

Experimental Study on the Evaluation of Ionoregulation Enzymes, Heat Shock Protein, DNA Oxidation and Apoptosis in Male Mice Heart Tissue in Exposure of Emamectin Benzoate

Özge Temiz*

Department of Biology, Faculty of Science and Letters, Cukurova University, Adana, Turkey

*Corresponding Author: Özge Temiz, Department of Biology, Faculty of Science and Letters, Cukurova University, Adana, Turkey.

Received: January 02, 2020; Published: February 07, 2020

Abstract

Emamectin benzoate (EMB) is a biopesticide of natural origin used in agriculture, household pests and livestock. As a result of its wide range of use, it is easier to reach ecologically and affects human health. The aim of this study was to investigate the changes in apoptosis pathway in biochemical, ionoregulatory, and consequential damage caused by oxidative toxicity in heart tissue in EMB exposure in a male mice. At different doses of EMB; 25 mg/kg/day (E1), 50 mg/kg/day (E2), 100 mg/kg/day (E3) were administered by gavage to Swiss albino male mice for 14 days. Changes in heat shock protein 70 (HSP70) and thiobarbituric acid reactive substance (TBARS) levels, caspase 3 enzyme activity and DNA oxidation biomarker 8-hydroxy-2-deoxyguanosine (8-OHdG) levels, and Adenosine triphosphatase (ATPases) enzymes (Na^+/K^+ ATPase, Ca^{+2} ATPase, and Mg^{+2} ATPase) were investigated in heart tissue. Our results showed that at 14 days of exposure to EMB, it caused toxicity due to increased caspase 3 enzyme activity, HSP70 and TBARS levels in heart tissue. In addition, it were been shown to increase genotoxicity by increasing levels of DNA oxidation biomarker 8-OHdG. These changes in parameters indicate that the toxic effects of EMB are effective in the mice heart within 14 days.

Keywords: Emamectin Benzoate; Heart; Caspase 3; Stres Proteins; DNA Oxidation; ATPases; Male Mice

Introduction

Pesticides are biologically active chemicals, which are generally used in agriculture or in human health protection programs against harmful insects in agriculture and against vector diseases in human health. Pesticides which are used extensively in every field cause important health problems on ecology and human [1]. Avermectins are a variety used in pesticide groups in every field. The low toxic effect on mammals has been reported to have an effective toxic effect on target organisms. Emamectin benzoate is a semi-synthetic macrocyclic lactone produced naturally from the toxins of the soil microorganism *Streptomyces avermitilis* [2]. It shows selective activity which can be highly effective by increasing membrane chloride ion permeability and affinity to GABA receptors in living organisms with EMB application [3]. Exposure to EMB has been shown to reduce intracellular antioxidant activity in different organs in living organisms, leading to increased reactive oxygen species (ROS) and oxidative stress [4,5]. As a result of oxidative stress, cell structure; transport enzymes, fat, protein and DNA macromolecules. Abnormal gene formation as a result of DNA oxidation may result in carcinogenesis or cell death due to mutation. 8-OHdG is produced by oxidation of deoxyguanosine as a result of ROS attack in the DNA structure. The important biomarker indicating the formation of DNA oxidation is the 8-OHdG level [6]. 8-OHdG levels have been shown to play a role in cell death as a result of various induction [7]. Cell death is programmed death caused by exposure of the cell to oxidative stress in the physiological process. Caspase 3 activities are an important enzyme in apoptosis pathway in case of physiological damage of cell [8]. As a result of oxidative stress,

