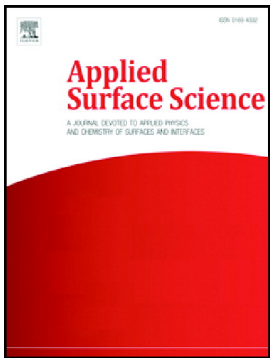

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Chekuri Poornavaishnavi, Reddivary Gowthami, Koigoora Srikanth, P.V. Bramhachari, N. Venkatramaiah

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Nickel nanoparticles induces cytotoxicity, cell morphology and oxidative stress in Blue gill sunfish (BF-2) cells

Chekuri Poornavaishnavi[1], Reddivary Gowthami[1], Koigoora Srikanth[1 2]*,
P.V. Bramhachari[3], N. Venkatramaiah[4]

[1]*Department of Biotechnology, VIGNAN'S Foundation for Science Technology & Research, deemed to be University, Vadlamudi-522213, A.P, India*

[2]*CESAM-Centre for Environmental & Marine Studies and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*

[3]*Department of Biotechnology, Krishna University, Machilipatnam 521001, A.P, India*

[4]*Department of Chemistry, SRM Institute of Science and Technology, Kattankulathur 603203, Chennai, TN, India*

***Corresponding author.**

E-mail address: koigooras@ua.pt, Telephone: +351 234401527 ext. 33503,

Fax: +351 234370084

ABSTRACT

The rationale of the current study was to assess the suitability of BF-2 cell line as a model to assess nanotoxicity in the caudal fin cells of bluegill sunfish *in vitro*. The current study investigates the potential toxicity, morphological changes and oxidative stress of nickel nanoparticles (Ni NPs) in blue gill sunfish cells (BF-2) using mitochondrial, neutral red uptake and lactate dehydrogenase assays. Results indicated a concentration dependent cytotoxic effect after 24 h in mitochondrial, lysosomal and lactate dehydrogenase activities. BF-2 cells morphology was altered when exposed to 30 μg ml^{-1} concentrations of Ni NPs for 24 h. Dose dependent increase of oxidative stress was evidenced in BF-2 cells when exposed to Ni NPs, showed significant escalation in peroxidation of lipids (LPO), protein carbonyl (PC), glutathione sulfo-transferase (GST) and glutathione peroxidase (GPX) as compared to their experimental controls. However, the catalase (CAT) and total glutathione content (TGSH) was found to decrease dose dependently in BF-2 cells exposed to Ni NPs. The current study demonstrated that BF-2 cell lines may serve as a sensitive indicator for aquatic contaminant evaluations in toxicological research.

Keywords Blue gill sunfish, nickel nanoparticles, cytotoxicity, morphology, oxidative stress

1. Introduction

The raw materials used in various nano-technology based devices/products include different nanoparticles (NPs) which are usually ranging from 1-100 nm [1]. These materials usually do occur in the environment and as byproducts of industrial process [2]. The safety of these NPs has also been receiving growing attention by the scientific community due to wide range of uses which include nano-electronics, aerospace engineering, medical, health care and environmental remediation [3]. The physiochemical characters such as size, shape, surface charge are very important for toxicity evaluation of NPs [4-8]. The aquatic systems are vulnerable (directly or indirectly) to pollution by NPs, and it is essential to assess their toxicity. *In vitro* cell assays are considered as the most competent substitute to animal testing [9].

Nanoparticles of nickel (Ni NPs) are novel materials with unique characteristics, which include low melting point, high magnetism and high level of surface energy [10-11] and with innumerable application which usually include gas sensors, alkaline batteries, paint formulations, displayers [3]. Moreover, nano rings of Ni are used as memory cells [12] and as electrodes in ceramic capacitors [13]. Ni NPs originating from different sources are reaching the coastal areas, thus becoming a significant source of contamination to seawaters [14]. NPs are directly deposited on surface waters by aerial emission, leakages, spills and indirectly through surface runoff from land-based sources [15-16]. Hence, Ni NPs have raised concern regarding their probable risk related to the health and safety of human and environment [3, 6].

Ni NPs are known to exert their toxicity on *Paramecium caudatam*, *Chlorella vulgaris* Beijer, *Daphina magna* and *Danio rerio* [17]. The cytotoxicity of Ni NPs was reported on epidermal cells of mouse [18], on leukemia cells [19]. [20] Reported that nanowires of Ni NPs were cytotoxic to human fibroblasts cells (WI-38). [21] confirmed the developmental morphological defects in zebrafish exposed to various shape (spheres and dendritic particles) and size (30, 60 and 100 nm) of Ni NPs. Cytotoxicity studies using Ni NPs were evaluated by means of mammalian models [22].

Studies conducted using fish models could be useful when comparing with the toxicological data of mammalian systems [23]. Ni NPs toxicity was not broadly assessed using fish models: apart from a few studies *in vivo* using zebrafish (larvae and adult) [16, 21] and

Oreochromis mossambicus [24], there are no other evidences on Ni NPs toxicity using fish cells *in vitro*. The evaluation of metal and metal oxide NPs toxicity by using fish cell lines is becoming a popular tool [25-27]. It is generally accepted that there exist species-dependent differences in the sensitivity to contaminants and there is increasing affirmation that this may also hold true for NPs. Significant differences in fish and mammalian cell lines were noticed upon exposure to NPs [28], most probably due to the difference in culture conditions/methods, composition of medium and the tissues from which they are isolated [29-30]. The cells of mammals and fish usually differ in their lipid and protein compositions of plasma membrane (PM) which helps them to adapt during variation in external environmental conditions [31]. The association of PM with different nanomaterials is dependent on lipid composition of the PM [32]. There exist differences in the toxicity of NPs to mammalian and fish cells to cope with the oxidative stress experienced by them. Based on the available literature it is evident that only few reports evaluated the cytotoxicity of Ni NPs so far. The bluegill sunfish is widely distributed and consumed by humans making it potentially important for evaluating human exposures to aquatic contaminants. Bluegill sunfish also serves as a sensitive indicator for contaminants evaluation in the environment and also for toxicological research [33].

