Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Therapeutic and protective effect of selenium nanoparticles against experimentally induced hepatotoxicity in rats

Afaf Desouky Abdel Magid; Hussien Abdelmaksoud Ali; and Marwa A. Ahmed*

Biochemistry Department, Faculty of Veterinary Medicine, Benha University, Egypt.

ARTICLE INFO

Keywords

Hepatotoxicity
Nano selenium
Cyclophosphamide
Antioxidant enzymes
Cytokines

Received 27/03/2021
Accepted 01/04/2021
Available On-Line 01/07/2021

ABSTRACT

Selenium nanoparticles have received wide attention because of their importance in nutrition compared to other forms of selenium used in food fortification. Within recent study, selenium nanoparticles (SeNPs) were prepared, characterized, and evaluated in albino rats to identify their hepatic protective effect against cyclophosphamide-induced hepatotoxicity. Fifty white albino rats were divided randomly into five groups. Group I was used as a negative control, group II was administrated daily dose of CP (5 mg/kg body weight/8 weeks) orally by gavage, group III were administrated SeNPs orally at a dose of (2 mg/kg body weight / 12 weeks) three times weekly. Group IV was administrated CP at a dose of (5 mg/kg body weight/8 weeks) daily orally then administrated SeNPs orally at a dose of (2 mg/kg body weight / 4 weeks) three times weekly. Group V (protected group) was administrated SeNPs orally at a dose of (2 mg/kg body weight/4 weeks) three times weekly, then administrated daily dose of CP (5 mg/kg body weight/8 weeks) orally with continuous administration of SeNPs till the end. Based on results obtained, CP administration in group II showed significant increase in serum liver enzymes activity (AST, ALT, and ALP), which was followed by a substantial significant decrease in serum albumin conc. and tissue antioxidants (GPx, SOD, and CAT) activity, with significant increase in cytokine levels (IL6, IL1β). Such biochemical changes were significantly improved by Nano-Se treatment, particularly in the protected rats. The current study found that Nano-Se reduces oxidative stress in liver tissue caused by CP administration.

1. INTRODUCTION

The liver is a crucial organ, and because of its strategic position, blood supply, and important function in metabolism, it is susceptible to drugs and chemicals to which we are constantly exposed (Gu and Manautou, 2012) Furthermore, the detoxification process in the liver eliminates the effects of xenobiotics with the aid of various microsomal enzymes. Hepatocytes have mitochondria more than other cells because they need more ATP to perform a variety of functions, resulting in higher ROS output within hepatocytes. As a result, hepatocytes are more vulnerable to oxidative stress-mediated toxic injuries. (Cichoz-Lach and Michalak, 2014, Dwivedi and Jena, 2018).

Cyclophosphamide (CP) is an immunosuppressant drug, and it is commonly used also to treat a number of cancers. (Fraiser et al., 1991, Khan and Jena, 2014, Patwa et al., 2020,) However, due to significant adverse effects and toxicities in major organs such as the bladder, reproductive system, and liver, CP's clinical use is restricted (Patwa et al., 2020). Hepatic microsomal P450 oxidases metabolize CP in the liver, producing two main metabolites: phosphoramide mustard and acrolein (Ramirez et al., 2019). To control the CP-related adverse toxic effects, several methods have been used recently, including the use of Mesna (antioxidant) or alternative CP analogues, as well as a low dose of CP combined with another anticancer drug (Fisusi and Akala, 2019). These techniques, however, are insufficient and unsuitable for a wide variety of applications (Basu et al., 2014). As a result, an efficient and sufficient chemoprotective agent is urgently needed to minimize the toxic effects of CP while also increasing its therapeutic uses.

Many biological processes, including enzymatic reactions, require selenium as a trace element. Due to its protective potential against the reactive oxygen species (RSO), Selenium plays an important function in lowering chronic disease risks such as disorder of cancer neurodegenerative and hepatotoxicity (Teodor et al., 2011, Khan et al., 2012). At present, Se nanoparticles (SeNPs) are of great importance in medicine field because of their promising characteristics and excellent bioactivities, wherever they showed substantial impact as antitumor, toxic-free and biocompatible operators in comparison to the other selenium forms such as selenite (SeO3-2) and selenate (SeO4-2) compounds (Menon et al., 2018).

