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Protective effect of zinc acetate and Zinc - *Aristolochia longa* Extract Nanoparticles against nickel induced acute liver and kidney injury in rats

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ABSTRACT

The objective of this work was to study the protective effect of zinc acetate and Zinc Nanoparticles biosynthesized by *Aristolochia longa* extract against variations in biochemical parameters and oxidative stress in rats exposed to nickel. Twenty female Wistar rats divided into four groups (n=5); Control, Nickel, Nickel + zinc, Nickel + ZnNPs. Nickel (20mg / kg feed), Zinc (231 mg/kg feed) as ZnSO4 7H2O and ZnNPs (100mg/kg feed) were added to the feed of the animals in groups for 20 days. Results show. In this study, there was also a significant rise in relative liver and kidney weight ($p<0.05$)., blood glucose, lipid profile, urea, creatinine, uric acid concentration transaminases and a considerable decrease ($p<0.05$) in body weight gain in rats contaminated with nickel compared to control. The results also revealed an increase in liver Ni, tissue MDA and a decrease in liver zinc, tissues GSH and SOD levels. The addition of zinc and zinc nanoparticles to the diet of rats exposed to nickel partially corrected previous parameters. The experiment demonstrates the great effectiveness of zinc, especially in the nano- form bio-synthesized by *Aristolochia longa* extract, in reducing the toxic effects of nickel on the liver and kidneys of rats.

Keywords : Nickel, Zinc, ZnNPs, , Oxidative stress, Wistar rates.

Introduction

Many harmful heavy metals have been exposed to man and his surroundings as a result of the rapid development of technological sciences, industries (chemical and metallic), medicine and agriculture [1]. As the main problem with these toxic elements is their presence as polluting elements for water or human food, which characterizes the most prevalent cases in developing countries [2]. The nature of their toxicity can vary depending on the form in which they enter the body (metal itself, vapor, inorganic or organic derivatives, water-or fat-soluble, etc [3]. Nickel comes in different chemical forms, but it only effectively enters cells in the bivalent cationic form (Ni2) or in the form of nickel chloride or sulfate [4]. The nickel ion can cause direct damage to all cellular components: peroxidation of lipids, proteins and nucleic acids, due to attacks by free radicals generated by nickel [5]. Numerous studies indicate a massive production of oxidizing species and the inhibition of the activities of the main antioxidant enzymes due to the cytotoxicity of nickel in a cell can favor an excessive cell death or a tumor evolution [6]. Zinc is part of a biotherapy system characterized by its high antioxidant power which plays a role in reducing the production of ROS [7]. The progress made in time is evident from the development in technology that has revealed the ability of minerals to perform specific functions better than the shape of metals [8]. The use of naturally occurring reagents such as plant extracts and microbes as reductant and capping agents in biosynthesis to create nanoparticles could be deemed appealing for nanotechnology [9]. Because of its unique catalytic and antibacterial capabilities, as well as its inexpensive cost and wide range of uses, zinc oxide nanoparticle is considered one of the most promising and revolutionary materials [10]. The biological methods for synthesis of ZnO NPs by using plants and their extracts have been suggested as the effective eco-friendly methods of nanoparticles synthesis. [11]. Plant materials, in compared to microbial and chemical approaches, are unquestionably more benign for the manufacture of zinc oxide nanoparticles since they pose

no threat of bacterial and dangerous chemical contamination and need less energy [12]. The aim of present study is to evaluate the toxic effects of nickel in rats and to study the effectiveness of therapeutic systems based on zinc acetate and a new form of zinc; nanoparticle zinc (ZnNPs) biologically synthesized from Aristolochia longa on biochemical blood parameters alteration as well as the evaluation of parameters of oxidative stress in liver and kidney.

Materials and methods

Animals and experiment design

Our study carried out on twenty female Wistar type rats, from the Pasteur Institute of Algiers, eight weeks old with a weight of 184.84 ± 8.48. The animals are bred at the pet store in the Faculty of Nature and Life Sciences at Echahid University HammaLakhdar-El-Oued. They submit to a period of adaptation to the conditions of the animal house for about a month at a temperature of 18 ±0.18°C. The rats are housed in plastic cages and fed a standard diet. The experiment was conducted over a period of 20 days. After a period of adaptation, the animals, at the age of 08 weeks, were divided into four experimental groups of 5 animals each:

Groups 1 (T): Healthy rats (control).