Cytotoxicity studies performed using BF-2 cells with different contaminants had shown good correlation with *in vivo* acute toxicity tests of *Oncorhynchus mykiss* and *Lepomis macrochirus* [34-35]. The major objectives of the current study include cytotoxicity of Ni NPs and mechanism underlying Ni NPs toxicity on fibroblastic adherent cell line derived from the caudal fin of *L. macrochirus* or Bluegill sunfish. To know the potential toxicity of Ni NPs the following biomarkers were investigated i) cytotoxicity evaluation using three different assays (MTT, NRU, LDH) ii) morphological changes iii) oxidative stress was evaluated by measuring both protein or reactive carbonyl (PC) and peroxidation of lipid (LPO) iv) antioxidant defense assessed using total glutathione content (TGSH), glutathione sulfo-transferase (GST), glutathione peroxidase (GPX) and catalase (CAT).

2.0 Materials and Methods

2.1 Chemicals and reagents

Fetal bovine serum (FBS), Eagles minimum essential medium (EMEM), streptomycin, penicillin, glutathione, reduced nicotinamide adenine dinucleotide (NADH), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazoliumbromide (MTT), neutral red dye, reduced glutathione (GSH), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA) were Purchased from Sigma chemicals (St. Louis, USA). Rest of the chemicals was purchased from other commercial sources of highest purity.

2.2 Synthesis of Ni NPs

In the current study, Ni NPs were prepared by the method of co-precipitation technique. Chemicals $NiCl_2.6H_2O$, $NaBH_4$ and $C_2H_6O_2$ were purchased from Sigma-Aldrich Chemicals, USA. The preparation was attained by the following procedure (i) 0.2 gm of $NiCl_2.6H_2O$ was dispersed in 20 mL of $C_2H_6O_2$ and the solution was mixed using magnetic stirrer with simultaneous heating (ii) change in the color of the solution to light green was observed as the temperature reached 140^{o}C (iii) then, 0.1 gm of $NaBH_4$ was placed in the solution and indication of a reaction is given by a color change to black. The precipitate is cleaned with deionized water and acetone for several times and dried at 80^{o}C for 24 h and finally weighed. The procedure mentioned above produces 0.07 gm of precipitate and it is repeated several times for obtaining the bulk quantity of Ni NPs.

2.3 Characterization of Ni NPs

The characterization of synthesized Ni NPs was characterized using X-ray diffraction (XRD). The pattern for the XRD was obtained using Siemens D-500 X-ray diffractometer equipped with a Ni filter. The SEM pictures were obtained using a dilute sample of Ni NPs suspension which was dried at room temperature on a glass slide which is coated with evaporated carbon. Using Hitachi S4100 which was operational at an accelerating voltage of 25kV the images of Ni NPs were captured. The morphology of NPs were examined by Transmission Electron Microscopy (TEM) (JEOL 2200F, 200 kV). The hydrodynamic size of NPs was measured using Malvern ZS nano S analyzer, UK. The mean hydrodynamic size of Ni NPs in Milli-Q water and EMEM was measured by dynamic light scattering (DLS, Malvern ZS nano S analyzer, UK). In brief NPs were placed in Milli-Q water and EMEM at a concentration of 1 mg ml^{-1} and then after diluted to

5, 10, 20 and 30 µg ml^{-1} using same medium. This suspension was sonicated (Bandelin, Sonorex, Digitech, Germany) at room temperature for 20 min at 40W and then the size measurements were performed with highest concentration (30 µg ml^{-1}) using DLS both in Milli-Q water and culture medium at a time interval of 0 and 24 h at 25^{o}C. The size of the Ni NPs are reported based on the mean of three independent measurements per sample, with each measurement consisting of three individual readings, and calculated using the software Zetasizer automated particle characterization system, version 7.12.

2.4 Cell culture and treatment of Ni NPs

The BF-2 cells were obtained from American Type Culture Collection (ATCC # CCL91). Cells were maintained in culture medium with non-essential amino acids supplemented with 2 mM glutamine, 1.25 µg ml^{-1} fungizone, 100 µg ml^{-1} streptomycin, 100 µg ml^{-1} penicillin and 10% fetal bovine serum. The cells were incubated at 23 ± 2 ^{o}C supplied with 5% CO_2 and maintained in 75 cm^2 culture flask obtained from Corning. Prior to exposure of cells, the NPs were dispersed in EMEM at 1 mg ml^{-1}. The dispersion of NPs was done using water bath sonicator for 15 min at 40 W to avoid agglomeration. Moreover, the culture medium used for treating the control cells was also subjected to sonication. BF-2 cells were treated with 5, 10, 20 and 30 µg ml^{-1} of Ni NPs for 24 h and cells without any NPs treatment served as controls.

2.5 Cell viability assay

The viability of BF-2 cells was evaluated using MTT assay described by [36]. The mitochondrial dehydrogenase enzyme of viable cells which are seen reducing the yellow MTT into water insoluble purple formazan product. After 24 h of cells exposure to NPs the cells were incubated with MTT (5 mg ml^{-1}) and left for 4 h in dark at 21^{o}C. Later the medium was removed and fresh DMSO was added to each well to dissolve purple formazan product. The interferences of NPs were corrected by taking their absorption effects. The supernatant (100 µl) was then transferred to fresh plates and the absorbance measured at 570 nm. Effects of Ni NPs on cell viability were calculated using control cells (without nanoparticles).

2.6 Neutral red uptake assay

The neutral red uptake assay was performed based on the original protocol mentioned by [37], which detects the accumulation of neutral red in the lysosomes of the BF-2 cells. BF-2 cells after treatment, medium was removed and 90 µl of culture medium with 10 µl of neutral red solution (0.33%) was added and incubated for 2 h. Later the cells were washed with PBS to remove the excess dye and de-stained with solution containing water, glacial acetic acid and ethanol. The optical density was measured using UV-visible spectrophotometer supported by SOFT max PRO-3.0.

