UDC 615.849.19:616.36 *https://doi.org/10.31861/biosystems2021.01.014*

THE EFFECT OF LOW-LEVEL LASER IRRADIATION ON FREE RADICAL PROCESSES IN THE MITOCHONDRIAL FRACTIONS INDUCED BY THE BISPHENOL A ADMINISTRATION

V. BORSCHOVETSKA, M. MARCHENKO, V. IVANTSIV

Biochemistry and Biotechnology Department
Yuriy Fed'kovych Chernivtsi National University,
Ukraine, Kotsyubynsky 2, Chernivtsi, 58012
e-mail: v.borschovetska@chnu.edu.ua

Bisphenol A (BPA), the xenoestrogen and plasticizer, can induce mitochondrial dysfunction via the shift in the balance between oxidants and antioxidants. Low-level laser irradiation may influence oxidative stress parameters by changing the activity of antioxidant enzymes and the production of ROS. Our study aimed to investigate the effect of low-level laser irradiation on oxidative stress parameters in hepatic mitochondrial fractions of rats under the conditions of BPA administration. The BPA was administered per os daily for 3 days at a dose of 50 mg/kg body weight. Low-level laser irradiation was performed after each or last administration of xenobiotic. The activity of antioxidant enzymes and the content of free radicals was spectrophotometrically determined in the mitochondrial fraction of the liver. Short-term BPA exposure results in the induction of free radical processes in hepatic mitochondria by the enhanced generation of $O_2^{\bullet -}$ and decreased activity of antioxidant enzymes. At the same time, low-level laser irradiation reduces the prooxidant effect of this xenobiotic in mitochondria by the enhancement of the antioxidant activity, which is primarily associated with conformational changes induced by a short-term increase in the temperature of light-absorbing biomolecules. This effect was observed only in the case of LLLI after BPA exposure.

Keywords: low-level laser irradiation, bisphenol A, antioxidant system, free radicals

Introduction. Low-level laser irradiation/therapy (LLLI/LLLT), phototherapy or photobiomodulation refers to light therapy that uses either coherent light sources (lasers) or non-coherent light sources consisting of filtered lamps or light-emitting diodes (LED) or, on occasion, a combination of both. These treatments were use of photons at a non-thermal irradiance and originally referred to as "low-level laser" because the light is of low intensity compared with other forms of medical laser treatment, which are used for ablation, cutting, and coagulation (Avci et al. 2013; Zecha et al. 2016). Among many possible mechanisms potentially responsible for the LLLI effects at the cellular level has been associated with the primary reactions involving absorption of specific wavelengths of light by specific photo acceptor molecules, the activation of signalling pathways and transcription factors (De Freitas and Hamblin 2016).

The most of current evidence suggests that mitochondria are responsible for the cellular response to red visible and NIR light. The effects of laser irradiation on mitochondria isolated from rat liver have included increased proton electrochemical potential, more ATP synthesis, increased RNA and protein synthesis, increases in oxygen consumption, membrane potential, and enhanced synthesis of NADH and ATP. These effects are the result of absorption of red and NIR light by mitochondrial chromophores, in particular components of the mitochondrial respiratory chain such as cytochromes, cytochrome oxidase, and flavin dehydrogenases. But it was proposed that cytochrome c oxidase (Cox) is the primary photo acceptor for the red-NIR range in mammalian cells and plays a vital role in the activation of the diverse biological cascade observed subsequently to laser irradiation. Cox is a terminal enzyme of the electron transport chain and plays a vital role in the bioenergetics of a cell. The absorption of photons by Cox leads to electronically excited states and consequently can lead to the quickening of electron transfer reactions and results in changes in reduction-oxidation reaction (REDOX) status. These primary reactions stimulate a cascade of secondary reactions at the cellular level, involving photodissociation of inhibitory nitric oxide from CCO leading to enhancement of enzyme activity, electron transport, mitochondrial respiration and adenosine triphosphate (ATP) production. In turn, altered the cellular redox state by LLLI induces the activation of numerous intracellular signalling pathways, and alters the affinity of transcription factors concerned with cell proliferation, survival, tissue repair and regeneration. Consequently, a cascade of events occurs in the mitochondria, leading to biostimulation of various processes (Farivar, Malekshahabi, and Shiari 2014; Avci et al. 2013; Baxter et al. 2017; Jówko et al. 2019).

The main medical applications of LLLT are reducing pain and inflammation, augmenting tissue repair and promoting regeneration of different tissues

and nerves, and preventing tissue damage in situations where it is likely to occur (Avci et al. 2013; Tomazoni et al. 2019). In addition, previous literature reviews indicated promising effects of LLLT (PBM) for liver injury correction. For example, therapy effect of LLLI was observed in a murine model of colorectal liver metastases (Lin et al. 2011), rat model with hepatocellular carcinoma(Thompson et al. 2013), intraoral wound healing in rat liver (Erdemli et al. 2018), hepatic ischemia-reperfusion injury (Takhtfooladi, Takhtfooladi, and Khansari 2014), liver regeneration following acute hepatectomy (Oron et al. 2010) included the in the xenobiotic-induced liver injury. In particular, *Oliveira-Junior et al.* in the study shown the beneficial effects of LLLI on liver function and structure in an experimental model of CCl_4 -induced cirrhosis (Oliveira-Junior et al. 2013).

Persistent organic pollutants (POPs) are organic compounds resistant to degradation and can bioaccumulate in the environment, affecting human health. Today, as in the past, many POPs are used to produce fertilizers, pharmaceuticals, and pesticides. As a consequence, these chemicals have contaminated water, air, and soil, and high concentrations of POPs have been found in animal and human tissues, milk, and blood (Cimmino et al. 2020). Bisphenol A (BPA) is a near-ubiquitous substance and one of the highest volume chemicals produced worldwide. The global bisphenol A market is projected to reach approximately 7,348K tons by the end of 2023 (Sonavane and Gassman 2019). It is widely used for manufacturing epoxy resins, which are found in the protective lining of plastic food containers, healthcare equipment, steel drums and pipes. BPA is a food contact material and is thus practically ubiquitous in household kitchenware and canned food items. BPA is also found in eye-ware, optical devices and medical equipment. BPA is also an additive in the manufacture of polyvinyl chloride plastics, which have wide applications in healthcare consumables, piping, wire insulation and construction materials (Wazir and Mokbel 2019).

The liver is the primary organ responsible for BPA metabolism in humans and animals. Therefore, it could be largely exposed to BPA and could be susceptible to lower doses, than other organs. It is known that BPA affects the liver through the induction of oxidative stress by the enhanced generation of free radicals. Increased ROS from BPA exposure also induces antioxidant depletion, impacts mitochondrial function, alters cell signalling pathways, and can induce cell death. Both nanomolar and micromolar doses of BPA can accumulate in the mitochondria of cells, due to the lipophilic nature of BPA, and induce mitochondrial dysfunction by increasing mitochondrial ROS and altering mitochondrial membrane potential (Sonavane and Gassman 2019; Mahdavinia et al. 2019; Eid, Eissa, and El-Ghor 2015).