Groups 2 (Ni): Rats exposed to nickel (20 mg / kg weight of rat) for 20 days

Groups 03 (Ni + Zn): Rats exposed to nickel and treated with zinc (231 mg / kg feed) For 20 days

Groups 04 (Ni +Zno): Rats exposed to nickel and treated with zinc oxide nanoparticles (100mg / kg feed).

The 20mg / kg doses for nickel according to the Tebani study (2013) [13] and the zinc dose according to Derouiche (2016) [14].

Preparation of serum and tissue samples

The rats are anesthetized with chloroform (94%) after 16h of fasting and are sacrificed (part decapitation). the blood sample is taken at the time of the sacrifice of the rats collected in dry tubes previously labeled and numbered for each rat then separated by centrifuge at 3000 rpm for 15 minutes, the serum obtained is separated by centrifuge at 3000 rpm for 15 minutes, the serum obtained is kept in a freezer until the time of biochemical analyzes.

Biochemical parameters analyses

Serum glucose, urea, uric acid, creatinine, serum protein and lipid levels in serum were determined using the commercial kit from Spinreact, Spain (ref: glucose-20121, urea-20141, uric acid-20091, creatinine-20151, total protein-1001291 and cholesterol-20111, triglyceride-20131,). And for enzymes TGO and TGP are also measured by the use of commercial kits (Spinreat, ref: GOT-20042, GPT-20046).

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Tissues nickel and zinc analyses

The dry calcination of the organs (1g) is carried out in a muffle furnace increasing the temperature in successive stages up to 450 ° C for 4h in order to avoid sudden ignitions of the sample and which would cause losses The ash obtained is dissolved by an attack of 03 ml of pure nitric acid (HNO3) by heating slightly on a hot plate. The liquid obtained is filtered on filter paper in a 15 ml flask and made up to its final volume with demineralized water. For zinc and nickel using an atomic absorption spectrophotometer (Shimadzu AA-6200; Somerset, New Jersey, USA).

Oxidative stress markers analyses

The concentration of malondialdehyde (MDA) was determined using the method published by Yagi et al. [15]. Weckbecker's method [16] was used to determine the concentration of reduced Glutathione (GSH), while Beauchamp's method [17] was used to determine superoxide.

Statistical analysis

The results obtained are expressed as the mean ± mean deviation. The analysis of the data was carried out by application of the Student's T test, which is based on the comparison between two means, using MINITAB-13 software.

Results

Initial body weight, body weight gain and relative liver weight

Results showed that Nickel treatment at a dose (20mg/kg b.w) caused a decrease ($p<0.05$) in body weight gain and an increase in kidney and liver Relative weight in the rats compared to the control rats. Whereas the animals that received the zinc acetate and Zinc nanoparticles showed a correction of this change (table 1).

Table 1. Body weight and relative kidney and liver weight in control and experimental groups.

Parameter	Control (n=5)	Nickel (n=5)	Nickel + Zn (n=5)	Nickel +ZnO (n=5)
Initial Body weight (g)	194.80±6.26	167.8±16.4	192.60±7.12	193.40±4.62
Weight gains (g / j / rat)	1.05±0.0845	0.03±0.30***	1.27±0.890*c	1.01±0.001c

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Relative Liver weight(g)	2,56±0,166	2,92±0,260**	2,702±0,148[a]	2,60±0,108[a]
Relative kidney weight(g)	0,59± 0,0136	0,70±0,0581*	0,702±0,148*	,47±0,105*[b]

* p<0.05, ** p<0.01: significantly different from control group. a p<0.05, b p<0.01: significantly different from Ni group. Values are mean ± SEM, n=number of observations.

Nickel and Zinc concentrations

Figure (1) shows a significant increase (p <0.05) in liver nickel concentration and a significant decrease in the liver zinc level in nickel group compared to the control rats. On the other hand, we notice a highly significant decrease in nickel and increase in zinc levels in groups treated with ZnO and acetate Zn in comparison with the Ni group.

