain insignificant as compared to control.

However, GR (glutathione reductase) (Fig. 5f) showed significant (**p < 0.01) depletion in GR activity in the group received high doses of ZNP treated group as compared to control, whereas those received extracts only reflects significant (*p < 0.05) increment in GR activity as compared to those who received high doses of ZNP only, but no changes as compared to control.
3.7. Hematological study

ZNP mediated alterations and pharmacological interventions of Pyrus malus fruit extract was evaluated on hematologic parameters shown in (Table 1), where Hemoglobin levels were found to decreased (**p < 0.01, * p < 0.05) in rats treated with a high dose of ZNP as compared to control and low dose treated group respectively. Also, those groups received a high dose of ZNP along with extract continue to show decreased Hb content as compared to control (**p < 0.001, *p < 0.05) decrease as compared to those exposed to high dose of ZNP only as well as a high dose of ZNP along with extract. RBCs showed insignificant changes in all the groups as compared to control. Further, MCV decreased significantly (**p < 0.01) in high dose ZNP treated groups as compared to control also those groups received a high dose of ZNP along with extract continue to show significant (*p < 0.05) decrease as compared to control. Whereas, those received extract only showed significant (**p < 0.01) increase to both groups, high dose of ZNP as well as a high dose of ZNP along with extract.

A significant (**p < 0.01) increase in neutrophils occurs in a dose-dependent manner to the group exposed to the high dose of ZNP but those animals received a high dose of ZNP along with fruit extract showed significant (**p < 0.01) decrease as compared to those who received a low dose of ZNP. Whereas groups received extract only exhibited a significant decrease to both low and high dose ZNP treated group but changes remain insignificant as compared to control. In addition to this eosinophil followed a similar pattern as in neutrophil however these changes remained insignificant as compared to control.

Whereas, significant (**p < 0.01, ***p < 0.001) decrease in lymphocyte was observed in a dose-dependent manner in the groups received a high dose of ZNP treated group as compared to control, but significant elevation has been observed in both high dose of ZNP along with extract and extract only treated group as compared to high dose ZNP treated group but changes remains insignificant as compared to control.

3.8. Histopathological study of the liver

No histopathological changes observed in control groups hepatocytes, as they are found in regular arrangement along the sinusoids with spherically localized nuclei (Fig. 6a). However, groups exposed to low doses of ZNP showed abnormal architecture along with dilated sinusoids, binucleated nuclei and shrinkage of hepatocytes can be seen (Fig. 6b) such histopathological changes are more prominent as the dose increases (Fig. 6c) where further dilation of sinusoids are increased, further shrinkage of hepatocytes are more prominent and vacuolization can be easily seen.

 Whereas those rats exposed to a low dose of ZNP along with fruit extract (Fig. 6d) showed comparatively less shrinkage in hepatocytes, less sinusoidal dilation as compared to those received low dose ZNP
only, but those animals received high doses of ZNP along with extract still showed binucleated nuclei, further hepatocyte enlargement can be seen which may be due to vacuolization (Fig. 6e) but animals received only extract (Fig. 6f) showed normal cellular architecture as in control.

4. Discussion

To meet normal daily requirement, Zn needs to be supplemented daily, as it fulfills not only the normal mineral requirement but also a part of various enzymes, essential for normal growth [23] and reproduction [24,25]. However, a diet supplemented with zinc oxide leads to environmental pollution as it is poorly absorbed in the intestine and its major amount excreted unabsorbed [26–28]. The introduction of the nano form of zinc oxide leads to the reduction in the amount of zinc oxide [29] to be added in the diet to fulfill the nutritional requirement [30]. Apart from this, being used widely in various other fields including food and cosmetic industry, agriculture and textile, raises the alarming bell as various experiments conducted worldwide reporting cytotoxic and genotoxic effect of ZnO NP [31] on both in vivo [32–35] and in vitro [8,36,37]. There are several mechanisms found to be responsible for nanotoxicity such as the release of ionic form, protein denaturation, inflammation, lipid peroxidation, mitochondrial perturbation, DNA damage, ROS generation [38].