Based upon the oxidative stress that is induced by BPA exposure in the hepatic mitochondria and the antioxidant effects of LLLI, in the current study, we decided to evaluate the effects of LLLI on oxidative stress markers in hepatic mitochondria of BPA-exposure rats. We evaluated the generation of superoxide, hydroxyl radical and the content of hydrogen peroxide, the content of markers of lipid peroxidation and protein oxidative damage, catalase, superoxide dismutase and glutathione peroxidase enzyme activities in mitochondria of rats' liver under the conditions of BPA administration and LLLI in case of different mode irradiation. Thus, this study may provide information about the antioxidant mechanisms of LLLI in liver diseases.

Materials and Methods. Male Wistar rats (10-12 weeks of age and with body weight 120±10 g) were maintained at 22°C with a 12-h:12-h light/dark cycle and had free access to a normal chow diet and tap water. Animal handling and manipulation were conducted in accordance with regard to NIH Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 2011).

To investigate the effect of LLLI on the toxicity of BPA, a total of 50 rats were randomly divided into 5 groups consisting of 10 rats. Group I was administered daily 3 μl/kg/day body weight of pure corn oil (control group, C). Group II was administered daily 50 mg/kg/day body weight of BPA for 3 days (once a day, BPA was dissolved in corn oil) (National Toxicology Program U.S. Department of Health and Human Services and Center 2008). Group III was administered daily 50 mg/kg/day of BPA and irradiated with laser after 6 hours each (BPA+LLLI group) administration of xenobiotic, group IV was administered daily 50 mg/kg/day body weight of BPA for 3 days and then LLLI was conducted (BPA+$LLLI_a$ group). A laser diode (50 mW) with 650 nm continuous wavelength was applied to the skin surface at the anatomical site of the liver. The experimental groups were irradiated with a dosage of 1,5 J/cm^2 and power density 12.5 mW/cm^2, and the duration of irradiation was selected 120 s. The distance between the laser source and the surface of the application was 10 cm.

At 24 hours after receiving the last BPA dose or irradiation, animals were anesthetized with an intraperitoneal injection of ketamine (87.5 mg/kg) and xylazine (12.5 mg/kg) mixture (S. Bliss 2012). The liver was excised, rinsed with physiological saline, weighed, flash-frozen in liquid nitrogen, and stored at 80°C until.

The procedure yields mitochondria and is performed according to the methods described by *Kitaga-*

wa and *Sugimoto* (Kitagawa, Y., Sugimoto 1980). A piece of liver weighing 1 g was homogenized in 10 ml of a medium containing 0.25 M sucrose, 5 mM Tris-HCl (pH 7.4) and 1 mM EDTA (sucrose medium) using a Potter homogenizer. After removing the pellet sedimenting at 900 g for 10 min, the mitochondrial fraction was sedimented at 10000 g for 10 min from the homogenate. The mitochondrial pellet was suspended in 20 ml of sucrose medium and was frozen overnight at -20°C.

The protein concentration in all samples was determined by the method of *Lowry et al*. using bovine serum albumin (BSA) as standard (Lowry et al. 1951). The purity of the mitochondrial fraction was assessed by estimating succinate dehydrogenase activity, which was determined by the method of *Slater et al.* (Slater and Borner 1952).

The catalytic activity of catalase (CAT) was assessed by the spectrophotometric method according to *Goth* (Goth 1991), which measures the stable complex formation of hydrogen peroxide with ammonium molybdate. The optimal conditions for the assay were as follows: 0.2 ml samples were incubated in 1.0 ml substrate (65 mM hydrogen peroxide in 60 mM sodium-potassium phosphate buffer, pH 7.4) at 37°C for 60 s. The enzymatic reaction was stopped with 1.0 ml of 32.4 mM ammonium molybdate and the yellow complex of molybdate and hydrogen peroxide was measured at 405 nm.

Glutathione peroxidase activity (GPx) was measured based on the nonenzymatic oxidation of reduced glutathione (GSH) (Paglia, D.E., Valentine 1967). The reaction mixture contained 1 ml of 300 mM sodium phosphate buffer, pH 7.4, that contains 6 mM EDTA, 12 mM NaN_3, and 0.5 ml of 2.5 mM reduced form of glutathione (GSH). After the addition of 0.2 ml of samples to the reaction mixture, the enzymatic reaction was initiated by the addition of 0.5 ml of 1.8 mM H_2O_2. The enzymatic reaction was stopped after 2 min with 1.0 ml of 10% TCA and centrifuged at 3000 g for 15 min. The supernatant was measured at 260 nm.

Superoxide dismutase (SOD) activity was measured by the method of *Misra* and *Fridovich* (Misra and Fridovich 1972), based on the ability of SOD to inhibit the autoxidation of adrenaline to adrenochrome in an alkaline medium. 0.1 ml of SOD sources were added to epinephrine (0,18%), and 0.2 M sodium carbonate at pH 10.65 and the rate of adrenochrome formation was recorded at 347 nm.

The detection of the intensity of superoxide anion radical generation in mitochondrial fractions based on the reduction of nitroblue tetrazolium by superoxide anion radical. The technique has been described previously in detail (Auclair C., Voisin 1985). The reaction mixture contained of the sample (1.5 mg of protein) and 3% NADH The mixtures were kept in a water bath at 37°C for 10 min. After incubation 0.2% NBT in 0.1 M Tris-HCl buffer (pH=7.4) was added to the reaction mixture and was incubated at 37°C for 5 min. Then 2 ml of chloroform:dimethylsulfoxide solution at v/v of 1:2 was added and the mixture stirred vigorously for 1 min and centrifuged for 5 min at 1500 g. The supernatant was measured at 540 nm. Determination of the amount of superoxide radicals formed was performed according to the calibration graph constructed using solutions of different concentrations of diformazan obtained by oxidation of NBT.

The intensity of hydroxyl radical was determined by the method of *Halliwell et al.* (Halliwell and Gutteridge 1989). The assay is based on the quantification of the deoxyribose degradation product, which forms a pink chromogen upon heating with TBA. The reaction mixture (in total volume was 0,5 ml) contained sodium phosphate buffer (20 mM, pH 7.4), 20 mM deoxyribose, 1mM H_2O_2 and sample (20 mg of protein). The mixtures were kept in a water bath at 37°C for 30 min. After incubation 0.5 ml of 1% cold TCA in 50 mM, NaOH was added to the reaction mixture followed by 0.5 ml of 2.8% trichloroacetic acid. The mixture was heated at 100°C for 20 min and then cooled down with water. The absorbance of the solution was measured at 532 nm with a spectrophotometer. The intensity of OH-radical generation was expressed in μmol/min/mg of protein.