* Corresponding author: dr.marwa_lab@yahoo.com

Therefore, the present research was designed to understand the ameliorative characteristics of SeNPs on hepatotoxicity triggered experimentally in albino rats.

2. MATERIAL AND METHODS

2.1. Experimental Animals

The experimental design of this study used fifty white male albino rats that were (10-12 weeks old) and weighed (140–160 g). Rats were collected from the laboratory animal's research center, Moshtohor, Benha University's faculty of veterinary. Rats were housed in standard light and temperature conditions and given free access to a standard pellet diet containing 21% protein, as well as tap water. Prior to the start of the experiment, the rats were given ten days to adjust.

2.2. Chemicals

Chemicals of analytical grade were obtained from trusted commercial suppliers. In the recent work chemicals used were:

2.2.1. Cyclophosphamide

Commercially available CP tablets (Endoxan® 50mg, Baxter Oncology GmbH).

Preparation of Cyclophosphamide (CP): The accurate doses of the drugs were dissolved in saline solution daily and shortly before administration at a dose of (5 mg/kg body weight) daily.

2.2.2. Sodium selenite from Sigma-aldrich, Egypt

Synthesis of nano-selenium

One ml of 25 mM sodium selenite (Sigma-Aldrich, Egypt) was combined with four ml of 25 mM glutathione containing either two mg or twenty mg of bovine serum albumin. To produce red elemental selenium and oxidized glutathione, the pH of the mixture was changed to 7.2 with 1.0 M sodium hydroxide. To isolate oxidized glutathione from selenium nanoparticles, the red solution was dialyzed for 96 hours at 4°C against double distilled water, which was replaced every 24 hours. The final solution, which included selenium nanoparticles and bovine serum albumin, was kept refrigerated at 4°C, in which, the selenium nanoparticle solution made with a low concentration of bovine serum albumin was stable for months, whereas the selenium nanoparticle solution made with a high concentration of bovine serum albumin was stable for years. (Abd-Allah and Hashem, 2015).

Preparation of nano-selenium

Nano-Se was synthesized and dissolved in saline (0.9 % NaCl) every day, just before being taken orally at a dose of (2mg/kg body weight) orally three times per week.

2.3. Experimental Design

Rats of the experiment randomly divided into five groups, 10 rats of each as follow:

2.3.1. Group (1): Negative control:

Rats were fed a normal diet without any medication for the duration of the work.

2.3.2 Group (2): Cyclophosphamide group "Positive group". Rats received CP orally at a dose of (5 mg/kg body weight) daily via gavage for 8 weeks (Gad El-Karim and El-Amrawi (2019).

2.3.3 Group (3): protective group" Nanoselenium group:"

Rats received SeNPs (2 mg/kg body weight) orally three times per week via gavage during entire experimental period (Bhattacharjee, et al., 2014).

2.3.4 Group (4): Treated group "Cyclophosphamide + Nanoselenium group" Firstly rats received CP orally at a dose of (5 mg/kg body weight/8 weeks) daily via gavage, followed by receiving SeNPs (2mg/kg body weight/ 4 weeks) orally three times per week via gavage.

2.3.4 Group (5): treated group "Nanoselenium group + Cyclophosphamide" Firstly rats received SeNPs 2mg/kg body weight/4 weeks) orally three times per week via gavage as protector then, followed by orally at a dose of (5 mg/kg body weight/8 weeks) daily via gavage, with continuous administration with SeNPs.

2.4. Sampling

Rats in each group were fasted overnight and then euthanized after 12 weeks. At the end of the experiment, blood samples and liver tissue specimens were taken from all rat groups.

2.4.1 Blood samples:

Blood sample were collected from retro-orbital plexus of eye. Letting blood to clot, then, it was centrifuged at 3,000 rpm for 15 minutes. Sera were aspirated by automated pipette in Eppendorf. and kept at -20 °C in a deep freezer until biochemical parameters were determined.

