POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 39(3): 151–165, Y. 2024

DOI: 10.31648/pjns.10258

EMAMECTIN BENZOATE EXPOSURE IMPAIRED GERM CELL MATURATION IN THE TESTES OF ADULT ALBINO RATS*

***Assia Kamel*[1], *Samira Akdader-Oudahmane*[2], *Zohra Hamouli-Said*[3]**

[1] ORCID: 0000-0001-8461-0639
[2] ORCID: 0000-0003-0959-9112
[1–3] Department of Biology and Physiology of Organisms
University of Sciences and Technology Houari Boumediene (USTHB), Bab Ezzouar, Algiers, Algeria
[2] Department of Biology
Faculty of Biological Sciences and Agricultural Sciences
University of Mouloud Mammeri, Tizi-Ouzou, Algeria

Key words: EMB, oxidative stress, toxicity, testis.

Abstract

Certain pesticides represent a substantial risk to reproductive function, and their possible reprotoxic effects have been the focus of numerous research. Emamectin Benzoate (EMB), a biopesticide widely used in agriculture, household items, and veterinary health, can cause tissue damage in the male reproductive system due to oxidative toxicity. The purpose of this study was to investigate the effects of Emamectin Benzoate toxicity in male Wistar rats. Fourteen rats were divided into two groups of seven each. For 15 days, the EMB group received a daily dose of EMB corresponding to 20 mg/kg by gavage. Distilled water was given to the control group. Oxidative stress, DNA fragmentation, histopathology and germ cell degeneration of testis were investigated. Emamectin administration induced oxidative stress in testicle evidenced by elevated levels of MDA, protein carbonyl and percentage of DNA fragmentation with depletion of the defense system (CAT and SOD activities). Emamectin produced several histopathological changes in the testis, these results suggesting that the subchronic exposure to emamectine benzoate affects the redox state which contribute to testicular impairment.

Introduction

Pesticides, also known as plant protection products, are a class of chemicals that are commonly used in agriculture to preserve crops and

Address: Assia Kamel, University of Sciences and Technology Houari Boumediene (USTHB), Bab Ezzouar, Algiers, Algeria, e-mail: assia.kamel_fsb@usthb.edu.dz

* This work was supported by scientific research project of Ministry of Higher Education and Research (Grant number 200220183-Prfu).

prevent hazardous organisms in order to increase the quantity and quality of food produced (PARRON et al. 2011). As a result, characterizing the hazards presented by these pollutants has become a major ecotoxicological task. Pesticides are now thought to be very hazardous to both human health and the environment. Because of their extensive usage, persistence, and bioaccumulation along the trophic chain, they are among the most harmful contaminants in the environment. EMB is a potent neurotoxic agent from the Avermectin family that is produced through the fermentation of *Streptomyces avermitilis*. B1a ($C_{49}H_{75}NO_{13}C_7H_6O_2$, 90%) and B1b ($C_{48}H_{73}NO_{13}C_7H_6O_2$, 10%) are EMB's active homologues. Radiolabeled emamectin B1a benzoate or MAB1a is rapidly absorbed, distributed and excreted following oral and intravenous administration. In rat, More than 98% of the total radioactivity was found in faeces, with <0.4% in urine and <1.6% in the tissues (MUSHTAQ et al 1996). Tissue distribution and bioaccumulation appeared minimal, the metabolism of MAB1a benzoate appears to involve primarily N-demethylation to AB1a. AB1a was the only metabolite detected in the faeces, liver, kidneys, muscle and fat, the amount of this metabolite represented about 1 to 2% of the radioactivity one day post -treatment, but increased to 19% of radioactivity on 7 post -treatment . The biotransformation of MAB1a results in formation of the metabolite AB1a, which is present in lower amounts compared with the parent compound in various tissues and is excreted mainly via faeces (WOLTERINK et al. 2012). EMB is a plant protection product that is anti-parasitic, antibacterial, and insecticidal. Significant amounts move through the soil, migrate, and are likely to wind up in agricultural commodities, particularly animal foodstuffs (WANG et al. 2006). Emamectin is structurally similar to abamectin and ivermectin. EMB acts as a disruptor of neurotransmitter activity (chloride channel activator) in the target organism and results in paralysis and death. Although the data about the effects of EMB on antioxidant status is insufficient, many studies have confirmed that EMB insecticides created oxidative stress in intoxicated animals (TEKELI et al. 2023, TEMIZ 2020). EMB caused elevation of malondialdehyde and reduction of antioxidant enzymes in rats, and/also it has been reported that emamectin induced single and double-strand DNA breaks in human liver cells (ZHANG and ZHAO 2017) and chromatin condensation with nuclear fragmentation in leukemia K562 and Molt-4 cells in vitro (YEN et al. 2017). Studies have shown that workers who have been exposed to pesticides for a long time may experience oxidative damage to their various organs and decreased sperm quality (LATCHOUMYCANDANE and MATHUR 2002). EMB exposure, whether chronic or subchronic, causes reproductive and fertility problems (AITKEN et al. 2022). This study aims

to evaluate the redox state, DNA fragmentation and testicular toxicity induced after subchronic exposure to EMB in albinos Wistar rats.