Determination of hydrogen peroxide concentration was determined by the method of *Gupta* (Gupta 1973). The basic principle of this method is the oxidation of ferrous ions by the sample oxidizing agents to ferric ions, which bind with xylenol orange to give a colored complex. The optimal conditions for the assay were as follows: 0.05 ml of mitochondrial fraction was added to 0.95 ml FOX reagent (100 μM xylenol orange, 250 μM ferrous ammonium sulphate, 100 mM sorbitol, 25 mM H_2SO_4), vortexed and incubated for 40 min at room temperature. The absorbance was read at 560 nm and H_2O_2 content was expressed in μmol/mg of protein.

The extent of lipid peroxidation in subcellular fractions of the liver was assessed by measuring the content of thiobarbituric acid-reactive substances (TBARS) according to the method of *Ohkawa H. et al.* (Ohkawa, Ohishi, and Yagi 1979). 0.1 ml of mitochondrial suspension and 2.5 ml of 25 mM tris-HCl (pH=7,4), that contained 0,175 M KCl were put into a test tube. 1 ml of 17% TCA was added and a mixture was centrifugated for 10 min at 4000 g. 2 ml of supernatant was added to 1 ml of 0,8% TBA and tubes were heated at 100°C for 10 min and then cooled down with water. The absorbance of the solution was measured at 532 nm with a spectrophotometer. The content of TBARS was expressed in nmol/mg of protein.

The extent of protein oxidation in subcellular fractions of the liver was assessed by measuring the

content of protein carbonyl groups, using 2,4-dinitrophenylhydrazine derivatization as described by *Levine et al.* (Levine et al. 1990). 0.1 ml of mitochondrial fraction was added to 1ml of 20% TCA and treated with 1 ml of 0.1 M DNPH or 1 ml of 2 N HCl (control tube). Samples were incubated at 37°C for 1,5 h and centrifuged for 10 min at 3000 g. Pellets were washed three times with 1 ml of the ethanol:ethyl acetate mixture (1:1, v/v). The pellet was then dissolved in 3 ml of 8 M guanidine hydrochloride and the carbonyl content was determined by reading the absorbance at 370 nm. The content of protein CO groups was expressed in nmol/mg of protein.

Protein thiol groups were measured by the method of *Murphy et al.* (Murphy and Kehrert 1989) using Ellman reagent. For these 0.1 ml of mitochondrial fraction was added to 1ml of 20% TCA and centrifuged for 10 min at 3000 g. The protein pellet was washed once with 2 ml of 0.3 M $HClO_4$ containing 5 mM-EDTA and then centrifuged for 10 min at 3000 g. The pellet was suspended in 3 ml of 8 M-guanidine hydrochloride and 3 ml of 0.05 M KH_2PO_4/5 mM-EDTA, pH 7.4. Tubes were heated at 100°C and centrifuged at 3000 rpm for 10 min and 2 ml of supernatant was mixed in a test tube with 1 ml of 0.05 M KH_2PO_4/5 mM-EDTA, pH 7.4, and 20 µl of 2 mM-DTNB (control tube – without DTNB). All samples were read after 30 min at 412 nm and the content of protein thiol groups was expressed as nmol/mg of protein.

Each result is expressed as mean±SD from three experiments (n=3). Differences among groups were then evaluated by one-way analysis of variance (ANOVA) followed by Tukey's HSD post hoc analysis for multiple comparisons. The results were considered statistically significant at $P<0,05$.

Results and discussion. In the present study, the effect of low-level laser irradiation on oxidative stress parameters in mitochondria of rats under the conditions of BPA administration was investigated. Bisphenol A (BPA), a key monomer in the production of polycarbonate plastics and epoxy resin, is widely used in a variety of products, including baby bottles, food storage containers, and dental sealants. A trace amount of BPA released from these products leads to human exposure and the development risk of an effect on metabolic processes in the organism. Many of the physiological effects of BPA have been described in the context of the ability of the active aglycone form to interact with classic estrogen receptors. But severe studies demonstrated effects on the liver, kidney and body weight at doses of 50 mg/kg bw and higher (Moon et al. 2012; "Toxicological and Health Aspects of Bisphenol A: Report of Joint FAO/WHO Expert Meeting 2-5 November 2010 and Report of Stakeholder Meeting on Bisphenol A, 1 November 2010 Ottawa, Canada" 2011). Toxic effects of BPA in liver tissues are mostly mediated by increased oxidative stress associated with elevated production of toxic free radicals such as bisphenol-3,4-quinone, 5-hydroxy BPA, isopropyl-hydroxyphenol, a glutathione conjugate of BPA, glutathionyl -phenol, glutathionyl 4-isopropyl phenol and BPA dimers (Amjad, Rahman, and Pang 2020; Kang, Katayama, and Kondo 2006). These intermediates cause oxidative stress in the hepatocytes (Ooe et al. 2005), moreover, as was shown in our previous study, the primary target of BPA radicals action is the components of endoplasmatic reticulums and cytosol, (Shmarakov, Borschovetska, and Blaner 2017). But oxidative stress in these compartments can induce mitochondrial damage, and damaged mitochondria can generate more ROS, which can be created during mitochondrial oxidative phosphorylation. Superoxide anion is produced mainly at complexes I and III of the ETC which release $O_2^{\bullet-}$ in the mitochondrial matrix and at the inter-membrane space, respectively (Ferraresi, Hamblin, and Parizotto 2012). In this study, administration of LOAEL dosage of BPA increased ROS production, as assessed by the measurement of the intensity of superoxide anion generation in hepatic mitochondria. As shown in Figure 2, a marked decrease in the intensity of superoxide anion radical generation in mitochondrial fractions was observed in BPA-treated animals. This indicator was higher at 6.2 times compared to the control group. In contrast, administration of BPA didn't have an effect on the generation of hydroxyl radical and content of hydrogen peroxide in hepatic mitochondrial fractions, which values were even lower than in the control group.

The other mechanism of inducing oxidative stress in a cell is the depletion of the antioxidant system. An antioxidant is a reducing agent that scavenges and neutralizes free radicals, thereby preventing oxidation reactions (Haider et al. 2020). In the present study, the evaluation of antioxidant enzyme activities in hepatic mitochondria under the BPA administration was demonstrated. The most decrease was recorded regarding the activity of SOD, which protects tissues from oxidative stress and damage by catalyzing the conversion of $O_2^{\bullet}$ to H_2O_2, a more stable ROS (Yin. et al. 2018) (Fig. 2 A). Possibly, BPA caused a marked oxidative impact on the enzyme's molecules. Along with a decrease in the activity of SOD, 50 mg/kg BPA administration caused a significant decrease in the CAT and GPx activity, which values were 1.7 and 1.4 times lower than the corresponding indicators of the control group (Fig. 2 B, C). The decrease in the activity of these enzymes under the BPA administration may be related to the decrease of the hydrogen peroxide, as the substrate, that was shown in our experiment.

