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## *In vitro* effects of cobalt nanoparticles on aspartate aminotransferase and alanine aminotransferase activities of wistar rats

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### ABSTRACT

Cobalt nanoparticles (Co-NPs) have been extensively used in clinical practices and medical diagnosis. In this study, the potential toxicity effects of Co-NPs with special emphasis over the biochemical enzyme activities, such as aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) in serum, liver, and kidney of Wistar rats were investigated. This toxicity measurement of nanomaterials can support the toxicological data. The biochemical enzymatic variations are powerful tools for the assessment of toxicity. ASAT and ALAT enzymes have been widely used to predict tissue-specific toxicities associated with xenobiotic. The biochemical changes induced by Co-NPs have significance in their toxicological studies because the alterations in biochemical parameters before clinical symptoms indicate either their toxicant safety or detrimental effect. Herein, Co-NPs with particle size <50 nm significantly activated ASAT and ALAT enzymes in the serum, liver, and kidney of rats at concentration-dependent order.

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## 1. Introduction

Nowadays, nanoparticle-based research is an expeditiously developing area with enormous potential in medicine. Cobalt nanoparticles (Co-NPs) are spherical in shape [1]. Co-NPs with magnetic properties shows their potential applications in biotechnology, biomedicine, material sciences, engineering, and environmental areas [2]. Co-NPs can be manufactured by using a laser evaporation process and the contaminants can be removed to produce high purity of Co-NPs [3,4]. Co element is also an indispensable constituent of human nutrients such as vitamin B<sub>12</sub> part and is used in drug production especially for cancer treatments [5]. The syntheses of Co-NPs with new shapes are suitable for clinical biomedical practice due to their accumulation in tumor tissue without the need of intratumor injection, high

photothermal conversion, excellent optical and photoacoustic imaging performances, and renal excretion [6]. But, the risk may be toxic or cure of cells that can be counted by the death rate of cells [6]. Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) are the enzymes found mainly in the liver, red blood cells, heart, kidneys, and pancreas cells [7]. The activities of hepatic ASAT, ALAT,  $\gamma$ -GT (gamma-glutamyl transpeptidase), and GPT (serum glutamic pyruvic transaminase) enzymes are commonly used as biomarkers for recent alcohol abuse and *in situ* toxic compounds [8–11]. The amount of ASAT and ALAT enzymes in the blood is directly related to tissue damages. More or fewer amounts of the enzymes lead to an increase or decrease in tissue damages, respectively [12]. Meanwhile, an increase in the concentration of NPs may cause damage to the cell morphology and structure. The inflammation and necrosis caused by hepatocyte damage can lead to an increase the permeability of the cell membrane. ASAT and ALAT enzymes are released into the body through the cell membrane and hence their concentration in the blood increases [13]. Thus, ASAT and ALAT enzymes are indicators of liver damage [14].

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The toxicity of Co-NPs belonging to the cluster of metal and metal oxide NPs may be attributed to either direct uptake of Co-NPs by cells or to the cessation of Co-NPs, leading to the increased level of cationic ions in the tissue with consequent effects on the cells [15]. Until now, there has been limited attention to gaining knowledge related to the potential biochemical enzymatic toxicity of Co-NPs. Co-NPs were found to exert oxidative stress in lung epithelial cells [16], DNA damage in fibroblasts [17], inflammatory response in endothelial cells [18], human mononuclear cells, and neutrophils cytotoxicity [19]. The morphological transformation and genotoxicity induced by Co-NPs were demonstrated in Balb3T3 cells [20]. Furthermore, it was shown that Co-NPs induced a genotoxic effect in human peripheral leukocytes [21]. However, a systematic survey of Co-NPs effects on the viability of non-identical cell types is missing.