Materials and Methods

Chemicals

Commercial formulation of emamectin benzoate was PROMED 5_{SG} (CAS No: 12052064), containing 5% emamectin benzoate, A pure reference standard of emamectin benzoate (Medmac, Agrimatco, Algeria) was purchased from the Ministry of Agriculture and Rural Development (Direction de la Protection des Végétaux et Contrôles Techniques DPVCT, Algeria). Tris base, EDTA, Triton x-100, NaCl and diphenylamine, thiobarbituric acid (TBA), 5, 5′-dithio-bis (2-nitrobenzoic) acid (DTNB) were obtained from commercial sources (Sigma Aldrich, France).

Animals

All experimental procedures were approved by the institutional animal care and use committee of the University of Houari Boumedienne in Algeria (USTHB) and were carried out in accordance with to the ethical approval number: 981-1 law of August 22, 1998, of the National Administration of Algerian Higher Education and Scientific Research. 14 adults male Wistar rat weighing (133 g ±38 g) was obtained from IPA (Algiers Pasteur Institute). The supplied rats were placed in cages. Maintained under controlled temperature conditions (21–23°C) and 12–12 h photoperiodic cycle, All the animals were fed with standard pellets (ONAB: Office National des Aliments de Bétail, Alger, Algeria) and water *ad libitum*.

Experimental design

Healthy adult rats (n = 7) were randomly divided into 2 groups (n = 7 per group) as follows: group 1 control (C) – received distilled water (placebo); group 2 (EMB) – received by oral gavage 20 mg/kg/day of EMB (equivalent to 1/3.75 th oral LD50) (WOLTERINK et al. 2012) for 2 weeks. EMB was prepared in distilled water. Animals were carefully observed during the 15 days of treatment and attention was paid to signs that would indicate neurotoxicity, including tremor, salivation, and diarrhea. Rats were sacrificed at the end of the experiment by decapitation. The testicle was quickly removed and immediately weighed and stored for biochemical analysis or fixed for histopathological studies.

Determining pro-oxidant/anti-oxidant status

Preparation of testicular homogenates. After decapitation, the testes were immediately excised and frozen at −20°C. Each testis was placed in a cold buffer containing 30 mmol/L monosodium hydrogen phosphate (NaH_2PO_4; pH 7.4), 0.1% Triton X-100, 0.12 mol/L NaCl and 0.3 mmol/L PMSF and was homogenized with a Staufen homogenizer. The homogenates were centrifuged at 1,000 g for 5 min at 4°C. The protein concentrations were determined using the Bradford method (BRADFORD 1976).

Assessment of oxidative markers. The level of lipid peroxidation was determined by measuring malondialdehyde using the BIRD and DRAPER (1984) TBARS (Thiols Barbituric Acid Reactive Species) test. The testicular protein carbonyl content (PCC) was determined by the method described by LEVINE et al. (1990).

Evaluation of enzymatic and non-enzymatic antioxidants. The method of MISRA and FRIDOVICH (1972) was used to determine testicular superoxide dismutase (SOD) activity by measuring the inhibition of epinephrine auto-oxidation at pH 10.2. Testicular catalase (CAT) activity was obtained using the method of AEBI (1984) by measuring the decrease in hydrogen peroxide (H_2O_2) concentration at 240 nm. Reduced glutathione (GSH) was assayed using 5, 5′-dithio-bis (2-nitrobenzoic) acid (DTNB). The absorbance of DTNB was measured at 412 nm in a microplate reader (BIO-TEK) because it forms a yellow complex with GSH.

DNA fragmentation assay. DNA fragmentation in the testicular homogenate was quantified by the diphenylamine reaction procedure described by PERANDONES et al. (1993). Slices of the testes were homogenized (1:9/w:v) in cold hypotonic lysis buffer containing (10 mM Tris base, 1 mM EDTA and 0.2% Triton X-100). The homogenates were centrifuged at 3,000 · g for 15 min. the supernatant and the pellet were used for quantification of fragmented DNA. The developed blue color was quantified at 578-nm. Percentage of DNA fragmentation in each sample was expressed by the formula: % DNA fragmentation = (O.D Supernatant / O.D Supernatant + O.D Pellet) ·100.

Histological/Histochemical study. The testis was embedded in paraffin after being fixed in 10% neutral buffered formalin and dehydrated in a graded series of ethanol. Each block was cut into four-micron-thick sections and stained using the following methodology: Hematoxylin and eosin (HE) stains were used to demonstrate the general histological fea-

tures, while the periodic acid-Schiff (PAS) technique allowed to show mucopolysaccharides in the testis and observed under Optika B-500 TPL "TS-View" light microscope.

Germ cell degeneration. Testis specimens were fixed with 4% glutaraldehyde, buffered with 0.2 M phosphate buffer (PB), pH 7.4 and post-fixed with 1% osmium tetroxide in the same buffer. They were dehydrated in an increased series of ethanol followed by propylene oxide and embedded in Epon 812. Semi-thin sections (0.5 μm) were cut on an ultra-microtome (LKB BROMMA) and stained with toluidine blue. Toluidine blue staining was performed for the identification of germ cell degeneration. Germ cell degeneration was expressed as a percentage of tubular cross-sections containing degenerating cells. 20 tubular cross-sections per animal were scored. All counts were performed at 1000× magnification in the light microscope (Optika B-500 TPL "TS-View).

