Toxicological Effects of 30-Day Intake of Malathion on the Male Reproductive System of Wistar Rats

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HIGHLIGHTS
- Weight of rat testes treated from Malathion (MAL) was significantly lower than the control group.
- No significant difference was found between body weight of the control and the treatment rats.
- MAL induced histopathological changes in testes in sex hormones levels.

ABSTRACT
Background: Malathion (MAL) is a choice insecticide in the stored grain in different countries. The aim of the present study is to evaluate toxicological effects of 30-day intake of MAL on the male reproductive system of Wistar rats.
Methods: This research was ethically approved by the local authority at Faculty of Medicine, Monastir, Tunisia. Three MAL treated groups received 1 ml corn oil containing 1.3, 13.7, and 137 mg/kg body weight/day, respectively. Body and testes weights, testosterone, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels were measured. Histopathological sections were prepared from testes of animals and stained with hematoxylin and eosin and also Masson trichrome. Statistical analysis was done using SPSS for windows version 10.
Results: A meaningful decrease (p<0.05) in the testes weight of treatment groups was observed compared to the control animals. However, no significant difference (p>0.05) was found between body weight of control and treatment groups. All the three MAL-treated rats had significantly (p<0.05) lower plasma FSH, LH, and testosterone levels than the control group. Some major histopathological changes were found in testes sections of treatment groups.
Conclusion: Our study revealed that subchronic and relatively short time exposure of male Wistar rats to MAL can induce histopathological changes in testes and disturbance in sex hormones levels in a dose-dependent manner.

Introduction
Pesticides as a heterogeneous group of chemical products are widely used in producing crops to control pests such as insects, rodents, weeds, mold, etc. Organophosphate pesticides, the most used insecticides, are widely used in agriculture having high exposure possibility for human or livestock especially through consumption of contaminated foodstuffs (Raghu et al., 2014; Rezg et al., 2010). Although, the primary toxicity associated with acute exposure to these pesticides is cholinergic symptom resulting from acetylcholinesterase inhibition, pesticides have usually numerous compound-specific chronic effects especially developmental abnormality, immunotoxicity, carcinogenesis, and also reproductive...
toxicity (Menezes de Carvalho et al., 2014; Dhouib et al., 2015; Sultatos, 1994; Tamura et al., 2001). Considerable increasing rate of testicular abnormalities over a short time is probably related to environment rather than the genetic risk factors (Giwercman et al., 1993; Oehninger, 2001). It is known that many chemical agents can affect the reproductive performance of mammalian (Sanchez-Pena et al., 2004).

Among organophosphate pesticides, Malathion (MAL) with chemical compound of S-1,2(bis ethoxycarbonyl) ethyl O,O dimethyl phosphorodithioate is the most commonly used. MAL is a choice in the stored grain in different countries because of its high potency to a wide range of pests and its relatively low toxicity in mammalian (Flehi-Slim et al., 2015; Rezg et al., 2010; Wang et al., 2014). So, the aim of the present study is to evaluate toxicological effects of 30-day intake of MAL on the male reproductive system of Wistar rats.

Materials and methods

Ethics

This research was ethically approved by the local authority at Faculty of Medicine, Monastir, Tunisia.

Chemicals

MAL (Fyfanon® 50 EC 500 g/L) of commercial grade was used in this study. All the other chemicals were of analytical grade and obtained from local commercial sources.

Animals

Male Wistar rats weighted 150-180 g and aged 6–8 weeks obtained from SIPHAT Company (Pharmaceutical Industrial Society of Tunisia, Ben Arous, Tunisia). Animals were acclimatized during one week before the experiments. They were randomly assigned to one control group (n=6) and three different treatment groups (n=6) and housed at 21±5 °C with a 12 h light/dark cycle as well as 55% humidity. The rats were fed a standard commercial pellet diet from Sico Company (Sfax, Tunisia) and drinking water ad libitum.

Treatment

Animals were treated for 30 days by gavages using stomach tube. The three MAL treated groups received 1 ml corn oil containing 1.3 (dose 1), 13.7 (dose 2), and 137 (dose 3) mg/kg body weight/day, respectively. Control groups received equal amount of corn oil. Body weight was recorded weekly through the experiment.

Blood sampling and sex hormone analysis

At the end of treatment period, each animal was anesthetized by ether and 5 ml of blood was sampled by heart tapping. Heparinized tubes containing blood were then centrifuged at 3000 rpm during 5 min and obtained plasma was conserved at -80 °C. Plasma testosterone, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) level were measured by electrochemiluminescence assay with automate Cobas 6000 (Roche Diagnostics, Switzerland).

Histopathology

Body and testes weights of each group were measured. The testes were dissected and tissue samples were fixed in Bouin’s solution, processed in a series of graded ethanol, and embedded in paraffin. Paraffin sections were cut at 5 µm thickness and stained with hematoxylin and eosin and also Masson trichrome for light microscopy.

Statistical analysis

The data for various biochemical parameters were expressed as mean±Standard Deviation (SD) and compared using T-test. Values were considered statistically significant when p<0.05. Statistical analysis was done using SPSS for windows version 10.