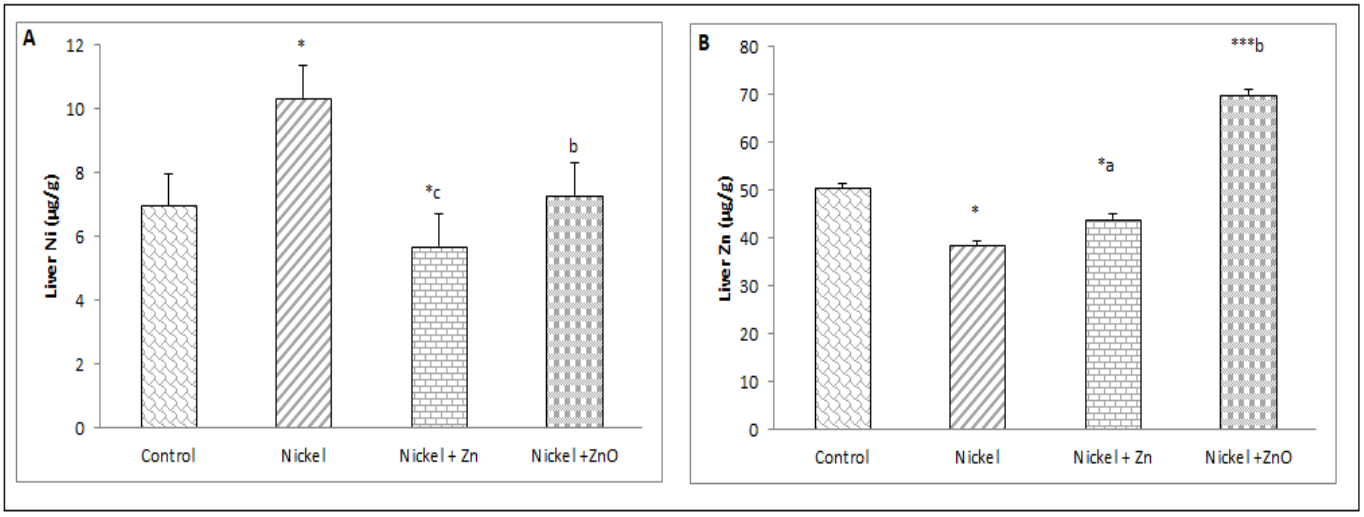

Figure 1. LiverNickel and Zinc concentrationsin control and experimental groups**.** Values are mean ± SEM, (n=5).* p<0.05, *** p<0.001: significantly different from control group.a p<0.05, b p<0.01,c b p<0.001: significantly different from Ni group.

Biochemical parameters

Our results show a significant increase in blood glucose (p <0.05), triglycerids (p <0.001), cholesterol (p <0.05) urea (p <0.01), uric acid (p <0.05) and Creatinine (p <0.05) and also a significant increase in transaminases acitities (p <0.001) in Ni contaminated group compared to the control. On the other hand, in the group treated with Zn andZnO we note a highly significant reduction in all previous parameters when we compared with the nickel group (table2).

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Table 2. Level of biochemical markers in control and experimental

Parameter	Control (n=5)	Nickel (n=5)	Nickel + Zn (n=5)	Nickel +ZnO (n=5)
Blood glucose (g/l)	1,11±0,107	1,47±0,0403*	1,03±0,113^{a}	1,29±0,199^{*a}
Serum Triglycerides (g/l)	0,48±0,04	0, 98± 0,05***	0,62±0,44^{***b}	0,71±0,42^{***b}
Serum total cholesterol (g/l)	2,19±0,172	2,41±0,176*	2,71±0,103^{b}	2,23±0,134^{a}
Serum urea (g/l)	0,48± 0,05	0,67±0,17**	0,47±0,05^{c}	0,48±0,123^{a}
Serum creatinine (mg/l)	7,42±0,91	7,91±0,69*	6,32±0,49^{**a}	13,29±0,76^{***c}
Serum uric acid (mg/l)	18,07±1,09	29,73±0,99*	19,23±2,23^{C}	17,79±1,23^{c}
Serum AST (U/l)	93.04±23,1	185,20±14,3***	175,40±15,4^{***a}	135±13,34^{***c}
Serum ALT (U/l)	38,61±8,13	54,07±6,63***	36,870±6,878^{c}	51,041±7,256^{***a}

Values are mean ± SEM, n=number of observations.* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group.
a p<0.05, b p<0.01,c b p<0.001: significantly different from Ni group.