Statistical analysis

All values are expressed as mean SEM (mean standard error of the mean) by Student's *t*-test. All statistical tests are performed using Graph pad prism Software version 5.

Results

Evaluation of body and testis weights

Body weight gain (or lost) (%) was calculated as follows: Final body weight – initial body weight · 100/initial body weight. Table 1 shows that body weight increased by 12%; ($p > 0.05$) after EMB treatment when compared with the control group. Relative paired testes weight was 1.06 ±0.03 g/100 g BW in the control group, decreased non significantly by 2% after EMB treatment (Table 1).

Table 1
Percentage body weight gain and relative testis weight, in rats from control and EMB-treated groups. Data are expressed as the means ±SEM of 7 animals for each group. Values are significantly different ($p < 0.05$).

Body weight gain [g]	Control	EMB
Initial body weight	133.51 ±11.13	146.89 ±8.37
Experimentation body weight	219.55 ±11.25	246.15 ±10.87
Body weight after treatment [%]	64%	67%
Relative testis weight [g/100 g BW]	1.06 ±003	1.04 ±0.05

Assessment of oxidant/antioxidant biomarkers

Administration of emamectin to rats at a dose level equivalent to 1/37.5 th oral LD_{50} for 2 weeks caused significant increase ($p < 0.05$) of MDA level (Figure 1*a*) and protein carbonyl content (PCC) ($p \leq 0.05$) (Figure 1*b*) while reduced activities of CAT (Figure 1c) and SOD (Figure 1*d*). There was no significant difference ($p > 0.05$) in testicular GSH concentration between EMB-exposed and control animals (Figure 1e).

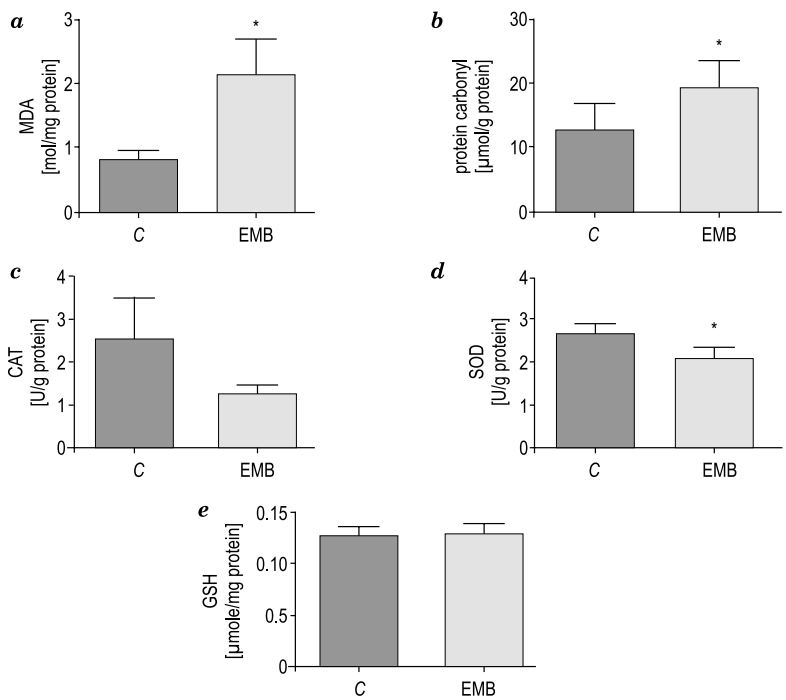

Fig. 1. Effects of EMB on oxidative stress parameters (oxidant /antioxidant): *a* – MDA level [nmol/g protein]; *b* – protein carbonyl [µmol/g protein]; *c* – CAT activity [U/g protein]; *d* – SOD activity [U/g protein]; *e* – GSH [µmol/mg protein] in the testis of rat. Values expressed as the means ±SEM of seven animals for each group; *$p < 0.05$; Values are significantly different

DNA fragmentation assay findings

The percentage of DNA fragmentation of emamectin-treated rats significantly increased in comparison to the control group. The estimated mean values were 17.57 ±2.05% in control group and 27.71 ±6.3% in EMB group (50% higher than control, ($p \leq 0.05$) indicating that the testes are vulnerable to the toxic insult of emamectin (Figure 2*a*).

