Indian Journal of Experimental Biology
Vol. 61, September 2023, pp. 712-716
DOI: 10.56042/ijeb.v61i09.5115

Determination of lithium toxicity by apoptosis and different biomarkers on *Carassius auratus* (Linn.) tissues

Sama Ammer Abbas El-Tekreti[1] & Aslı Çilingir Yeltekin[1]*

[1]Department of Chemistry, Faculty of Science, University of Van Yuzuncu Yil, 65080, Turkey

Received 31 December 2021; *revised 08 May 2023*

The widespread use of lithium, one of the heavy metals, in many industrial processes has significantly contaminated the groundwater as well as the surface water. Such contaminants enter into the food chain ecosystem and thereby challenge humans health. In fish, absorption of lithium is most likely through the default Na channel in the gills and muscle. In fish gills, the cell type primarily responsible for the regulation of atoms and ionic regulation are mitochondria-rich cells, also known as chloride cells. Here, we studied the effect of lithium toxicity on tissues of Goldfish, *Carassius auratus* (Linn.) as a model. We did spectrophotometric measurements to determine the effects of lithium dose (50 mg/L) in *C. auratus* at different times (24, 48, 72 and 96 h) of lithium dose. The results have shown LiCl induced alterations in CAT, SOD and GPx enzyme activities in the tissues of *C. auratus* depending on the concentration and duration of action at MDA, 8-OHdG and caspase-3 levels. Further, we observed increased levels of 8-OHG, modificationsof nucleotides, and DNA damage due to lithium toxicity.

Keywords: Antioxidants, Aquatic pollution, Caspase-3, DNA damage, Goldfish, Heavy metal contamination

Lithium (Li), more than 60 years after its therapeutic discovery, remains the standard treatment for bipolar disorder and has been widely used as a mood stabilizer due to its ability to reduce episodes of mania and depression, its efficiency in long-term mood stabilization, and its effectiveness in reducing patients' suicide risk[1].

Lithium occurs naturally in soil and water, and its uptake through plants enters the food chain. Research has shown that the levels of lithium in the blood plasma are roughly proportional to the ingestion of lithium. Further, its concentration is disorganized. Lack of regulation contrasts sharply with other cations of biological importance such as sodium, potassium, calcium, protons and magnesium whose blood concentration is closely regulated[1,2]. Recent studies confirm the protective effect of lithium on oxidative stress. Lithium causes various complex actions on the nervous system viz. effects on Bcl-2 neurotransmitter, fermentation of kinase-3-beta nerve tissue and regulation of gene transcription. Stellate cells are also a potential target for lithium, which explains their neuroprotective action[3]. Despite the use of lithium in the treatment of bipolar disorder, the rate of application has been decreasing recently. One of the reasons is the toxicity effect associated with the use of lithium[4]. The therapeutic level of lithium in the blood for normal effect is 0.6 mmol/L, but side effects can occur at levels of 1.2 mmol/L and more[5,6]. Lithium has toxic effects on many organs such as kidneys, liver, nervous system, etc. Studies indicate that a portion of the lithium toxicity is mediated by oxidative stress which results in high levels of lipid peroxide[7]. Some cases treated with the lithium doses intensively and for long periods showed significant toxic effects and led to progressive renal impairment[8,9]. Lithium is reported to affect brain, intestines, thyroid, liver function and also metabolism. In severe cases, it causes death[10].

Extensive use of lithium and its compounds in pharmaceuticals, dehumidifiers, air conditioning, ceramics, mineral processes, lubricants and several chemical and biological laboratories adds toxicity to the environment[11]. Different studies have reported concentration based effect of lithium toxicity on different aquatic species, Waste disposal or recycling facilities and chemical leakage are major sources of lithium entry into freshwater and groundwater[12]. Reports on the effects of lithium exposure on the development of fish in terms of treatment duration, doses, and conditions. Prolonged lithium exposure is known to affect vertebrate embryo development, the formation of the dorsoventral body axis. In many studies, lithium chloride was used, among other materials, to examine the toxic effects on fish, as they found that high concentrations of lithium chloride with a long stay longer, harmful effects of lithium were observed, including growth retardation, skeletal abnormalities, swimming is only reduced when a later time points[10,12,13].