Stress oxidative parameters

Our results illustrated in table (3) show a significant increase ($p < 0.05$) in lipid peroxidation levels and a significant decrease ($p < 0.05$) in GSH concentrations and SOD activity in liver and kidney of rats exposed to nickel group compared to the control. In contrast our results also show a significant decreased in MDA level and a significant increase in GSH concentration and SOD activity in Ni+ ZnO and Ni+Zn treated groups compared to Nickel group.

Table 3. oxidative stress markers in control and experimental groups

Parameter		Control (n=5)	Nickel (n=5)	Nickel + Zn (n=5)	Nickel +ZnO (n=5)
MDA (µmol/mg pro)	**Liver**	2,3810± 0, 04	2,54 ±0,33*	1,35 ±0,234***c	2,129±0,341^{**b}
	Kidney	1,79±0,0396	2,19±0,143*	1,85±0,159^{b}	2,12±0,09*
GSH(nmol/mg pro)	**Liver**	1,19±0,27	0,77±0,234*	0,83±0,154**	0,59±0,107^{**a}
	Kidney	0,10±0,01	0,047±0,003***	0,05±0,0143^{***c}	0,08±0,01**^{a}

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SOD(UI/mg pro)	**Liver**	7,25±0,13	3,13±0,645*	4,41±0,03*a	4,106±0,896***a
	Kidney	0,22925±0,005	0,175±0,028**	0,145±0,0342**	0,219±0,022a

* p<0.05, ** p<0.01, *** p<0.001: significantly different from control group. a p<0.05, b p<0.01,c b p<0.001: significantly different from Ni group. Values are mean ± SEM, n=number of observations.

Discussion

Through this study, we investigated the potential benefit of treatment with Zinc acetate and Zinc nanoparticules in reversing Ni- induced liver and Kidney oxidative stress in rats orally exposed to Ni. In Our study we observed that Nickel administration at a dose (20mg / Kg) induced a reduction in body weight gain of the rats. The rat's body weight loss is utilized as a measure of the rat's general health decline.. Whereas, treatment of rats with, ZnO and Zn resulted in improvement in body weights gain compared to rats exposed to nickel. Zinc could be attributed to its ability to reduce the accumulation of nickel in organs [18]. The successful mechanisms of zinc supplementation on weight loss could be related to zinc's role in appetite regulation via changes in hypothalamic neurotransmitter metabolism of the leptin system and its receptors; in other words, zinc can cause leptin production [19]. In addition, zinc may enhance levels of growth factor-1 (IGF-1); in particular, it may contribute to elevating serum testosterone. IGF-1 and testosterone are both anabolic substances that might help to acquire weight of rats. [20]. Regarding the relative weights of the organs, we notice nephromgaly and hepatic hyertrophy this is explained by the intense accumulation of this metal in these target organs [21]. This increase in relative organ weight may be due to nickel-induced necrosis [22]. However, the Zinc acetate and nanoparticule Zinc reverted partially this change. Our study is consistent with the study by Tizhe et al., 2020 [23], which showed a similar protective effect of zinc by improving the relative weights of animals. The therapeutic effect of nanoparticles of zinc oxide it can serve as a useful catalyst for the reduction or elimination of toxic chemicals [24]. Results show that zinc acetate and ZnO reduce the levels of liver nickel.The concentration of nickel in liver tissue has increased because the liver is the main target of environmental and occupational toxicity and the main site of detoxification [25].Regarding ZnO has a capacity for interaction with biological systems at the cellular level because the small size of nanomaterials promotes their penetration into the cell which give a protective effect to reduce the concentration of this nickel [26]. The results of the present study demonstrate that the level blood glucose, lipid profile, urea, uric acid and creatinine showed a significant increase in group exposed to nickel.The rats treatments with ZnO or Zn enhance these increases.Following zinc supplementation, several molecular processes are thought to be involved in the modulation of blood glucose and lipid profile levels. Zinc ions are known to activate protein tyrosine phosphatase 1B, a major regulator of the phosphorylation state of the insulin receptor. Zinc has been found in studies to improve peripheral insulin sensitivity by potentiating insulin, which stimulates glucose transport and lipid breakdown. [27]. ZnO-NPS is possesses an antioxidant capacity which prevents or slows down oxidation by neutralizing free radicals, thus improving the functioning power of the kidney to stop the disruption of the cell membrane [28]. The results demonstrated that the activities of liver TGO and TGP serum were increased after nickel poisoning. This is clearly explained by the hepatotoxic effect of nickel, which penetrates into cells and crosses the nuclear membrane [29]. The treatments of the rats with ZnO, Zn generated a decrease in the enzymatic activity of TGO and TGP compared to the rats exposed to nickel this

