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

Akhtar Rasool\textsuperscript{a,b,*}, Muhammad Zulfajr\textsuperscript{c}, Arif Gulzar\textsuperscript{d}, Marlia Mohd Hanafiah\textsuperscript{e,f}, Syeda Azeeem Unnisa\textsuperscript{b}, Mohammed Mahboob\textsuperscript{b}

\textsuperscript{a}Toxicology Unit, Applied Biology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, 500007, Telangana India
\textsuperscript{b}Department of Environmental Sciences, UCS, Osmania University, Hyderabad, 500007, Telangana, India
\textsuperscript{c}Department of Chemistry Education, Universitas Serambi Mekkith, Banda Aceh 23245, Aceh, Indonesia
\textsuperscript{d}Key Laboratory of Material Science and Chemical Engineering, Harbin Engineering University, Heilongjiang 150001, Harbin, China
\textsuperscript{e}Department of Earth Sciences and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, UKM Bangi 43600, Selangor, Malaysia
\textsuperscript{f}Centre for Tropical Climate Change System, Institute of Climate Change, Universiti Kebangsaan Malaysia, UKM Bangi 43600, Selangor, Malaysia

A R T I C L E   I N F O
Article history:
Received 28 September 2019
Received in revised form 23 March 2020
Accepted 6 April 2020

Keywords:
Biochemical
Enzyme
ASAT
ALT
Toxicity
Cobalt nanoparticles
Wistar rats
serum
Liver
Kidney

A B S T R A C T
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.

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 show 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\textsubscript{12} 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\textsuperscript{-}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].

* Corresponding author at: Toxicology Unit, Applied Biology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, 500007, Telangana India.
E-mail address: akhtarrasool01@gmail.com (A. Rasool).
https://doi.org/10.1016/j.btre.2020.e00453
2215-017X/© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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 mainly 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 predominant 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 % Na2CO3, 0.4 % NaOH, 0.16 % Na/K tartrate, and 1 % SDS. Reagent B: 4.0 % CuSO4 (copper reagent). Reagent C: 100 parts of reagent A with 1 part of reagent B. 2 N folins 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:

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

\[
\text{Tissue} = \frac{x}{mg\text{protein, }} \frac{1}{88} \times \frac{1}{88} = \mu\text{mol/mL}/h
\]

Where X stands for pyruvic acid formed in μg stands for volume of serum in ml and stand for molecular weight of pyruvic acid (for converting μg into μmoles)

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 IC50 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 IC50 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 IC50 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 IC50 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).
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 IC₅₀ was 0.27 ± 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 IC₅₀ was observed at 3.22 ± 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 Co²⁺ ions are known to be a health hazard to
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 Co²⁺ 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 Co²⁺ 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 Co²⁺ 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 CoFe2O4 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:

\[
\text{R–CHNH}_2\text{COOH} + \text{RC(COOH) } \rightarrow \text{H}_3\text{C.COOH} + \text{R.CH.NH}_2\text{COOH}
\]

\[
\alpha\text{-Oxoglutarate} + \text{l-aspartate} \rightarrow \text{l-glutamate} + \text{oxaloacetate}
\]

\[
\alpha\text{-Oxoglutarate} + \text{l-alanine} \rightarrow \text{l-glutamate} + \text{Pyruvate}.
\]

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.
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 major 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 IC₅₀ 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 IC₅₀ 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

Akhter Rasool: Conceptualization, Methodology, Investigation, Validation, Writing - original draft. Muhammad Zulfajiri: 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.

Acknowledgments

The authors thank the CSIR-Indian Institute of Chemical Technology, Hyderabad, India, for providing the lab facilities to conduct this work.

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.