The detection of ASAT and ALAT enzymes has been widely studied due to their clinical significance in monitoring patients with liver diseases [22,23]. The elevated level of ASAT enzymes may be induced by disorders that affect organs or tissues other than the liver with the most common being striated muscles. The elevated level of ASAT and ALAT up to 300 u/L is considered nonspecific. Normally elevated levels are occurred during the second trimester in asymptomatic normal pregnancy [24–27] whereas elevated levels of ASAT predominate in cirrhosis patients [28]. The toxicity of Co-NPs is one of the major challenges and with a green approach. Co-NPs as a biomarker can reduce the risk of xenobiotics and focus on the most recent emerging developments including synthesis methods, bioimaging, and biosensing applications. Then, it is very important to know the absorbance of ASAT and ALAT in terms of toxicity in different cells and response with the Co-NPs subjected to induced hepatotoxicity and indices of antioxidant status of liver tissues.

## 2. Materials and methods

### 2.1. Chemicals

Co-NPs were obtained from Sigma Aldrich, Germany. All other chemicals used in this study were of analytical grade and purchased from Sigma Aldrich (St. Louis, MO, USA). The BSA (bovine serum albumin) (mg/mL): 0.01 g of BSA dissolved in 100 mL of 0.5 M NaOH. Reagent A: the mixture of 2 % Na<sub>2</sub>CO<sub>3</sub>, 0.4 % NaOH, 0.16 % Na/K tartarate, and 1 % SDS. Reagent B: 4.0 % CuSO<sub>4</sub> (copper reagent). Reagent C: 100 parts of reagent A with 1 part of reagent B. 2 N folin reagent was dissolved in an equal volume of pure water and 1 N folin-phenol reagent was made.

### 2.2. Rat treatment

Wistar rats were purchased from the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. They were maintained under controlled conditions in the animal house of the Council of Scientific and Industrial Research, Indian Institute of Chemical Technology, Hyderabad, India, for a week before the experiment. The ethical clearance was obtained from the Animal Ethical Committee (Animal House Facility, Applied Biology Division, Indian Institute of Chemical Technology). The rats were maintained at 22 °C and relative humidity of 30–70 %. Lightening arrangements were 12 h in light and 12 h in dark.

### 2.3. Biochemical studies

The rats were sacrificed by cervical dislocation. Rat blood was collected without any anticoagulants before being sacrificed. Rat serum was obtained by centrifuging the blood at 1500 rpm for 10 min. The liver and kidney of rats were dissected out and quickly

homogenized separately in ice-cold sucrose solutions using Yarco speed homogenizer to make 10 % homogenate (w/v). The pellet was discarded and the supernatant was used as an enzyme source. Serum, kidney, and liver homogenates were used to determine *in vitro* ASAT and ALAT enzymes in control and nanomaterial-exposed organs.

### 2.4. Protein, ASAT, and ALAT estimations

The protein was estimated in the liver and kidney of control and exposed cultures with folin-phenol reagent using the standard procedure as described by Lowry and coworkers [29]. ASAT and ALAT activities in rat's serum, kidney, and liver were estimated by the spectrophotometer method of Yatzidis [30].

### 2.5. Statistical analysis

The data represents the mean and standard deviation of three biological replicates from each animal group. The data was analyzed by the Dunnett test to illustrate the significant concentration differences between the control and treated-culture groups. Statistical significance was considered at P < 0.05. Statistical analysis for Co-NPs concentrations in organs and biochemical examinations were calculated by:

$$\text{Serum} = \frac{X}{0.025} \times \frac{1}{88} = \frac{\mu\text{mol}}{\text{mL}}/\text{h}$$

$$\text{Tissue} = x/\text{mgprotein}, \frac{1}{88} \times \frac{1}{88} = \frac{\mu\text{mol}}{\text{mL}}/\text{h}$$

Where X stands for pyruvic acid formed in  $\mu\text{g}$   
stands for volume of serum in ml and  
stand for molecular weight of pyruvic acid (for converting  $\mu\text{g}$  into  $\mu\text{moles}$ )

