Turk. J. Fish.& Aquat. Sci. 20(8), 593-602
http://doi.org/10.4194/1303-2712-v20_8_02

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Oxidative and DNA Damage Potential of Colemanite on Zebrafish: Brain, Liver and Blood

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Article History
Received 21 November 2019
Accepted 11 March 2020
First Online 06 April 2020

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Keywords
Colemanite
Boron compounds
Zebrafish
Brain
Liver
Micronucleus assay
Blood
Oxidative stress
DNA damage

Abstract

Recently, boron has been used in animal feeding due to its significant biological roles. In this study, the action mechanism of colemanite (COL), a commercially important borate mineral, was aimed to investigate via evaluating parameters related to oxidative alterations on the brain, liver and blood tissues of zebrafish. For this purpose, zebrafish were exposed to different doses of COL (5, 10 and 20 mg/L) in a static test apparatus for 96 hours. Multiple biochemical analysis including determination of DNA damage (8-OHdG), apoptosis (Caspase-3), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), myeloperoxidase (MPO), paraoxonase (PON), arylesterase (AR) and lipid peroxidation (MDA) levels were performed in brain and liver tissues for assessing oxidative responses. In addition to micronucleus (MN) assay was performed in obtained blood tissues. The results indicated that low doses of COL supported antioxidant system and did not lead to oxidative stress in zebrafish brain and liver. Again, our results showed colemanite did not cause DNA damage or apoptosis at all tested concentrations. Besides the statistically insignificant changes ($P>0.05$) of MN rates of erythrocytes between the control and experimental groups revealed the non-genotoxic feature of COL on zebrafish. In conclusion, boron compounds especially COL can be used safely and provide positive impacts on aquatic environments.

Introduction

Boron is an element with the atomic number 5, which is in the first place in the 3A group on the periodic table, indicated by the symbol "B". Boron has compounds with calcium, magnesium and sodium elements, and several commercially important types; borax (BX) ($Na_2B_4O_7.10H_2O$), colemanite (COL) ($Ca_2B_6O_{11}.5H_2O$) and ulexite (UX) ($NaCaB_5O_9.8H_2O$). These compounds are also known as natural borates. The usage areas of boron compounds are quite high. In recent years, the biological importance of boron element has been understood and clinical - experimental studies have been started to investigate its effects on health. This mineral, which has serious reserves in our country, has widespread uses (glass, ceramics, cleaning, whitening, cosmetics, metallurgy, nuclear, computer, aircraft industry, energy industry,

agriculture and health) (Helvacı, 2003). Borax mineral is effective on hormone and lipid metabolism as well as free radicals of many enzymes (Comba, Oto, Mis, Özdemir, & Comba 2016; Acaroz *et al.,* 2019). One of the most common boron minerals, colemanite (COL), is a monoclinic crystalline calcium borate mineral with chemical formula $2CaO \cdot 3B_2O_3 \cdot 5H_2O$ (Kızılca & Copur, 2015). Boron minerals are effective in activity of many enzymes, as well as hormone and lipid metabolism (Comba *et al.,* 2016). Although their action mechanisms are not clear, it has been demonstrated that borates are not metabolized and control the damage of the organism by altering the oxidative stress parameters (Pawa & Ali, 2006; Acar, İnanan, Zemheri, Kesbiç, & Yılmaz, 2018; Alak et al. 2018; Alak *et al.,* 2019a; 2019b;2019c). Boron compounds exhibit important biological roles in the development of arthritis, plasma lipid profiles, brain function, mineral (potassium (K), calcium (Ca)) and vitamin (vitamin D) metabolism and hormone (Devirian & Volpe, 2003; Colak *et al.,* 2011; Ince, Kucukkurt, Acaroz, Arslan-Acaroz, & Varol, 2019). Borates have also been reported to have protective effects against many diseases such as cancer and neurodegenerative diseases (Gallardo-Williams, Maronpot, Wine, Brunssen, & Chapin, 2003; Barranco & Eckhert 2006). In addition, boron/borax in animal organisms has been reported to support the tissue antioxidant defense system by an unknown mechanism via affecting oxidative metabolism (Alak *et al.,* 2018, 2019a; 2019b; 2019c).