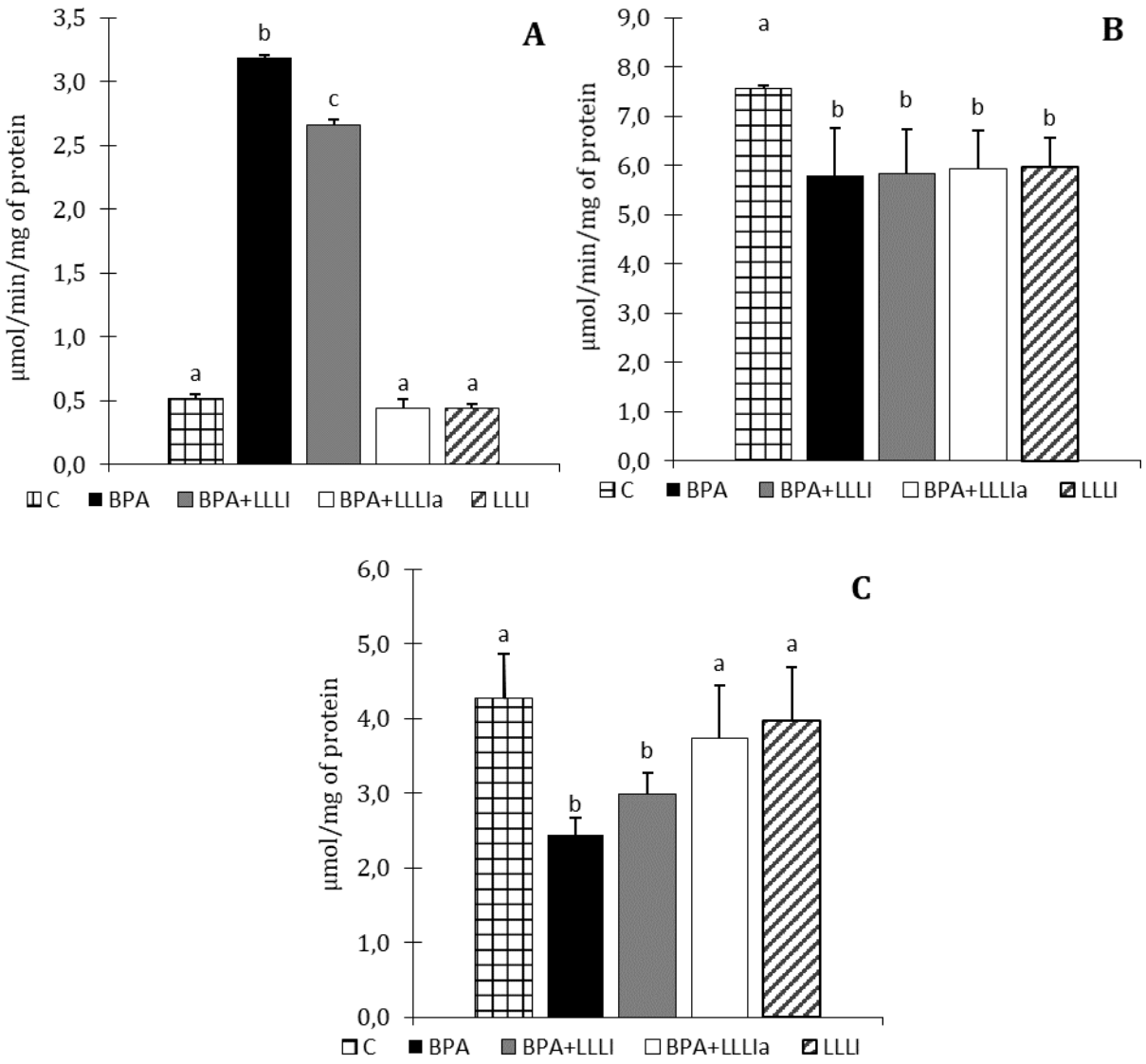

Fig. 1. The intensity of superoxide anion radical (A) and hydroxyl radical generation (B) and content of hydrogen peroxide (C) in mitochondrial fractions of rats' liver under the BPA-exposure and LLLI. Values marked with different letters (a, b, c) are statistically different, P<0.05. All values are given as the mean ± SD.

However, on the other hand, perhaps BPA negatively affects the antioxidant state in the cell? Indeed, the important role of enzymatic antioxidants, in overcoming the harmful effects of BPA has been highlighted in many studies (Hassan et al. 2012; Bindhumol, Chitra, and Mathur 2003; Kabuto et al. 2003). It was reported that treatment of rats with various doses of the BPA (0.1, 1, 10, and 50 mg/kg) led to changes in expression levels of antioxidant genes of glutathione, peroxidase (GSHPx), catalase (CAT), glutathione transferase (GST), and glutathione reductase (GR) in liver tissue. The expression of these antioxidant genes was decreased with increasing doses of the BPA (Kazemi et al. 2016). Severe oxidative stress resulted from exposure to BPA and could lead to DNA damage that can be the other mechanisms of decreased activity of antioxidant enzymes. One pathway of BPA metabolism is the hydroxylation of one of its symmetric phenyl rings to form its catechole, O-OH BPA, which can oxidize to O-quinone BPA [47] which in turn, react with DNA that causes it damage. O-quinone BPA forms predominantly depurinating adducts O-OH-BPA 6-N3 Ade and O-OH-BPA 6-N7Gua (Moustafa and Ahmed 2016). *Sakuma et al.* (Sakuma et al. 2010) detected that O-quinone BPA could increase ROS formation and oxidize the guanine moiety of deoxyguanosine in the DNA of primary rat hepatocyte culture.

The effects of the detected destruction of prooxidant-antioxidant balance by the BPA are the induction of lipid peroxidation, the increase of TBARS content in the mitochondria was evidenced of these processes. TBARS level was significantly increased by BPA administration (Fig. 3 A).