the cell also increases the levels of heat shock proteins (HSP). In the physiological process, HSPs play an important role in protein metabolism as molecular chaperone. HSPs are ATP-dependent and are involved in processes such as folding, repairing, and degrading proteins. HSPs are classified according to their different molecular weights and functions that are cytoprotectively preserved in eukaryotic and prokaryotic cells. The most preserved variety is HSP70. In their role in protein metabolism, HSP70 increases levels in the cell in response to stress in environmental, pathological and physiological stimuli. It has been shown that HSP70, which normally has a certain level of cytosol, increases due to environmental conditions due to the effect of *raison d'erte* effect [9]. As chaperone, HSP70 shows activity regulating cycles in the production of Adenosine triphosphatase (ATPase) enzymes. HSP70 is involved in functional binding, restoration and fixation of ATPase enzymes to the cell wall. ATPases and HSP70 have also been shown to interact in their evolutionary development [10]. ATPases enzymes in the cell play an important role in transmembrane ion transitions, osmotic balance and membrane permeability and electrical potential. It has been determined that it can be affected by many environmental and physiological factors. ATPases are Na^+/K^+ ATPase, Ca^{+2} ATPase and Mg^{+2} ATPase [11,12]. Na^+/K^+ ATPase is responsible for the elimination of Na^+ ions and K^+ ions into the cell [13,14]. ATPase enzymes, which are important in preserving transmembrane potential, are considered to be of great importance as biochemical biomarkers. K^+ , Mg^{+2} and Ca^{+2} ions have important roles in regulating osmotic and ionic regulation in intracellular and extracellular fluids. The tissue with high Ca^{+2} ATPase activity is largely stored in the heart stripe muscle sarcolem. Ca^{+2} ATPase is a membrane enzyme that intrinsically plays a role in the contraction of heart muscle tissue. Mg^{+2} ATPase plays an important role in the transport of Mg^{+2} , which is important for membrane integrity and permeability balance in the cell [12].

Objective of the Study

The objective of this study was to investigate the toxic effects of exposure to biopesticide EMB in the experimental model of albino male mice; DNA oxidation biomarker 8-OHdG level, stress protein HSP70 level, caspase 3 enzyme activation and ion regulation enzymes ATPases, Na^+/K^+ ATPase, Ca^{+2} ATPase and Mg^{+2} ATPase activities were determined.

Materials and Methods

Chemicals

Commercial formulation of Emamectin benzoate was Proclaim Opti UV 5 WG, Sygenta was purchased from Turkey distributor. The chemicals used were obtained from Sigma Aldrich and Merck.

Test animals

Experimental animals, 10 weeks old male Swiss albino mice (25 - 30 gr) were used in the laboratory of Çukurova University Faculty of Medicine Experimental Medicine Research and Application Center (ÇÜTF-DETAUM). The animals were acclimatized in their cages for 5 days with a suitable humidity and temperature for 12 hours day and night cycle. All albino mice were fed ad libitum with standard laboratory pellet feed and tap water and no specific diet was administered.

Experimental design

Albino mice were found to be 8 mice in each group, and 4 groups were formed together with the control group. The control group (C) was administered same amount of water under the same experimental conditions and by oral gavage. Experimental groups [15] Wolterink, *et al.* (2012) determined by the LD_{50} value; E1 group (25 mg/kg/day = $1/30 \text{ LD}_{50}$), E2 group (50 mg/kg/day = $1/15 \text{ LD}_{50}$), E3 group (100 mg/kg/day = $1/7.5 \text{ LD}_{50}$) administration daily oral gavage. The toxicity study was continued for 14 days. The animals were sacrificed by cervical dislocation under mild ether anesthesia. Heart tissues were frozen at -80°C for use until biochemical analysis.

Biochemical analysis

Stored heart tissue samples were divided into 2 parts, the first part was homogenized in PBS (phosphate buffered saline solution) containing 2.5 mM ATP for 3 minutes at 10000 rpm for 20 minutes at 4,000C for 4 minutes at 16000g. supernatant fractions were separated by centrifugation. In the supernatant; protein, TBARS, 8-OHdG and HSP70 levels; Na⁺/K⁺ ATPase, Ca⁺² ATPase and Mg⁺² ATPase enzyme activities were determined. The second part of the heart tissue was separated for caspase 3 enzyme activity measurement and homogenized for 1 minute at 10000 rpm with Ultra-Turrax homogenizer with special buffer 1/10 (w/v) in the kit. The homogenates were centrifuged at 13000 xg for 15 min. at +4°C.