Reactive oxygen species (ROS) are formed during normal metabolism in a healthy organism. However, in some cases involving inflammation, drugs, exogenous sources and radiation exposure cause increases of ROS production. As a result, changes in the ROS signal transduction pathway lead to oxidative damages on both lipids and proteins (Ciftci, 2017; Parlak, 2018). The hydroxyl radical and peroxynitrite are particularly damaging to cell membranes and lipoproteins. It initiates chemical chain reactions that cause the formation of malondialdehyde (MDA), which has cytotoxic and mutagenic nature. MDA is formed as a result of oxidative damage and reacted with bases in DNA structure and exhibits mutagenic properties. Endogenous and exogenous antioxidants protect cells from ROS-induced damage in long and short terms via neutralizing or inhibiting the effect by ROS (Borek, Ong, Mason, Donahue & Biaglow, 1986). However, when ROS is produced excessively or there is a significant decrease in antioxidant defense, the antioxidant defense system is suppressed and oxidative stress occurs. This oxidant-antioxidant balance in the organism depends on many factors. These are endogenous and exogenous factors that usually act together (Rahman, 2007).

To our best knowledge, the effects of COL exposure to aquatic organisms have not evaluated yet. Therefore, in this research the use of COL in fish and its possible protective mechanism considering oxidative stress response were investigated for the first time.

Material and Methods

Fish Material

Adult zebrafish (28-32 mm length, 0.40±0.03 g weight) were obtained from Atatürk University, Fisheries Faculty (Erzurum, Turkey). Colemanite (COL) applications were performed on the fish whose acclimation was completed in Atatürk University, Faculty of Fisheries, Zebrafish Research and Toxicology Trial Unit.

Colemanite

Colemanite (COL) ($CaB_3O_4(OH)_3 H_2O$; Cas no. 1318-33-8, purity 99.8%) was obtained from Eti Mine Works (Ankara, Turkey) and the tested concentrations were determined according to the previous study by Rowe, Bouzan, Nabili, & Eckhert (1998).

Trial Plan and Application of Chemical

The trial plan was composed according the static test rules in four replications (control 0 mg/l, and experimental groups that exposed to 5, 10 and 20 mg/L of COL. 288 zebrafish were placed in trial mediums randomly and each treatment groups had 24 zebrafish (three replicates). Zebrafish were treated for 96 hours and enzyme activities (SOD, CAT, GPx, PON, and AR) and MPO, MDA, 8-OHdG and Caspase-3 levels were analyzed in obtained zebrafish brain and liver tissues. In addition, MN assay was performed in blood samples taken at the end of the trial period.

Determination of Enzyme Activities and Lipid Peroxidation (MDA) Level

Homogenate Preparation

All group's fish were anesthetized in ice-cold water and subsequently sacrificed (Liu, Wang, Wei, Zhang, Xu, & Dai, 2008). After collecting six fish's tissues (brain and liver) from each treated and control groups (Yan, Wang, Zhu, Chen, & Wang, 2015) homogenized in phosphate buffer (0.1M phosphate buffer KH_2PO_4, pH 7.4) and centrifuged at 1300 rpm for 30 minutes (Yan et al. 2015; Alak et al. 2017a, 2017b, 2019c). Four homogenates were prepared at each group. Protein concentration was determined spectrophotometric at 595 nm according to the Bradford method (Bradford 1976). These obtained homogenates (SOD, CAT, GPx, MPO, PON, AR, MDA, Caspase-3 and 8-OHdG) were used in analysis.

Measurement of Superoxide Dismutase (SOD) Enzyme Activity

The color change observed as a result of the reaction of superoxide radicals formed by xanthine oxidase enzymatic reaction with NBT was measured by

spectrophotometer at 560 nm (Sun, Oberley, & Li, 1988).

Measurement of Catalase (CAT) Activity

The method of Aebi (1974) was taken into consideration and the reaction of hydrogen peroxide (H_2O_2) degradation by catalase enzyme was measured with the absorbance reduction rate determined at 240 nm.