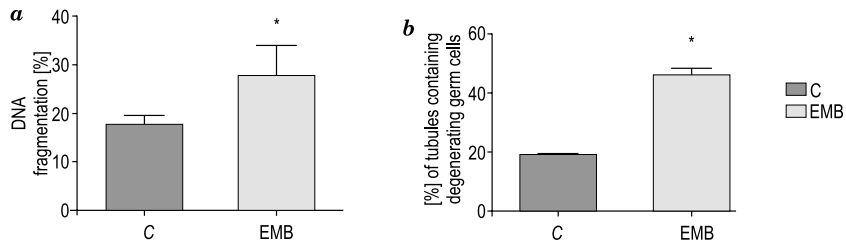

Fig. 2. Percentages of DNA fragmentation and tubules containing degenerating germ cell in the testes of rats from control and EMB-treated groups: *a* – DNA fragmentation [%]; *b* – % of tubules containing germ cell degenerating. Values expressed as the means ±SEM of seven animals for each group; *$p < 0.05$; values are significantly different

Germ cell degeneration finding

We observed that in treated rats, 46% of seminiferous tubule cross-sections containing degenerating germ cell. The incidence of tubules containing degenerating germ cells was 19.05 ±0.27 % in the control group, increased to 46.20 ±2.3% in EMB group (2.4-fold of control, $p < 0.05$) (Figure 2*b*). Tubular cross-sections containing degenerating cells, identified as having strongly condensed darkly stained nuclei in toluidine-blue-stained sections (Figure 3*b*), in addition to the basal germ cells showing loss of their cytoplasm with very dark nuclei characterized by pyknotic nuclei (Figure 3*b*), thus the nuclei of acrosomal caps of late spermatids are strongly stained and were seen in the basal level of the seminiferous epithelium, indicating failure of sperm relase (Figure 3*c*).

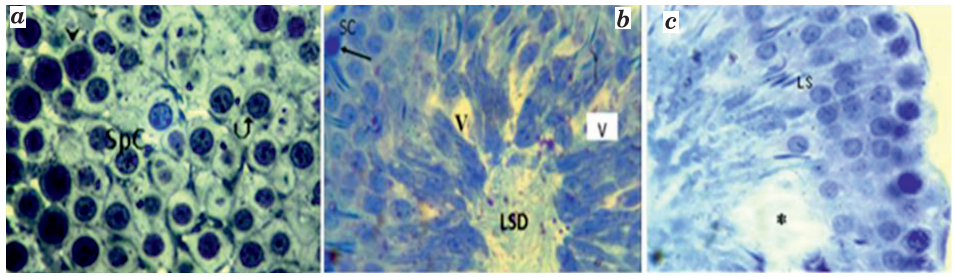

Fig. 3. A semithin section of a testis of a rat: *a* – control group showing normal tissue architecture; SpC – spermatogenic cell, primary spermatocytes (arrowhead), spermatids (curved arrow); *b*, *c* – EMB – treated group showing vacuolations (V); depletion, extensive vacuolization (V) of the germinal epithelium; sertoli cells (SC) resting on the basement membrane; LSD – low spermatozoa density; failure of late spermatid release (LS); lacunar spaces (*), pyknotic nuclei of basal germ cells (arrows). Toluidine blue ×1000

Source: own study, photo by Assia Kamel, Samira Akdader-Oudahmane and Zohra Hamouli-Said

Histopathological study

Hematoxylin and eosin

The microscopic analysis of cross sections of controls rats testes reveal a normal structural appearance of seminiferous tubules surrounded by a basal lamina and separated by interstitial tissue containing Leydig cells as well as blood vessels in between (Figure 4*a*). Each tubule was lined by germinal epithelium, which is a complex stratified epithelium containing germ cells at various stages of the spermatogenetic cycle, with cell types represented by spermatogonia, Sertoli cell, spermatocytes, round spermatids, late spermatids and variable numbers of mature sperms were present in the lumen of the tubules (Figure 4*b*, *c*).

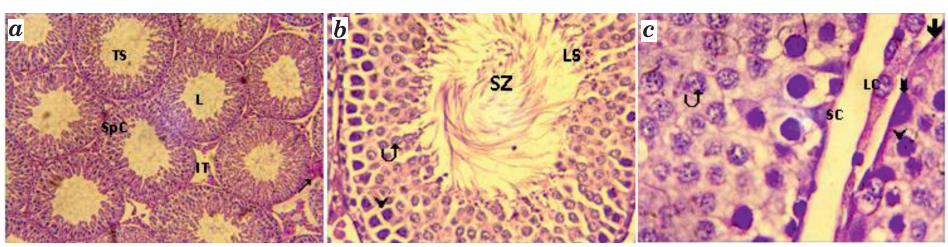

Fig. 4. Cross sections of the testis of the control group, showing: *a* – rounded seminiferous tubules (Ts) lined by spermatogenic cells (Spc) and separated by the interstitial tissue (IT) with blood vessels in between (arrow). H&E ×100; *b* – the seminiferous tubule containing germ cell, including spermatogonia (bifid arrow), primary spermatocytes (arrowhead), and spermatids (curved arrow), spermatozoa (Sz). H&E ×400; *c* – spermatogonia (bifid arrow), primary spermatocytes (arrowheads), spermatids (curved arrow), sertoli cells (SC), leydig cells (LC) and myoid cells (thick arrow). H&E ×1000

Source: own study, photo by Assia Kamel, Samira Akdader-Oudahmane and Zohra Hamouli-Said

Testis of rats from emamectin treated group showed disturbances in the normal architecture of the testis. Some of the seminiferous tubules showed abnormal shapes with indentation and contour irregularity, severe dilated congested blood vessels and mononuclear cells infiltration were also seen between the tubules (Figure 5*a*, *c*). In addition, reduced size of tubular lumen and degenerated cytoplasm of germ cell, karyolysis and pyknosis of some nuclei were also seen (Figure 5*a*, *d*, *e*) Regarding the interstitial tissue, it was more spaced, containing many vacuoles (Figure 5*d*) multifocal vacuolation of Sertoli cell cytoplasm were also observed, and the tubules were filled with dark acidophilic cells with fragmented nuclei (Figure 5*d*, e) Preserved Sertoli cells with pyramidal elongated nuclei and prominent nucleoli were also seen (Figure 5*e*) we denoted also reduction in the quantity of spermatozoa in the lumen of the seminiferous tubules (Figure 5*c*).