2.7 LDH leakage assay

The LDH assay is relied on determining the LDH enzyme which is located in the cytoplasm and is released into the culture medium when cells are ruptured/damaged. LDH is measured using a spectrophotometric method provided by the Sigma kit. The protocol included as follows. After the treatment, the medium was carefully aspirated and transferred to a fresh plate 96 well plate as mentioned in the protocol. After completion of the reaction the stop reagent was added, and the absorbance was read at 340 nm.

2.8 Cell morphology

Well-plates (96) were seeded with a cell density of $2x10^4$ cells. Later the cells were provided with fresh medium containing Ni NPs (30 µg ml^{-1}). The morphological changes in BF-2 cells were recorded after 24 h using the Nikon microscope at a magnification of 10X.

2.9 Protein carbonyl evaluation

Protein carbonyl was evaluated following the method described by [38]. The assay includes 200 µl of supernatant along with 300 µl of 10 mM 2,4 Dinitrophenyl hydrazine which is incubated for 1 h. Later, 300 µl of 10% Trichloro acetic acid was added to the following mixture and centrifugation was performed at 500xg for 5 min to precipitate the proteins. The obtained

protein was washed several times with ethanol/ethyl acetate mixture and suspended in 6M guanidine hydrochloride prepared in 20 mM PBS (pH 2.3) and the absorbance was measured at 370 nm.

2.10 Lipid peroxidation Assay

The lipid peroxidation was measured in the cellular extract using the method adopted by [39]. The reaction mixture consisted of 0.1 ml of cellular extract and sodium phosphate 0.1 M (1.9 ml). The whole reaction mixture was incubated at 37^{o}C for one hour. The reaction was terminated by the addition of TCA (5%) and later it was centrifuged at a speed of 2300xg for 15 min. The supernatant was collected the absorbance was measured at 532 nm.

2.11 Catalase Assay

Catalase activity was determined according to the method described by [40] which measured the decline of H_2O_2 concentration at 240 nm. Briefly the reaction mixture consisted of 100 µl of cell lysate, 0.8 ml of H_2O_2 and 100 µl of distilled water and the reaction was monitored at 240 nm.

2.12 Glutathione sulfo-transferase

Glutathione sulfo-transferase was determined in the PMS fraction using CDNB following the method of [41], the reaction mixture included sodium phosphate buffer (0.1 M), 16.4 mM CDNB and 16.4 mM GSH along with the cellular lysate. The absorbance was measured at 340 nm. Glutathione peroxidase was measured by the method described by [42], using H_2O_2 and NADPH as substrate. The transformation of NADPH to $NADP^+$ was monitored by measuring the absorption at 340 nm.

2.13 Total glutathione content

Protein content in the sample was precipitated using 5% of sulfosalicylic acid for 1h and later centrifuged at 13,400xg for 20 min at 4^{o}C. The TGSH was measured in the supernatant using

the enzymatic recycling method using GR excess, the sulfhydryl group of GSH reacts with dinitrobenzoic acid producing the thio2nitrobenzoic acid. The rate of thio2nitrobenzoic acid production is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of glutathione in the sample [43]. This product is measured spectrophotometrically at 412 nm.

2.14 Statistical analysis

The presented values are mean±SD of three independent experiments with at least three replicates each. Data were tested for normal distribution by performing the Shapiro-Wilk test and for variance homogeneity by using Leven's test prior to performing one-way analysis of variance (ANOVA). Post hoc Dunnett's test was applied to compare values between control and treated groups using SPSS and Origin 8 software. Difference, for which statistical comparison resulted in values of *p< 0.05, ** p < 0.01, *** p < 0.001 were considered as statistically significant.

3.0 RESULTS

3.1 Characterization of Ni NPs

The XRD patterns of Ni NPs at 2θ = 45, 52 and 77 corresponding to (111), (200) and (220) crystal plane. It indicates that nano powder contains pure nickel particles and there were no other particles (Fig. 1). The average particle size (d) was calculated using Sherrer's equation.

$$d = \frac{0.94\,\lambda}{B\,Cos\theta} \qquad (1)$$

Where (d) is the grain size, (λ) the wavelength of X-ray, B the full width at half maxima of the diffraction of the peak (θ) corresponds to the angle at maximum peak. For the maximum peak in XRD pattern (2θ) is observed as 44.43^{o}, and B (2θ) is 1.92^{o}. For a wavelength (λ) is 0.15148 nm the value of (d) was approximately 72 nm, as given in Eq. (1). The average hydrodynamic size of Ni NPs in Milli-Q water and culture medium at 0 and 24 h along with polydispersity index and zeta potential are featured in the Table 1. The Fig. 2 a, b and C shows

the typical Scanning and Transmission Electron Microscopic images of Ni NPs respectively. The average diameter of Ni NPs using TEM was measured over 100 particles in random fields of view and the size was approximately 73.94 ± 2.61 nm (Fig. 2c).

3.2 Nickel nanoparticles induced cytotoxicity

Viability of BF-2 cells was assessed using MTT and NRU assays after treatment with Ni NPs at the concentrations of 0, 5, 10, 20 and 30 µg ml^{-1} for 24 h. Strikingly, Ni NPs at 5 µg ml^{-1} concentration had no significant effect on cell viability using MTT assay when compared to their controls. However, as the Ni NPs concentration increased from 10 to 30 µg ml^{-1} they did produce significant decrease in cell viability in comparison to their respective control (Fig. 3a). Cell viability assessment using NRU assay confirm the results of MTT assay indicating a decline in BF-2 cell viability when treated with Ni NPs. It is interesting to note that, reduction in number of viable cells revealed dose dependent decline in the BF-2 cells after treatment with Ni NPs for 24 h as assessed by MTT and NRU assays respectively (Fig. 3 a & b). A significant increment in LDH leakage was noted in BF-2 cells after 24 h of exposure with Ni NPs at following concentrations 5, 10, 20 and 30 µg ml^{-1} (Fig 3 c).