Measurement of Glutathione Peroxidase (GPx) Activity

GPx activity was determined by measuring the difference in decrease of absorbance during the oxidation of NADPH to $NADP^+$ (Beutler 1984).

Measurement of Myeloperoxidase (MPO) Activity

It is based on the kinetic measurement of the absorbance of the yellowish-orange colored complex resulting from the oxidation of o-dianisidine with MPO in the presence of hydrogen peroxide at the wavelength of 460 nm (Bradley, Priebat, Christensen, & Rothstein, 1982).

Measurement of Paraoxonase (PON) Activity

Spectrophotometric absorbance change (in unit time) of P-nitrophenol formed by paraoxone hydrolysis was considered at 37 ° C and at 412 nm (Gülcü & Gürsü, 2003).

Measurement of Arylesterase (AR) Activity

ARE activity was determined by measuring the absorbance of phenol formed at 270 nm with the method using phenyl acetate as substrate (Gülcü & Gürsü, 2003).

Measurement of Lipid Peroxidation (MDA)

The level of lipid peroxidation was obtained by determining the level of malondialdehyde (MDA), a product of lipid peroxidation (Alak *et al.,* 2019d).

Determination of Apoptosis (Caspase-3) Level

The level of Caspase-3 in brain and liver tissues was determined with the help of a commercial kit (Fish (CASP3) ELISA Kit (Catalog No: 201-00-0031) (SunRed). According to prospectuse, the prospectus of a commercial kit had been considered. Caspase-3 standard was prepared by diluting with Standard diluent and left in Microelisa Strip Plate wells as 50 µl. One well was chosen as blank and the remaining wells were placed in 40 µl of samples. 50 µl Str-HRP-Conjugate Reagent was added to each well. 10 µl Biotin- (CASP3) Ab was added to the sample and the platen was covered. The plate was incubated in the plate shaker at 37°C for 1 hour. Plate wells were washed with 30X Wash Buffer (350 µl per well) and diluted 30 times with distilled water, waited for 1-2 minutes then this was repeated 5 times. After washing, 50 µl of Chromogen Solution A and 50 µl of Chromogen Solution B were added to each well and incubated for 10 min in the plate shaker at 37°C in the dark. As a result of the incubation, 50 µl Stop Solution was added to the plate wells and the measurement was made in ELISA (Plate Reader) device with 450 nm absorbance within 10 minutes (Alak et al. 2018).

Determination of DNA damage (8-OHdG) Level

The level of 8-OHdG in brain and liver tissues was determined with the help of a commercial kit (Fish (8-OHdG) Catalog No: 201-00-0041 / SunRed). In this method, standards prepared for 8-OHdG are added in Microelisa Strip Plate, as 50 µl. It is selected blank in one well and then 40 µl of samples are added to the other wells. 50 µl of Str-HRP-Conjugate Reagent is added to each well then only 10 µl of Biotin- (8-OHdG) Ab is added to the sample and the plate is covered. The plate is incubated at 37°C in the Plate Shaker for 1 hour. Plate wells are washed 5 times with 30X Wash Buffer (300 µl / well) diluted 30 times with distilled water, after waited 1-2 minutes. After washing, 50 µl of Chromogen Solution A was added and then adding 50 µl of Chromogen Solution B to each tube and incubate for 10 minutes in the Plate Shaker at 37°C in the dark. As a result of incubation, 50 µl Stop Solution is added to the plate wells and the measurement was done in ELISA (Plate Reader) device with an absorbance of 450 nm within 10 minutes (Alak et al. 2018).

Micronucleus (MN) Assay in Zebrafish Erythrocytes

MN test was performed with blood of 8 fish sampled from each of the treatment and control groups according to chance. For MN test, one drop of blood from each sample in groups was immediately smeared on clean glass slides, then glass slides were allowed to dry (Baršiene, Dedonyte, Rybakovas, Andreikenait & Andersen, 2006). The slides fixed with methanol for 10 min, then stained with 5% Giemsa (Merck, Germany) for 30 min. In zebrafish erythrocytes, the frequencies of MN were calculated by counting MN/1000 cells per fish at 100X magnification.