## 3. Results

In the present systematic study, Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT) activities in serum, liver, and kidney reacted with Co-NPs at different concentrations were evaluated. Co-NPs with particle size < 50 nm significantly activated Aspartate aminotransferase and Alanine aminotransferase in serum, liver, and kidney. Similarly, the ASAT and ALAT, significant changes and activation were observed with each concentration of Co-NPs. The significant activation of ASAT in serum was observed at the concentration of 1.0, 2.5, and 5.0 mM of Co-NPs (Table S1). The maximum activation was about 149 % at 5.0 mM concentration of Co-NPs (Fig. 1a) and the IC<sub>50</sub> was observed <1.00 mM (Fig. 1b). Similarly, the ALAT significant activation in serum was observed at 1.0, 5.0, and 10.0 mM concentrations of Co-NPs (Table S2) and the IC<sub>50</sub> was observed at 3.18 ± 0.18 mM of Co-NPs (Fig. 1b). The maximum activation was observed at 161 % at 10 mM Co-NPs (Fig. 1a).

Furthermore, ASAT significant changes in kidney were observed in the Co-NPs concentration of 0.5, 1.0, 2.5, 5.0, and 10.0 mM (Table S3). The IC<sub>50</sub> was observed to be 1.14 ± 0.17 mM Co-NPs (Fig. 1b). The maximum activation was 242 % at 10.00 mM of Co-NPs concentration (Fig. 1a) with the activity of 57.61 ± 0.61 (Fig. 2a). Meanwhile, the ALAT significant changes in kidney were observed at 1.0, 2.5, 5.0, and 10.0 mM Co-NPs and the observed IC<sub>50</sub> was 3.00 ± 0.10 mM (Table S4). A maximum activation was 175 % at 10.0 mM concentration of Co-NPs (Table S4 and Fig. 1). The activity of Co-NPs was increased from 0.5 mM to 10.0 mM compared to the control (Fig. 2b).

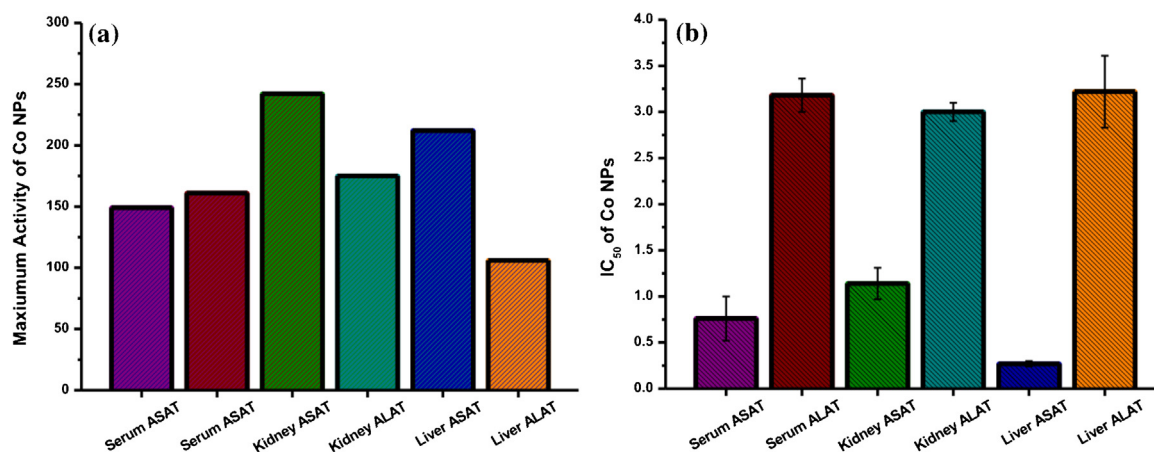


Fig. 1. (a) The maximum activation of Co-NPs (5 mM) for ASAT and ALAT in different samples. (b) IC<sub>50</sub> of Co-NPs for ASAT and ALAT in different samples.