3.3 Cell morphology

The figure 5 represents the morphology of unexposed BF-2 cells (Fig. 4 a) and cells treated with Ni NPs (30 µg ml^{-1}) for 24 h (Fig. 4b). The Ni NPs treated cells lost their contact from the surface of culture plate and became round as shown in the figure (indicated by white arrows). Strikingly few cells have lost their cellular contents and observed non-viable as indicated in red colour arrows (Fig. 4b).

3.4 Oxidative stress markers

The potential of Ni NPs to cause oxidative stress was monitored by assessing the reactive carbonyl content and LPO in BF-2 cells exposed to 0, 5, 10, 20 and 30 µg ml^{-1} of Ni NPs for 24 h. Surprisingly a significant increase in both reactive carbonyl content (PC) and LPO was

prominent at all the tested concentrations (Fig. 5 a). Current results revealed that the exposure to Ni NPs caused a significant increase in the activity of GST and GPX in BF-2 cells in comparison to that of controls (Figure 6 a). The CAT and TGSH content was evaluated in BF-2 cells treated with Ni NPs at a concentration of 0, 5, 10, 20 and 30 µg ml^{-1} for 24 h. The current results showed a significant decrease in the CAT and TGSH content among all the concentrations of Ni NPs (Fig. 5 b and 6 b).

4.0 DISCUSSION

Nanotechnology is increasing rapidly during the recent past showing its applicability in diverse applications for the advancement of human life. However, because of its increased fabrication and use both environment and humans pose a serious risk of contact to these NPs [44]. The wide spread use of Ni NPs in diverse applications from medical to consumer products has raised concern about their toxic effects to both humans and environment [45-46]. Most of the studies performed using Ni NPs or NiO NPs evaluated the cytotoxicity using mammalian cells but as such on piscine models are lacking [24]. Due to the paucity of information on cytotoxicity studies on fish cells using Ni NPs the following study was undertaken and the cellular responses of BF-2 cells upon exposure to Ni NPs were investigated. Interestingly, a unique morphological change in the BF-2 cells is a direct evidence of cytotoxicity induced by Ni NPs which is confirmed by the results of MTT, NRU and LDH assays. Nonetheless all assays confirmed that Ni NPs decreased the number of viable BF-2 cells in a concentration dependent manner with slight variation. However, Ni NPs only in MTT assay at 5 µg ml^{-1} concentration had not shown any significant cytotoxic effect in BF-2 cells. Cytotoxic studies conducted by Ahamed and Alhadlaq on MCF-7 cells using NPs of Ni also demonstrated comparable results. However, Ni NPs had shown cytotoxicity at concentrations >10 µg ml^{-1} in HepG2 cells [10]. Moreover, studies conducted by [45] revealed that the concentration of Ni NPs required for producing the cytotoxicity on adrenocarcinomic human alveolar cells is 2 µg ml^{-1}. Hitherto, these results depicted that A549 cells are vulnerable to Ni NPs exposure than BF-2 cells and less liable than HepG2 cells. Among the different cell lines, the cell viability behaviour is similar regardless of the shape of the nanomaterials. However, under similar conditions different cells show different cell viability [20].

The assays performed (MTT & NRU) in the present study signify that mitochondrial and lysosomal membrane damage eventually resulted in their cell death [47-48]. The LDH assay which is one of the markers for membrane damage revealed that BF-2 cells when treated with Ni NPs for 24 h induced membrane damage. It is noteworthy that current findings are in good agreement with the results of other researchers in mouse epidermal cells, leukaemia cancer cells [18-19], in A549 cells [45] and human breast carcinoma cells when treated with Ni NPs [18]. Similar finding was noted in human skin epidermal cells exposed to Ni NPs [49].

Nanoparticles induced oxidative stress and LPO are considered as the most trivial components of toxicity [44]. However, any alteration in the homoeostasis sparks the production of free radicals and resulting in oxidative stress and cause alteration in the antioxidants. Attributable to the noxious affects of nanoparticles/nanomaterials which results in excess ROS generation leading to oxidative stress causes oxidation of lipids and proteins. Furthermore, a significant hike in both LPO & PC was found in BF-2 cells treated with Ni NPs. Current results are corroborated by the studies of [45], evidenced a significant increase in LPO in the cells of A549 exposed to Ni NPs. Notably, similar increase in PC & LPO was observed in WAG cells, Zebra fish treated with titanium oxide zinc oxide NPs and their bulk counterparts [50]. In another example, Chinook salmon cells treated with CuO NPs had also shown similar responses [44]. Interestingly, the Medical Research Council strain 5 (MRC-5) cells exposed to SiO_2 nanomaterials also confirmed an inclination of PC & LPO similar to the current studies [51].

Carbonlation of proteins is an irrevocable ROS induced reaction which results in the increase of PC in BF-2 cells exposed to Ni NPs. It is noteworthy that different pathways are existing to acquaint the carbonyl groups into the protein, the ROS may react with carbohydrates, lipids or directly oxidise threonine, arginine, lysine and proline residues leading to the production of reactive carbonyl species which include 4-hydroxy-2-noneal, malondialdehye, ketoaldehyes and ketoamine which are interacting with proteins [52]. For this reason, the morphology, solubility, conformation and susceptiveness to protein breakdown, may be altered because of oxidative modification which leads to their modification in physiological functions. Consequently, an inclination in PC is observed in BF-2 cells exposed to Ni NPs. Additionally,

the marker LPO of oxidative stress was also found to increase in BF-2 cells treated with Ni NPs when compared with their respective controls. Remarkably, increased LPO caused membrane damage which is indicated by the increase in LDH release in the cells exposed to Ni NPs. Ni NPs toxicity was noticeable by the morphological changes which are perceptible in BF-2 cells.