Statistical Analyses

All data are given with standard deviation and mean values. The differences among the groups were determined by one-way (ANOVA) variance analysis and then evaluated by Duncan's test via SPSS program ($P<0.05$).

Results

The potential regulatory effect of COL and the reference range for fish were investigated in three different categories and multiple biochemical analyzes in 96-hour administration of adult zebrafish brain and liver tissue.

Enzyme Activities and Lipid Peroxidation Level

The alterations in antioxidant enzyme activities were observed in brain and liver tissues after the administration of COL (5 10 and 20 mg/l) to zebrafish adults (after 96 hours) ($P<0.05$). According to the data obtained from the Table1, brain tissue and Table 2 liver tissue results, it was found that high dose (20 mg) colemanite administration significantly increased the MDA level for both tissues compared to the control. This situation has been interpreted as triggering oxidative stress and a similar situation had been observed in liver tissue. In the liver tissue findings, it was found that cleomonite treatment as 5mg / l gave statistically similar and close results in some antioxidant enzyme activities.

It was determined that high concentration application (20 mg / l) of COL in both tissues has an inhibitory effect compared to control in SOD, CAT, GPx, PON and AR enzyme activites (Tables 1 and 2).

DNA Damage and Apoptosis Level

After the administration of COL to zebrafish adults (at the end of 96th hour), significant alterations in 8-OHdG levels in brain tissues were observed among the groups ($P<0.05$). The low doses of COL (5, 10 and 20 mg/l) led to lower levels of 8-OHdG in comparison to control group (Figure 1). Similar situation was observed in Caspase-3 activity and the difference between the groups was statistically significant ($P<0.05$) (Figure 2).

Micronucleus Test in Zebrafish Erythrocytes

MN rates that observed in each experimental groups are shown in Figures 3 and 4. All tested COL doses did not alter the MN rates as compared to the control fish. The insignificant alterations ($P>0.05$) among the control and MN treatment groups revealed a non-genotoxic nature of COL on zebrafish blood.

Discussion

Enzyme Activities and Lipid Peroxidation Level

In our study, it was determined that low dose of COL supported antioxidant system. In addition, low concentrations of COL yielded lower results in brain MDA levels as compared to control group. Likewise, the determined enzyme activities for these groups (5 and 10 mg/l) supported our findings that oxidative stress did not occur due to COL exposure. As supporting the present findings, Türkez, Geyikoğlu, Tatar, Keleş, & Özkan, (2007) reported that boron compounds including boric acid, borax, ulexite and COL supported antioxidant enzyme activities and showed non-genotoxic damage potentials. In this line, the result of study by Geyikoğlu & Türkez (2008) also supported our findings which revealed that boron compounds increased antioxidant enzyme activities at relatively low concentrations (<20 mg/l) and decrease at high concentrations(>160mg/l). In another study, it was reported that the antioxidant defense system of the rats was strengthened with oral borax supplementation (Pawa & Ali, 2006). Similarly, Nielsen (1994) reported that boron led to increases of SOD levels. In this study, increased SOD activities could be effective by increasing antioxidant capacity (Hunt 1998; Alak *et al.,* 2013). When the results obtained from this study were examined, it was found that borax (a borate like COL) supported antioxidant activity due to increased SOD activity. In addition, the increase in GPx activity in brain tissue is thought to be an adaptive response to boron intake (Mohora, Boghianu, Muscurel, Dute, & Dumitrache, 2002; Alak et al. 2019d). Some researchers had reported that the effects of boron derivatives on CAT enzyme may differ even in different tissues of animals (Verbitskaya, 1975; Hunt, Herbel, & Idso, 1993). Paraoxonase enzymes exhibit a highly athero-protective effect by hydrolyzing lipid hydroperoxides and prevent oxidative damage formation (Draganov & La Du 2004). Our study is the first report in aquaculture that COL gives higher results in enzyme activities as compared to control, statistically these increases are important and at the same time, COL promotes antioxidant enzyme activities. Boron compounds are known to have negative effects on GPx at lower levels than those associated with other enzymes. At this point, a clear dose-response relationship between boron compounds and this enzyme can be established. It has been reported that selenium has significant roles on the activity of this enzyme (Al-Saleh & Al-Doush, 1997; Turkez et al. 2007). However, although we know that boron compounds do not only interact with selenium, we cannot say that the effects on the antioxidant enzymes of boron compounds are solely caused by selenium. The protective effect of COL was investigated and positive impact were observed in this study. As known, the increase in ROS causes a rise in lipid peroxidation and MDA levels. Free radicals affect lipids, proteins, carbohydrates and important cell structures. Lipid peroxides rapidly decompose to form reactive carbon compounds. One of the important structures that occur is MDA and the increasing of MDA has been accepted as a physiologically important indicator of oxidative stress (Nielsen *et al.,* 1997; Alak *et al.,* 2017a; 2017b; 2019d). MDA level was found to be lower in low and medium dose COL applied groups than in high dose group and it has been determined that this situation is parallel with previous studies that performed on different organisms or with different borate compounds (Ince, Kucukkurt,