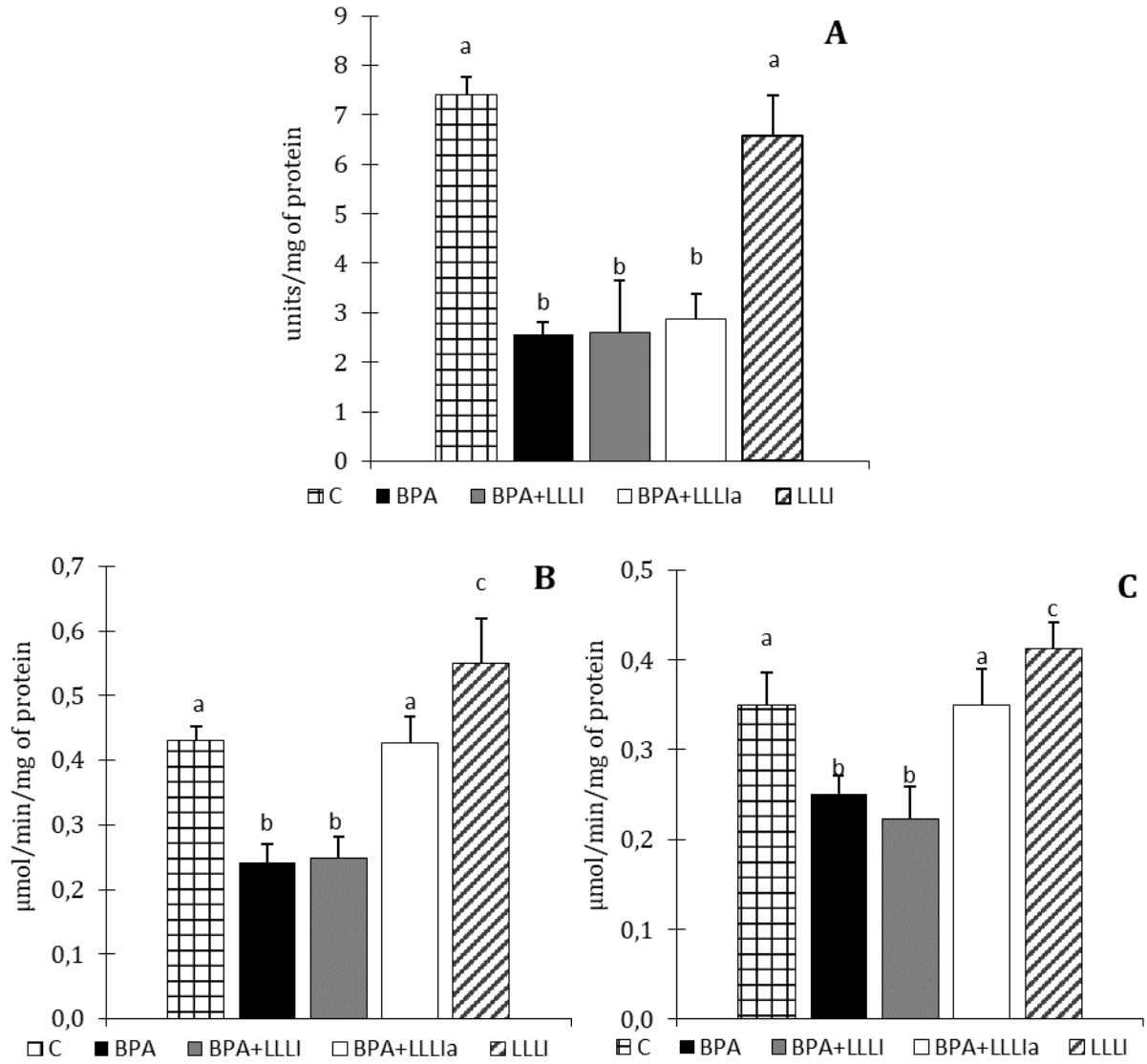

Fig. 2. The SOD (A), CAT (B) and GPx (C) activities in mitochondrial fractions of rats' liver under the BPA-exposure and LLLI. Values marked with different letters (a, b, c) are statistically different, P<0.05. All values are given as the mean ± SD.

In addition, the protein oxidative damage in this subcellular fraction occurs under these experimental conditions, primarily the oxidation of SH-groups and protein fragmentation. The level of protein carbonyl groups in mitochondrial fraction increased at 1,8-fold (Fig. 3 B) and the level of protein thiol groups decreased by 40% compared to the control group (Fig. 3 C).

Thus, the short-term administration of BPA in the 50 mg/kg of body weight accompanied the development of oxidative stress in mitochondria of rats by the enhanced free radical's generation and decreased activities of the antioxidant system. Accumulation of oxidative damage in the mitochondria can induce mitochondrial dysfunction, mitochondrial DNA depletion, and cell apoptosis.

Currently, for the correction of different metabolic abnormalities, LLLI is used. The infrared radiation emitted by LLLI seems to act on cellular energy metabolism, stimulating photochemical and photophysical events in the cell mitochondria. LLLT causes a change in the biochemical cell activity due to the transient heating of the chromophore molecules. This happens with the small dose of energy, minimally supplied if there is no increase in tissue temperature (a slight increase of 1°C is attributed to the activation of cellular metabolism) (Ferraresi et al., 2018). Photochemical and photophysical changes may result in increased mitochondrial membrane potential and higher enzyme activity in the respiratory chain. The structural change includes the formation of giant mitochondria through the merging of membranes of smaller and neighboring mitochondria. These changes enable mitochondria to provide higher levels of respiration and ATP to cells.