2.4.2. Tissue sample:

The liver was rapidly removed, washed with ice-cold salt, snapped in fluid nitrogen directly and held at -80°C .

One gramme of liver tissue was cut and minced into small parts, then homogenized with a glass homogenizer in 9 volumes of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates, then centrifuged for 15 minutes at 6000 r.p.m. at 4°C. The antioxidant activity of the supernatant was measured directly.

2.5 Analysis

2.5.1 Biochemical analysis:

Biochemical parameters were estimated to evaluate liver enzymes activity as following: alanine aminotransferase (ALT) according to Fischbach, et al., (1992), aspartate amino transferase (AST) according to Fischbach, et al., (1992) and Alkaline Phosphatase (ALP) according to Z.Klin, (1970) by using Human kits for diagnosis (Germany). Also, albumin level was determined according to Gendler (1984) by using diagnostic kit supplied by (Diamond, Egypt).

2.5.2 Antioxidant estimation:

Furthermore, the supernatant of hepatic tissue homogenate (10%) was used for estimation of catalase (CAT) activity according to Fossati.et al., (1980), Superoxide dismutase (SOD) activity according to Nishikimi et al., (1972) and Glutathione peroxidase (GPx) activity according to (Paglia and Valentine et al.,1967) by using Biodiagnostic kit (Cairo, Egypt).

2.5.3 Cytokines estimation:

IL6 was measured by using Rat IL-6 Immunoassay, qantikine Elisa kit, (USA), Rat IL-1 beta ELISA Kit, Ray Biotech (USA) according to (Hirano, T. 1998) and (Auron et al., 1984) respectively.

2.6. Statistical analysis

SPSS software (version 19) was used to evaluate all gathered data. Differences between group means was identified by using (ANOVA), one-way analysis of variance, followed by the least significant difference (LSD) test. The minimum level of significance P-values were considered to be < 0.05.

3. RESULTS

3.1 Biochemical results

Administration of Cyclophosphamide to normal rats exhibited a significant increase in serum ALT, AST and Alp activity after 2 months of administration period when compared with negative control group. This elevation was reduced in SeNPs treated group when compared with untreated hepatotoxic group. However, Serum Albumin showed significant decrease in CP administrated group when compared to negative control, this reduction improved significantly in protected and treated SeNPs groups, data shown in table (1).

3.2 Antioxidants results

The results presented in our study revealed that, CP administration resulted in significant decreased liver SOD, catalase and GPx activity when compared with the control rats. SeNPs administration as a treatment or as a protection lead to significantly increased liver SOD and Catalase and GPx activity when compared to positive control, data shown in table (2).

3.3 Cytokines results

Increased inflammation of liver in CP administered group was reported by increased levels of IL-1β and IL6 in comparison to negative control. However, the amount of these inflammatory parameters in protected and treated with SeNPs groups decreased considerably when compared to CP treated group, such data shown in table (2).

4. DISCUSSION

Nanotechnology, the design and manipulation of materials at the atomic scale, has the potential to deliver considerable benefits to society. The novel properties that emerge as materials reach the nanoscale open the door to innovations in energy, manufacturing, and medical treatment. At the same time, these novel properties may pose new risks to workers, consumers, the public, and the environment. The limited data now available demonstrate the potential for some nano-materials to be persistent and mobile in the environment and in living organisms; to cross multiple physiologic barriers (including lung-blood, blood-brain, and placental barriers, and cell membranes) (Abd-Allah and Hashem, 2015). Selenium is an essential dietary trace element, which has an antioxidant role in the protection of the cell from oxidative damage. The most important metabolic roles of selenium in mammalian cell are due to its function in the active site of many antioxidant enzymes, e.g., thioredoxin reductase, glutathione (GPx) and GR (Flora et al., 2002).