Table 1. Alteration in oxidative parameters after exposure to COL on zebrafish *(Danio rerio)* brain (Mean±SD)

Treatments	CAT (EU/mg)	SOD (EU/mg)	GPx (EU/mg)	PON (U/ml)	AR (U/ml)	MPO (U/ml)	MDA (nmol/protein)
Control	0.734±0.44^{a}	1.069±0.36^{a}	1.175±0.25^{a}	116.310±7.35^{a}	101.221±7.84^{a}	65.344±18.29^{c}	0.149±0.04^{c}
5mg/l	0.459±0.19^{b}	0.735±0.14^{b}	0.626±0.108^{b}	97.281±12.51^{b}	90.862±4.34^{ab}	138.918±37.60^{b}	0.249±0.08^{bc}
10mg/l	0.344±0.13b^{c}	0.686±0.15^{b}	0.592±0.07^{bc}	87.956±6.98^{b}	79.651±12.30^{bc}	142.488±22.64^{b}	0.401±0.14^{b}
20mg/l	0.243±0.10^{c}	0.506±0.19^{b}	0.413±0.17^{c}	36.753±6.62^{c}	70.796±12.78^{c}	181.469±23.21^{a}	0.735±0.21^{a}

Lowercase superscripts (a, b, c, d) indicate significant differences among same column within each experimental treatment group, P<0.05

Table 2. Alteration in oxidative parameters after exposure to COL on zebrafish *(Danio rerio)* liver (Mean±SD)

Treatments	CAT (EU/mg)	SOD (EU/mg)	GPx (EU/mg)	PON (U/ml)	AR (U/ml)	MPO (U/ml)	MDA (nmol/protein)
Control	0.658±0.35^{a}	1.138±0.12^{a}	0.871±0.13^{a}	127.679±22.48^{a}	99.590±9.80^{a}	6.687±2.02^{b}	0.318±0.02^{b}
5mg/l	0.502±0.20^{ab}	1.155±0.10^{a}	0.532±0.10^{b}	67.224±14.56^{b}	97.750±6.03^{a}	12.899±7.84^{b}	0.241±0.09^{b}
10mg/l	0.399±0.18^{b}	0.894±0.16^{b}	0.434±0.14^{b}	50.285±13.09^{c}	83.960±8.00^{b}	18.306±14.09^{ab}	0.418±0.03^{ab}
20mg/l	0.380±0.15^{b}	0.654±0.09^{c}	0.230±0.06^{c}	19.104±7.07^{d}	79.380±7.46^{b}	29.449±20.53^{a}	0.621±0.02^{a}

Lowercase superscripts (a, b, c, d) indicate significant differences among same column within each experimental treatment group, P<0.05