*Correspondence:
E-Mail: aslicilingir@yyu.edu.tr

Goldfish, *Carassius auratus* (Linn.), an ornamental species, due to its wide availability and easy handling, is a suitable model for neurology, endocrinology, reproduction and ecotoxicity studies[14,15]. In this study, we have evaluated the effect of lithium on *C. auratus* for morphological changes, if there any, and functional responses and enzyme activities in tissues.

Materials and Methods

Fish

Carassius auratus fish was purchased from a licensed commercial company. Forty-eight fishes (1.80 ± 0.05 g) were distributed randomly in two aquarium glass tanks with a capacity of 10.0 L and were exposed to 12 h night/light period. Water temperature was 25.0 ± 1°C. It was studied in the pH range of 7.8 ± 0.1. Lithium chloride (LiCl) was added @50 mg/L to one tank with 28 fishes (treatment group), and the other tank with 20 fishes served as the control group. Fishes were collected for sampling from both the tanks after 24, 48, 72 and 96 h, and anesthetized using MS 222 (0.1 g/L). The tissues were homogenized and frozen at −18°C until analysis.

Biochemical analysis

Superoxide dismutase (SOD) activity was measured using the Randox-Ransod enzyme group at 505 nm and 37°C under the UV spectrum (Shimadzu UV-1201, UV-Vis Japan)[16-18]. CAT enzyme activity was measured using the UV spectrophotometer method of Aebi[19] below 240 nm based on the destruction of hydrogen peroxide (H_2O_2). The MDA level was determined following Placer *et al*.[20] and Yeltekin[21] as a UV spectrophotometer to absorb pink under 532 nm, observed as a result of thioparpetoric acid TBA and MDA interactions. GSH-Px enzyme activity was measured using the Randox-Ransod enzyme kit at 340 nm and 37°C under the UV spectrum[22]. In the study, Fish (8-OHdG) ELISA kit (catalogue no: 201-00-0041) (SunRed brand) was used to determine 8-hydroxy-2-deoxyguanosine (8-OHdG) levels. Half an hour before starting the study, the kit materials were brought to room temperature and the kit procedure was applied with the specified materials[23]. Fish (CASP3) ELISA kit (catalogue no: 201-00-0031) (SunRedtrade) was used for determining caspase-3 levels of fish in each tissue[24].

Statistical analysis

Data were announced as mean values ± standard error of the mean (SEM). Students 't' test was used to compare study groups. Statistically significant differences were contemplated significant at P <0.05. Statistical analysis of all data was achieved using SPSS (version 23.0 Inc).

Results and Discussion

Effect of lithium toxicity on (SOD, GSH-Px and CAT) activity in *C. auratus* gill and muscle tissues

The effect of lithium on SOD, GSH-Px and CAT activity in *Carassius auratus* gill and muscle are given in Table 1. Compared to the control group, the treated group showed decreased SOD at (24, 48 and 72 h) and increased after 96 h of exposure. GSH-Px was decreased at (24 and 48 h) and a marked increase begins at (72 and 96 h), CAT was significantly decreased (P <0.05). Lithium had a clear effect on SOD, GSH-Px and CAT activity over the whole period.

Similarly in the muscle tissue, SOD and GSH-Px activities were found significantly decreased (P <0.05) in treated group compared to the control group. Lithium had a clear effect on SOD and GSH-Px activity over the whole period. However, CAT activity significantly increased (P <0.05) in the treated group compared to the control group.

Effect of lithium toxicity on MDA, 8-OHdG and caspase-3 level in *C. auratus* gill and muscle tissues

Results showed that the MDA, 8-OHdG and caspase-3 levels increased in both the gills and muscle tissue with exposure to the lithium significantly across the groups (P <0.05) (Table 2).

Table 1 — Oxidative parameters after exposure to lithium on Goldfish (*Carassius auratus*) gill and muscle tissues (mean ± SD)