Cells treated with 30 µg ml^{-1} concentration of Ni NPs appear to be spherical after they detach from the substratum. As the time of exposure increased some of the cells had blurred cell margins, cytoplasm shrinking, and few cells were dead which is apparent from the presented figure. Identical results were noted in JB6 cells treated with Ni NPs [53]. Similar type of loss was noted in cellular morphology of Chinook salmon cells treated with Al_2O_3 & CuO NPs [54, 44] Similar type of results was noticed in leukemia K562 cells exposed to Ni NPs (20 µg ml^{-1}) for 24 h [55]. Skin epithelial cells (A431) exposed to Ni NP at 2, 4, 8 and 20 µg ml^{-1} for 48 h has also shown morphological abnormalities similar to our studies [49]. The TGSH content and CAT activity were considered as biomarkers for antioxidant status. CAT levels decreased significantly in BF-2 cells exposed to Ni NPs in a concentration dependent manner when compared to controls. Similar observation was observed in CHSE-214 cells treated with Al_2O_3 NPs and TiO_2 NPs [54, 56]. It is noteworthy that hike in CAT activity was evident in cells of WAG treated with TiO_2 and NPs of ZnO in comparison to that of their respective control values [50]. CAT enzyme which exists in peroxisomes is involved in elimination of H_2O_2 which is metabolized to molecular oxygen and water. GPX enzyme also plays a deciding role in detoxification of H_2O_2. GPX as one of the crucial enzyme helps in catalysing H_2O_2 and other lipid hydroperoxides reduction into nontoxic products. The elevation of GPX in BF-2 cells exposed to NPs of Ni may be due to elimination of H_2O_2 and prevent the cells from oxidative damage.

Dose dependent decrease in TGSH content was noticed in BF-2 cells treated with Ni NPs. Similar decrease in TGSH was evident in WAG cells exposed to ZnO NPs and TiO_2 NPs [50]. The present study suggests that TGSH depletion of BF-2 cells exposed to Ni NPs accompanied by augmented LPO is reported to be highly correlated with increased ROS levels. The reduction of peroxides by GST enzyme assists in protecting BF-2 cells from peroxidative damage. Similar responses were illustrious when BF-2 cells exposed to Ni NPs. The increase of

GST and GPX dose dependent behaviour may be correlated with a sharp decline in TGSH content of BF-2 cells exposed to Ni NPs. The decrease in TGSH content may be clarified by the fact that it is used as substrate by GPX. The enzyme GPX in the gill cells of *A. anguilla* exposed to iron oxide NPs showed a similar increase [57]. Moreover, identical responses were noted in Chinook salmon cells exposed to CuO NPs [44]. BF-2 cells treated with Ni NPs declined in TGSH content combined with increased LPO suggesting that primary mechanism for toxicity is oxidative stress. Our results primarily confirm that NPs of Ni induce cytotoxic effects in BF-2 cells as clearly evident from the assays of MTT, NRU and LDH. The current results indicated that Ni NPs prompt oxidative stress as apparent from inclination in LPO and PC followed by the decrease of TGSH. Strikingly, the morphological alterations in cells were noticed in BF-2 cells when treated with Ni NPs at higher concentration. In addition to that, dose dependent increase in GST and GPX followed by decline in CAT and TGSH was also noted in BF-2 cells treated with Ni NPs. The current study serves as a model system for risk assessment of Ni NPs studies using BF-2 fish cells.

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Conflict of interest

We declare we do not have any conflict of interest

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FIGURE LEGENDS

Figure 1. The characterization of nickel nanoparticles (Ni NPs) using X-ray diffraction.

Figure 2. (a) The characterization of nickel nanoparticles (Ni NPs) using low magnification scanning electron microscopy. (b) Transmission electron microscopy image of nickel nanoparticles (Ni NPs). (c) The transmission electron microscopy of single nickel nanoparticles (Ni NPs).

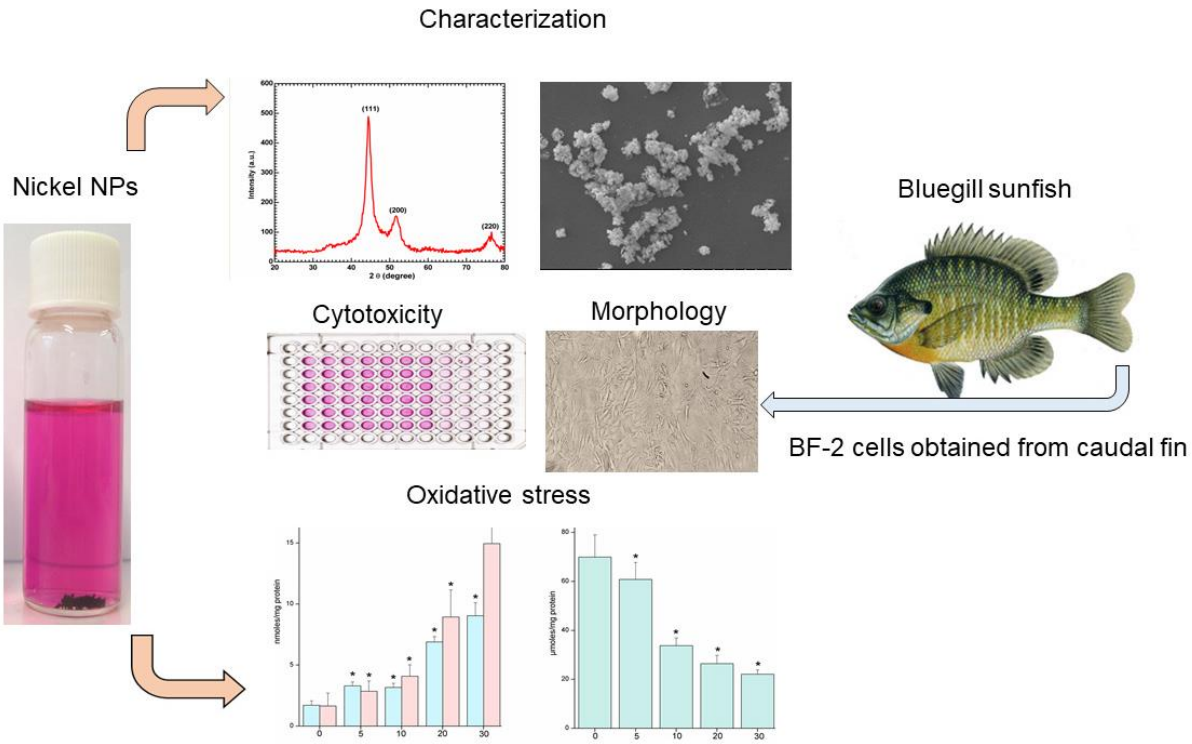
Figure 3. Cytotoxicity in blue gill sunfish (BF-2) cells exposed to nickel nanoparticles (Ni NPs). Cells were treated with Ni NPs at 0, 5,10, 20 and 30 µg ml^{-1} up to 24 h later the cytotoxicity was evaluated using (a) MTT, NRU and (b) LDH assays. Data were presented as mean ±SD (n=3) of three independent experiments of three replicates each. Statistically significant differences compared to controls are indicated by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Figure 4. Morphological changes in blue gill sunfish (BF-2) cells exposed to 30 µg ml^{-1} concentration of nickel nanoparticles (b) and control cells (a).