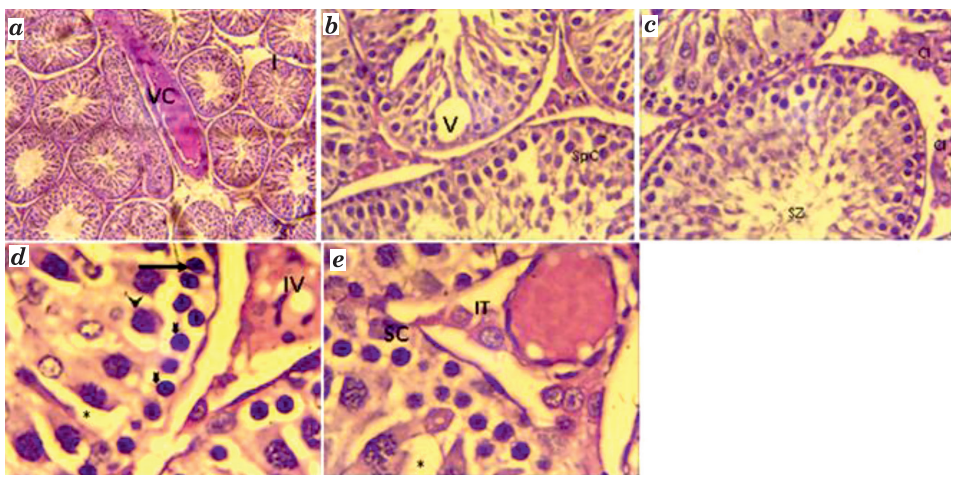

Fig. 5. Cross sections of a rat testis of EMB – treated group, showing: *a* – Irregular contour tubules (I), and vascular congestion (VC), with decreasing size of tubular lumen (L). H&E ×100; *b*, *c* – loss of spermatogenic cells (Spc), vacuolization (V), cell infiltration (CI) and reduction in spermatozoa count (SZ). H&E ×400; *d*, *e* – lacunar spaces (*), basal germ cells with very dark nuclei (arrow), vacuolated spermatogonia (bifid arrows) and vacuolated primary spermatocytes (arrowhead); vacuolated interstitial tissue (IV), sertoli cell (SC); interstitial tissue (IT). H&E ×1000
Source: own study, photo by Assia Kamel, Samira Akdader-Oudahmane and Zohra Hamouli-Said

Periodic Acid-Schiff's (PAS)

The control group showed an apparent strong PAS-positive reaction in the basement membrane of the seminiferous tubules, acrosomal caps of spermatids and spermatozoa, as well as in the interstitial tissue in between the seminiferous tubules (Figure 6*a*) whereas primary and secondary

spermatocytes showed negative affinity. These findings suggested that spermatid and spermatozoa contained more glycogen and carbohydrates. Sections of the EMB group showed a depletion of carbohydrate content, which was concentrated as a faint coloration at the basal lamina and interstitial tissue, an apparent weak PAS-positive reaction in Sertoli cell (Figure 6*b*).

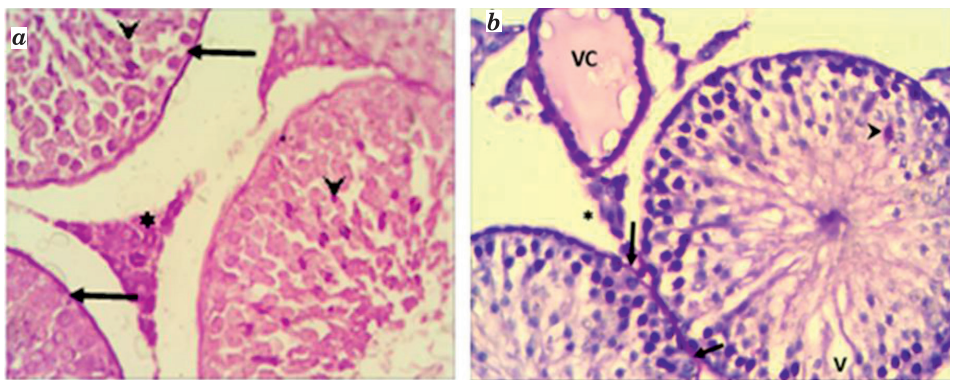

Fig. 6. Cross sections of rat testis from control and EMB – treated groups showing the PAS reaction: *a*–*b* basal lamina (arrow), acrosomal cap (arrowhead), and interstitial tissue (*); vascular congestion (VC); vacuole (V). PAS ×400; *a* – control, *b* – EMB
Source: own study, photo by Assia Kamel, Samira Akdader-Oudahmane and Zohra Hamouli-Said

Discussion

Emamectin benzoate is a macrocyclic lactone insecticide derived from the avermectin series of natural. The accumulation of avermectin in the environment makes avermectin a most important molecules for toxicological studies (ZHU et al. 2013, MA et al. 2014). The present investigation demonstrated that subchronic administration of emamectin to male Wistar rats for 2 weeks caused some moderate symptoms such as reduced activity, increasing weakness and hair loss.