Determination of total protein level

The supernatants and bradford reagent were mixed and allowed to stand at room temperature for 15 minutes. As standard, bovine serum albumin (BSA) was read against the standard values prepared in the microplate reader at a wavelength of 595 nm, the results compared with the calculation of mg/ml [16].

HSP70 Level

The non-competitive enzyme-linked immunosorbent assay (ELISA) method measures the absorbance of the orange colored product at 492 nm in 2 replicates for each sample in a microplate reader [17].

TBARS Level

TBARS, a secondary product of lipid peroxidation, was a lipid peroxidation product as a biomarker. It was measured by spectrophotometric method at a wavelength of 532 nm, indicating damage in lipid metabolism [18].

Activity of caspase-3 enzyme

Caspase-3 enzyme activity was determined by the method specified in the caspase-3 assay kit produced by Sigma commercial company.

8-OHdG level

DNA oxidation measurement was measured at a wavelength of 450 nm by the competitive ELISA method [19].

ATPase enzyme activities

For the measurement of Na⁺/K⁺ATPase activity, 40 mM Tris-HCl for Ca⁺² ATPase activity with media containing 40 mM Tris-HCl, 120 mM NaCl, 20 mM KCl, 3 mM MgCl₂, pH 7.7 and 1 mM ouabain. Incubation medium (pH 7.7) containing 4 mM MgCl₂, 1 mM CaCl₂ and 1 mM EGTA were used. The homogenates were centrifuged at 1000g for 15 minutes so that they were separated from the residues. ATPase activity was performed immediately with the resulting supernatant. Ca⁺² ATPase activity was calculated by subtracting Mg⁺² ATPase (ouabain-containing) activity from total-ATPase (ouabain-free) activity. This method was described by Atkinson., *et al.* (1973) [20].

Statistical analysis

Statistical analysis of biochemical analysis data were performed using the One Way Anova-Duncan test (P < 0.05) in the SPSS 22.0 (SPSS Inc., Chicago, IL) package program.

Result and Discussion

In this study, we found a 34%, 53% and 76% increase in heart HSP70 level (Figure 1), 14-day exposure in E1, E2 and E3 groups, respectively. Increases in TBARS levels; The E1, E2 and E3 groups were 14%, 34% and 55% respectively (Figure 1, P < 0.05).