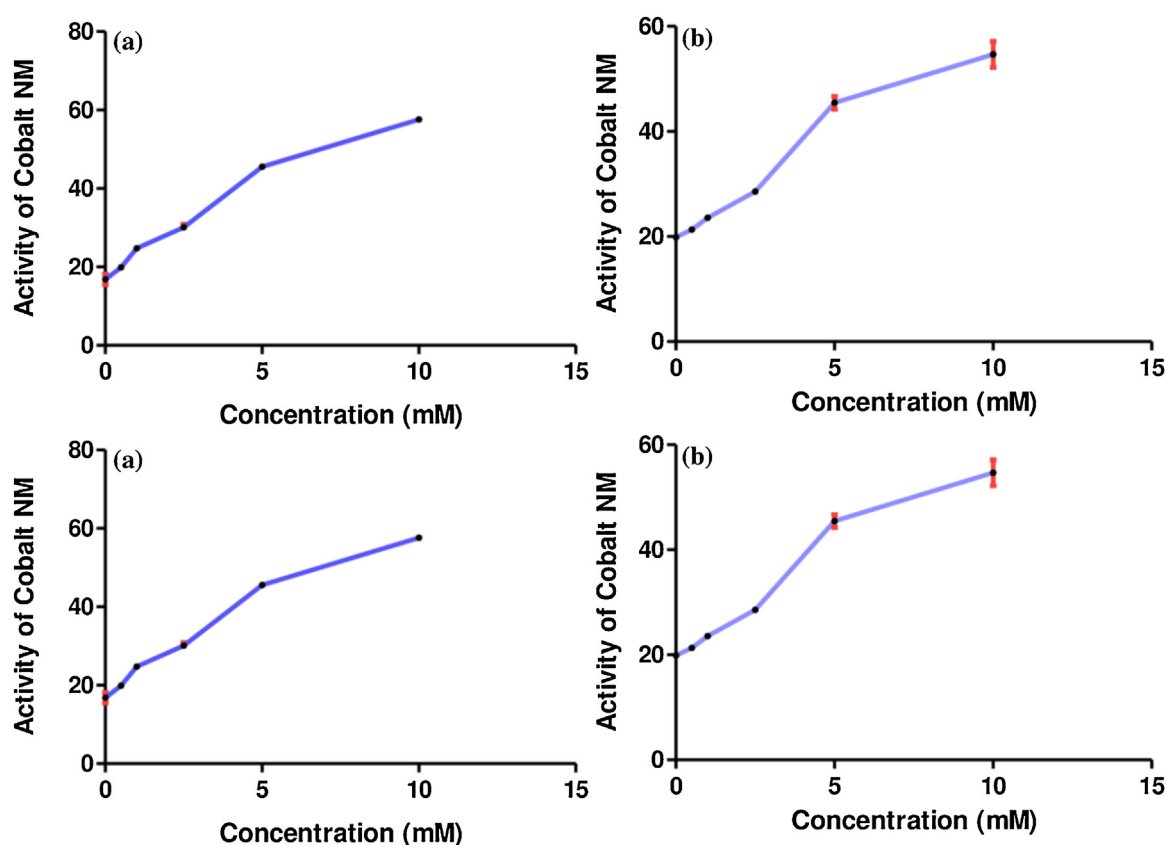


Fig. 2. The activity of Co-NPs with different concentrations on the ASAT (a) and ALAT (b) in the kidney of rats.

The ASAT in the liver was significantly activated at 0.5, 1.0, 5.0, and 10.0 mM concentrations of Co-NPs and the observed IC<sub>50</sub> was  $0.27 \pm 0.03$  mM (Table S5). Further, 212 % activation was observed at 10.0 mM concentration of Co-NPs with increasing their activity (Fig. 3a). Similarly, the ALAT activation in the liver was significant for all concentrations including 0.5, 1.0, 2.5, 5.0, and 10.0 mM concentrations of Co-NPs (Table S6). The IC<sub>50</sub> was observed at  $3.22 \pm 0.39$  mM Co-NPs. The maximum increase of their activity was observed with the maximum concentration of Co-NPs at 10.0 mM (Fig. 3b).