	24 h cont.	24 h	48 h cont.	48 h	72 h cont.	72 h	96 h cont.	96 h
Gill								
SOD (U/mg prot.)	45.242±1.22^{b}	43.585±1.53^{b}	45.063±3.01^{b}	29.957±1.96^{a}	43.557±1.29^{b}	31.932±2.39^{a}	44.172±2.62^{b}	44.224±3.14^{b}
GSH-Px (EU/mg prot.)	30.461±2.14^{ab}	25.689±2.52^{a}	28.255±1.88^{ab}	24.185±0.96^{a}	30.428±1.26^{ab}	34.278±2.09^{b}	28.895±1.22^{ab}	35.751±2.33^{b}
CAT(EU/mg)	0.651±0.31^{b}	0.489±0.33^{ab}	0.625±0.03^{b}	0.424±0.02^{ab}	0.604±0.04^{b}	0.318±0.03^{a}	0.605±0.03^{b}	0.460±0.04^{ab}
Muscle								
SOD (U/mg prot.)	45.420±1.24^{b}	47.307±2.13^{b}	43.100±3.63^{b}	36.113±2.79^{ab}	45.242±4.29^{b}	30.333±1.99^{a}	45.242±3.42^{b}	43.535±3.16^{b}
GSH-Px(EU/mg prot.)	24.801±1.62^{b}	22.085±1.02^{ab}	24.468±1.06^{b}	16.585±1.66^{a}	23.648±1.98^{b}	14.88±0.92^{a}	22.838±1.58^{b}	14.613±0.97^{a}
CAT (EU/mg)	0.292±0.13^{a}	0.344±0.09^{ab}	0.305±0.11^{a}	0.431±0.07^{b}	0.292±0.11^{a}	0.432±0.13^{b}	0.297±0.09^{a}	0.4640±0.14^{b}

[Different letters indicate significant differences between the groups (P <0.05). Each value is the mean±SEM.]

Table 2 — Changes in lipid MDA, DNA damage (8-OHdG) and apoptosis (caspase-3) parameters after exposure to lithium on Goldfish (*Carassius auratus*) gill and muscle tissues (mean ± SD)

	24 h cont.	24 h LiCl	48 h cont.	48 h LiCl	72 h cont.	72 h LiCl	96 h cont.	96 h LiCl
Gill								
MDA (nmol/g)	0.117±0.02^{a}	0.127±0.03^{a}	0.120±0.02^{a}	0.138±0.02^{a}	0.118±0.01^{a}	0.199±0.02^{ab}	0.142±0.02^{a}	0.253±0.03^{b}
8-OHdG (ng/mL)	2.547±0.81^{a}	2.566±0.36^{a}	2.563±0.25^{a}	2.589±0.64^{a}	2.564±0.59^{a}	2.622±0.54^{ab}	2.559±0.64^{a}	2.659±0.71^{b}
Caspase-3 (ng/mL)	0.759±0.07^{a}	0.799±0.09^{ab}	0.754±0.08^{a}	0.823±0.09^{b}	0.762±0.07^{a}	0.847±0.08^{ab}	0.761±0.06^{a}	0.857±0.09^{ba}
Muscle								
MDA (nmol/g)	0.034±0.01^{a}	0.045±0.01^{a}	0.034±0.02^{a}	0.061±0.03^{ab}	0.036±0.02^{a}	0.069±0.02^{ab}	0.036±0.01^{a}	0.089±0.03^{b}
8-OHdG (ng/mL)	2.479±0.67^{a}	2.482±0.88^{a}	2.480±0.70^{a}	2.489±0.68^{a}	2.482±0.83^{a}	2.496±0.98^{b}	2.482±0.82^{a}	2.502±0.91^{b}
Caspase-3 (ng/mL)	0.476±0.04^{a}	0.498±0.05^{a}	0.504±0.04^{a}	0.586±0.04^{ab}	0.504±0.04^{a}	0.652±0.05^{ab}	0.503±0.04^{a}	0.701±0.04^{b}

[Different letters indicate significant differences between the groups (P <0.05). Each value is the mean±SEM.]

Mineral induced changes in the physiology and survival of aquatic organisms under mineral stress are complex because these changes vary from mineral to mineral, and from species to species. In studies with *C. auratus*, exposure to Li caused defects in the activities of gill and muscle tissues enzymes with escalating severity with increased lithium concentrations. The abnormally high lithium concentrations in water resources contaminated by alkaline mining have caused changes in the ultrastructure and lipid composition of the fish gill. Fish gills play a major role between an organism and its environment[25]. In addition to their role in gas exchange, elimination of nitrogenous wastes, and acid-base balance, gills play a central role in ion exchange and osmotic regulation processes. Basal cell membranes contain channels through which ions, such as sodium and potassium, are pumped selectively and against concentration gradients by the activity of Na^{+}, K^{+} -ATPase. Apical membrane channels are involved in acid-base regulation, and the apical osmotic gradient is very important for gill cells. Other authors have reported cases of lithium toxicity on fish gills but did not observe such a severe effect of Li[26,27].