Although both EMB-treated and control rats exhibited body weight gain during the experimentation period, EMB exposure increased body weight after 2 weeks of treatment. Our findings agree with those of KHALDOUN-OULARBI et al. (2014) who reported that emamectin administration (5 mg/kg BW) for 14 days to rats had a significant increase in body weight. Duration of exposure to EMB and dose may influence the body weight. The stress caused by the pesticide, according to AGBOHESSI et al. (2014), could explain the differences in body weight. Stress affects growth by influencing feeding behavior and food conversion efficiency.

Our results indicate that 15 days treatment with EMB decreased the relative testicular weight. Our findings coincide with the findings of DAWOUD et al. (2021) who showed a decrease in liver, testis, spleen and heart weights in rats treated with EMB (5 mg/kg BW diet) for 8 weeks.

The tissue loss seen in our study, which was characterized by the presence of degenerative lesions, could explain the decrease in relative testicular weight. Furthermore, subchronic administration of emamectin to Wistar male rats for 2 weeks caused significant elevation of malondialdehyde (MDA) and protein carbonyl levels in testis while the activities of CAT and SOD were reduced, which indicated that emamectin could induce oxidative stress. Our findings agree with the findings of TEKELI et al. (2023) who found that EMB dramatically raised MDA concentrations in the testis. Similar findings were reported that emamectin benzoate increased TBARS levels at 28 days in rat liver (EL-SHEIKH and GALAL 2015). These findings also showed consistent results with previous studies in the avermectin group emamectin benzoate and abamectin pesticides made by other authors (ABOUZEID et al. 2017, ZHANG et al. 2020). This could lead to the creation of hypermetabolic state by enhancing mitochondrial respiration, leading to an excess generation of reactive oxygen species (ROS) and the induction of oxidative stress. Indeed, lipid peroxides promote damage to testicular cell membranes, and the testes have been considered susceptible to oxidative damage because of the high content of polyunsaturated fatty acids. On the other hand, the testes possess an elaborate range of enzymatic (SOD, CAT, γGT, G6PD) and nonenzymatic antioxidant defenses (e.g., GSH) to detoxify ROS. The activities of SOD and CAT are related to metabolism of superoxide radicals and hydrogen peroxide. The increase in testicular level of protein carbonyl may be an indication of protein oxidation by ROS. ROS have been shown to alter or inactivate proteins in both reversible and irreversible ways (MANAWADI and KALIWAL, 2010). Inhibition of antioxidant enzymes (CAT, SOD) indicate that cellular defense mechanisms in intoxicated animals failed to protect against the overproduction of ROS (ZHANG and ZHAO 2017, MOHAMED and ABDELRAHMAN 2019).

Our present study revealed that subchronic administration of emamectin to rats caused significant increase in DNA fragmentation and germ cell degeneration in testis of intoxicated animals. DNA is an important germ plasm in organisms, and the damage of DNA can induce cell apoptosis or death (HONG et al. 2017). Apoptosis is responsible for the cytotoxic effect induced by many chemicals characterized by fragmentation of nuclear DNA into internucleosomal fragments (ZHANG et al. 2020, ZHANG and ZHAO 2017). EMB causes both apoptosis and the breakage of both sin-

gle- and double-strand DNA on Tn5B1-4 cells, and it also causes the death of QSG7701 cells, which may occur via mitochondrial-mediated intrinsic apoptotic pathways (LUAN et al. 2017). The apoptosis induced by emamectin may be attributed to ROS generation which in turn causes activation of the mitochondrial-dependent intrinsic pathway resulting in disruption of mitochondrial function and subsequent mitochondrial membrane potential collapse and release of cytochrome-c.

In the current study, the EMB – treated group revealed irregularities in the outline of the seminiferous tubules. These irregularities may be due to tubular shrinkage of the degenerated seminiferous tubules (MOHAMED and ABDELRAHMAN 2019). Moreover, dilated congested blood vessels appeared between the tubules This finding was attributed to EMB toxicity. Thus, vacuolated cytoplasm of Sertoli cells and degenerated cytoplasm of germ cell were detected. These findings were in accordance with previous studies (TAKELI et al. 2023, KHALDOUN et al. 2015). These pathological manifestations could be the result of the increased oxidative damage induced by Avermectin. ROS generated by EMB can damage membrane components of the cell and lead to the leakage of cytoplasmic enzymes, correlated with other alterations at molecular level, involving the DNA fragmentation. Additionally, basal insertion of sperm in between spermatogenic cells may be due to rapid disruption of the junctions between Sertoli and germ cells (ABARIKWU et al. 2010). Some authors attributed this to the change in the proportion of myoid cells and collagen fibers, which may hinder the proper spermatozoa release into the lumen (EL-SHAFAI et al. 2011).

