Study of Oxidative Stress and Cytotoxicity in Reproductive Tissues of Adult Male Rats Exposed to Insecticides Proteus and Biscaya

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ABSTRACT

Insecticides, which are composed of endocrine disrupting compounds, lead to reduction of hormones and disturbance in the reproduction and growth of animals by interfering with endocrine systems. Also, reduced spermatogenesis activity and reproduction of animals are among other effects of insecticides. Considering that these compounds affect testicular activities and steroidogenesis by altering the activity of the relevant enzymes, and given the role of lactate dehydrogenase (LDH) enzyme in the function of spermatogenesis as well as presence of malondialdehyde (MDA) under oxidative stress conditions caused by tissue degradation, this study aimed to investigate changes in the two LDH and MDA enzymes influenced by the use of the insecticides Proteus and Biscaya, separately and in combination, and their effect on the sexual activity process in adult male rats.

Method: In this experimental study, 110 adult male 80-90-days-old Wistar rats with a mean weight of 200 g were selected and randomly divided into 11 equal groups including a control group (without receiving any substance), a control group (receiving distilled water), experimental groups 1, 2, 3 (respectively receiving 2.75, 5.5 and 11 mg/kg/Bw Proteus), experimental groups 4, 5, 6 (respectively receiving 1.5, 3, 6 mg/kg/Bw Biscaya), and experimental groups 7, 8, 9 (respectively receiving 1.5 mg/kg/Bw Biscaya+2.75 mg/kg/Bw Proteus, 3 mg/kg/Bw Biscaya+5.5 mg/kg/Bw Proteus, 6 mg/kg/Bw Biscaya+11 mg/kg/Bw Proteus). The LD50 method was used to evaluate the effect and amount of use of each of the insecticides. All the injections were performed intraperitoneally and the duration of the experiment was two weeks. At the end of the experiment period, blood samples were taken from the rats and the serum concentration of the LDH and MDA enzymes was measured using the ELISA kit. Statistical analysis was performed using SPSS version 16 and Duncan's test at a significant level of 0.05 ($P<0.05$).

Findings: Comparison between the groups receiving different concentrations of the insecticides Proteus and Biscaya separately and in combination with the control group in the mean serum level of LDH and MDA showed that the maximum concentration of the insecticide Proteus and the medium and maximum concentrations of the insecticide Biscaya as well as different concentrations of a combination of these two types of insecticide significantly increased the level of LDH and MDA in a dose-dependent form.

Conclusion: Increased serum levels of LDH and MDA in the groups that received different doses of these insecticides separately and in combination were probably because the insecticides caused cytotoxicity and production of reactive oxygen species (ROS) in the dose-dependent form, which resulted in tissue degradation and elevated LDH. Moreover, by causing oxidative stress that was associated with increased MDA, these insecticides led to the production of nitric oxide (NO) and hydrogen peroxide (H2O2); these molecules, in turn, caused Leydig cell atrophy by their signaling function.

Key Words: *LDH, MDA, Insecticide, Proteus, Biscaya, Rat.*

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INTRODUCTION

Endocrine disrupters are compounds that, in equilibrium, can cause changes in reproduction, growth or behavior, and thus affect animals or humans [1, 2]. These compounds destroy or inhibit endocrine systems, thus reducing or increasing the amount of hormones. Among endocrine disrupters are industrial and disposable products as well as pesticides including insecticides [3, 4]. Thiacloprid (THIA) from neonicotinoids and deltamethrin (DEL) from pyrethroids are among synthetic insecticides commonly used singly or in combination [5-9]. The insecticide Proteus, commercially known as DEL+THIA, and the insecticide Biscaya, commercially known as THIA, are two types of insecticide that have been widely used in agriculture in recent years; also, high levels of use of such pesticides have led to severe and varied effects on human health [10-13].

It has also been shown that these chemical compounds cause disturbances in the steroidogenesis mechanism in testicular tissues [14, 15]. It has been found that there is a high correlation between these factors (disrupting compounds) and reduction in sperm count, mobility and fertility as well as production of defective spermatozoids in animals [16]. Moreover, organochlorine pesticides have been reported to exert their effect by altering the activity of the enzymes involved in testicular activities [17, 18]. It has also been reported that the anti-androgenic effect caused by the use of glyphosate in animals increases the height of the epithelium of the tubes and reduces the cavity diameter of seminiferous tubules [19]. Another report states that imidacloprid increases intracellular space and atrophy of seminiferous tubules, reduces weight of the testicles, and reduces Leydig cells and their hypertrophy [20].