A patho-morphology analysis on heart, liver, spleen, lung, and kidney in different groups was conducted to estimate the toxicity of the Co-NPs. The spleen, heart, and lung were optional data for hemotoxylin and eosin (H&E) stain. The H&E stained images of the

samples are given in Fig. 4. In addition, the cell viability report of the samples at different concentrations of Co-NPs is shown in Fig. 5. The results showed that the Co-NPs were predominantly accumulated with ASAT and ALAT, suggesting the transferring of Co-NPs to the samples.

#### 4. Discussion

The liver is the primary organ where exogenous synthetic substances are utilized and in the long run, discharged; as a result, liver cells are presented significantly with these chemical substances, which lead the liver brokenness, cell damage, and even organ failure [31]. Cobalt metals are known to be genotoxic *in vitro*, whereas Co<sup>2+</sup> ions are known to be a health hazard to

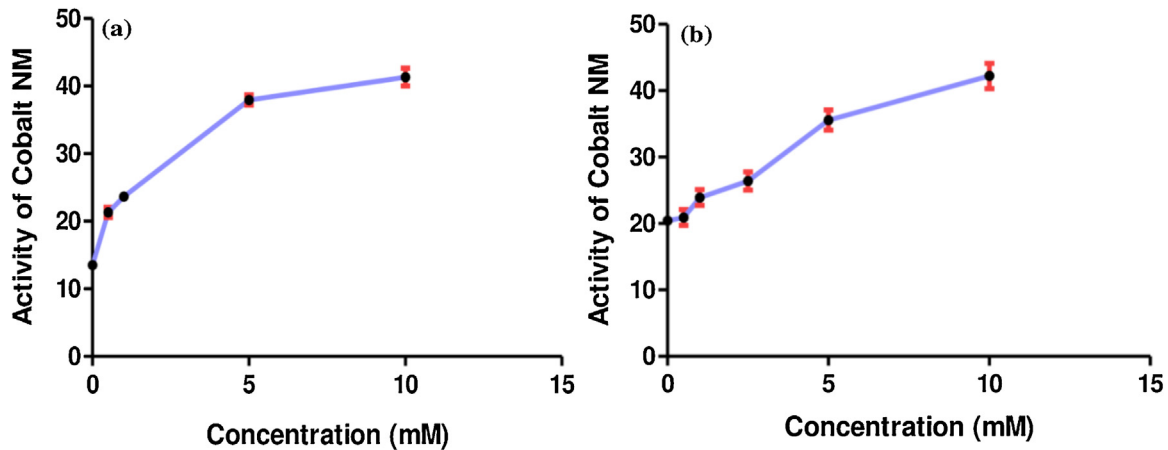


Fig. 3. The activity of Co-NPs with different concentrations on the ASAT (a) and ALAT (b) in the liver of rats.