In this work, some seminiferous tubules contained only a few spermatozoa in their lumen. These findings are in agreement with those of KHALDUN et al. (2015). These results can be explained by ROS formation leading to to defect in the process of spermatogenesis, with a decreased number of mature sperm. There seems to be good evidence to suggest a causative chain of events involving, EMB toxicity, oxidative stress, sperm DNA damage, and impaired sperm function. The results of this study affirm the toxicity of Promed ® on laboratory animals. Biochemical and histopathological biomarkers showed the effects of EMB on sperm maturation. However, it is necessary to study the potential detoxification of pesticides by certain medicinal plants and test the phototherapeutic property on the toxic effects of EMB. Study design and short study periods are important limitations. This could have led to an overestimation of the effect. Future research should reconfirm these findings by conducting larger-scale studies.

Conclusion

From this study, it can be concluded that EMB caused structural changes in the testes of adult albino rats and imbalance of the enzymatic antioxidant system associated with massive production of ROS, which cause DNA fragmentation, germ cell degeneration and induced multiple sperm abnormalities, suggesting male infertility. Subchronic exposure of male Wistar rats to emamectin benzoate impairs testicular redox system, which is considered the key molecular mechanism.

Acknowledgements. The authors thank the Algerian Ministry of Agriculture and Rural Development (Direction de la Protection des Végétaux et des Contrôles Techniques, DPVCT) for providing the emamectin benzoate standard.

Accepted for print: 9.09.2024

References

ABARIKWU S.O., ADESIYAN A.C., OYELOJA T.O., OYEYEMI M.O., FAROMBI E.O. 2010. *Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine*. Arch. Environ. Contam. Toxicol., 58: 874–882. https://doi.org/10.1007/s00244-009-9371-2.

ABOU-ZEID S.M, ABUBAKR H. O., MOHAMED M. A., EL-BAHRAWY A. 2017. *Ameliorative effect of pumpkin seed oil against emamectin induced toxicity in mic*e. Biomed. Pharmacother., 98: 242. https://doi.org/10.1016/j.biopha.2017.12.040.

AEBI H. 1984. *Catalase in vitro*. Methods. Enzymol., 105: 121–126. https://doi.org/10.1016/s0076-6879(84)05016-3.

AGBOHESSI T.P., TOKO I.I., N'TCHA I., GEAY F., MANDIKI S.N.M., KESTEMONT P. 2014. *Exposure to agricultural pesticides impairs growth, feed utilization and energy budget in African Catfish Clarias gariepinus (Burchell, 1822) fingerlings*. Int. Aquat. Res., 6: 229–243. https://doi.org/10.1007/s40071-014-0083-5.

AITKEN R.J., BROMFIELD E.G., GIBB Z. 2022. *Oxidative stress and reproductive function: The impact of oxidative stress on reproduction: A focus on gametogenesis and fertilization.* Reproduction., 164(6): F79-F94. https://doi.org/10.1530/REP-22-0126.

BIRD R., DRAPER H. 1984. *Comparative studies on different methods of malonaldehyde determination*. Methods. Enzymol., 105: 299–305. https://doi.org/10.1016/s0076-6879(84)05038-2.

BRADFORD M. 1976. *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein- dye binding*. Anal. Biochem., 72: 248–254. https://doi.org/10.1006/abio.1976.9999.

DAWOUD S.F., AL-AKRA T.M., ZEDAN A.M. 2021. *Hepatoprotective effects of chitosan and chitosan nanoparticles against biochemical, genetic, and histological disorders induced by the toxicity of emamectin benzoate.* Rep. Biochem. Mol Biol., 10(3): 506. https://doi.org/10.52547/rbmb.10.3.506.

EL-SHAFAI A., ZOHDY N., EL- MULLA K., HASSAN M., MORAD N. 2011. *Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of Ascorbic Acid*. Food. Chem. Toxicol., 49: 734–743. https://doi.org/10.1016/j.fct.2010.11.033.

El-Sheikh S.A., Galal A.A. 2015. *Toxic effects of sub-chronic exposure of male albino rats to emamectin benzoate and possible ameliorative role of Foeniculum vulgare essential oil*. Environ Toxicol. Pharmacol., 39: 1177–1188. https://doi.org/10.1016/j.etap.2015.04.008.

Hong Y.X., Yang G., Yan Y., Huang F., Zuo Y., Shen Y., Ding., Cheng Y. 2017. *Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, Eriocheirsinensis*. Fish Shellfish. Immunol., 71: 19–27. https://doi.org/ 10.1016/j.fsi.2017.09.062.

Khaldoun O.H., Allorgec D., Richevalc C., Lhermittec M., Djenase N. 2015. *Emamectin benzoate (Proclaim®) mediates biochemical changes and histopathological damage in the kidney of male Wistar rats. (Rattus norvegicus)*. Toxicol Anal. Clin., 27(2): 72–80. https://doi.org/10.1016/j.toxac.2014.11.002.

Khaldoun O.H. 2014. *Biochemical and histopathological changes in the kidney and adrenal gland of rats following repeated exposure to Lambda-Cyhalothrin*. J. Xenobiot., 4(1): 2240. https://doi.org/10.4081/xeno.2240.