The use of dicofol at high and low doses has been reported to significantly reduce lactate dehydrogenase (LDH), which is a major enzyme for spermatogenesis, in testicular tissues. This reduction could be attributed to the interference of dicofol with energy metabolism in testicular tissues [21].

There are also reports on the impact of pyridabine on the increased levels of malondialdehyde (MDA) and nitric oxide (NO) [22]. Accordingly, it has been reported that testicular oxidative stress may be associated with decreasing testicular testosterone levels, and an increase in the NO level, similar to an oxidative index, results in inhibition of testosterone production [23].

On the other hand, it has been reported that pesticides lead to production of high levels of reactive oxygen species (ROS) in both extracellular and intracellular spaces, which is associated with a reduction in sperm count and fertility in humans and animals [24].

Therefore, considering the probable role of these toxins in the cytotoxicity of reproductive organs and their interference with the function of hormones [25], as well as the role of these pesticides in the production of high ROS levels in both extracellular and intracellular spaces followed by a reduction in sperm count [24] and the richness of spermatozoa from unsaturated fatty acids that makes them suitable for oxidative stress [26], in this study, we attempted to measure the LDH level as a cytotoxicity marker and the MDA level as an oxidative stress indicator and also, to investigate the impact of use of the insecticides Proteus and Biscaya separately and in combination on these two enzymes.

MATERIALS AND METHOD

This is a completely randomized experimental study. The protocol of the study was established in accordance with the international regulations on the protection of laboratory animals and approved by the University's Ethics Committee under the number IR.miau13951206.

Animals and their grouping: In this research, all ethical considerations regarding the maintenance and use of laboratory animals were observed throughout the research period. In this experimental study, 110 adult male 80-90-days-old Wistar rats weighing 200-220 grams were used. The rats were kept at the animal breeding room of the Jahrom University of Medical Sciences for a period of one week to adapt to the environment. Throughout the study, the rats were maintained under a 12:12-h light:dark cycle at the environment temperature of 23±2°C and relative humidity of 50-55%. The rats were kept in shelves made of transparent makrolon polycarbonate and had free access to water and food.

The LD50 method was utilized to study the effect and amount of use of each of the insecticides [21]. The rats were randomly divided into 11 groups of 10, as described in Table 1.

Table 1. The different groups of the studied rats

Groups	Treatment
Control	Getting water and food naturally
Sham	Getting solvent of toxins (distilled water)
Experimental I	P 2/75 mg/kg/BW
Experimental II	P 5/5 mg/kg/BW
Experimental III	P 11 mg/kg/BW
Experimental IV	B 1/5 mg/kg/BW
Experimental V	B 3 mg/kg/BW
Experimental VI	B 6 mg/kg/BW
Experimental VII	B 1/5 + P 2/75 BW/kg/mg
Experimental VIII	B 3 + P 5/5 BW/kg/mg
Experimental IX	B 6 + P 11 BW/kg/mg

mg = milligram/ p = Proteus/ Kg = kilogram/ B = Biscaya/ BW = Body Weight

All the injections were performed intraperitoneally by insulin syringes. The injection rate was 0.4 cc in all the groups and the duration of the experiment was two weeks. The insecticides were manufactured by the Bayer CropScience Company.

Blood collection

One day after the last injection, blood samples were collected directly from the heart. Then, with the aid of a centrifuge machine (3000 rpm for 15 minutes), blood serums were collected and kept in the freezer at -20 ° C until further use. To measure the LDH and MDA enzymes, the ELISA kit for rats manufactured by the Crystal Day Company of China (LOT = 2015 10-14-2016 10 13) was used.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to analyze the data. According to the Kolmogorov-Smirnov test, the data distribution was normal and thus, analysis of parametric tests was used in the next stages. In cases where the statistical difference between the different groups was significant, Duncan's test was used to find out the difference between the means.