may be explained by the important role of ZnO which has a hepato-protective effect by trapping free radicals or by increased activity of antioxidants, which then detoxify free radicals through an antihepatotoxic effect [30]. Zinc treatment results in correction of the enzyme activity of plasma TGO and TGP, which could be attributed to the anti-free radical / anti-oxidant and chelating efficacy of the metals of this element and stabilization of the cell membrane [31]. The results of the present study demonstrate that the levels of endogenous antioxidants, both enzymatic and non-enzymatic (GSH and SOD) are significantly lowered SOD, GSH and increased MDA in the group treated with nickel compared to normal control rats. In our results we show the increase in renal MDA levels: This is explained by the adverse effects of the action of nickel in the body and the induction of the formation of reactive oxygen species (ROS) and increased lipid peroxidation in cells [32]. In our experimental study, the results show a significant decrease in the level of glutathione and SOD activity in the liver, kidney, in rats contaminated with nickel. The sulfhydryl group of glutathione's cysteine component has a strong affinity for metals, which explains this result. Otherwise, due to nickel's interaction with free radicals, GSH can be oxidized which can cause a number of deleterious effects due to the accumulation of superoxide radicals. Administration of nickel to rats in the present study may lead to the generation of a peroxy radical, $O2^{-}$, associated with inactivation of SOD [33]. Our results showed a restoration of the level of MDA and an improvement of antioxidant systems thanks to the effectiveness of therapeutic system (ZnO and Zn) against the oxidative deterioration induced by nickel. Zn is an excellent antioxidant which inhibits the synthesis of oxygen free radicals which are responsible for oxidative stress [34]. While NP-ZnO is able to protect cell membrane integrity against oxidative stress damage induces nickel to increase antioxidant enzyme levels [35]. NPs-ZnO by eliminating free radicals, or by enhancing the activity of antioxidants, which then detoxify free radicals.ZnO has specific characteristics such as size, distribution and morphology, also The surface / volume ratio of NPs is very high, which increases their reactivity compared to larger parts of the same compound and also a greater capacity of penetrate cells [36, 27].

Conclusion

The study clearly demonstrated the supplementation of zinc and zinc nanoparticles to rats exposed to nickel protect against decrease the zinc status, metabolic and physiological alterations, which play an important role antioxidant reducing the renal and hepatic injury.

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References

[1]Das, K.K.,&Dasgupta, S. (2002). Effect of nickel sulfate on testicular steroidogenesis in rats during protein restriction. *Environ. Health Perspect. 110(9)*: 923-6.

[2]Harber, L.T., Diamond, G.L., Zhao, Q.,Erdreich, L.,&Dourson, M.L. (2000). Hazard identification and dose response of ingested nickel-soluble salts.Regul. .Toxicol.Pharmacol. 31(2): 231-241.

[3]Audrey, F.,Dumax-Vorzet, M., Tate, R.W.,Rhod, H., Elder, A.,&Povey, C (2015). Cytotoxicity andgenotoxicity of urban particulate matter in mammalian cells. Mutagenesis 30(5): 621–633.

[4]Jan, A.T.,Azam, M.,Siddiqui, K., Ali, A., Choi, I.,&Haq, Q.M. (2015). Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants. Int J Mol Sci. Dec 10,16(12):29592-630.