Latchoumycandane C., Mathur P. 2002. *Induction of oxidative stress in the rat testis after short-term exposure to the organochlorine pesticide methoxychlor*. Arch. Toxicol., 76: 692–698. https://doi.org/10.1007/s00204-002-0388-9.

Levine R.L., Garland D., Oliver C.N., Amici A., Climent I., Lenz A.G., Stadtman E.R. 1990. *Determination of carbonyl content in oxidatively modified proteins*. Methods. Enzymol.,186: 464–478. https://doi.org/10.1016/0076-6879(90)86141-h.

Luan S.X., Yun W., Rao C., Xiao Z., Xu J., Lang., Huang Q. 2017. *Emamectin benzoate induces ROS-mediated DNA damage and apoptosis in Trichoplusia Tn5B1-4 cells*. Chem. Biol. Interact., 273: 90–98. https://doi.org/10.1016/j.cbi.2017.06.004.

Ma J., Zhou C., Li Y., Li X. 2014. *Biochemical responses to the toxicity of the biocide abamectin on the freshwater snail Physa acuta*. Ecotoxicol. Environ. Saf., 101: 31–35. https://doi.org/10.1016/j.ecoenv.2013.12.009.

Manawadi S., Kaliwal B. B. 2010. *Methomyl induced alteration in mice hepatic-oxidative status. Int J Biotechnol*. Appl. International., 2(2): 11–19. http://dx.doi.org/10.9735/0975-2943.2.2.11-19

Misra H.P., Fridovich I. 1972. *The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase*. J. Biol. Chem., 247(10): 3170–3175.

Mohamed D.A., Abdelrahman S.A. 2019. *The possible protective role of zinc oxide nanoparticles (ZnOnPs) on testicular and epididymal structure and sperm parameters in nicotine-treated adult rats (a histological and biochemical study)*. Cell Tissue. Res., 375: 543–558. http://dx.doi.org/10.1007/s00441-018-2909-8.

Mushtaq M., Syintsakos L., Krieter P., Colletti A., Arison B,. Crouch L,. Wislocki P. 1996. *Absorption, tissue distribution, excretion, and metabolism of 3H and 14C-labeled emamectin benzoate in rats*, J. Agric. Food. Chem., 44: 3342–3349.

Parrón T., Requena M., Hernández A.F., Alarcón R. 2011. *Association between environmental exposure to pesticides and neurodegenerative diseases*. Toxicol. Appl. Pharmacol., 256(3): 379–385. https://doi.org/10.1016/j.taap.2011.05.006.

Perandones C.E., Illera V.A., Peckham D., Stunz L.L., Ashman R.F.1993. *Regulation of apoptosis in vitro in mature murine spleen T cells*. J. Immunol., 151: 3521–3529.

Tekeli M.Y., Eraslan G., Bayram L.Ç., Aslan C., Çalimli S. 2023.*The protective effects of baicalin and chrysin against emamectin benzoate-induced toxicity in Wistar albino rats*. Environ. Sci. Pollut. Res. Int., 30(18): 53997–54021. https://doi.org/10.1007/s11356-023-26110-5.

Temiz Ö. 2020. *Biopesticide emamectin benzoate in the liver of male mice: evaluation of oxidative toxicity with stress protein, DNA oxidation and apoptosis biomarkers*. Environ. Sci. Pollut. Res. Int ., 27(18):23199-23205. https://doi.org/10.1007/s11356-020-08923-w.

Wang X., Piao X., Chen J., Hu J., Xu F. Tao. 2006. *Organochlorine pesticides in soil profiles from Tianjin. China*. Chemosphere., 64(9):1514–1520 https://doi.org/10.1016/j.2005.12.052.

Wolterink G., Van Kesteren P., McGregor D. 2012. *Emamectin benzoate. Pesticide residues in food*. 2011, p. 211.

Yun W., Rao C., Xiao C., Huang Q. 2017. *Apoptosis of leukemia K562 and Molt-4 cells induced by emamectin benzoate involving mitochondrial membrane potential loss and intracellular Ca2+ modulation*., Environ. Toxicol. Pharmacol., 2: 280–287. https://doi.org/10.1016/j.etap.2017.04.013.

ZHANG Z., ZHAO X., QIN X. 2017. *Potential genotoxic and cytotoxicity of emamectin benzoate in human normal liver cells. Oncotarge*t., 8(47): 82185. https://doi.org/10.18632/oncotarget.18988.

ZHANG Y., KONG C., CHI H., LI J., XING J., WANG F., ZHAI, Q. 2020. *Effect of a beta-cypermethrin and emamectin benzoate pesticide mixture on reproductive toxicity in male mice in a greenhouse environment.* Toxicol. Mech. Methods., 30(2): 100–106. https://doi.org/10.1080/15376516.2019.1669241.

ZHU W.J., LI M., LIU C., QU J.P., MIN Y.H., XU S. W., LI S. 2013. *Avermectin induced liver injury in pigeon: mechanisms of apoptosis and oxidative stress.* Ecotoxicol. Environ. Saf., 98, 74–81. https://doi.org/10.1016/j.ecoenv.09.021.