References

[1] L. Biao, G. Jian-guo, W. Qi, Z. Qing-jie, Preparation of nanometer cobalt particles by polyol reduction process and mechanism research, Mater. Trans. 46 (2005) 1665–1667.
[2] Y. Ha, S. Ko, I. Kim, Y. Huang, K. Mohanty, C. Huh, J.A. Maynard, Recent advances incorporating superparamagnetic nanoparticles into immunocass, ACS Appl. Nano Mater. 1 (2018) 512–521.
[3] M. Faraji, Y. Yamini, M. Rezaee, Magnetic nanoparticles: synthesis, stabilization, functionalization, characterization, and applications, J. Iran. Chem. Soc. 7 (2010) 1–37.
[4] P. Tartaj, MdE P. Morales, S. Veintemillas-Verdaguer, T. Gonzalez-Carrero, C.J. Serna, The preparation of magnetic nanoparticles for applications in biomedical, J. Phys. D Appl. Phys. 36 (2003) 182–197.
[5] K. Czarneck, S. Terpilowski, A.K. Siwicki, Selected aspects of the action of cobalt ions in the human body, Cent. J. Immunol. 40 (2015) 236–242.
[6] H.B. Rubins, S.J. Robbins, D. Collins, C.L. Pye, J.W. Anderson, M.B. Elam, F.H. Faas, E. Linares, E.J. Schaefer, G. Schectman, T.J. Wilt, J. Wittes, Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol, N. Engl. J. Med. 341 (1999) 410–418.
[7] X.J. Huang, Y.K. Choi, H.S. Im, O. Yarimaga, E. Yoon, H.S. Kim, Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques, Sensors 6 (2006) 756–782.
[8] C.S. Lieber, D.G. Weiss, R. Groszmann II, Veterans affairs cooperative study of polyenylphosphatidylcholine in alcoholic liver disease, Alcohol. Clin. Environ. Res. 27 (2003) 1765–1772.
[9] C.S. Lieber, Alcoholic liver disease: new insights in pathogenesis lead to new treatments, J. Hepatol. 32 (2000) 113–128.
[10] M. Mowe, T. Behmer, Increased levels of alcohol markers (γGT, MCV, ASAT, ALAT) in older patients are not related to high alcohol intake, J. Am. Geriatrics Soc. 44 (1996) 1136–1137.
[11] I.A. Liappas, C. Nicolau, S. Chatzipanagiotou, E.O. Travellas, C. Piperi, C. Papageorgiou, F. Roufoudou, P. Bajos, C.R. Soldatos, Vitamin B12 and hepatic enzyme serum levels correlate with interleukin-6 in alcohol-dependent individuals without liver disease, Clin. Biochem. 40 (2007) 781–786.
[12] S. Gowda, P.B. Desai, V.V. Hull, A.K. Math, S.N. Vernekar, S.K. Kulkarni, A review on laboratory liver function tests, Pan Afr. Med. J. 3 (2009) 1–11. extrnl.nih.gov/ articlerender.fcgi?artid=PMC2984286.
[13] E.G. Giannini, R. Testa, V. Savarino, Liver enzyme alteration: a guide for clinicians, Can. Med. Assoc. J. 172 (2005) 367–379.
[14] P. Hall, J. Cash, What is the real function of the liver “function” tests? Ulster Med. J. 81 (2012) 30–36.
[15] L. Horev-Azaria, C.J. Kirkpatrick, R. Korenstein, P.N. Marche, O. Maimon, J. Ponti, R. Romano, F. Rossi, U. Golla-Schindler, D. Sommer, C. Uboldi, R.E. Unger, C. Villiers, Predictive toxicology of cobalt nanoparticles and ions: comparative
in vitro study of different cellular models using methods of knowledge discovery from data, Toxicol. Sci. 122 (2011) 489–501.

[16] C. Monteleone, L. Tran, W. MacNee, S. Faux, A. Jones, B. Miller, K. Donaldson, The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area, Occup. Environ. Med. 64 (2007) 609–615.

[17] I. Papageorgiou, C. Brown, R. Schin, S. Singh, R. Newson, S. Davis, J. Fischer, E. Ingham, C.P. Case, The effect of nano- and micro-sized particles of cobalt-chromium alloy on human fibroblasts in vitro, Biomaterials. 28 (2007) 2946–2958.

[18] K. Peters, R.E. Unger, C.J. Kirkpatrick, A.M. Gatti, E. Monari, Effects of nano-scaled particles on endothelial cell function in vitro Studies on viability, proliferation and inflammation, J. Mater. Sci. Mater. Med. 15 (2004) 321–325.

[19] E. Papis, R. Gornati, M. Prati, J. Ponti, E. Sabbioni, G. Bernardini, Gene expression in nanotoxicology research: analysis by differential display in BALB3T3 fibroblasts exposed to cobalt particles and ions, Toxicol. Lett. 170 (2007) 185–192.

[20] R. Colognato, A. Bonelli, J. Ponti, M. Farina, E. Bergamaschi, E. Sabbioni, L. Migliore, Comparative genotoxicity of cobalt nanoparticles and ions on human peripheral leukocytes in vitro, Mutagenesis. 23 (2008) 377–382.

[21] D.M. Goldberg, M.A. Remtulla, V. Lustig, The Diagnostic accuracy of three recommended methods for serum aspartate aminotransferase assays in patients suspected of myocardial infarction and hepatobiliary diseases, Clin. Biochem. 21 (1988) 323–328.

[22] F.J. Gella, T. Oriella, M.C. Pastor, J. Arenas, R. Moreno, R. Durban, J.A. Gomez, A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate, Clin. Chim. Acta 153 (1985) 241–247.

[23] V. Lustig, A. Papanastasiou-Diamandis, D.M. Goldberg, Evaluation of commercially formulated aspartate aminotransferase and alanine aminotransferase activity determinations by the scandinavian committee on enzymes and IFCC methods as modified for use with automated enzyme analysers, Clin. Biochem. 21 (1988) 283–290.

[24] S.H. Vagvala, S.D. O’Connor, Imaging of abnormal liver function tests, Clin. Liver Dis. 11 (2008) 128–134.

[25] R.M. Green, S. Flamm, AGA technical review on the evaluation of liver chemistry tests, Gastroenterology. 123 (2002) 1367–1384.

[26] K. Jung, K.D. Grützmann, Comparative determinations of aminotransferase activities in serum with so-called “optimized” methods, Clin. Chim. Acta 81 (1977) 299–304.