Results

Changes detected in body and testes’s weight in experimental and control groups are summarized in Table 1. A meaningful decrease (p<0.05) in the testes weight of treatment groups was observed compared to the control animals. To make a functional comparison of weight gain or loss, tissue weight per 100 g body weight was calculated. The testes showed significant (p<0.05) weight reduction per 100 g body weight among the three treated groups. However, no significant difference (p>0.05) was found between body weight of control and treatment groups. Table 2 shows that all the three MAL-treated rats had significantly (p<0.05) lower plasma FSH, LH, and testosterone levels than the control group.

Fig. 1A and Fig. 2A represent photomicrographs of testes from MAL treated and a general reduction in the size of seminiferous tubules (Fig. 1B and Fig. 2B). Sections
from MAL treated animals with 13.7 mg/kg body weight/day revealed stopped spermatogenesis and discontinuity or dyscohesive basal germ cells. In addition, basal germ cells, and primary and secondary spermatocytes showed typical features of apoptosis. An abnormal aspect of spermatozoa flagella into a large number of seminiferous tubules was also observed (Fig. 1C and Fig. 2C). After the third administrated of MAL dose of 137 mg/kg body weight/day, we observed that most tubules contained apoptotic bodies. There are also absence of spermatozoa in the lumen of seminiferous tubules, thickness of basement of spermatozoa in the lumen of seminiferous tubules, and thickness of basement membrane accompanied by an important regression of interstitial tissue and Leydig cells. We recorded some abnormal tubules conformity in comparison with sections obtained from testes of control group. The highest MAL dose induced an important deformation as well as a dyscohesive aspect of whole tubules (Fig. 1D and Fig. 2D).

Table 1: Body and testes weights of rats after administration of different doses of MAL for 30 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Absolute testes weight (g)</th>
<th>Relative testes weight (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>166.65±9.62</td>
<td>187.06±8.89</td>
<td>2.34±3.76</td>
<td>1.25±6.21</td>
</tr>
<tr>
<td>Dose 1</td>
<td>164.96±7.65</td>
<td>186.08±6.88</td>
<td>2.12±3.40</td>
<td>1.14±2.60</td>
</tr>
<tr>
<td>Dose 2</td>
<td>168.41±8.11</td>
<td>179.43±9.48</td>
<td>1.87±5.25</td>
<td>1.04±7.76</td>
</tr>
<tr>
<td>Dose 3</td>
<td>166.1±9.42</td>
<td>184.69±7.91</td>
<td>1.68±5.22</td>
<td>0.91±3.12</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD; n=6 for each treatment group.

Table 2: Plasma levels of FSH, LH, and testosterone of rats after administration of different doses of MAL for 30 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testosterone (ng/L)</th>
<th>FSH (U/L)</th>
<th>LH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.91±0.19</td>
<td>2.87±7.09</td>
<td>8.52±6.26</td>
</tr>
<tr>
<td>Dose 1</td>
<td>3.63±0.12</td>
<td>2.39±7.79</td>
<td>8.18±4.09</td>
</tr>
<tr>
<td>Dose 2</td>
<td>2.97±4.98</td>
<td>1.33±6.75</td>
<td>5.87±4.09</td>
</tr>
<tr>
<td>Dose 3</td>
<td>2.41±7.95</td>
<td>1.01±6.26</td>
<td>5.34±2.8</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD; n=6 for each treatment group.

**Discussion**

In the current research, even though MAL was given at relatively low levels of 1/1000, 1/100, and 1/10 of the oral LD$_{50}$, it was found some pathological changes in animals, although no rat died during the period of the study. It was found a significant decrease in absolute and relative testes weight in a dose-dependent manner. In
Organophosphate pesticides may also affect male reproductive function by decreasing FSH, LH, and testosterone levels. Similar to our findings, significant alterations in FSH, LH, and testosterone levels have been reported after exposure to other pesticides such as abamectin (Elbetieha and Da’as, 2003) and methyl parathion (Maitra and Mitra, 2008). However, Mahgoub and El-Medany (2001) found that pesticides increase FSH and LH levels. LH and FSH activity depends both on the quantity of these hormones and the frequency of specific receptors in the testis. It has been reported that exposure to some environmental hazards affects testicular function through reduction of pituitary LH secretion as well as Leydig cell steroidogenesis (Akingbemi et al., 2004; Murugesan et al., 2007). In the current study, FSH and LH levels in all the three doses-treated rats have been significantly decreased compared to control rats. This could be the result of the fact that some insecticides like chlorpyrifos, as an organophosphate, were reported to affect hypothalamic Gonadotrophic Releasing Hormone (GnRH) gene expression and reduce LH and FSH (Gore, 2001; Pudungtod et al., 1998; Smallridge et al., 1991). On the other hand, some investigations indicated that the major location of androgenic hormone biosynthesis is in testicular Leydig cells. So, this observation is consistent with earlier reports which had shown that factors could inhibit steroidogenesis by reducing cAMP formation in Leydig cells (Stocco and Clark, 1996). It is worth noting that Valenti et al. (1997) and Ronco et al. (2001) concluded that fenvalerate pesticide may disturb the cAMP formation to make the negative action on steroidogenesis.

Conclusion
In conclusion, our study revealed that subchronic and relatively short time exposure of male Wistar rats to MAL can induce histopathological changes in testes and disturbance in sex hormones levels in a dose-dependent manner.

Conflicts of interest
The authors have not any conflicts of interest to declare.

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References