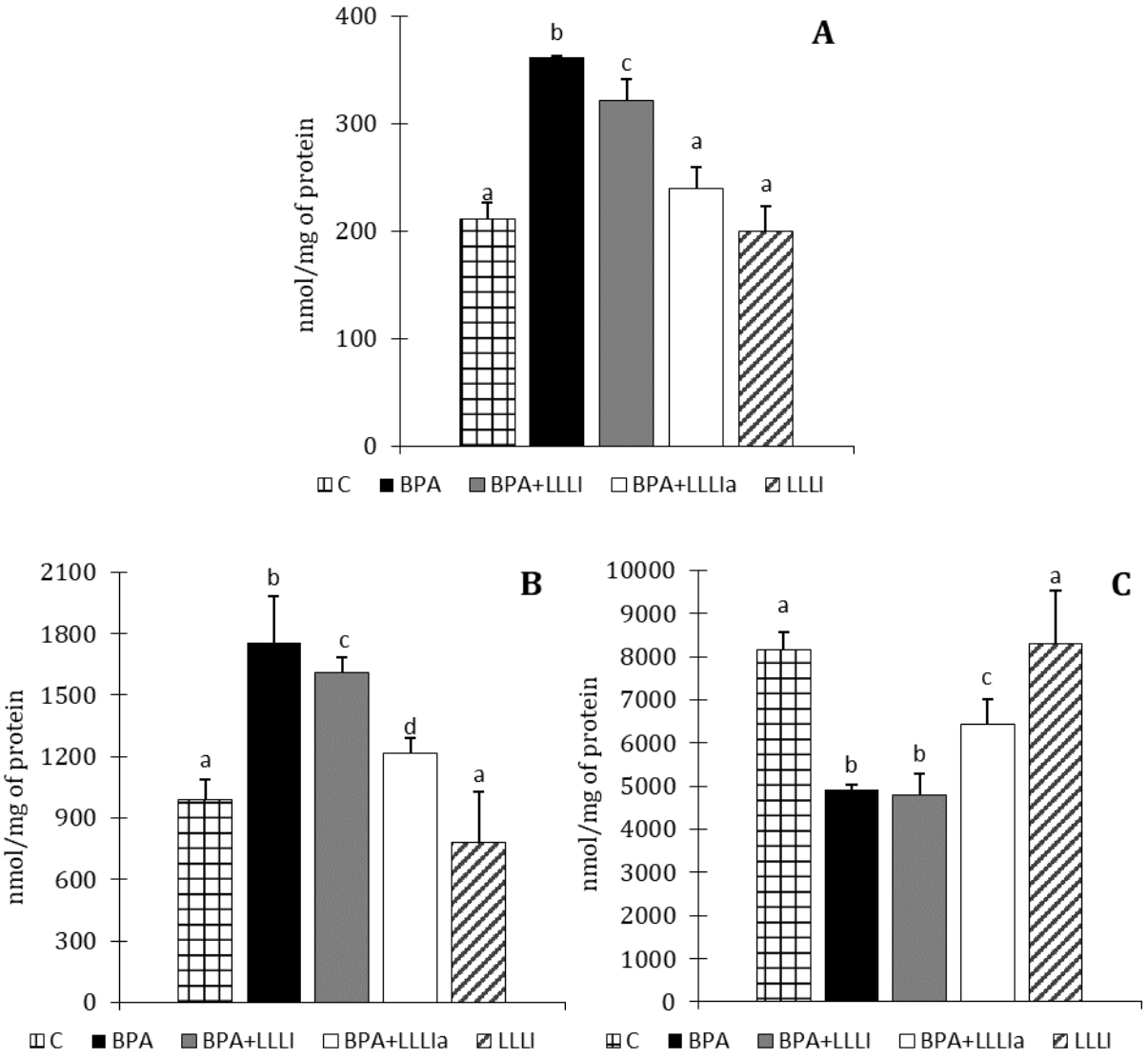

Fig. 3. Effects of LLLI on the severity of oxidative stress in hepatic mitochondria. A – the content of TBARS, B – the content of protein carbonyl groups, C - the content of protein thiol groups. Values marked with different letters (a, b, c) are statistically different, P<0.05. All values are given as the mean ± SD.

In the present study for the correction of the prooxidant effect of BPA in mitochondria, we conducted the irradiation of BPA-treated rats with the low-level diode laser simultaneously with the administration of xenobiotic. But this manipulation did not have positive results. The applicated mode irradiation did not decrease the generation of ROS and effect on the activity of antioxidant activity in hepatic mitochondria of BPA-treated animals. Values of SOD, CAT and GPx activity did not statistically significantly differ from corresponding values in the BPA-exposed group and were lower than in the control group at 2.8, 1.7 and 1.6-fold, respectively.

These, in turn, causes a higher count of markers of oxidative lipid and protein damage in mitochondria of this animals' group. The level of protein SH-groups in this group did not statistically differ from the corresponding indicator in the BPA-exposure group and was lower at 41% compared to the control group. Some change in the content of TBARS and protein carbonyl groups was recorded in the group of BPA-treated animals which simultaneously irradiated with a laser diode. These indicators were lower at 11% and 8% compared to the BPA-treated animals but were still much higher (51% and 62%) than the indicators in the control group.

But since laser therapy is characterized by a cumulative effect these results possibly was accompanied by the duration of irradiation. On the other hand, our results were probably, was accompanied by the synergistic effect of the simultaneous action of BPA and LLLI. As was noted above, enhanced formation of free radicals occurs during BPA exposure. In addition, some studies reported that the LLLI increases the availability of electrons for the reduction of molecular oxygen in the catalytic site of cytochrome c oxidase, increasing the mitochondrial membrane potential (MMP) and a short burst of ROS is produced (De Freitas and

Hamblin 2016; Ferraresi, Hamblin, and Parizotto 2012; Jackson, Roche, and Wisler 2010). The absorption of laser light accelerates electron transfer (respiratory chain) and induces an initial production of ROS, specifically by increasing the production of superoxide anion (Denadai et al. 2015).

That is why we decided to change the mode of irradiation and investigate the prooxidant effect of BPA in rats, which irradiate with laser after the BPA treatment. This result shows the positive effect of LLLI on BPA induced free radical processes. LLLI of these animals is accompanied by the increase in CAT and GPx activities, which is primarily due to conformation changes, which are induced by a short-term increase in the temperature of biomolecules, which absorb the light. This, in turn, can induce/initiate activation or inhibition of enzymes [20]. CAT and GPx activities in hepatic mitochondria were higher at 1.7 and 1.6-fold compared with the corresponding indicators of the BPA-treated group. In addition, CAT and GPx activities in mitochondria fraction of rats' liver this group was at the level in the control group and did not statistically significantly differ from these indicators in hepatic mitochondria of intact animals.

But the LLLI at applicated mode irradiation didn't affect the SOD activities in hepatic mitochondria. SOD activity in BPA+LLLI$_a$ groups did not statistically significantly differ from corresponding values in the BPA-exposed group and was lower at 2.6 times compared with the control group. These results, possibly, associated with different effects of red light on the activity of different isoforms of this enzyme, primary due to the metal in the active site of enzymes. This suppose is partly confirmed by the fact that the SOD activity in the animal of the LLLI-group was at the level of the control group. The high activity of antioxidant enzymes and low intensity of free radical generation after LLLI were shown on the amount of products of protein oxidative damage and lipid peroxidation in the hepatic mitochondrial fraction of this experimental group of animals.

The level of TBARS, as a marker of lipid peroxidation, decreased at 1.5-fold compared to BPA treated animals and did not statistically significantly different from the control group. Similar results were obtained regarding protein oxidative damage in hepatic mitochondria of this animals' group. The level of protein carbonyl groups and thiol groups, as markers of protein peroxidation in the liver, was lower at 1.4-fold and higher at 1.3-fold, respectively, compared with the BPA-treated group.

Thus, the low-level laser irradiation may be used as an instrument in the correction or therapy prooxidant effect of xenobiotic. But the selection of correct parameters LLLI is the main point of the effectiveness of the treatment because there are significant variations in dosimetry parameters: wavelength, irradiance or power density, pulse structure, coherence, polarization, energy, irradiation time and repetition regimen. And the lower dosimetric parameters can result in reduced effectiveness of the treatment and higher ones can lead to tissue damage.

Conclusions. The administration of bisphenol A was accompanied by the induction of free radical processes in the mitochondrial fraction of the liver due to increased generation of free radicals and a decrease in the main enzymatic activities of the antioxidant system. Irradiation of animals after each administration of xenobiotics did not significantly affect the activity of antioxidant enzymes and the intensity of free radical generation, which is probably due to the synergistic effect of BPA and LLLI. The effect of low-level laser irradiation of the red spectrum on free radical processes in the mitochondrial fraction of the liver is mode dependent.