Studies on the impact of lithium on the behaviour in *Carassius auratus* are limited. The present results show that lithium can slow down and disrupt locomotor activity in *C. auratus* in a manner similar to that reported for mammals. This study has demonstrated that Li causes changes in CAT, SOD and GPx enzyme activities in gill and muscle tissue of *C. auratus* depending on concentration and duration of action at MDA, 8-OHdG and caspase-3 levels. In fish, the gills are the first organs affected by environmental pollution. Large areas of fish gills make toxic substances pass easily[28,29]. It has been reported that in toxicity situations, DNA fragmentation, nucleotide modifications, and 8-OHdG levels are increased. Contaminants have been known to cause oxidative stress by negatively affecting antioxidant and lipid peroxide enzymes in fish and mammals[30,31]. In the aqueous environment, the accumulation of harmful substances in the cell membrane disrupts the lipid structure. Depending on the degradation of a hydrophobic reaction between protein and lipids, enzyme activities may change[32]. Here, lithium chloride inhibited SOD after 72 h of exposure and the toxic effect of lithium chloride got reduced after exposure for 96 h. For GSH-Px enzyme, it was inhibition after 48 h of exposure which had increased after 72 and 96 h. CAT enzyme activity, reduced enzyme activity can be illustrated by high lipid peroxide. MDA, 8-OHdG and caspase-3 are commonly used parameters to determine oxidative damage and apoptosis. Increasing lipid peroxide affects the activities of antioxidant enzymes. At the same time, the increase in DNA damage triggers apoptosis and initiates chain reactions. Increased lipid peroxide affects the activities of antioxidant enzymes.

Environmental chemicals may increase or inhibit the antioxidant enzymes activities[33,34]. In this study, SOD and GSH-Px activities in the tissues were decreased by the concentration of the exposure to lithium in all treatment groups. These decreases in the tissue SOD and GSH-Px activities can be attributed to the inhibition of superoxide radical formation[35,36]. The marked decrease in antioxidant enzyme activities increases DNA damage[23,37]. Because of these changes, the production and inhibition of O_2 have an effect on the enzyme activity of some substances[38]. In this work, lithium chloride concentration significantly (P <0.05) increased CAT activity in tissues when compared to the control group. MDA is regarded as an important indicator of the oxidative stress level; therefore, it has been widely used to confirm a state marker of oxidative stress in the aquatic toxicology. In our study, a significant elevation of MDA concentration was observed with the increasing lithium concentrations in all exposure time, which

showed that Li exposure causes oxidative stress in *C. auratus*. Oxygen radical-producing agents and carcinogens can increase the level of 8-OHdG formation[31,39-41]. In the present study, it was observed that lithium chloride increased 8-OHdG and caspase-3 levels in general. The toxicity of Li increased lipid peroxidation through increased tissues MDA level and decreased tissues GPx and SOD activities. In this study, it was determined that lithium increases the oxidative DNA damage and apoptosis (caspase-3).

Conclusion

This study demonstrated that lithium (Li) has physiological and biochemical effects on Godfish *Carassius auratus*, suggesting that the Li compound alters antioxidant parameters in fish. Therefore, these parameters may be involved in the toxicity mechanism of lithium on *C. auratus.* In addition, the increase in MDA 8-OHdG and caspase 3 levels indicates the damage caused by high-dose lithium in tissues. It is necessary to study the possible relationship between oxidative stress and chemical-physiological response with different aquatic organisms using longer exposure time.

Acknowledgement

This study was supported by the project of Van Yuzuncu Yil University, Scientific Research Projects with FBA-2021-9332 project.

Conflict of interest

Authors declare no competing interests.

References

1 De Roos NM, de Vries JH & Katan MB, Serum lithium as a compliance marker for food and supplement intake. *Am J Clin Nutr*, 73 (2001) 75.

2 Richelson E, Lithium ion entry through the sodium channel of cultured mouse neuroblastoma cells: a biochemical study. *Science*, 196 (1977) 1001.

3 Gould TD, Chen G & Manji HK, *In vivo* evidence in the brain for lithium inhibition of glycogen synthase kinase-3. *Neuropsychopharmacology*, 29 (2004) 32.

4 Kessing LV, Vradi E & Andersen PK, Nationwide and population-based prescription patterns in bipolar disorder. *Bipolar Disorders*, 18 (2016) 174.