Figure 5. (a) Protein carbonyl (PC), lipid peroxidation (LPO) and (b) Catalase (CAT) in blue gill sunfish (BF-2) cells exposed to nickel nanoparticles (Ni NPs) at 0, 5,10, 20 and 30 µg ml^{-1}. Data

were presented as mean ±SD (n=3) of three independent experiments of three replicates each. Statistically significant differences compared to controls are indicated by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

Figure 6. (a) Glutathione sulfo-transferase (GST), glutathione peroxidase (GPX) and total glutathione content (b) in blue gill sunfish (BF-2) cells exposed to nickel nanoparticles (Ni NPs). Data were presented as mean ±SD (n=3) of three independent experiments of three replicates each. Statistically significant differences compared to controls are indicated by * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.



Graphical abstract

Research highlights

- This study highlights the role of Ni NPs cytotoxicity on Bluegill sunfish cells
- Morphology of BF-2 cells was altered when exposed to Ni NPs
- Ni NPs caused induction of LPO and PC in BF-2 cells
- Ni NPs enhanced the levels of GST and GPX
- NPs of Ni were found to be cytotoxic and induce oxidative stress in BF-2 cells

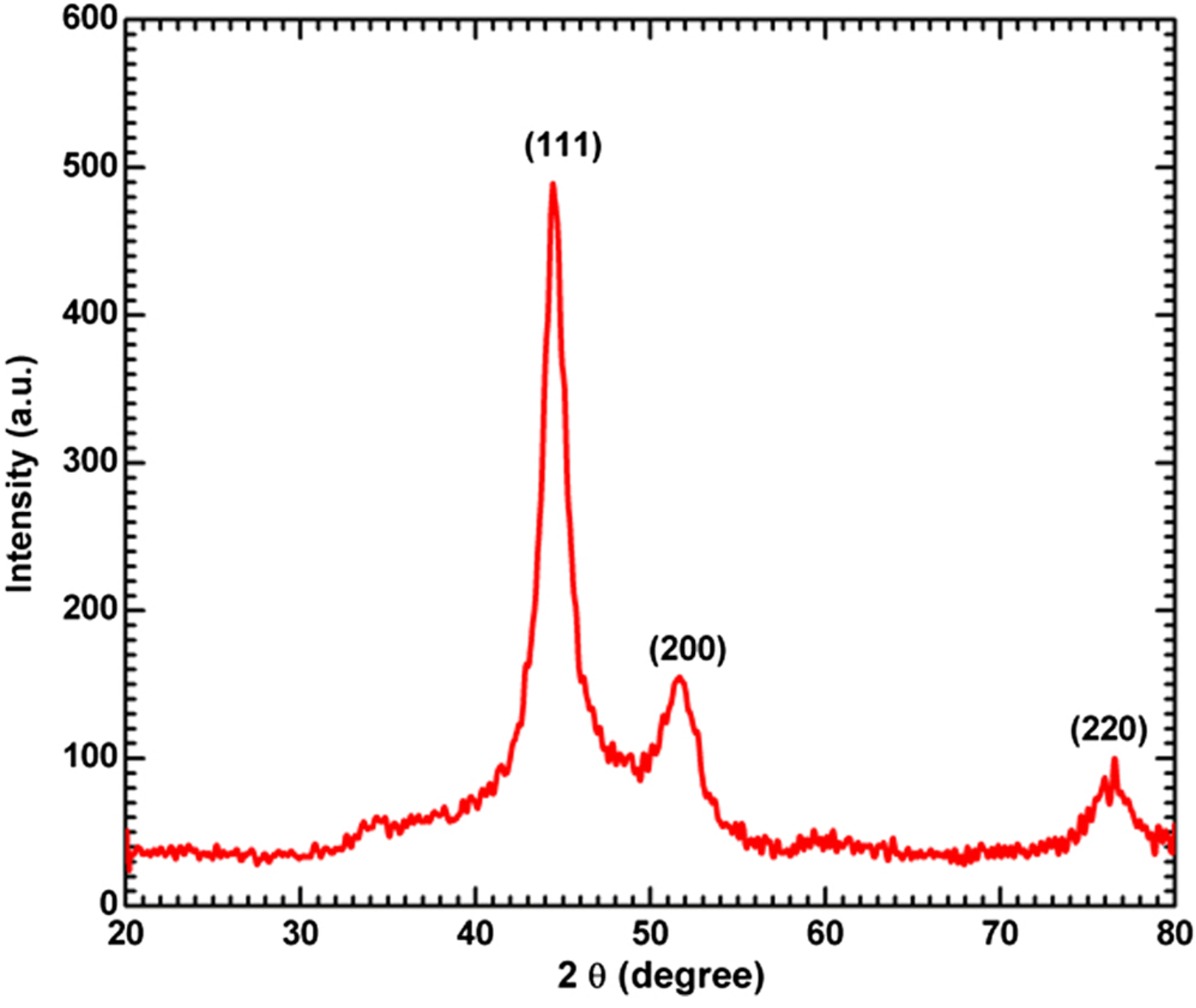

600
500
400
300
200
100
0
Intensity (a.u.)
(111)
(200)
(220)
20
30
40
50
60
70
80
2 θ (degree)

Figure 1

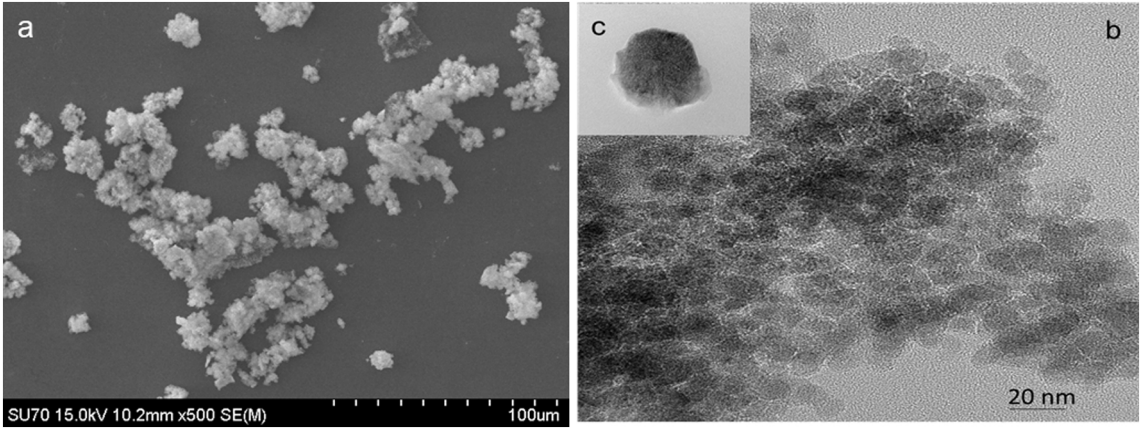


Figure 2

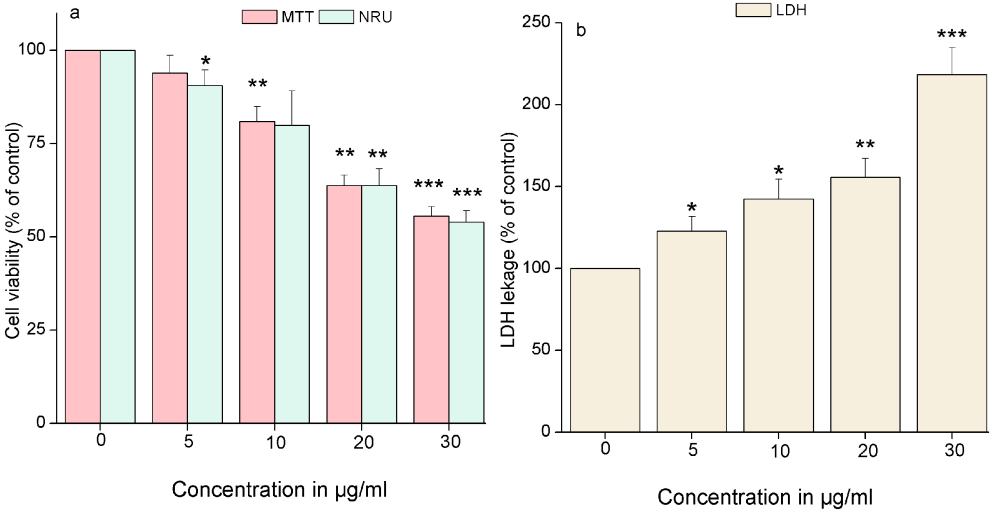

a
MTT
NRU
Cell viability (% of control)
100
75
50
25
0
*
**
**
**
***
***
0
5
10
20
30
Concentration in µg/ml
b
LDH
LDH lekage (% of control)
250
200
150
100
50
0
*
*
**
***
0
5
10
20
30
Concentration in µg/ml