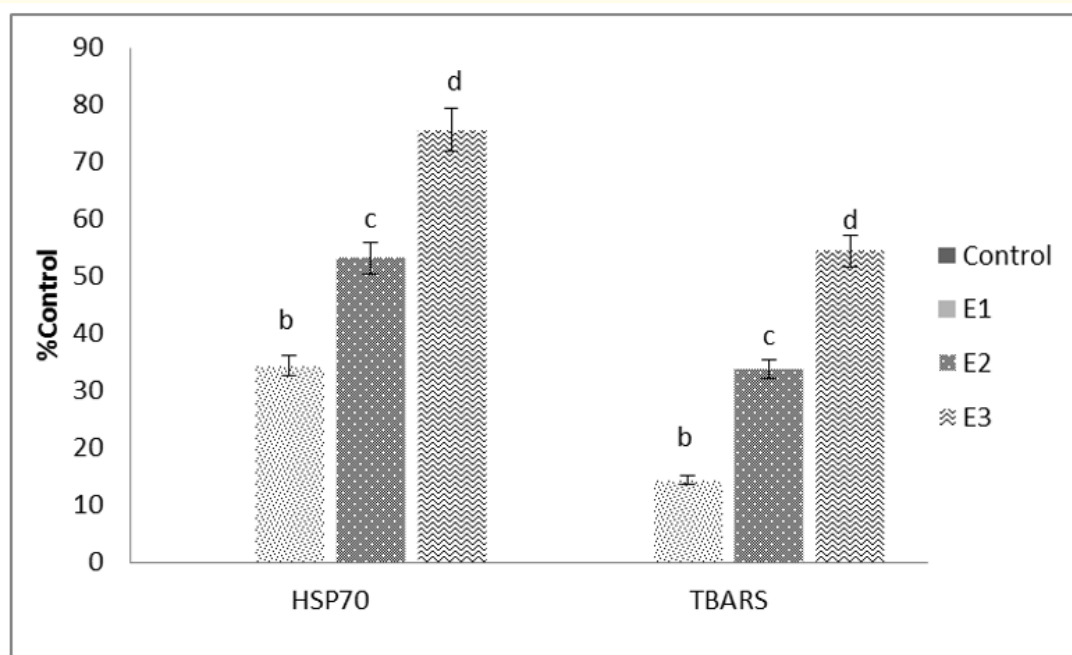


Figure 1: Effects of emamectin benzoate biochemical biomarkers in the heart of Swiss albino mice are given in percentages of different doses compared to the control group, HSP70 level (ng/HSP70µg protein) and TBARS level (nmol/mg protein). Values expressed as mean ± SE, n = 8. The letters above the columns (a, b, c, d) mean significant at P < 0.05.

Increased expression of HSP72 and HSP73 belonging to the HSP 70 family has been reported in rat testicular tissue in acute exposure to methoxychlor [21,22]. The induction of HSP70 has been associated with the degree of stress. HSPs regulate cellular homeostasis and provide intracellular stress tolerance by regulating protein-protein interactions [23]. Diazinon-induced rat heart has been reported to cause lipid peroxidation due to insufficient antioxidant system as a result of excessive ROS formation [24]. Although ROS can damage various macromolecules, DNA is critical because it can lead to base modifications, base regions, and double-strand breaks, all of which can change the information content of cells [25]. In this study, caspase 3 enzyme activities (Figure 2) were increased in E1, E2 and E3 groups by 27%, 64% and 102% respectively. 8-OHdG levels were significantly increased in E1, E2 and E3 groups by 24%, 80% and 101% (Figure 2, P < 0.05).

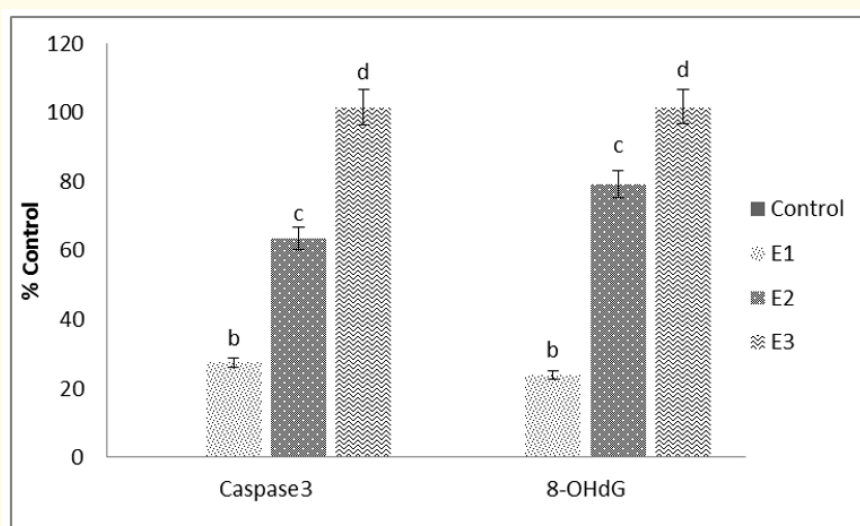


Figure 2: The effects of emamectin benzoate apoptosis and DNA oxidation in the heart of Swiss albino mice are given in percentages of different doses compared to the control group, caspase 3 enzyme activity (µU/mg protein) and 8-OHdG level (ng/8-OHdG µg protein). Values expressed as mean ± SE, n = 8. The letters above the columns (a, b, c, d) mean significant at P < 0.05.

