Histopathology of Male Swiss Albino Mice reproductive system due to Toxic effects of Thiamethoxam

Salema L Hasan, Khalid K Kadhim, Abdulkarim J Karim and Jenan M Khalaf

Abstract
The present study aimed to investigate the effect of thiamethoxam on the physiological and histological status of male mice reproductive system. This study was carried out at College of Veterinary Medicine-Baghdad, from August to December 2016. Fifty adult males Swiss albino mice, aged two months, were divided into five equal groups. First group immunized with B. abortus antigen. Second group was dosed orally thiamethoxam. Third group were subjected to same treatments in the 1st and 2nd group. The 4th group injected virulent B. abortus. The 5th group acted as negative controls. All animals were euthanized and blood samples were taken for hormonal analysis and pieces of testes, epididymis were fixed in 10% formalin for routine histopathological examination. Results showed that thiamethoxam significantly decreased the sperm viability, motility and testosterone level. In conclusion, thiamethoxam possess toxic effect for spermatogenesis in mice. To avoid its harm on human, the use of thiamethoxam should be expertised.

Keywords: Histopathology, Testis, Testosterone, Thiamethoxam

Abbreviations: TMX, Thiamethoxam; CFBAs, Culture filtrated B abortus antigen; ROS, reactive oxygen species; nAChRs, nicotinic acetylcholine receptors; IP, intraperitoneal.

1. Introduction
Thiamethoxam (TMX), a relatively new pesticide, belongs to second-generation of neonicotinoid pesticides, a group of several different insecticides, with mostly applicable, imidacloprid: thiacloprid and clothianidin [5]. Pesticides are classified according to their target, their mode or period of action, or their chemistry [2]. The wide use of TMX is attributed to its efficacy at low doses, variety of application methods, and long half-life [8]. It is bioactivated by cytochrome P450 enzymes through desulphuration to its corresponding Oxon derivative [4]. Close resemblance of TMX chemical structure to nicotine: render it as agonist at mammalian and insect nicotinic acetylcholine receptors (nAChRs) [6]. Although these insecticides are believed to be of low toxicity to mammals because their lower interaction with vertebrate nAChRs compared to insect’s [6], ingestion of a large amount may develop severe poisoning mainly targeted the reproductive system [7]. The effects on male fertility may directly damage spermatozoa, alter Sertoli cell or Leydig cell function, or disrupt the endocrine function in any stage of hormonal regulation (hormone synthesis, release, storage, transport, and clearance; receptor recognition and binding; thyroid function; and the central nervous system) [3]. Chemical changes in sperm nuclear proteins (protamines), which DNA during the last steps of spermatogenesis are part of the cause to male reproductive toxicity [5].

Insecticides that used in commercial formulation could be toxic and harmful to the developing embryo and in-vitro fertilization [9]. They frequently alter human health or development, typically because they have endocrine agonist or antagonist activities and alter hormone-regulation and gene expression [10]. In some epidemiological studies, decreased semen quality has been reported in agricultural workers [12]. In animal models, results showed that single doses of diazinon (8.125mg/Kg, IP) administered to mice alter sperm viability, motility, morphology and fertilization ability [12].

In vitro, TMX induces lipid peroxidation, alterations the activities of antioxidant enzymes and DNA damage [13]. Sublethal doses may lead to reduced growth and reproduction of aquatic.
invertebrate, interference with algae-invertebrate interactions, reduced egg-production, delayed sexual maturity, adverse behavioral modifications in mammals and disrupt ovarian structure [14]. Some studies showed that exposure to environmental chemicals such as diazinon and malathion result in cancers or reduced reproductive functions in animals and human [15]. The present study was designed to investigate the effects of TMX on the structure of testis and levels of sex hormones in adult male mice.

2. Materials and Methods

2.1 Chemicals, Bacterial Isolate and Experimental Mice

Thiamethoxam (Sigma, St. Louis, MO) and other chemicals were of analytical grade. Virulent culture of B. abortus was obtained from Dept of Microbiology, College of Veterinary Medicine-University of Baghdad. Fifty adult males Swiss Albino mice at the age of two months were purchased from Institute of Vaccine and Sera, Ministry of Health, Baghdad. Mice were kept in plastic cages for 5 days at the Animal House for adaptation before the experiment started. Pellets and water were supplied ad libitum. All experiments were carried out in Lab of Pathology, College of Veterinary Medicine-University of Baghdad, from August to December 2016.