Animal Groups	ALT activity (U/L)	AST activity (U/L)	ALP activity (U/L)	Albumin Conc. (g/dl)
Control group	28.01 ± 1.33^{d}	32.54 ± 1.89^{d}	154.80 ± 6.77^{d}	3.69 ± 0.15^{a}
CP group	65.12 ± 1.93^{a}	70.51 ± 3.55^{a}	255.47 ± 7.32^{a}	1.87 ± 0.09^{e}
protective SeNPs	25.02 ± 1.52	29.23 ± 1.75^{d}	149.83 ± 5.34^{d}	3.44 ± 0.11^{bc}
Treated CP + SeNPs	44.04 ± 3.13^{b}	51.46 ± 3.18^{b}	182.15 ± 2.81^{b}	2.80 ± 0.06^{d}
Treated SeNPs + CP	35.03 ± 2.59^{bc}	37.83 ± 1.14^{c}	163.80 ± 3.44^{c}	3.05 ± 0.06^{c}

Table 1 Effect of NanoSelenium administration on serum ALT, AST, ALP activity (U/L) and Albumin concentration (g/dl) in normal and hepatotoxicity groups of male albino rats, in comparison with control and hepatotoxicity untreated groups.
Data are presented as (Mean ± S.E), S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P<0.05)

Table(2) Effect of NanoSelenium administration on liver CAT, SOD and GPx activity (U/g. tissue) and proinflammatory markers (IL6-LI1 β) (pg/ml) in normal and hepatotoxicity groups of male albino rats, in comparison with control and hepatotoxicity untreated groups. comparison with control and hepatotoxicity untreated groups.

Animal Groups	Antioxidants Activity			Cytokines level	
	CAT (U/g.)	SOD (U/g.)	GPx (U/g. tissue)	IL6 (pg/ml)	IL1β (pg/ml)
Control group	128.53 ± 10.07^{a}	6.36 ± 0.21^{a}	86.93 ± 4.90^{a}	16.73 ± 1.31^{e}	32.70 ± 2.42^{d}
CP group	55.73 ± 4.39^{d}	1.34 ± 0.24^{d}	41.16 ± 3.39^{e}	146.50 ± 5.58^{a}	132.30 ± 4.09^{a}
protective SeNPs	134.30 ± 7.90^{a}	6.67 ± 0.18^{a}	93.42 ± 3.21	14.07 ± 1.73^{e}	32.66 ± 2.05^{d}
Treated CP + SeNPs	101.53 ± 5.89^{c}	4.35 ± 0.40^{c}	62.03 ± 3.92^{cd}	74.20 ± 4.34^{b}	50.80 ± 3.50^{bc}
Treated SeNPs + CP	103.53 ± 6.92^{c}	5.28 ± 0.34^{b}	72.40 ± 2.83^{b}	32.63 ± 2.11^{d}	46.70 ± 3.72^{c}

Data are presented as (Mean ± S.E), S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at (P<0.05).

In this study we have shown that CP usage on rats had resulted in a significant increase in serum activity ALT, AST and ALP which indicating hepatocellular injury. The release of these enzymes from the liver cytoplasm in blood circulation could theoretically be the cause of this increase. However, the Nano-Se treatment has returned ALT, AST and ALP to normal patterns. Pre-treatment with Nano-Se has been more successful than post-treatment group with hepatocellular injury. Our results were come in accordance with Bhattacharjee et al., (2014) who documented that treatment and protection with Nano Se reduced the hepatic damage and toxicity extent that affect directly liver enzymes. Moreover, Abdou and Sayed, (2019) who documented that the administration of the SeNPs had the ability to modulate the elevated levels of liver enzymes to be almost identical to negative control which may be attributable to the preservation of integrity or hepatocyte regeneration of damaged hepatocytes. The first line of defense against the reactive intermediates within the body can be considered CAT and SOD antioxidants. SOD is the first responsible for scavenging the superoxide radicals first responsible is SOD while CAT essential function is to neutralize the radical of hydrogen peroxide (Abdou and Sayed, 2019). Oxidative damage to DNA has been confirmed to be caused by cyclophosphamide's hydroperoxide by the production of H_2O_2 (Murata et al., 2004). The significant reduction of hepatic antioxidants (SOD and CAT) in a recent study can reveal their use in combating the pro-oxidants produced in CP metabolism and serve as a marker of tissue degeneration and injury (Al-Salih et al., 2020). A single selenocysteine (Sec) residue is needed for enzyme activity in selenium-containing glutathione peroxidase (GPx) (Talas et al., 2010). GPx uses GSH to catalyse the reduction of hydroperoxidase, shielding mammalian cells from oxidative injury. Glutathione metabolism is, in fact, one of the most important antioxidative defence mechanisms (Bhattacharjee et al., 2014). The inability of the liver to produce the antioxidant enzyme GPx can explain the lower activity of this enzyme in our CP-treated community. Treatment with SeNPs stopped GPx activity from being depleted in the liver, shielding the cell membrane from oxidative harm.