Fipronil exposure may mediate the initiation of the apoptosis pathway in *in vivo* models through ROS production. After male rats were exposed to Fipronil (5 and 10 mg/kg/day), the percentage of apoptotic sperm increased significantly after the formation of increased ROS compared to the control group [26,27]. Significant increase in serum levels of 8-OHdG in the Parkinson's disease group. The highly active hydroxyl radical, which is a member of the ROS, reacts with guanine through hydroxylation to produce 8-OHdG and DNA breaks [28]. Since 8-OHdG is water-soluble, it is secreted from the cell after being extracted from the DNA helix. Thus, monitoring extracellular 8-OHdG may provide information on oxidative DNA damage [29]. Oxidation occurs most easily in guanine residues due to its high trajectory energy. Since the increase in 8-OHdG levels is an oxidized DNA product that exhibits oxidative cellular damage, it is a parameter indicating increased oxidative stress. This observation shows that 8-OHdG measurement may be a marker of oxidative DNA damage in animal model studies [30].

In this study, Na⁺/K⁺ ATPase activity was reduced by 22%, 29% and 42% in the E1, E2 and E3 groups at 14 days of exposure (Figure 3). A decrease of 15%, 26% and 36% was observed in Ca⁺² ATPase enzyme activities in E1, E2 and E3 groups, respectively (Figure 3, P < 0.05). Mg⁺² ATPase enzyme activities in the E1, E2 and E3 groups were reduced by 12%, 30% and 39%, respectively (Figure 3, P < 0.05). The cell membrane is believed to be the domain of insecticides by altering the structural and functional integrity of the cell membrane and also affects membrane-bound enzymes such as total ATPase, Na⁺/K⁺ ATPase and Mg⁺² ATPase [31]. Insecticides such as methyl paration and paration have been reported to inhibit the activity of ATPases [32]. Archana., *et al.* (2001) [33] reported that an OP herbicide anilofos causes inhibition of total ATPase, Na⁺/K⁺ ATPase, Mg⁺² ATPase enzymes in rats brain and liver. And suggested that inhibition of ATPase could affect ionic transport across the cell membrane, leading cellular dysfunction. Kiran and Varma (1990) [34] showed that endosulfan causes a significant reduction in erythrocyte membrane Na⁺/K⁺ ATPase and Mg⁺² ATPase activities in mice. At the same time, Ca⁺² ATPase in parallel with the determination of cell enzymes and intracellular calcium levels. It works as a signal exchanger regulator [11]. Lindane has been reported to increase membrane-bound Ca⁺² ATPase activity, while reducing both Na⁺/K⁺ ATPase and Mg⁺² ATPase activities [12].

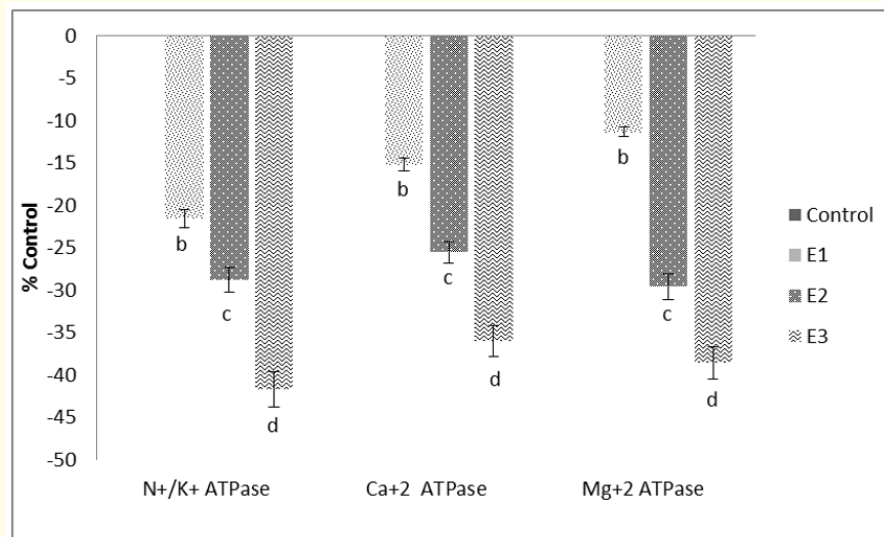


Figure 3: The effects of emamectin benzoate ATPase enzymes in the heart of Swiss albino mice are given in percentages of different doses compared to the control group, Na⁺/K⁺ ATPase, Ca⁺² ATPase and Mg⁺² ATPase. Enzyme activities was given μmol Pi/mg protein/h. Values expressed as mean ± SE, n = 8. The letters above the columns (a, b, c, d) mean significant at P < 0.05.