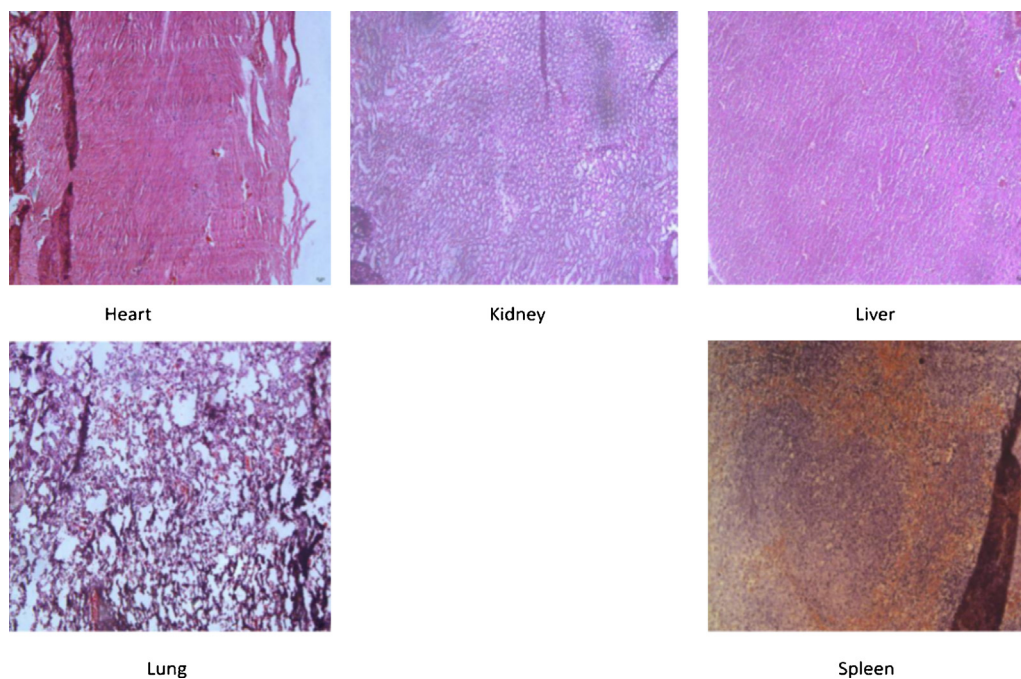
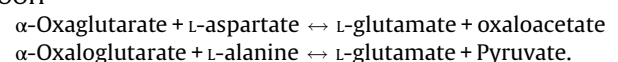
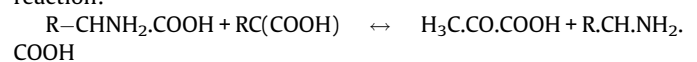


Fig. 4. H&E stained images of the liver, kidney, heart, lung, and spleen.

rodents [32]. Co-NPs induced greater cytotoxicity and genotoxicity in liver cells, cell membrane damage, oxidative stress, immune inflammation, and DNA damage, which may play an important role in the effects of Co-NPs on the liver cells [33]. It is proven that Co-NPs induce time and concentration-dependent cytotoxicity. While, it was unknown that whether the  $\text{Co}^{2+}$  ions release from the prostheses or by corrosion of wear particles, the mechanism of Co cytotoxicity is encompassed in that oxidative pressure assumes a vital role [34–36]. A recent study demonstrated that NPs can cross cytomembranes with no evident damage to cell integrity [37]. Young-min Kwon et al. reported the potential toxicity of Co-NPs in macrophages, although  $\text{Co}^{2+}$  in the culture medium resulting from the corrosion of Co-NPs cannot have significant effects [38]. The latest study suggested that the cytotoxicity of Co-NPs was probably mediated by Co-NPs rather than metal ions dissolved by nanoparticles in the extracellular culture medium. Although the toxicity of  $\text{Co}^{2+}$  and Co-NPs has been confirmed, the specific mechanism has not been explained in the study [39]. This study by Akhtar et al. shows that PEG-coated  $\text{CoFe}_2\text{O}_4$  synthesized by hydrothermal technology is an excellent model of

drug carrier and can use curcumin, which is a natural chemical and has no side effects and no other diseases reported [40]. The accumulation of Co in the liver causes alterations of hepatic function [41]. Degeneration, inflammation, and necrosis prevented by hepatocyte damage can increase the permeability of the cell membrane. Subsequently, ASAT and ALAT as clinical biomarkers are released into the body through the cell membrane and hence their concentration in the blood increases. ASAT and ALAT are indicators of liver damage [42]. Recent findings *in vitro* studies suggest that Co-NPs can penetrate cell membranes and other biological dams [43]. The ASAT and ALAT catalyze the following reaction:



The oxaloacetate in the presence of aniline citrate is converted into pyruvate, in both the cases of pyruvate reacts with 2,4-DNPH and form a red-colored complex dinitrophenylhydrazine which was be measured on a spectrophotometer.