Results also showed that CP increased inflammatory damage, as evidenced by increased serum levels of pro-inflammatory cytokines IL-1β and IL-6. Toxic materials induce inflammation by stimulating macrophages and inducing the release of pro-inflammatory cytokines (Kang et al., 2012).

Abdou et al., (2019) stated that IL-6 is usually produced due to inflammatory response induction and progression. As inflammatory reactions are mediated with IL-1 β through neutrophil activation (Segel et al., 2011). These findings are in consistence with Gangemi et al., (2016) and Ali et al., (2018), who conclude that the activation of redox-sensitive transcription factors that regulate the gene expression of pro-inflammatory mediators and antioxidants is linked to oxidative stress in the pathogenesis of inflammation.

Our findings showed that administering SeNPs reduced elevated liver enzymes and inflammatory markers to levels that were virtually identical to the control group; these findings may be due to the maintenance of hepatocytes or the regeneration of damaged hepatocytes (Patrick-Iwuanyanwu, et al., 2007). In addition, when compared to the Cp group, treatment with SeNPs increased antioxidant enzyme activity (CAT, SOD, and GPx) in liver tissue. These results support the restorative effects of SeNPs on liver tissue, which could be due to SeNPs' ability to reduce Cp-induced oxidative stress by inhibiting free radical chain reactions by reducing free radical output. Other researchers have confirmed SeNPs' antioxidant activity (Khalaf, et al., 2018, Zachara, 2015). Many studies have shown that nano Se has important protective effects against oxidative stress, DNA damage, and apoptosis, which are linked to Selenium's important function in improving antioxidant defense mechanisms and free radical scavenging ability within the cell (Fahmy et al., 2016).

5. CONCLUSION

Based on our results, we can conclude that Nano-Se is a promising Se formulation for the prevention of CP-induced hepatic injury. This research could pave the way for new ways to use Nano-Se as a hepatoprotective in the field of medicine.