Statistical calculations were performed using SPSS version 16 and the significance level was considered to be P<0.05. The data in the results section are given as Mean±SEM.

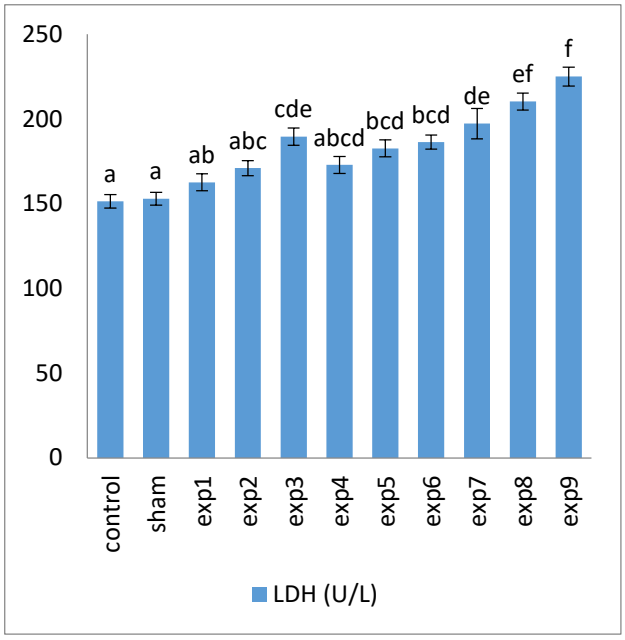

Figure 1. Evaluation of the LDH mean in the studied groups

- According to the Duncan's test, if there is at least one common letter in each column, groups in the columns are not significantly different from one another.
- U/l= Unit per Liter/ exp= expeimental

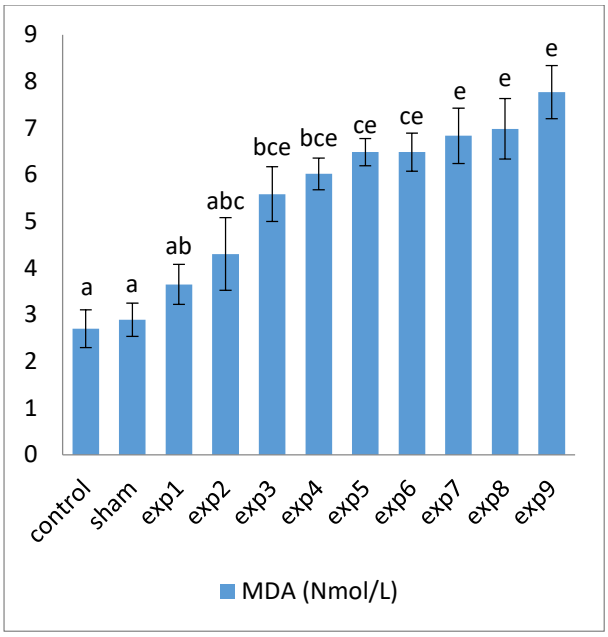

Figure 2. Evaluation of the MDA mean in the studied groups

- According to the Duncan's test, if there is at least one common letter in each column, groups in the columns are not significantly different from one another.
- Nmol/l= Nanomolar/ exp= expeimental

Table 2. The parameters studied in the different groups (Mean±SEM)

Groups	MDA	LDH
Control	2.70±.40 a	151.48±3.92 a
Sham	2.89±.35 a	152.95±3.78 a
Experimental 1 (P 2.75 mg/kg)	3.65±.42 ab	162.66±5.05 ab
Experimental 2 (P 5.5 mg/kg)	4.30±.77 abc	171.00±4.38 abc
Experimental 3 (P 11 mg/kg)	5.58±.58 bce	189.56±5.03 cde
Experimental 4 (B 1.5 mg/kg)	6.01±.33 bce	172.90±4.95 abcd
Experimental 5 (B 3 mg/kg)	6.48±.29 ce	182.66±4.95 bcd
Experimental 6 (B 6 mg/kg)	6.48±.40 ce	186.36±4.15 bcd
Experimental 7 (P 2.75+B 1.5 mg/kg)	6.83±.59 e	179.31±8.95 de
Experimental 8 (P 5. 5+B 3 mg/kg)	6.98±.65 e	210.30±5.03 ef
Experimental 9 (P 11+B 6 mg/kg)	7.76±.56 e	225.05±5.52 f