2.2 Experimental design

Experimental procedure was approved by the Animal Care and Use Committee (approval no. 168/2016). Mice were divided into five equal groups, 10 each. The 1st group (G1) consist of mice immunized with CFBAGs (protein concentration 4.2mg/ml), two IP doses at two weeks intervals, the 2nd group (G2) dosed orally daily with TMX (83.7mg/Kg BW). Mice of the 3rd group were administered both treatments in G1 and G2 for 9 weeks, and IP injected with 0.3 ml of bacterial suspension containing 1 x 10^8 CFU/ml of viable virulent B. abortus. Mice in the 4th group (G4) were IP injected with 0.3ml of bacterial suspension containing 1 x 10^9 CFU/ml of viable virulent B. abortus, one month after the experiment started and served as positive control. The 5th group (G5) administrated orally with 0.3 ml of normal saline served as negative control.

2.3 Median lethal dose (LD50) of Thiomethoxam

"Up-and-down" method [16] was used for determination of median lethal dose (LD50) of TMX. Six albino mice weighed (20-25) gm were orally gavaged 750-850 mg TMX (40mg/ml DW) per each Kg bodyweight. The difference in doses was 50mg/Kg BW. LD50 was calculated after 24 hours observation of lethality in the dosed animals. The LD50 is calculated by using the equation

LD50 = xF + kd

xF = The last dose administrated
k = factor value from appendix
d = difference between dose levels

2.4 Assay

Culture filtrated B. abortus antigen (CFBAgs) was used for immunization animals. This antigen was prepared according to Quin et al. [17]. Hormonal assay was done according to radioimmunoassay as described by Schulster et al. [18]. Sperm Abnormality Assay: Spermatogenesis in mice was performed according to Bruce and Heddle [19]. The caput and cauda epididymis excised from the male mice were placed in a petridish containing 1ml of physiological saline and then minced and teased carefully well with fine scissors and forces to release the spermatozoa. After gentle pipetting, the suspension is separated from the tissue fragments and to this suspension was added a drop of 1% Eosin Y solution (10: 1) for 30mins. Air-dried smears were prepared on clean grease-free glass slides using another clean slide angularly positioned at 300 to spread the drop through the whole length of the slide. The slides were then coded, randomized and examined cytoligically under 40x binocular light microscopy. Eight separate slides were prepared for each mouse randomly. The percentage of viability sperms was estimated as in the following equation:

Viability = [Number of live sperms / Total Number of sperm] x 100

Motility = [Number of motile sperms / Total Number of sperm] x 100

2.5 Histopathology

Pieces of testes and epididymis were fixed in 10% normal buffer formalin for 72 hours for routine histopathological examination [20].

2.6 Statistical Analysis

The data were analyzed by one way ANOVA using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). The significance level was designated at P < 0.05.

3. Results

A significant reduction in sperm counts and spermatogenesis, and a decrease in serum testosterone concentration were observed (Table, 1). Histopathological examination of testes showed degenerative changes in seminiferous tubules, Leydig and Sertoli cells compared to the control groups. Seminiferous tubules of testes of Mice in G1 showed normal stage of spermatogenesis (Fig. 1). Epididymis filled with sperm (Fig. 2). Mice in G2 showed that inflammatory cells mainly neutrophil infiltrated in sub the epithelial layer of the Epididymis and in the interstitial tissue (Fig. 3) as well as focal hyperplasia of the epithelial lining cells of the Epididymis. In addition, there were few or no sperm in the Epididymis and incomplete mature spermatogenesis. Homogeneous material and cellular debris were observed in the lumen (Fig. 4). The main lesion in the testes of mice in G3 characterized by few or no sperm in the seminiferous tubules of epididymis with round multi nuclei cell in the lumen (Fig. 5) accompanied by homogeneous material and cellular debris (Fig. 6). Mice in G4 (positive control group) showed lesion characterized by few or no sperm in the epididymis (Fig. 7) and incomplete mature spermatogenesis (Fig. 8). Vice versa, there was no significant microscopic findings in G5 (negative control) mice.

Table 1: Testosterone concentration, sperm motility and viability in mice at 9 weeks post–treatment with TMX.

<table>
<thead>
<tr>
<th>G</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testosterone (ng/ml)</td>
<td>77.8±0.3*</td>
<td>19.7±0.22f</td>
<td>55.1±0.214*</td>
<td>60.0±0.57b</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>32±0.23*</td>
<td>25.0±0.69*</td>
<td>28.0±0.5b</td>
<td>20.0±0.3*</td>
<td>20.0±e2.3*</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>38.2±0.5*</td>
<td>37.0±0.6*</td>
<td>5.0±0.3*</td>
<td>3.0±0.8*</td>
<td>40.1±1.2*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SE. Different superscript within rows refer to significant differences at p<0.05.
Fig 1: Histopathological section in the testes of mice of immunized with CFBAgs. Note the seminiferus tubules (red arrow) with normal stage of spermatogenesis (H&E stain, 40X).