[27] Y. Bacq, O. Zarka, J.F. Bréchot, N. Mariotte, S. Vol, J. Tichet, J. Weill, Liver function tests in normal pregnancy: a prospective study of 103 pregnant women and 103 matched controls, Hepatology 23 (1996) 1030–1034.

[28] P.S. Kamath, Clinical approach to the patient with abnormal liver test results, Mayo Clin. Proc. 71 (1996) 1089–1095.

[29] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[30] H. Yatzidis, New method for direct determination of “True” creatinine, Clin. Chem. 20 (1974) 1131–1134.

[31] D.H. Adams, C. Ju, S.K. Ramaiyal, J. Uetrecht, H. Jaeschke, Mechanisms of immune-mediated liver injury, Toxicol. Sci. 115 (2010) 307–321.

[32] M. De Boeck, M. Kirsch-volders, D. Lison, Cobalt and antimony: genotoxicity and carcinogenicity, Mutat. Res. 533 (2003) 135–152.

[33] Y.K. Liu, X.X. Deng, H.L. Yang, Cytoxicity and genotoxicity in liver cells induced by cobalt nanoparticles and ions, Bone Jt. Res. 5 (2016) 461–469.

[34] C. Machado, A. Appelbe, R. Wood, Arthroprosthetic coxal bunion and coxal bunionopathy, Hear. Lung Circ. 21 (2012) 759–760.

[35] M. Oldenburg, R. Wegner, X. Baur, Severe cobalt intoxication due to prosthesis wear in repeated total hip arthroplasty, J. Arthroplasty 24 (2009) 825 e15–825 e20.

[36] D. Pelcova, M. Sklenkys, P. Janicek, K. Lach, Severe cobalt intoxication following hip replacement revision: clinical features and outcome, Clin. Toxicol. 50 (2012) 262–265.

[37] E. Bossi, et al., Cobalt oxide nanoparticles can enter inside the cells by crossing plasma membranes, Sci. Rep. 6 (2016) 22254, doi: http://dx.doi.org/10.1038/ srep22254.

[38] Y.M. Kwon, Z. Xia, S. Glyn-Jones, et al., Dose-dependent cytotoxicity of clinically relevant cobalt nanoparticles and ions on macrophages in vitro, Biomater. 4 (2009)025018.

[39] Ya-ke Liu, et al., Toxicity and bioactivity of cobalt nanoparticles on the monocytes, Orthop. Surg. 7.2 (2015) 168–173, doi:http://dx.doi.org/10.1111/ os.12180.

[40] Shahnaz Akhtar, et al., Toxicity of PEG-Coated CoFe 2 O 4 nanoparticles with treatment effect of curcumin, Nanoscale Res. Lett. 13.1 (2018) 52, doi:http://dx. doi.org/10.1186/s11671-018-2468-7.

[41] J.G. Nikolov, N. Joki, S. Vicca, N. Patey, D. Auchère, J. Benchitrit, J.P. Finois, M. Ziol, P. Beaune, T.B. Drieuè, B. Lacour, Tissue accumulation of lanthanium as compared to aluminum in rats with chronic renal failure: Possible harmful effects after long-term exposure, Nephron - Exp. Nephrol. 115 (2010) e112–e121.

[42] S.A. Kumar, P. Madhusudhanacharya, A.K. Patilolla, P.B. Choumouw, Hepatotoxicity and ultra structural changes in wistar rats treated with Al2O3 nanoparticles, Trends Cells Mol. Biol. 11 (2016) 77–88.

[43] A.M. Schnard, M.F. Rahman, S.M. Hussain, J.J. Schlager, D.A. Smith, A.F. Syed, Metal-based nanoparticles and their toxicity assessment, WIREs Nanomedicine and Nanobiotechnology. 2 (2010) 544–568.

[44] S. Harrison, C.P. Page, D. Spina, Airway nerves and protein phosphatases, Gen. Pharmacol. 32 (1999) 287–298.

[45] S.S. Jakobsen, C. Danscher, M. Stoltenberg, A. Larsen, J.M. Bruun, T. Mygind, K. Kemp, K. Soballe, Cobalt-chromium-molybdenum alloy causes metal accumulation and metallotheonin up-regulation in rat liver and kidney, Basic Clin. Pharmacol. Toxicol. 101 (2007) 441–446, doi:http://dx.doi.org/10.1111/j.1742-7843.2007.0137.x.

[46] C.P. Day, Genes or environment to determine alcoholic liver disease and non-alcoholic fatty liver disease, Liver Int. 26 (2006) 1021–1028.

[47] S. David, C. Passirani, N. Carmoy, M. Morille, M. Mevel, B. Chatin, J.P. Benoist, T. Montier, B. Pitard, DNA nanocarriers for systemic administration: characterization and in vivo bioimaging in healthy mice, Mol. Ther. - Nucleic Acids. 2 (2013) e44.

[48] W. Ding, S. Manley, H. Ni, The emerging role of autophagy in alcoholic liver disease, Exp. Biol. Med. 236 (2011) 546–556.