Figure 3

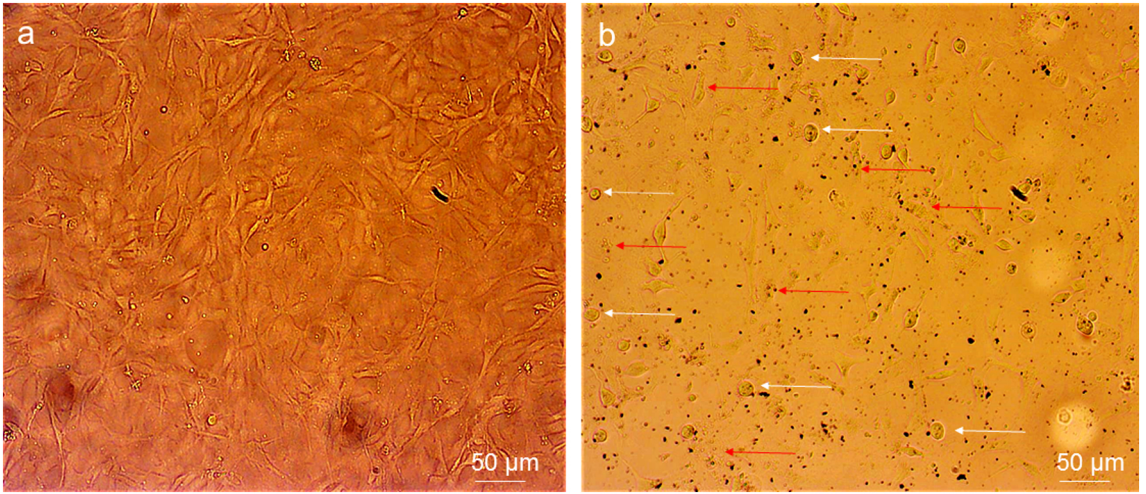


Figure 4

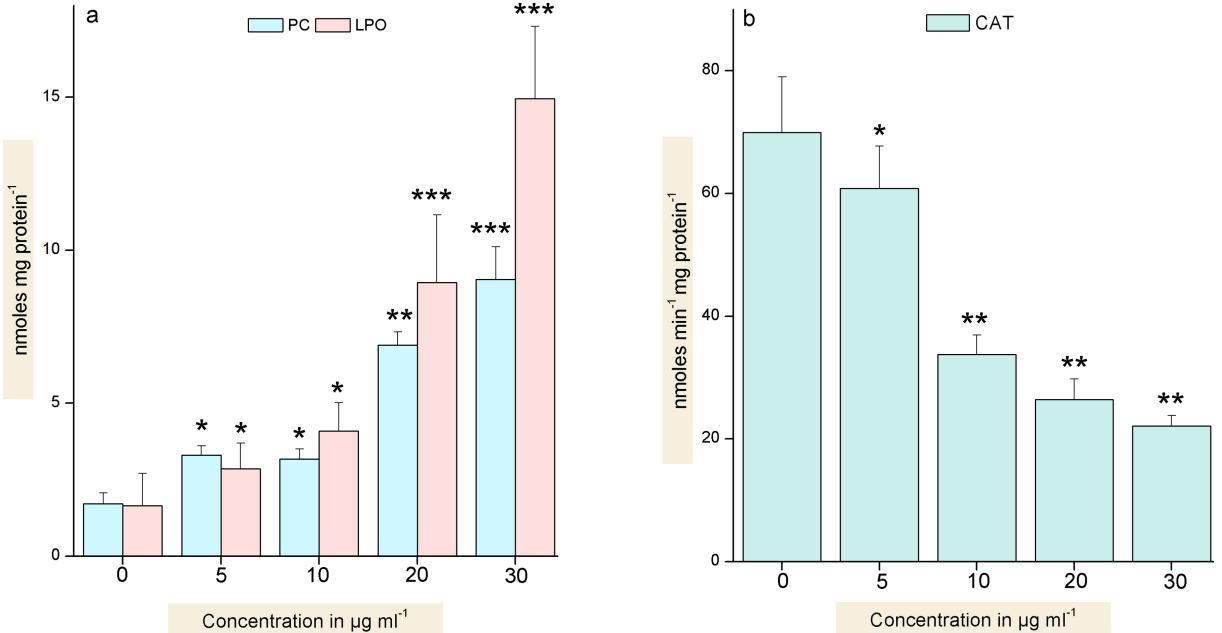

a
PC
LPO
nmoles mg protein-1
0
5
10
15
0
5
10
20
30
Concentration in µg ml-1
b
CAT
nmoles min-1 mg protein-1
0
20
40
60
80
0
5
10
20
30
Concentration in µg ml-1

Figure 5

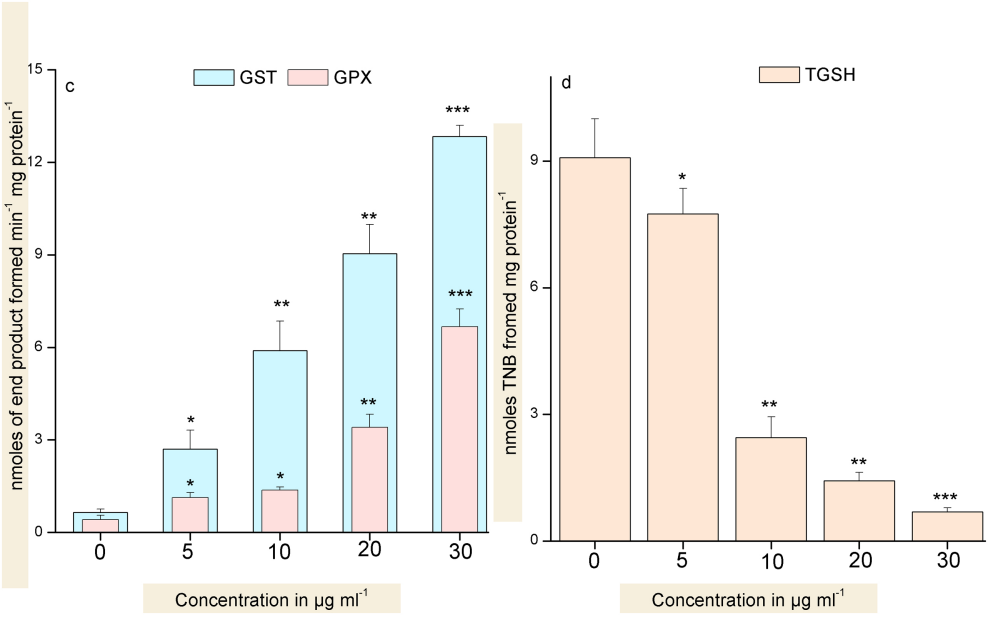

c
GST
GPX
nmoles of end product formed min-1 mg protein-1
15
12
9
6
3
0
*
*
**
*
**
**
***
***
0
5
10
20
30
Concentration in µg ml-1
d
TGSH
nmoles TNB fromed mg protein-1
9
6
3
0
*
**
**
***
0
5
10
20
30
Concentration in µg ml-1

Figure 6