- The mean of groups with dissimilar letters have significant difference.
- Data means are presented as Mean±SEM.
- The level of p<0.05 is considered statistically significant.
- P = Proteus, B= Biscaya

FINDINGS

MDA: The comparison between the group receiving the maximum concentration of Proteus and the control group showed a significant increase in the mean serum concentration of MDA ($P<0.05$). Also, the comparison of the groups receiving the minimum, medium and maximum concentrations of Biscaya with the control group revealed a significant increase in the mean serum concentration of MDA ($P<0.05$).

In addition, the comparison between the groups receiving minimum, medium and maximum concentrations of a combination of Proteus and Biscaya with the control group showed a significant increase in the serum concentration of MDA, which was in a dose-dependent form so that the maximum concentration of the Proteus and Biscaya combination exerted a higher impact in the increase of the MDA serum concentration ($P<0.05$).

LDH: A significant increase was observed in the mean serum concentration of LDH in the group receiving the maximum concentration of Proteus, as compared to the control group ($P<0.05$). Also, the comparison between the groups that received different Proteus concentrations showed a significant increase in the LDH serum concentration in the groups receiving the minimum and medium concentrations of Proteus, as compared to the group receiving the maximum dose ($P<0.05$).

Furthermore, the comparison between the groups receiving different Biscaya concentrations with the control group in terms of the LDH serum concentration showed that there was a significant difference between the groups receiving the medium and maximum Biscaya concentrations with the control group ($P<0.05$). On the other hand, a significant increase was observed in the LDH level in a dose-dependent manner in the groups receiving the minimum, medium and maximum concentrations of a combination of Proteus and Biscaya, as compared to the control group ($P<0.05$).

DISCUSSION

The results of this study showed that the maximum doses of Proteus alone and the medium and maximum doses of Biscaya alone, as well as different doses of a combination of Proteus and Biscaya significantly increased the mean serum levels of the MDA and LDH enzymes in the dose-dependent form. Increased serum levels of the MDA and LDH enzymes in this study are consistent with the results of other researchers. In the studies by Ebadi Manes, it has been reported that pyridabine led to the increased MDA and NO levels [22]. It has also been reported that presence of LDH is one way to detect cell death, and this cytoplasmic enzyme is released when the cell membrane is damaged, such as in cytotoxicity conditions [27]. Moreover, it has been reported that MDA is found in human and animal tissues as a lipid peroxidation end product, and is a byproduct of biosynthesis of prostaglandins and thromboxanes [28].

Farag et al. reported that dimethoate has different effects on reproductive activities in male rats, including sperm density, mobility and vitality [29-31]. Also, Ngoula et al. reported that the pirimiphos-metyle insecticide reduced the number of Leydig cells [32].

Yet in other studies, it has been reported that testicular oxidative stress may be associated with lower testicular testosterone levels, and the increase in NO levels as an oxidative stress indicator results in inhibition of testosterone production [23].

CONCLUSION

Significant increase in the serum levels of the MDA and LDH enzymes between the groups that used different concentrations of Proteus and Biscaya separately or in combination with the control group was due to the degenerative effects of these pesticides [33], which caused atrophy and degeneration of the base cells as well as inhibition of spermatogenesis [29]. Hence, the increase in the LDH enzyme is probably due to cytotoxicity or cell death caused by these toxins. Moreover, these toxins may have caused Leydig cell apoptosis and atrophy in a dose-dependent manner [34]. The testicular oxidative stress associated with increasing NO levels has led to testosterone inhibition [23], and since NO and H_2O_2 can act as signaling molecules that results in oxidative cell death [35, 36], the LDH and MDA levels have increased. Therefore, considering that the reproductive hormones imbalance may contribute to reducing the amount of testicular antioxidants [37, 38], these toxins reduce testicular testosterone concentrations and, by producing high levels of ROS, stimulate lipid peroxidation and reduce enzymatic activities of antioxidants, and subsequently, cause apoptosis of base cells, degradation of spermatogenesis and elevation of LDH and MDA.

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