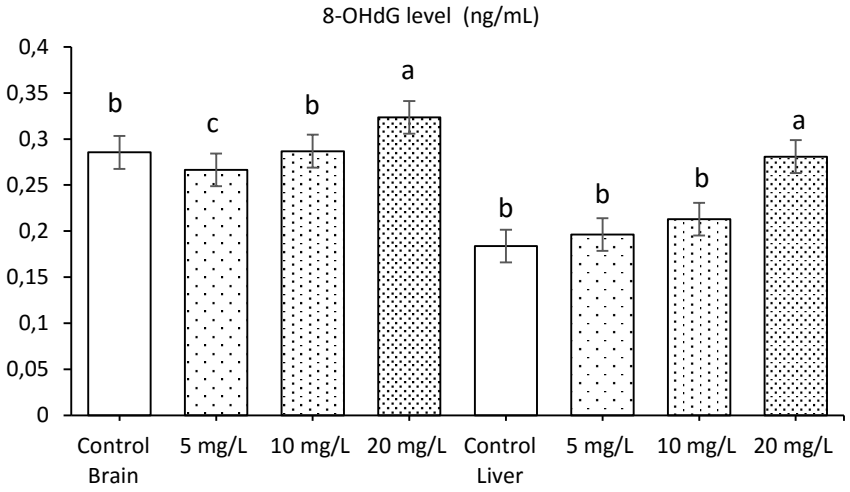

Figure 1. The effect of COL on 8-OHdG level in zebrafish (*Danio rerio*) brain and liver. Lowercase superscripts (a, b, c) indicate significant statistical differences among the experimental groups, P<0.05.

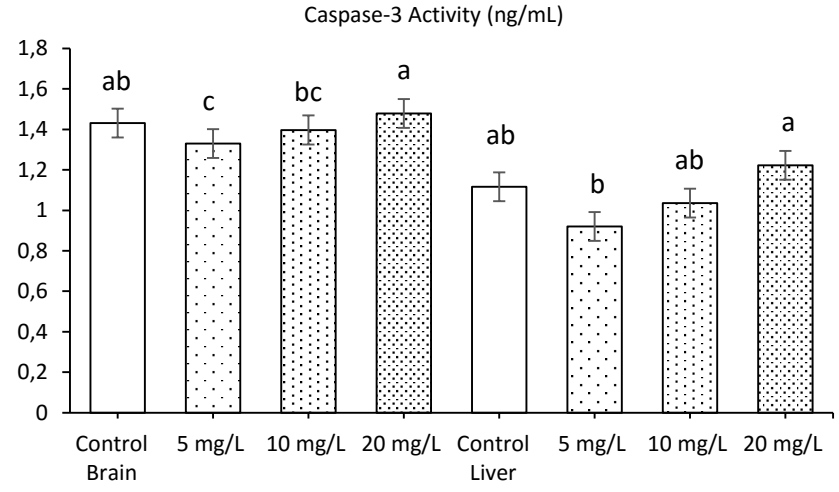

Figure 2. The effect of COL on Caspase-3 activity in zebrafish (*Danio rerio*) brain and liver. Lowercase superscripts (a, b, c, d) indicate significant statistical differences among the experimental treatment groups, P<0.05.

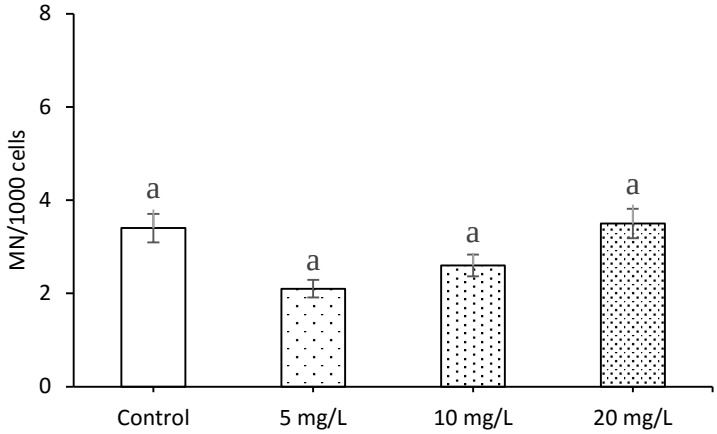

Figure 3. Micronucleus formation rates in *Danio rerio* erythrocytes after treatment with different colemanite doses. Values are presented as mean ± SD of four repetition, P<0.05.