[5]Lloyd, G.K. (1980). 'Dermal absorption and conjugation of nickel in relation to the Induction of allergic contact dermatitis Preliminary results'. Brown SS Sunderman FW Jr Nickel toxicology London UK Academic Press 145-148.

[6]Tiwari, B.K.,Pandey, K.B.,Abidi, A.B.,&Rizvi, S.I. (2013). Markers of Oxidative Stress during Diabetes Mellitus. *Journal of Biomarkers, 2013*: 1–8.

[7]Fernandez, E., Gustafson, A., Anderson, M., Hellman, B.,&Dencker L. (2003). Cadmium-Induced Changes in Apoptotic Gene Expression Levels and DNA Damage in Mouse Embryos Are Blocked by Zinc. Toxicological Sciences, 76(1): 162–170.

[8]Derouiche, S.,Guemari, I.Y.,&Boulaares I. (2020). Characterization and acute toxicity evaluation of the MgO Nanoparticles Synthesized from Aqueous Leaf Extract of Ocimumbasilicum L. Algerian Journal of Biosciences 1(1): 001–006.

[9] Atoussi, O.,Chetehouna, S.,&Derouiche, S. (2020). Biological properties and Acute Toxicity Study of Copper oxide nanoparticles prepared by aqueous leaves extract of Portulacaoleracea (L). Asian J Pharm Res. 10(2):89-94.

[10] Chetehouna, S.,Atoussi, O.,&Derouiche, S. (2020). Biological Activity and Toxicological Profile of Zinc Oxide Nanoparticles Synthesized by Portulacaoleracea (L) Leaves Extract. AdvNanomedNanotechnol Res, 2(2): 125-133.

[11] Naseer, M., Aslam, U., Khalid, B.,& Chen, B. (2020). Green route to synthesize Zinc Oxide Nanoparticles using leaf extracts of Cassia fistula and Meliaazadarach and their antibacterial potential. Sci Rep. 10(1):9055.

[12] Philip, D.,Unni, C.,Aromal, S.A.,&Vidhu, V.K. (2011). Spectrochim. Acta, Part A, 78: 899–904.

[13] Storeng, R.,&Jonsen, J. (1981). Nickel toxicity in early embryogenesis in mice. Toxicology. 20(1):45-51.

[14] Samir, D.,Kawther, A.,Manel, D.,Soumya, B.A.,&Kechrid, Z. (2013). The effects of copper supplement on zinc status, enzymes of zinc activities and antioxidant status in alloxan-induced diabetic rats fed on zinc over-dose diet. Int J NutrMetab, 5(5): 82-87.

[15] Yagi, K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. *Biochemical Medicine, 15(2):* 212–216.

[16] Weckbecker, G.,& Cory, J.G. (1988). Ribonucleotidereductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. *Cancer Letters, 40(3)*: 257–264.

[17] Beauchamp, C.,&Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry. Volume 44(1)*: 276-287.

[18] Pardeep, S.,Garg, M.L.,&Dhawan, D.K. (2005). Zinc Protects Rat Liver Histo-architecture from Detrimental Effects of Nickel. BioMetals,, 19(3): 301–313.

[19] Samir, D.,Manal, D.,&kaouther, A. (2017). Beneficial Effect of Zinc on diabetes induced kidney damage and liver stress oxidative in rats. J Adv Biol., 10(1): 2050-5055.

[20] Derouiche, S.,&Kechrid Z. (2013). Influence of calcium supplements on zinc status, carbohydrate metabolism and the liver activity of detoxifying glutathione enzymatic system in alloxan induced diabetic rats. J ExpBiolAgr Sci. 1(6): 424-429.

[21] Li, Q., Liu, H.,Alattar, M., Jiang, S., Han, J., Ma, Y.,& Jiang, C (2015).. The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM2.5 in rats. *Scientific Reports*, 5(1). doi:10.1038/srep16936

[22] Ibrahim, N.M.,Eweis, E.A., EI-beltagi, H.S.,& Abdel-Mobody , Y.E. (2011).The Effect of Lead Acetate Toxcicity on Experimental Male Abino Rat. *Biological Trace Element Research, 144*: 1120-1132.