Conclusion

In conclusion, this study demonstrates that increased TBARS levels in EMB exposure induce oxidative stress in heart tissue of albino male mice. Stress protein HSP70 levels and caspase-3 enzyme activity were found to be unable to prevent apoptosis pathway. HSP70 tried to generate an adaptive response to EMB-induced oxidative stress, but its oxidative effects demonstrated that Hsp70 could not be eliminated. Inhibition of ATPase enzymes (Na^+/K^+ ATPase, Ca^{+2} ATPase and Mg^{+2} ATPase) resulted in a significant blockage in ion regulation, and the toxicity of EMB toxicity in the heart with muscle structure was determined by a different biochemical pathway. It was determined that EMB may have genotoxic effect and increased DNA oxidation biomarker 8-OHdG level. This study determined that EMB shows cardiotoxicity with important biochemical and genotoxic parameters on environment and human health.

Bibliography

1. NSW EPA. What Are Pesticides and How Do They Work? (2013).
2. Parsaeyan., *et al.* "Toxicity of emamectin benzoate and cypermethrin on biological parameters of cotton bollworm, *Helicoverpa armigera* (Hübner) in laboratory conditions". *Journal of Crop Protection* 2.4 (2013): 477-485.
3. Horvat Alka JM., *et al.* "Analysis, occurrence and fate of anthelmintics and their transformation products in the environment". *Trends in Analytical Chemistry* 31 (2012): 61-84.
4. El-Sheikh El-Sayed A and Azza AA Galal. "Toxic effects of sub-chronic exposure of male albino rats to emamectin benzoate and possible ameliorative role of *Foeniculum vulgare* essential oil". *Environmental Toxicology and Pharmacology* 39.3 (2015): 1177-1188.
5. Abou-Zeid Shima M., *et al.* "Ameliorative effect of pumpkin seed oil against emamectin induced toxicity in mice". *Biomedicine and Pharmacotherapy* 98 (2018): 242-251.
6. Kasai, Hiroshi. "Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis". *Mutation Research/Reviews in Mutation Research* 387.3 (1997): 147-163.
7. Kinoshita Anna., *et al.* "Formation of 8-hydroxydeoxyguanosine and cell-cycle arrest in the rat liver via generation of oxidative stress by phenobarbital: association with expression profiles of p21WAF1/Cip1, cyclin D1 and Ogg1". *Carcinogenesis* 23.2 (2002): 341-349.
8. Porter Alan G and Reiner U Jänicke. "Emerging roles of caspase-3 in apoptosis". *Cell Death and Differentiation* 6.2 (1999): 99.
9. Clark Paul R and Antoine Ménoret. "The inducible Hsp70 as a marker of tumor immunogenicity". *Cell Stress and Chaperones* 6.2 (2001): 121.
10. Riordan Michael., *et al.* "HSP70 binding modulates detachment of Na-K-ATPase following energy deprivation in renal epithelial cells". *American Journal of Physiology-Renal Physiology* 288.6 (2005): F1236-F1242.
11. Bers Donald M., *et al.* "Intracellular Na^+ regulation in cardiac myocytes". *Cardiovascular Research* 57.4 (2003): 897-912.
12. Temiz Ozge., *et al.* "Influence of chlorantraniliprole toxicity on ionic regulation of gill and muscle ATPase activity of Nile fish (*Oreochromis niloticus*)". *Fresenius Environmental Bulletin* 27 (2018): 5027-5032.
13. Ahmed Hanaa H., *et al.* "Protective effect of Ginkgo biloba extract and pumpkin seed oil against neurotoxicity of rotenone in adult male rats". *Journal of Applied Sciences Research* 5.6 (2009): 622-635.
14. Carvalho Fabiano B., *et al.* "Inhibitory effect of *Scutia buxifolia* extracts, fractions and ursolic acid on Na^+ , K^+ -ATPase activity in vitro in membranes purified from rat hearts". *Journal of Ethnopharmacology* 179 (2016): 45-54.