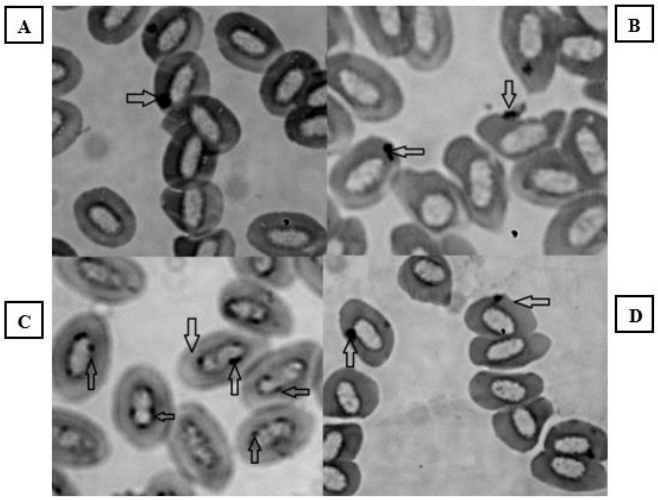

Figure 4. The sample micronucleus images of the experimental groups. A. 5 mg/L of COL; B. 10 mg/L of COL; C. 20 mg/L of COL; D. Control (Arrows show the MN formation).

Cigerci, Fidan, & Eryavuz, 2010; Alak *et al.,* 2018; 2019a; 2019b; 2019c). Boron had been reported to create protective effects by both blocking LPO and increasing antioxidant defense system activity (Küçükkurt, Acaröz, Demirel, İnce, & Eryavuz, 2017). Reduction of MDA modulation and strengthening of antioxidant system are thought to be effective in PON activation in COL treated groups (Ozdulger et al. 2003; Prıce, Uras, Banks, & Ercal,2006; Jasna, Anandbabu, Bharathi, & Angayarkanni, 2014). The results of arylesterase enzyme activity in brain and liver tissue in all COL administrated groups are consistent with paraoxonase activity results and may have emerged with similar mechanisms.

Although the biochemical function of boron is not yet fully known, it has been reported to act as an indirect proton donor, which has a specific effect on cell membrane structure and function (Barr, Barton, & Schull, 1996). Accordingly, it has been reported that boron compounds (boric acid and borax) may affect oxidative phosphorylation metabolism in mitochondria by increasing cAMP levels and inhibit hydrolytic enzyme activities (Hall *et al.,* 1980). In addition, it was reported that increases in cAMP levels may increase antioxidant enzyme activities (Sugino, Karube-Harada, Sakata, Takiguchi, & Kato, 2002). Again, in another study, boron was reported to prevent oxidative damage, by increasing glutathione and analogues or other neutralizing agents (Turkez, Geyikoglu, & Colak, 2011).

DNA Damage Grade and Apoptosis Level

Alterations in enzyme activity and DNA damage are widely used as important biomarkers to reduce the geno-toxicity and oxidative stress of natural and / or man-made chemical materials (Celikezen, Toğar, Özgeriş, Izgi, & Türkez, 2016). Increased levels of MDA and MPO obtained at the highest dose of COL (20 mg/l) indicated the occurance of oxidative stress. The increased 8-OHdG levels are confirmed by MDA increase. Diffusible MDA reacts with the nitrogen bases of the DNA (Frank, & Massaro, 1980; Freeman, & Crapo, 1982; Niki, Yoshida, Saito & Noguchi, 2005). It is thought

that, boron containing compounds exhibit modulating effect on 8-OHdG levels as determined in applications with low and medium doses of COL (5 and 10 mg/l) (Alak et al. 2018).