To counteract the adverse effect of oxidative stress induced by

![Fig. 5. Effect of pharmacological intrusion on ZNP exposed rats treated with Pyrus malus fruit extract (E) reflected by Oxidative stress biomarkers a) NADPH Cyt P450 b) NADH Cyt b5 R c) SOD activity d) CAT activity e) GPX activity f) GR activity. The result presented as Mean ± SEM. *Significant (p < 0.05) **Significant (p < 0.01), ***Significant (p < 0.001). Where, control = received DW, ZNP50=received 50 mg/kg ZNP, ZNP250= received 250 mg/kg ZNP, ZNP50E = received 50 mg/kg ZNP along with fruit extract (E), ZNP250E = received 250 mg/kg ZNP along with fruit extract (E), and E only = received fruit extract only.

Table 1

|                  | Control       | ZNP50        | ZNP250       | ZNP50E       | ZNP250E       | Extract only |
|------------------|---------------|--------------|--------------|--------------|--------------|--------------|
| Hb               | 17.25 ± 0.16  | 16.10 ± 0.16 | 13.70 ± 0.00 | 16.47 ± 0.57 | 14.70 ± 0.85 | 17.52 ± 0.34 |
| RBC              | 9.07 ± 0.16   | 8.77 ± 0.05  | 8.55 ± 0.40  | 8.95 ± 0.21  | 8.82 ± 0.51  | 9.32 ± 0.12  |
| MCV              | 55.20 ± 0.48  | 51.15 ± 0.30 | 48.35 ± 2.1  | 52.45 ± 0.73 | 49.07 ± 0.77 | 55.95 ± 0.44 |
| Neutrophil       | 17.25 ± 0.79  | 27.00 ± 4.4  | 33.00 ± 3.20 | 19.50 ± 1.43 | 22.75 ± 0.79 | 13.00 ± 1.4 |
| Eosinophils      | 1.00 ± 0.0    | 2.50 ± 0.46  | 5.00 ± 1.60  | 3.00 ± 0.65  | 2.50 ± 1.10  | 1.00 ± 0.0   |
| Lymphocytes      | 77.25 ± 0.0   | 64.75 ± 4.58 | 50 ± 2.13    | 70.75 ± 0.79 | 67.50 ± 0.80 | 74.25 ± 1.32 |

Note: Unit were taken as Hb = g/dl, RBC = million/cu mm, MCV = fm, Neutrophil, Eosinophils and Lymphocytes in (%), Doses were given as (mg/kg b. wt.). *Significant (p < 0.05) **Significant (p < 0.01), ***Significant (p < 0.001). Where, control = received water, ZNP50= received 50 mg/kg ZNP, ZNP250= received 250 mg/kg ZNP, ZNP50E = received 50 mg/kg ZNP along with fruit extract (E), ZNP250E = received 250 mg/kg ZNP along with fruit extract (E), and E only = received fruit extract only.
nanoparticles, cells are evolved with the endogenous antioxidant system, which is a nanomaterials, cells are evolved with the endogenous antioxidant system, which is a mesh-like compartmentalized system, includes both enzymatic (SOD, CAT, GPx, etc.) as well as non-enzymatic (GSH, NADPH, thioredoxin, etc.) system found to be well dispersed in organelles as well as in the cytoplasm, as they are important in maintaining a fine balance between cellular redox system thus, diminish the detrimental damage induced by ROS [39,40]. Apart from this, to counteract the damaging effect induced by ROS, various sets of antioxidants found in fruits and vegetables to reduce or nullify the effect. Apple (*Pyrus malus*), reported having the highest fraction of free phenolics as compared to other fruit, serves as a good reserve of antioxidants and displayed various activity such as antiproliferative, inhibition of lipid peroxidation, cholesterol-lowering effect, etc. [41,42]. These activities retained in apple is due to the presence of diverse phytochemicals such as epicatechin, catechin, cyanidins reflect strong antioxidant activity, chlorogenic acid has high peroxyl radical (ROO•) scavenging activity [42]. Quercetin checks oxidative injury and cell death by scavenging free radicals, quenching singlet oxygen, contributing hydrogen compound, chelating metal ions and preventing lipid peroxidation [40].