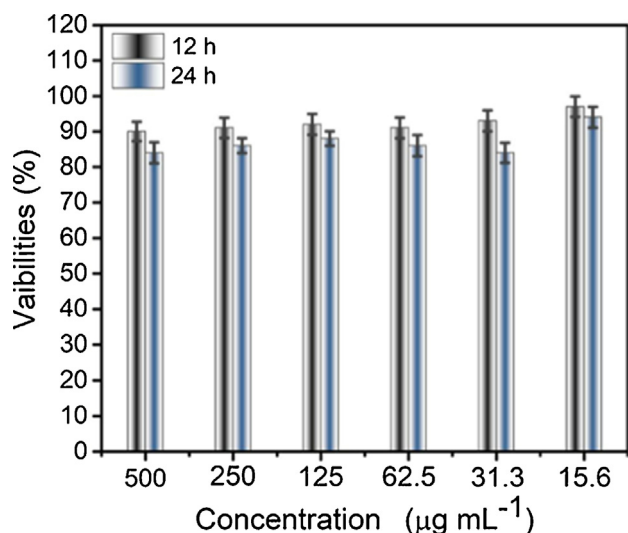


Fig. 5. Cell viability at different concentrations of Co-NPs.

In this study, the activities of ASAT and ALAT enzymes increased significantly their activation in the serum, liver, and kidney, when compared with the control. Thus, these results were further compared with the results that show consistency with the previous studies. The current study shows the significance of Co-NPs to increase the activity of ALAT and ASAT enzymes in the serum of exposed tissues in a concentration-dependent manner suggesting possible injuries to the liver tissue. ALAT enzyme is one of the main groups of the family of phosphatases [44]. The dysfunction of the heart and liver is associated with the elevation of ALAT and ASAT in the serum [45,46].

The present study shows the results of the liver, kidney, and serum of exposed tissues with 0.5, 1.0, 2.5, 5.0, and 10.0 mM concentrations of Co-NPs. Moreover, the data reveals that Co-NPs induced a concentration-dependent increase. A significant concentration-dependent of Co-NPs increase in pro-inflammatory was also found in the previous study [47]. Several reports are available on light microscopy studies of rat liver subjected to Co-NPs [48]. Co-NPs were at concentration-dependent order to activate both ASAT and ALAT activities in serum, liver, and kidney. These findings suggest that Co-NPs could induce concentration-dependent and the maximum increase of 106 % was observed at 10.0 mM concentration of Co-NPs for ALAT in the liver. The relative toxicity based on  $IC_{50}$  showed that liver ASAT was more potent in causing the toxicity followed by serum ASAT, kidney ASAT, kidney ALAT, serum ALAT, and liver ALAT (Table S7). The probable mechanisms of toxicity could be Co-NPs induce adverse biological effects through the activation of the immune response.

## 5. Conclusion

The biochemical enzymes of ASAT and ALAT increased significantly in serum, liver and kidney tissues exposed to Co-NPs. The elevated levels of ASAT and ALAT enzymes were reported in serum, kidney, and liver with toxic chemicals. These enzymes increased in serum and simultaneously increased in tissues like liver and kidney due to increased permeability of the plasma membrane. This is also suggestive of increased synthesis of these enzymes as an adaptive mechanism due to the chemical stress. The relative toxicity based on  $IC_{50}$  showed liver ASAT was more potent in causing toxicity than serum ASAT, kidney ASAT, kidney ALAT, serum ALAT, and liver ALAT. These results suggest that the *in vitro* exposure of Co-NPs caused alteration in the serum and in cellular

activities of vital organs like liver and kidney, inducing the changes in the physiological and metabolic activities of the individual.

## CRedit authorship contribution statement

**Akhtar Rasool:** Conceptualization, Methodology, Investigation, Validation, Writing - original draft. **Muhammad Zulfajri:** Visualization, Validation, Writing - review & editing. **Arif Gulzar:** Validation, Data curation. **Marlia Mohd Hanafiah:** Software, Writing - review & editing. **Syeda Azeem Unnisa:** Formal analysis, Project administration. **Mohammed Mahboob:** Supervision, Methodology, Validation.

## Declaration of Competing Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that there are no conflicts 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.btre.2020.e00453>.

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