Fig 2: Histopathological section in the testes of mice immunized with (CFBAgs). Note the epididymes (red arrow) filled with sperm (H&E stain, 40X).

Fig 3: Histopathological section in the testes of mice showed the effect of daily administration of thiamethoxam. Note the inflammatory cells mainly neutrophil infiltration (red arrow) in sub epithelial layer of the Epididymis and in the interstitial tissue (H&E stain, 40X).

Fig 4: Histopathological section in the testes of mice showed the effect of daily administration of thiamethoxam. Note the focal hyperplasia (red arrow) of the epithelial lining cells of the Epididymes. In addition to few or no sperm in the Epididymis and incomplete mature spermatogenesis (black arrow) (H&E stain, 40X).

Fig 5: Histopathological section in the testes of mice immunized with CFBAgs and daily administration of Thiamethoxam. Note, few or no sperm in the seminiferus tubules of Epididymis red arrow). In addition to round multi nuclei cell (yellow arrow) in the lumen (H&E stain, 40X).

Fig 6: Histopathological section in the testes of mice immunized with CFBAgs and daily administration of Thiamethoxam. Note the few homogeneous material and cellular debris (red arrow) in the lumen (H&E stain, 40X).
motility and concentration which is mainly affected by serum Testosterone in mice treated with TMX as comparable with their levels in other groups. This evidence is in consistency with Maxwell et al. [25] who showed that acetamiprid significantly decreased the body weight and the weight of the testis, epididymis, seminal vesicle, and prostate. Furthermore, acetamiprid also significantly reduced the serum testosterone concentration, and decreased sperm count, viability and motility.

Sperm count is strongly correlated with malformations and histological effects, both of which are important indicators of early-stage testicular toxicity due to chemicals [26]. Abnormalities may be attributed to the genetic material in the head of sperm or perhaps because of an abnormal chromosome complement. Severe pathological lesions in the testes of mice treated with TMX, as presented by our study, may be due the generation of ROS, which cause severe pathological changes in the reproductive tracts. This idea is consistent with Dutta and Meijer [27] who reported immature histological appearance of the testis, manifested by marked reductions in spermatogenesis, moderate tubular atrophy and increased incidences of spermatic giant cells occurred in the testes of all males treated with TMX. Shortage of testosterone level, which is needed along with the Sertoli cells to carry out spermatogenesis revealed an utmost importance [17]. Congested blood vessels with neutrophils in their lumen that were recorded in the testes (Fig. 3) may be due to relaxed smooth muscle of blood vessels induced by the NO, a free radical. This idea agrees with Pierce et al. [28] who recorded that over production of NO can cause toxic effects and vasodilatation effects. Moreover, the pathological lesions in reproductive tract are resulting from lipid peroxidation, inhibition protein synthesis and depletion of ATP by NOS [29].

The current result showed that the serum levels of Testosterone, sperm motility and concentration in immunized and immunized-TMX mice were significantly higher than those in mice treated with TMX only. This result may give an indication that immune response may stimulate enzymatic antioxidant against toxic effects of TMX. This observation is consistent with Marri and Richner [30] who showed that birds can avoid stress caused by a transient increase in reactive species generated by immune activation. Coinciding to present results, Colleen et al. [31] found that GnRH (FSH, LH), E2 and progesterone with vaccination can increase reproductive steroid hormonal levels, and the total per ejaculate sperm number was correlated with testosterone concentration [32].

The present finding explained that the serum levels of Testosterone, sperm motility and concentration in mice infected with Brucella were lower comparable with other groups. This may indicate a severe degree of infection of the testes. Several infectious conditions resulted in epididymitis and orchitis in male [33]. These results are in consistence with Depuydt et al. [34] who showed that ROS might be increased in chronic urogenital infections associated with increased leukocyte numbers and involvement of various cytokines accompanying epididymitis which influence sperm function. Several studies have investigated the association between interleukin (IL) concentration, leukocytes, and sperm function [34]. These epididymal changes are often associated with mild to moderate testicular degeneration and interstitial infiltration of lymphocytes and macrophages with accumulation of neutrophils in the lumen of the epididymis [35]. These lesions contribute to the poor spermatogenic quality and increased frequency of spermatogenic defects associated with infection [36].
5. Conclusion
In conclusion, thiamethoxam possess toxic effect for spermatogenesis in mice. Therefore, application of thiamethoxam should be limited to a designed program.

6. Acknowledgment
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7. References
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