5 Ng F, Berk M, Dean O & Bush AI, Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol*, 11 (2008) 851.

6 Öter Ç, Yeltekin AÇ & Ammer Abbas El-Tekreti S, Removal of lithium from aqueous solutions by solid-phase extraction using sawdust loaded with magnetite nanoparticles and study of apoptosis, MDA and 8-OHdG caused by lithium toxicity in fish brain. *Toxicol Ind Health*, 39 (2023) 158.

7 Nautiyal DA, Prasad B, Nautiyal, YVK & Chandra M, Variation in morphological, biochemical and antioxidant properties of Lilium polyphyllum, *Indian J Biochem Biophys*, 60 (2023) 237.

8 Aslanoglu F & Sahin E, N-Acetylation of methyl hydrazones with acetic acid in presence of $PdCl_2$. *Synth Commun*, 53 (2023) 713.

9 van Melick EJM, Souverein P, den Breeijen JH, Tusveld CE, Egberts ACG & Wilting I, Leeftijd als determinant van in stabiliteit van lithium concentraties in serum. *Pharm Weekbl*, A1534 (2015).

10 Gershon S, *Lithium: Its role in psychiatric research and treatment*. Kindle Edition. (Springer, New York, USA), 2012.

11 Öter Ç, Preparation a Magnetic Molecular Imprinted Polymer for Specific Adsorption of Pharmaceutical Pollutants. *Chemistryselect*, 8 (2023) 1.

12 Shahzad B, Mughal MN, Tanveer M, Gupta D & Abbas G, Is lithium biologically an important or toxic element to living organisms an overview. *Environ Sci Pollut Res*, 24 (2017) 103.

13 Manisha K, Prasanti S & Neelima S, Evaluation of anti-anxiety effects of the hydromethanolic extract of *Boerhaavia diffusa* L. roots in mice exposed to unpredictable chronic mild stress. *Indian J Nat Prod Res*, 14 (2023) 249.

14 Blanco AM & Unniappan S, Goldfish (*Carassius auratus*): biology, husbandry, and research applications. In: *Laboratory Fish in Biomedical Research*, (D'Angelo L & de Girolamo Pa; Academic Press, New York, USA), 2022, 373.

15 Khosrovyan A, Melkonyan H, Rshtuni L, Gabrielyan B & Kahru A, Polylactic Acid-Based Microplastic Particles Induced Oxidative Damage in Brain and Gills of Goldfish *Carassius auratus*. *Water*, 15 (2023) 2133.

16 Xia E, Rao G, Van Remmen H, Heydari AR & Richardson A, Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. *J Nutr*, 125 (1995) 195.

17 Flohe L & Otting F, Superoxide dismutase assays. *Meth Enzymol*, 105 (1984) 93.

18 Yeltekin AÇ, Oğuz AR, Kankaya E, Özok N & Güneş İ, Hematological and biochemical response in the blood of *Alburnus Tarichi* (Actinopterygii: Cypriniformes: Cyprinidae) exposed to tebuconazole. *Acta Ichthyol Piscat*, 50 (2020) 373.

19 Aebi H, *Catalase*. In: *Methods of Enzymatic Analysis*. Vol. 2, Second Edn., (Bergmeyer HU, Academic Press, New York, USA), 1974, 673.

20 Placer ZA, Cushman LL & Johnson BC, Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal Biochem*, 16 (1966) 355.

21 Yeltekin AÇ, Effect of Fungicide Toxicity on Apoptosis, DNA Damage, and Antioxidant Enzymes in Van Fish. *Pak J Zool*, 55 (2023) 355.

22 Yeltekin AÇ, Uçar A, Parlak V, Özgeriş FB, Türkez H, Esenbuğa N, Atamanalp M & Alak G, Borax exerts protective effect against ferrocene-induced neurotoxicity in *Oncorhynchus mykiss*. *J Trace Elem Med Biol*, 72. (2022).

23 Yeltekin AÇ & Oğuz AR, Antioxidant responses and DNA damage in primary hepatocytes of Van fish (*Alburnus*

tarichi, Güldenstadt 1814) exposed to nonylphenol or Octylphenol. *Drug Chem Toxicol*, 41 (2018) 415.

24 Cilingir Yeltekin AÇ, Toxic Effects of *Lactococcus garvieae*, *Staphylococcus epidermidis*, and *Bacillus subtilis* Bacteria on the Physiology of Rainbow Trout. *Biol Bull*, 49 (2022) 184.