[23] Tizhe, E.V., Ibrahim, N.D-G.,Fatihu, M.Y.,Ambali, S.F.,Igbokwe, I.O.,&Tizhe, U.D. (2020). Effect of zinc supplementation on chronic hepatorenal toxicity following oral exposure to glyphosate-based herbicide (Bushfire®) in rats. *Journal of International Medical Research*. doi:10.1177/0300060520925343

[24] Hassan, A.,AtefHowayda, M., El-Shafei.,& Mahmoud, H.H.(2013). Effect of zinc oxid nanoparticles on the growth of some mycotoxigenicmoulds j. studies in chemical process technology (SCPT), *American Society of Science and Engineering, 1(3)*: 16-25.

[25] Das, K.K., Das, S.N.,& Gupta, S. D. (2001). Influence of ascorbic acid against nickel-induced hepatic lipid peroxidation in rats. *Journal of Basic and Clinical Physiology and Pharmacology, 12(3*): 187-195.

[26] Sakai, N., Matsui, Y., Nakayama, A.,Tsuda A.,&Yoneda. M. (2011). Functional-dependent and size-dependent uptake of nanoparticles in PC 12. *Journal of Physics Conference Series 304(1)*: 1-10.

[27] Derouiche, S.,&kechrid, Z. (2016). Zinc Supplementation Overcomes Effects of Copper on Zinc Status, Carbohydrate Metabolism and Some Enzyme Activities in Diabetic and Nondiabetic Rats. *Canadian Journal of Diabetes*, 40(4): 342–347.

[28] Santhoshkumar, S.,Rajeshkumar V.,& Kumar, S. (2017). Phyto-assisted synthesis, characterization and applications of nanoparticles–A review. *Biochemistry and Biophysics Reports*, 11:46-57.

[29] Patierno, S.R, Sugiyama, M.,& Costa, M. (1987). Effect of nickel (II) on DNA-protein binding, thymidine incorporation, and sedimentation pattern of chromatin fractions from intact mammalian cells. *Journal of Biochemical Toxicology*, 2(1), 13–23. doi:10.1002/jbt.2570020103

[30] Hambidge, K.M.,& Krebs, N.F. (2007): Zinc deficiency: a special challenge. *The Journal of Nutrition*. 137(4): 1101 –1105.

[31] Djemli., S.,&Kechrid Z. (2013). Preventive effect of zinc on nickel-induced oxidative liver injury in rats. *African Journal of Biotechnology*, 12(51): 7112-7119.

[32] Pardeep, S.,Garg, M.L.,&Dhawan, D.K. (2004). Protective role of zinc in nickel induced hepatotoxicity in rats. *Chemico-Biological Interactions*, 150(2):199–209.

[33] Sidhu, P.,Garg, M.L.,&Dhawan, D.K. (2004). Protective role of zinc in nickel induced hepatotoxicity in rats. ChemBiol Interact, 150(2):199-209.

[34] Derouiche, S., Rezzag-mohcen, O.S., Serouti, A.(2018). Triazinone herbicide metribuzin induced acute liver injury: A study of animal model. *Journal of Acute Diseases*; 7(4): 152-157..

[35] Djouadi, A.,&Derouiche, S. (2021). Spinach mediated synthesis of zinc oxide nanoparticles: Characterization, In vitro biological activities study and in vivo acute toxicity evaluation. *Current Research in Green and Sustainable Chemistry*, 4: 100214.

[36] Nabeshi, H.T., Yoshikawa, K., Matsuyama, Y.,Nakazato, A.,Arimori, M.,Isobe, S., Tochigi, S.,Kondoh, T., Hirai, T.,Akase, T., Yamashita, K., Yamashita, T., Yoshida, K., Nagano, Y., Abe, Y., Yoshioka, H.,Kamada, T.,lmazawa, N.,Itoh, S.,Tsunoda, I.,&Tsutsumi, Y. (2010). Size-dependent cytotoxic effects of amorphous silica nanoparticles on Langerhans cells". *An International Journal of Pharmaceutical Sciences*, 65(3): 199-201.

[37] Sakai, N., Matsui, Y., Nakayama, A.,Tsuda, A.,&Yoneda. M. (2011). Functional-dependent and size-dependent uptake of nanoparticles in PC 12. *Journal of Physics Conference Series, 304(1)*: 1-10.