6. REFERENCES

1. Abd-Allah,S. and Hashem, K.S. 2015. Selenium nanoparticles increase the testicular antioxidant activity and spermatogenesis in male rats as compared to ordinary selenium. International Journal 3, 792-802.
2. Abdou, R.H. and Sayed, N. 2019. Antioxidant and Anti-Inflammatory Effects of Nano-Selenium against Cypermethrin-Induced Liver Toxicity. CellBio, 8, 53-65.
3. Abdou, R.H., Basha, W.A. and Khalil, W.F. 2019. Subacute Toxicity of Nerium oleander Ethanolic Extract in Mice. Toxicological Research, 35, 233-239.
4. Ali, S.I., Gaafar, A.A., Abdallah, A.A., El-Daly, S.M., ElBana, M. and Hussein, J. 2018. Mitigation of Alpha-Cypermethrin-Induced Hepatotoxicity in Rats by Tribulus terrestris Rich in Antioxidant Compounds. Jordan Journal of Biological Sciences, 11, 517-525.
5. Al-Salih, H.A., Al-Sharafi, N.M., Al-Qabi, S.S and Al-Darwesh, A.A. 2020. The Pathological Features of Cyclophosphamide Induced Multi-Organs Toxicity in Male Wister Rats. Sys Rev Pharm; 11(6): 45 49.
6. Auron, .PE., Webb, A.C., Rosenwasser, L.J., Mucci, S.F., Rich, A., Wolff, S.M. and Dinarello, C.A. 1984. "Nucleotide sequence of human monocyte interleukin 1 precursor cDNA". Proc. Natl. Acad. Sci. U.S.A. 81 (24): 7907–11
7. Bhattacharjee, A., Basu, A., Ghosh, P., Biswas, J., and Bhattacharya, S. 2014. Protective effect of Selenium nanoparticle against cyclophosphamide induced hepatotoxicity and genotoxicity in Swiss albino mice. J Biomater Appl, 29(2), 303-317 .
8. Basu, A., Bhattacharjee, A., Roy, S. S., Ghosh, P., Chakraborty, P., Das I. and Bhattacharya, S. 2014. anadium as a chemoprotectant: effect of vanadium (III)-L-cysteine complex against cyclophosphamide-induced hepatotoxicity and genotoxicity in Swiss albino mice J. Biol. Inorg. Chem. 19, 981.
9. Cichoz-Lach, H. and Michalak, A. 2014. Oxidative stress as a crucial factor in liver diseases.World J. Gastroenterol. 20, 8082.
10. Dwivedi, D. K. and Jena, G. B. 2018. Glibenclamide protects against thioacetamide-induced hepatic damage in Wistar rat: investigation on NLRP3, MMP-2, and stellate cell activation, Naunyn Schmiedebergs Arch. Pharmacol.,391, 1257.
11. Fahmy, A.A., Abd El-Azim, A.S. and Gharib, G.A. (2016) Protective Effect of Q-3 Fatty Acids and or Nano-Selenium on Cisplatin and Ionizing Radiation Induced Liver Toxicity in Rats. Indian Journal of Pharmaceutical Education and Research ,50, 649-655.
12. Fischbach, F. and Zawata, B. 1992. Klin. Lab. 38, 555-561.
13. Fisusi,F.A. and Akala, E. O.2019. Drug Combinations in Breast Cancer Therapy.Pharm. Nanotechnol. 7, 3.
14. Flora, S.J.S., Kannan, G.M., Pant, B.P. and Jaiswal, D.K. .2002. Combined administration of oxalic acid, succimer and its analogue for the reversal of gallium arsenide induced