Funding: This work was financed by the research project "Biochemical and laser-polarimetric parameters of complex prediction of metabolic disorders" (No. 0119U100717).

References:

1. Amjad S, Rahman S, Pang M. Role of Antioxidants in Alleviating Bisphenol A Toxicity. *Biomolecules*. 2020;10(1105):1-26. doi:10.3390/biom10081105
2. Auclair C., Voisin E. Nitroblue tetrazolium reduction. In: Greenwald RA, ed. *Handbook of Methods for Oxygen Radical Research*. Boca Raton, Fla.: CRC Press; 1985:123-132.
3. Avci P, Gupta A, Sadasivam M, et al. Low-level laser (light) therapy (LLLT) in skin: Stimulating, healing, restoring. *Semin Cutan Med Surg*. 2013;32(1):41-52.
4. Baxter GD, Liu L, Petrich S, et al. Low level laser therapy (Photobiomodulation therapy) for breast cancer-related lymphedema: a systematic review. *BMC Cancer*. 2017;17(1):833-846. doi:10.1186/s12885-017-3852-x
5. Bindhumol V, Chitra KC, Mathur PP. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Toxicology*. 2003;188(2-3):117-124. doi:10.1016/S0300-483X(03)00056-8
6. Cimmino I, Fiory F, Perruolo G, et al. Potential mechanisms of bisphenol a (BPA) contributing to human disease. *Int J Mol Sci*. 2020;21(16):1-22. doi:10.3390/ijms21165761
7. Denadai AS, Aydos RD, Silva IS, et al. Acute effects of low-level laser therapy (660 nm) on oxidative stress levels in diabetic rats with skin wounds. *J Exp Ther Oncol*. 2015;11:85-89.
8. Eid JI, Eissa SM, El-Ghor AA. Bisphenol A induces oxidative stress and DNA damage in hepatic tissue of female rat offspring. *J Basic Appl Zool*. 2015;71:10-19. doi:10.1016/j.jobaz.2015.01.006
9. Erdemli ME, Ekhteiari Salmas R, Durdagi S, et al. Biochemical changes induced by grape seed extract and low level laser therapy administration during intraoral wound healing in rat liver: an experimental and in silico study. *J Biomol Struct Dyn*. 2018;5:1-16. doi:10.1080/07391102.2017.1305297
10. Farivar S, Malekshahabi T, Shiari R. Biological effects of low level laser therapy. *J Lasers Med Sci*.

2014;5(2):58-62. doi:10.22037/2010.v5i2.5540
11. Ferraresi C, Hamblin MR, Parizotto NA. Low-level laser (light) therapy (LLLT) on muscle tissue: Performance, fatigue and repair benefited by the power of light. *Photonics Lasers Med*. 2012;1(4):267-286. doi:10.1515/plm-2012-0032
12. De Freitas LF, Hamblin MR. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J Sel Top Quantum Electron*. 2016;22(3):1-37. doi:10.1109/JSTQE.2016.2561201
13. Goth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta*. 1991;196(2-3):143-151. doi:10.1016/0009-8981(91)90067-M
14. Gupta BL. Microdetermination techniques for H2O2 in irradiated solutions. *Microchem J*. 1973;18(4):363-374. doi:10.1016/0026-265X(73)90059-3
15. Haider K, Haider MR, Neha K, Yar MS. Free radical scavengers: An overview on heterocyclic advances and medicinal prospects. *Eur J Med Chem*. 2020;204:112607. doi:10.1016/j.ejmech.2020.112607
16. Halliwell B, Gutteridge J. *Free Radicals in Biology and Medicine*. Clarendon.; 1989.
17. Hassan ZK, Elobeid MA, Virk P, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. *Oxid Med Cell Longev*. 2012;2012:1-6. doi:10.1155/2012/194829
18. Jackson RF, Roche GC, Wisler K. Reduction in Cholesterol and Triglyceride Serum Levels Following Low-Level Laser Irradiation : A Noncontrolled, Nonrandomized Pilot Study. *Am J Cosmet Surg*. 2010;27(4):177-184.
19. Jówko E, Maciej P, Cie M, Sacewicz T, Cie I, Jarocka M. The effect of low level laser irradiation on oxidative stress, muscle damage and function following neuromuscular electrical stimulation. A double blind, randomised, crossover trial. *BMC Sports Sci Med Rehabil*. 2019;11(38):1-14.
20. Kabuto H, Hasuike S, Minagawa N, Shishibori T. Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. *Environ Res*. 2003;93(1):31-35. doi:10.1016/S0013-9351(03)00062-8
21. Kang JH, Katayama Y, Kondo F. Biodegradation or metabolism of bisphenol A: From microorganisms to mammals. *Toxicology*. 2006;217:81-90. doi:10.1016/j.tox.2005.10.001
22. Kazemi S, Mousavi SN, Aghapour F, Rezaee B, Sadeghi F, Moghadamnia AA. Induction effect of bisphenol a on gene expression involving hepatic oxidative stress in rat. *Oxid Med Cell Longev*. 2016;2016:12-14. doi:10.1155/2016/6298515
23. Kitagawa, Y., Sugimoto E. Estimation of the in vivo translational activity of rat liver mitochondria without use of an antibiotic. *J Biochem*. 1980;88(3):689-693.
24. Levine RL, Garland D, Oliver CN, et al. Determination of Carbonyl Content in Oxidatively Modified Proteins. *Methods Enzymol*. 1990;186:464-478. doi:10.1016/0076-6879(90)86141-H
25. Lin WX, Fifis T, Malcontenti-Wilson C, et al. Induction of Th1Immune responses following laser ablation in a murine model of colorectal liver metastases. *J Transl Med*. 2011;9(1):83. doi:10.1186/1479-5876-9-83
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol Reagent. *J Biol Cemistry*. 1951;193(1):265-275. doi:10.1016/0304-3894(92)87011-4
27. Mahdavinia M, Alizadeh S, Atefeh Raesi V, et al. Effects of quercetin on bisphenol A-induced mitochondrial toxicity in rat liver. *Iran J Basic Med Sci*. 2019;22(5):499-505. doi:10.22038/ijbms.2019.32486.7952
28. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. Published online 1972.
29. Moon MK, Kim MJ, Jung IK, et al. Bisphenol A Impairs Mitochondrial Function in the Liver at Doses below the No Observed Adverse Effect Level. *J Korean Med Sci*. 2012;27:644-652.
30. Moustafa GG, Ahmed AAM. Impact of prenatal and postnatal exposure to bisphenol A on female rats in a two generational study: Genotoxic and immunohistochemical implications. *Toxicol Reports*. 2016;3:685-695. doi:10.1016/j.toxrep.2016.08.008
31. Murphy ME, Kehrert JP. Oxidation state of tissue thiol groups and content of protein carbonyl groups in chickens with inherited muscular dystrophy. *Biochem J*. 1989;260(2):359-364. doi:10.1042/bj2600359
32. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals*. 8th Ed. National Academies Press; 2011. doi:10.2307/1525495
33. National Toxicology Program U.S. Department of Health and Human Services, Center. *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A*. Center for The Evaluation of Risks To Human Reproduction; 2008.
34. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;95(2):351-358. doi:10.1016/0003-2697(79)90738-3
35. Oliveira-Junior MC, Monteiro AS, Leal-Junior ECP, et al. Low-level laser therapy ameliorates CCl4-induced liver cirrhosis in rats. *Photochem Photobiol*. 2013;89(1):173-178. doi:10.1111/j.1751-1097.2012.01211.x
36. Ooe H, Taira T, Iguchi-Ariga SMM, Ariga H. Induction of reactive oxygen species by bisphenol A and abrogation of bisphenol A-induced cell injury by DJ-1. *Toxicol Sci*. 2005;88(1):114-126. doi:10.1093/toxsci/kfi278
37. Oron U, Maltz L, Tuby H, Sorin V, Czerniak A. Enhanced liver regeneration following acute hepatectomy by low-level laser therapy. *Photomed Laser Surg*. 2010;28(5):675-678. doi:10.1089/pho.2009.2756
38. Paglia, D.E., Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967;70:158-169.
39. S. Bliss. Rodent Anesthesia. *Cornell Univ Institutional Anim Care Use Committee, Acup 10102*. Published online 2012:1-8.
40. Sakuma S, Nakanishi M, Morinaga K, Fujitake M, Wada SI, Fujimoto Y. Bisphenol A 3,4-quinone induces the conversion of xanthine dehydrogenase into oxidase in