25 Uçar A, Parlak V, Özgeriş FB, Yeltekin AÇ, Alak G & Atamanalp M, Determination of Fipronil toxicity by different biomarkers in gill and liver tissue of rainbow trout (*Oncorhynchus mykiss*). *In Vitro Cell Dev Biol-Animal*, 56 (2020) 543.

26 Malathi ST & Anuradhaf V, Lithium Induced Toxicity Profile of Oxygen Consumption, Haematological Parameters and Biochemical Profiles of *Channa punctatus* and *Oreochromis niloticus*. *Nat Environ Pollut Technol*, 19 (2020) 677.

27 Uçar A, Yeltekin AÇ, Köktürk M, Calimli MH, Nas MS, Parlak V, Alak G & Atamanalp M, Has PdCu@GO effect on oxidant/antioxidant balance? Using zebrafish embryos and larvae as a model. *Chem-Biol Interact*, 378 (2023) 110484.

28 Pereira B, Carneiro A, Carvalho A, Colodette J, Oliveira A & Fontes M, Influence of Chemical Composition of Eucalyptus Wood on Gravimetric Yield and Charcoal Properties. *Bioresources*, 8 (2013) 4574.

29 Alak G, Türkez H, Uçar A, Yeltekin AÇ, Özgeriş FB, Parlak V & Atamanalp M, Neuroprotective properties of borax against aluminum hydroxide-induced neurotoxicity: Possible role of Nrf-2/BDNF/AChE pathways in fish brain. *Brain Res*, 1803 (2023) 148241.

30 Yeltekin AÇ, Oguz AR, Kankaya E, Ozok N & Guneş, I, Hematological and Biochemical Response in The Blood of *Alburnus Tarichi* (Actinopterygii: Cypriniformes: Cyprinidae) Exposed To Tebuconazole. *Acta Ichthyol Piscat*, 50 (2020) 373.

31 Alak G, Parlak V, Aslan ME, Ucar A, Atamanalp M & Turkez H, Borax Supplementation Alleviates Hematotoxicity and DNA Damage in Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Copper. *Biol Trace Elem Res*, 187 (2019) 536.

32 Jorge MI, Soler-Rovira P, Casermeiro MA, de la Cruz MT & Polo A, Seasonal changes in microbial activity in a semiarid soil after application of a high dose of different organic amendments, *Geoderma*, 206 (2013) 40.

33 Atamanalp M, Parlak V, Özgeriş FB, Yeltekin AÇ, Ucar A, Keleş MS & Alak G, Treatment of oxidative stress, apoptosis, and DNA injury with N-acetylcysteine at simulative pesticide toxicity in fish. *Toxicol Mech Methods*, 31 (2021) 224.

34 Oruç EÖ, Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. *Pestic Biochem Physiol*, 96 (2010) 160.

35 Kokturk M, Yıldırım S, Nas MS, Ozhan G, Atamanalp M, Bolat I, Calimli MH & Alak G, Investigation of the Oxidative Stress Response of a Green Synthesis Nanoparticle (RP-Ag/ACNPs) in Zebrafish. *Biol Trace Elem Res*, (2021) 120.

36 Yonar SM, Toxic effects of malathion in carp, *Cyprinus carpio carpio*: protective role of lycopene. *Ecotoxicol Environ Saf*, 97 (2013) 223.

37 Dong L, Gao J, Xie X & Zhou Q, DNA damage and biochemical toxicity of antibiotics in soil on the earthworm *Eisenia fetida*. *Chemosphere*, 89 (2012) 44.

38 Alak G, Ozgeris FB, Yeltekin AÇ, Parlak V, Ucar A, Caglar O, Turkez H & Atamanalp M, Hematological and Hepatic Effects of Ulexite in Zebrafish. *Environ Toxicol Pharmacol*, 80 (2020) 103496.

39 Peng T, Li LQ, Peng MH, Liu ZM, Liu TW, Guo Y & Yan LN, Evaluation of oxidative stress in a group of adolescents exposed to a high level of aflatoxin B1amulti-center and multi-biomarker study. *Carcinogenesis*, 28 (2007) 2347.

40 Kasai H, Analysis of a form of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res Rev Mutat Res*, 387 (1997) 147.

41 Jahromi BN, Keshavarz M, Koohpeyma F, Badr P, Poordast T, Najib SF, Bakhtari A & Bahmanpour S, Protective effect of loboob (a Persian traditional remedy) on sexual hormones, antioxidant activities and stereological changes of testis tissue on busulfan induced oligospermia in rats. *Indian J Exp Biol*, 61 (2023) 175.