15. Wolterink, G., *et al.* "Emamectin benzoate". *Pesticide Residues in Food* 2011 (2012): 211.
16. Bradford and Marion M. "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". *Analytical Biochemistry* 72.1-2 (1976): 248-254.
17. De Boeck, *et al.* "Effect of cortisol treatment and/or sublethal copper exposure on copper uptake and heat shock protein levels in common carp, *Cyprinus carpio*". *Environmental Toxicology and Chemistry: An International Journal* 22.5 (2003): 1122-1126.
18. Ohkawa Hiroshi, *et al.* "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". *Analytical Biochemistry* 95.2 (1979): 351-358.
19. Yin Baoyun, *et al.* "Determination of 8-hydroxydeoxyguanosine by an immunoaffinity chromatography-monoclonal antibody-based ELISA". *Free Radical Biology and Medicine* 18.6 (1995): 1023-1032.
20. Atkinson A., *et al.* "The determination of inorganic orthophosphate in biological systems". *Biochimica et Biophysica Acta (BBA)-General Subjects* 320.1 (1973): 195-204.
21. Vaithinathan S., *et al.* "Methoxychlor-induced alteration in the levels of HSP70 and clusterin is accompanied with oxidative stress in adult rat testis". *Journal of Biochemical and Molecular Toxicology* 23.1 (2009): 29-35.
22. Hartl F Ulrich. "Molecular chaperones in cellular protein folding". *Nature* 381.6583 (1996): 571.
23. Kiang, Juliann G and George C Tsokos. "Heat shock protein 70 kDa: molecular biology, biochemistry and physiology". *Pharmacology and Therapeutics* 80.2 (1998): 183-201.
24. Akturk O., *et al.* "The effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and ameliorating role of vitamin E and vitamin C". *Cell Biology and Toxicology* 22.6 (2006): 455.
25. Cadet Jean Thierry Douki and Jean-Luc Ravanat. "Oxidatively generated base damage to cellular DNA". *Free Radical Biology and Medicine* 49.1 (2010): 9-21.
26. Khan Saleem, *et al.* "Fipronil induced spermotoxicity is associated with oxidative stress, DNA damage and apoptosis in male rats". *Pesticide Biochemistry and Physiology* 124 (2015): 8-14.
27. Junn, Eunsung and M Maral Mouradian. "Apoptotic signaling in dopamine-induced cell death: the role of oxidative stress, p38 mitogen-activated protein kinase, cytochrome c and caspases". *Journal of Neurochemistry* 78.2 (2001): 374-383.
28. Salama and Afrah F, *et al.* "Epigenetic study of Parkinson's disease in experimental animal model". *International Journal of Clinical and Experimental Neurology* 3 (2015): 11-20.
29. Cheng Keith C., *et al.* "8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G---T and A---C substitutions". *Journal of Biological Chemistry* 267.1 (1992): 166-172.
30. Yasuhara, Takao, *et al.* "Increased 8-OHdG levels in the urine, serum and substantia nigra of hemiparkinsonian rats". *Brain Research* 1133 (2007): 49-52.
31. Shao Qiming, *et al.* "Inhibition of cardiac sarcolemma Na⁺/K⁺ ATPase by oxyradical generating systems". *Molecular and Cellular Biochemistry* 147.1-2 (1995): 139-144.
32. Blasiak, Janusz. "Inhibition of erythrocyte membrane (Ca²⁺/Mg²⁺)-ATPase by the organophosphorus insecticides parathion and methylparathion". *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology* 110.2 (1995): 119-125.

33. Hazarika Archana., *et al.* "Subacute toxicity of anilofos, a new organophosphorus herbicide in male rats: effect on lipid peroxidation and ATPase activity". (2001).
34. Kiran R and MN Varma. "Age related toxic effects of endosulfan on certain enzymes of rat erythrocytes". *Indian Journal of Experimental Biology* 28.7 (1990): 694-696.

Volume 8 Issue 3 March 2020

©All rights reserved by Özge Temiz.