vitro. *Food Chem Toxicol*. 2010;48(8-9):2217-2222. doi:10.1016/j.fct.2010.05.051
41. Shmarakov IO, Borschovetska VL, Blaner WS. Hepatic detoxification of Bisphenol A is retinoid-dependent. *Toxicol Sci*. 2017;157(1):141-155. doi:10.1093/toxsci/kfx022
42. Slater EC, Borner WD. The effect of fluoride on the succinic oxidase system. *Biochem J*. 1952;52:185-196. doi:10.1042/bj0520185
43. Sonavane M, Gassman NR. Bisphenol A co-exposure effects: A key factor in understanding BPA's complex mechanism and health outcomes. *Crit Rev Toxicol*. 2019;49(5):371-386. doi:10.1080/10408444.2019.1621263
44. Takhtfooladi MA, Takhtfooladi HA, Khansari M. The effects of low-intensity laser therapy on hepatic ischemia-reperfusion injury in a rat model. *Lasers Med Sci*. 2014;29(6):1887-1893. doi:10.1007/s10103-014-1603-7
45. Thompson SM, Callstrom MR, Knudsen BE, et al. Molecular bioluminescence imaging as a noninvasive tool for monitoring tumor growth and therapeutic response to MRI-guided laser ablation in a rat model of hepatocellular carcinoma. *Invest Radiol*. 2013;48(6):413-421. doi:10.1097/RLI.0b013e31827a4a3f
46. Tomazoni SS, Machado CDSM, De Marchi T, et al. Infrared Low-Level Laser Therapy (Photobiomodulation Therapy) before Intense Progressive Running Test of High-Level Soccer Players: Effects on Functional, Muscle Damage, Inflammatory, and Oxidative Stress Markers - A Randomized Controlled Trial. *Oxid Med Cell Longev*. 2019;2019:1-11. doi:10.1155/2019/6239058
47. *Toxicological and Health Aspects of Bisphenol A: Report of Joint FAO/WHO Expert Meeting 2-5 November 2010 and Report of Stakeholder Meeting on Bisphenol A, 1 November 2010 Ottawa, Canada*.; 2011.
48. Wazir U, Mokbel K. Bisphenol A: A concise review of literature and a discussion of health and regulatory implications. *In Vivo (Brooklyn)*. 2019;33(5):1421-1423. doi:10.21873/invivo.11619
49. Yin. W, Robyn. B, Alycia. N, Siegfired. H. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *J Cell Biol*. 2018;217(6):1915-1928.
50. Zecha JAEM, Raber-Durlacher JE, Nair RG, et al. Low level laser therapy/photobiomodulation in the management of side effects of chemoradiation therapy in head and neck cancer: part 1: mechanisms of action, dosimetric, and safety considerations. *Support Care Cancer*. 2016;24(6):2781-2792. doi:10.1007/s00520-016-3152-z.Low

ВПЛИВ НИЗЬКОІНТЕНСИВНОГО ЛАЗЕРНОГО ОПРОМІНЕННЯ НА ВІЛЬНОРАДИКАЛЬНІ ПРОЦЕСИ В МІТОХОНДРІАЛЬНІЙ ФРАКЦІЇ ІНДУКОВАНИХ ВВЕДЕННЯМ БІСФЕНОЛУ А

В. Л. Борщовецька, М. М. Марченко, В. С. Іванців

Бісфенол А (BPA), ксеноестроген і пластифікатор, що може викликати дисфункцію мітохондрій через зсув балансу між окислювачами та антиоксидантами. Низькоінтенсивне лазерне опромінення може впливати на параметри окисного стресу, змінюючи активність антиоксидантних ферментів і синтез АФК. Метою дослідження було дослідити вплив низькорівневого лазерного опромінення на параметри окисного стресу у мітохондріальній фракції печінки щурів за умов введення BPA. Ксенобіотик вводили per os щодня протягом 3 днів у дозі 50 мг/кг маси тіла. Низькоінтенсивне лазерне опромінення проводили після кожного або останнього введення ксенобіотика. У мітохондріальній фракції печінки проводили спектрофотометричне визначення активностей антиоксидантних ферментів та вміст вільних радикалів. У роботі встановлено, що короткочасне введення BPA супроводжується індукцією вільнорадикальних процесів у мітохондріях печінки за рахунок посиленого утворення $O_2^{\bullet -}$ і зниження активності антиоксидантних ферментів. У той же час лазерне опромінення низького рівня зменшує прооксидантну дію цього ксенобіотика в мітохондріях за рахунок посилення антиоксидантної активності, що пов'язано насамперед з конформаційними змінами, спричиненими короткочасним підвищенням температури світлопоглинаючих біомолекул. Цей ефект спостерігався лише у випадку опромінення тварин після впливу BPA.

Ключові слова: низькорівневе лазерне опромінення, бісфенол А, антиоксидантна система, вільні радикали

Отримано редколегією 07.04.2020