The present investigation revealed that purchased ZNP was of various shape and size, also the average particle size is 28.4 nm, however, most of the particles fall in the range of 10–20 nm. Zeta potential of ZNP is approximately +26 mV which does not fall in the stability zone. Considering in-vitro and in-vivo toxicity, it is the ionic form that is easily taken up by the tissue which further ensures its accumulation causing the toxic effect. [42](Amara et al., 2014). However, in the systemic circulation, they retain their particulate form [43].

Body and organ weight variation are liable indicators for the potential toxicity of various chemicals on animals [44]. The present study revealed that there were no significant changes in relative body weight and organ coefficient. However, water intake, food intake and feces excretion altered as compared to control. Bio-distribution of nanoparticles is majorly dependent upon the animal, exposure route and the physicochemical property of the nanoparticles. Unlike other metal oxides like cerium oxide, iron oxide, and titanium dioxide, Zinc oxide nanoparticles are not very stable and tend to dissolve in aqueous solutions releasing metal ion Zn2+ from the particles [45]. Other factors such as concentration, pH, particle size and presence of organic compounds play a significant role in the solubility of ZnO NPs [43,46–48] contributing its uptake, distribution, bioavailability and fate of zinc oxide nanoparticles. Thus, the behavior of nanomaterials is greatly influenced by their environment in which they are released, further controlling their extent of toxicity [49].

Intrapерitoneal ZNP exposed rats in the present study revealed that there was a significant increase in Zn concentration in the liver was observed in both groups receiving a high dose of ZNP with or without fruit juice in comparison to control. Increased bioaccumulation was also observed in Wang et al and Sharma et al in liver at high doses [50,51]. And exposure to toxic metal concentration or metabolism of almost any type of xenobiotics generates free radicals [52–55] resulting in oxidative stress. However, biotransformation of endogenous and exogenous compounds operates majorly at three phases namely Phase I, Phase II and Phase III. Phase I biotransformation engross three important reactions explicitly oxidation, reduction, and hydrolysis which augment the polarity of xenobiotics by addition or deletion of functional groups facilitating their removal from the body through excretion [56,57]. The present study showed a general trend of increase in Phase I enzyme NADPH-cytochrome P450 reductase in a dose-dependent manner but reduced and reaches the normal level to the group exposed to ZNP along with extract in low dose. However, remains elevated in consideration of high dose ZNP treated group with extract as compared to the control group. In general, cells respond to oxidative stress by escalating the protective response which can be easily determined by assessing the antioxidants activity, therefore considered as a sensitive biochemical indicator, apart from that dietary supplement can further boost the antioxidant level [57,58]. Whereas, chronic exposure results in depletion inactivity of enzymes like SOD and CAT [28,59]. These antioxidants have a role to transform the generated free radicals into a harmless form such as H2O2 into H2O and O2 in presence of CAT, however excess production of ROS, activity of these antioxidants was inhibited [60]. In the present study, it has been observed that increasing concentration of ZNPs causes depletion in antioxidant activity whereas, those groups exposed with ZNPs along with fruit extract exhibited its protective effect against the activity of the enzyme by trying to bring back their activity to the basal level. Glutathione reductase (GR) is mainly associated with recycling of glutathione disulfide thus maintain the glutathione in its reduced form [61,62] and present study reflects the reduced activity of GR observed in those groups exposed to...
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5. Conclusion

The present study revealed that even once a week intraperitoneal exposure of ZNP leads to the alteration in antioxidant, hematological, histological parameters, however, groups treated with ZNP followed by fruit juice tries to reduce the damaging effect induced by ZNP, which may be due to the presence of phytochemicals present in fruit juice having free radical scavenging property although these effects are not effectively reduced. Thus, more study needed to affirm the protective role of fruit juice on nanoparticles exposure, along with other variables like dosage concentration, treatment mode, and exposure time, also the physicochemical property of nanoparticles has an important role in the induction of oxidative stress which further determines the target organ for bioaccumulation and their impact.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.03.009.

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