oxidative stress in rats. Arch Toxicol., 76:269-76.
15. Fossati, P., et.al. 1980 Clin. Chem. 26, 227 - 231
16. Fraiser, L. H., Kanekal, S., and Kehrer, J. P. 1991. Cyclophosphamide toxicity. Characterising and avoiding the problem. Drugs, 42,781.
17. Gad El-Karim, D. R. and El-Amrawi, G. A. 2019. Cyclophosphamide hepatotoxicity: the role of cytochrome C oxidase and the possible protective effect. Slov Vet Res 2019; 56 (Suppl 22): 15–23
18. Gangemi, S., Gofita, E., Costa, C., Teodoro, M., Briguglio, G., Nikitovic, D., Tzanakakis, G., Tsatsakis, A.M., Wilks, M.F., Spandidos, D.A. and Fenga, C. 2016. Occupational and Environmental Exposure to Pesticides and Cytokine Pathways in Chronic Diseases (Review). International Journal of Molecular Medicine, 38,1012-1020.
19. Gendler, S.; Kaplan, A. et al., (1984): Clin Chem Yhe C.V Mosby Co. st Louis. Toronto. Princeton; 1268-1273 and 425.
20. Gu, X. and Manautou, J. E. 2012. Expert Rev. Mol. Med., 14 e4.
21. Fisusi, F.A. and Akala, E. O. 2019. Drug Combinations in Breast Cancer Therapy, Pharm. Nanotechnol. 2019, 7, 3.
22. Hirano, T. 1998. Interleukin 6 in The Cytokine Handbook, 3rd. ed. Academic Press,New York, p. 197.
23. Jeelani, R., Khan, S. N., Shaeib, F., Kohan-Ghadr, H. R., Aldhaheri, S. R.,Najafi, T., Thakur, M., Morris, R., Abu-Soud, H. M. 2017. Cyclophosphamide and acrolein induced oxidative stress leading to deterioration of metaphase II mouse oocyte quality Free Radic. Biol. Med.2017, 110, 11.
24. Khalaf, A.A., Ahmed, W., Moselhy, W.A., Abdel-Halim, B.R. and Ibrahim, M.A. 2018. Protective Effects of Selenium and Nano-Selenium on Bisphenol-Induced Reproductive Toxicity in Male Rats. Human and Experimental Toxicology, 38, 398-408.
25. Kang, G.J., Kang, N.J., Han, S.C., Koo, D.H., Kang, H.K., Yoo, B.S. and Yoo, E.S. 2012. The Chloroform Fraction of Carpinus tschonoskii Leaves Inhibits the Production of Inflammatory Mediators in HaCaT Keratinocytes and RAW264.7 Macrophages. Toxicological Research, 28, 255-262.
26. Khan, S. and Jena, G. 2014. Sodium valproate, a histone deacetylase inhibitor ameliorates cyclophosphamide-induced genotoxicity and cytotoxicity in the colon of mice. J. Basic Clin. Physiol. Pharmacol., 1.
27. Khan, M. S., Dilawar, S., Ali, I., and Rauf, N. 2012. The possible role of selenium concentration in hepatitis B and C patients. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association, 18(2), 106-110.
28. Menon, S., Ks, S. D., R, S., Humar, R., and S, Kumar, V. 2018. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids and Surfaces B: Biointerfaces, 170, 280-292.
29. Murata, M., Suzuki, T., Midorikawa, K., Oikawa, S. and Kawanishi, S. 2004. Oxidative DNA damage induced by a hydroperoxide derivative of cyclophosphamide. Free Radic Biol Med.;37(6): 793-802.
30. Nishikimi, M., Roa, N.A., and Yogi, K. 1972. Biochem. Bioph. Res. Common., 46, 849 – 854.
31. Paglia, D.E. and Valentine W. N. 1967 J. Lab. Clin.Med. 70: 158 – 169.
32. Patrick-Iwuanyanwu, K.C., Wegwu, M.O. and Ayalogu, E.O. 2007 Prevention of CCl4-Induced Liver Damage by Ginger, Garlic and Vitamin E. Pakistan Journal of Biological Sciences, 10, 617-621.
33. Patwa, J., Khan, S. and Jena, G.2020. Nicotinamide attenuates cyclophosphamide-induced hepatotoxicity in SD rats by reducing oxidative stress and apoptosis. J Biochem Mol Toxicol.; 34:e22558.
34. Ramirez, D. A., Collins, K. P., Aradi, A. E., Conger, K. A. and Gustafson, D. L.2019. Kinetics of Cyclophosphamide Metabolism in Humans, Dogs, Cats, and Mice and Relationship to Cytotoxic Activity and Pharmacokinetics Drug Metab. Dispos., 47, 257.
35. Segel, G.B., Halterman, M.W. and Lichtman, M.A. 2011. The Paradox of the Neutrophil'sRole in Tissue Injury. Journal of Leukocyte Biology, 89, 359-372.
36. Talas, Z.S., Ozdemir, I. and Gok, Y., 2010. Role of selenium compounds on tyrosine hydroxylase activity, adrenomedullin and total RNA levels in hearts of rats. Regul Pept 2010; 159: 137–141
37. Teodor, V., Cuciureanu, M., Filip, C., Zamosteanu, N., and Cuciureanu, R. 2011. Protective effects of selenium on acrylamide toxicity in the liver of the rat. Effects on the oxidative stress. Rev Med Chir Soc Med Nat Iasi, 115(2), 612-618.
38. Z. Klin. Chem. Klin. 1972.Biochem.8, 658(1970), 10,182
39. Zachara, B.A. 2015. Selenium and Selenium-Dependent Antioxidants in Chronic Kidney Disease. Advances in Clinical Chemistry, 68, 131-151.