The last step in the mechanism of apoptotic damage is activation of caspase 3. Caspase 3 is induced by some caspase genes such as *CASP1, CASP8, CASP9* and *CASP12*. In parallel with this information, the highest dose of COL increased the caspase 3 level. The main issue that needs to be clarified here is to what extent the caspase 3 is activated by the application of COL.

The increase in caspase 3 in brain tissues with high dose (20 mg/l) is also parallel to the decrease in enzymes activity in the same group. It can be said that this situation causes an increase in apoptotic cells due to disruption of enzyme balance in the body and change of apoptosis pathway. These data were supported by high levels of MDA in tissues as well as 8-OHgD level and MPO activity.

As in the present study, boron containing (ulexite, colemanite, borax, etc.) agents used in therapy might induce apoptosis that activates caspase 3 and suppressed certain mechanisms (Hazman, Bozkurt, Fidan, Uysal, & Çelik, 2018). Apoptosis suppressing by borax has also been reported in a small number of aquaculture studies investigating the anti-apoptotic effect of borax on toxicity models (Alak et al. 2018; 2019a; 2019b; 2019c). Boron supplementation was found to reduce tissue antioxidant defense but also geno-toxicity (Yazıcı, Aksit, Korkut, Sunay, & Çelik, 2014). In our study, it was determined that COL, in low doses, had no negative effect on apoptosis and DNA damage. We can evaluate this situation as strengthening tissue antioxidant defense with COL supplementation. Similarly, Khaliq et al. (2018) reported that low doses of boron regulate cellular apoptosis by overcoming oxidative stress. Similar to our results, it had been reported that 10 mg / L boron supplement inhibits apoptosis in a previous study (Jin *et al.,* 2017).

Micronucleus in Zebrafish Erythrocytes

There is no performed investigation on evaluation of geno-toxic damage potential by COL on aquatic organisms. Therefore, in this study, geno-toxic evaluations were performed by using MN assay to provide more reliable information on COL. The obtained data showed that there was no significant increase in MN rates in erythrocytes exposed to COL as compared to control values. These findings are consistent with previous evaluations (Celikezen, Turkez, Togar, & Izgi, 2014; Celikezen *et al.,* 2016). At the end of the study, it was determined that COL had no geno-toxic damage potential. These results are in line with the previous findings showing that boron compounds (such as borax, boric acid) are non-genotoxic in cultured human blood cells (Turkez *et al.,* 2007, Turkez, Tatar, Hacimuftuoglu, & Ozdemir, 2010; Geyikoglu & Turkez 2008; Turkez, 2008; Celikezen *et al.,* 2014; 2016). Gülsoy, Yavas, & Mutlu (2018), was expressed in their study; the same type of borax and boric acid compounds may differ significantly depending on the time (increase in toxicity at 24 hours and decrease in 48, 72 and 96 hours). At 96 hours, this rate was still higher than the negative control level at all doses administered. They attributed the determined reductions to the repair of cytoprotective and tolerance mechanisms or damaged DNA in the cell.

These previous data and the present findings clearly revealed that COL and other boron compounds have no DNA damaging action or genotoxic damage potentials in both *in vitro* and *in vivo* conditions. Gulsoy et al. (2018) were assumed that boron compounds administered in vitro are not sufficient to show their genotoxic potential separately in short periods (1-2 hours) and low concentrations (1.25-5mg / L).

Conclusion

This study clearly demonstrated that COL, one of the most important commercial boron compounds, promoted antioxidant capacity at 5 and 10 mg/l concentrations in fish liver. Our results also showed that low concentrations of COL did not cause to oxidative stress, DNA damage and apoptosis generations in the brain and liver. In addition, low doses of COL (5 and 10 mg/l) were found to be more effective in preventing oxidative stress than higher dose (20 mg/l). Finally, we revealed that COL did not induce geno-toxicity zebrafish, there was no significant increase in MN frequency, oxidative stress, apoptosis and DNA damage levels.

As a conclusion, boron compounds especially COL can be used safely, but it would be beneficial to consider the cellular damages that are likely to develop due to oxidative stress at its excessive doses. The findings may be useful to explain possible side effects of boron compounds at excessive amounts or their effectiveness in treatment protocols.

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