INTRODUCTION

Thiamethoxam (TMX) is one of the second generation neonicotinoid insecticides, a group of new class of the synthetic insecticides that acts selectively on the insect nicotinic acetylcholine receptors (nAChRs), with only a little action on the mammalian nAChRs1. Rats treated with the TMX at different doses showed an increase in the anxiety behavior and there was a significant decrease in the activities of ALT, PON-1, and AchE were decreased. The concentration of creatinine was increased, while the concentration of thyroxine hormone was decreased. Hydropic degeneration in hepatocytes, hyaline casts in the lumen of renal tubules, sharp edge outlines vacuoles in the sarcoplasm of the degenerated cardio-mycocytes, and depletion in germinal epithelium of seminiferous tubules were the major histopathological alterations detected in intoxicated rats. TMX exposure was not only associated with pronounced deleterious effects on the hepatic, renal, cardiac and testicular functions, but also the disruption in the activity of thyroid gland.

Keywords: Hydropic degeneration, Paraoxonase, Thyroxine, Thiamethoxam

MATERIAL AND METHODS

Chemicals and Kits: Thiamethoxam (TMX) 25% WS (Actara®) with chemical name 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-[1, 3, 5] oxadiazinan-4-yllidene-N-nitroamine was obtained commercially from local market of pesticides. Paraxon, CaCl; and tris base were obtained from Sigma Chemical Co. St., Louis, MO, USA. Biochemical diagnostic kits for alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), gamma glutamyltransferase (GGT), total acid phosphatases (ACP), prostatic acid phosphatase, urea, uric acid, and creatinine were obtained from Vitro Scient Co. Egypt. ELISA diagnostic kits for testosterone and thyroxine (T₄) obtained from Dima Gesell-schaft Fur Diagnostika [GmbH], Germany. Acetylcholinesterase

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Table 1: Effect of TMX on biochemical parameters of rats

<table>
<thead>
<tr>
<th></th>
<th>ALT (U/l)</th>
<th>AST (U/l)</th>
<th>CK (U/l)</th>
<th>ALP (U/l)</th>
<th>GGT (U/l)</th>
<th>LDH (U/l)</th>
<th>PON 1 (U/l)</th>
<th>AchE (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>26.9±2.9*</td>
<td>164.3±4.6*</td>
<td>468.1±7.1</td>
<td>276.1±10.4*</td>
<td>9.1±0.3*</td>
<td>526.8±18.1*</td>
<td>30.4±4.0*</td>
<td>119.0±10.1*</td>
</tr>
<tr>
<td>Control</td>
<td>33.5±1.1</td>
<td>91.1±4.3</td>
<td>430.7±14.0</td>
<td>171.5±8.0</td>
<td>7.9±0.5</td>
<td>149.6±5.6</td>
<td>53.8±2.3</td>
<td>149.3±5.4</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM (n=10).

*p<0.05 compared with control group.

Table 2: Effect of TMX on biochemical and hormonal parameters of rats

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dl)</th>
<th>Uric Acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>T4 (ng/ml)</th>
<th>Testosterone (ng/ml)</th>
<th>ACP (U/l)</th>
<th>PACP (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>0.34±0.03*</td>
<td>0.11±0.01</td>
<td>0.94±0.02*</td>
<td>2.6±0.42*</td>
<td>1.4±0.14</td>
<td>13.4±0.36*</td>
<td>4.2±0.13*</td>
</tr>
<tr>
<td>Control</td>
<td>0.46±0.02</td>
<td>0.08±0.01</td>
<td>0.70±0.02</td>
<td>4.1±0.22</td>
<td>1.7±0.20</td>
<td>10.0±0.25</td>
<td>3.2±0.17</td>
</tr>
</tbody>
</table>

Data represented as mean ± SEM (n=10).

*p<0.05 compared with control group.

RESULTS

Clinical Picture, Biochemical and Hormonal Analysis: No mortalities were detected among intoxicated rats. Diarrhea and loss of hair were the major signs of toxicity which were observed in some of the TMX-intoxicated animals. As illustrated in Tables 1 and 2, activities of AST, ALP, GGT, LDH, total ACPs, and prostatic ACP in the serum of the rats treated with TMX were significantly higher than those of control. On the contrary, the activities of ALT, PON-1, and AchE were significantly decreased in TMX-treated...
Histological examination of the myocardium of control rats showed normal structures (Figure 3-a). Myocardium of intoxicated rats showed sharp edge outlines vacuoles in the sarcoplasm of degenerated myocytes (Figure 3-b) and mild to moderate epicardial lymphocytic infiltration (Figure 3-c). Moreover, there were segmental lytic necrosis and hemorrhage (Figure 3-d).

The testes of control rats showed well-organized seminiferous tubules. Also, all stages of transformation of the seminiferous epithelium from spermatogonia to spermatozoa could be seen (Figure 4-a). The encountered lesion in treated rat was marked thickening of the interstitium and edema that was represented by faint eosinophilic albuminous material (Figure 4-b). Some tubules showed depletion of germinal epithelium with hyalinization of the luminal contents (Figure 4-c). Lumina of the majority of seminiferous tubules contained sloughed degenerated germinal epithelial cells and giant cell formations beside the most of seminiferous tubules had single or double cell layers and devoid of spermatids and spermatozoa (Figure 4-d).

Epididymis of treated rats showed that some of epididymal ductules had very low sperm density and vacuolation of few epithelial cells (Figure 5-a). Cauda epididymal ductules had very low sperm density (Figure 5-b).

**DISCUSSION**

Thiamethoxam (TMX) is one of seven neonicotinoid insecticides currently sold in the market. Neonicotinoids are the most important new class of insecticides that acts...
selectively as agonists of the insect nicotinic acetylcholine receptors (AChRs). The objective of the present study is to investigate the multi-dose, short term toxic effect of TMX on biochemical and hormonal parameters, as well as on the histological structure of selected organs in albino rats.

The obtained results clearly showed that TMX has the potential to cause severe disturbances in rats' liver functions as evidenced biochemically. Such biochemical results were in harmony with our histopathological examination which revealed presence of severe hydropic degeneration of the majority of hepatocytes and focal hepatic necrosis with inflammatory cell infiltration. Also, our data is consistent with those obtained by Shalaby et al who suggested that TMX after 5 and 10 days of treatment caused impairment of some biochemical parameters and histological structure in albino rats. Regarding the analysis of enzymes, AST and ALT are enzymes which catalyze the transfer of α-amino groups from aspartate and alanine to the α-keto group of ketoglutaric acid to generate oxalacetic and pyruvic acids respectively, which are important contributors to the citric acid cycle. Both enzymes require pyridoxal-5'-phosphate (PLP) in order to carry out this reaction. It has been reported that the effect of PLP deficiency is greater on ALT activity than on that of AST.

We suggested that TMX perhaps decrease PLP which contributed to decreased ALT activity. This explanation is consistent with Diehl et al who reported that in the alcoholic liver disease, PLP deficiency may decrease serum ALT activity and contributed to the increase in the AST/ALT ratio. In the same trend, Hoffmann and Solter mentioned that AST enzyme is quite widely distributed in the body. It is used to investigate the muscular, cardiac and hepatic damage. Elevation of the AST activity in TMX-treated animals may be also attributed to the myocardial damage as shown by our histopathological results as sharp edge outlines vacuoles in the sarcoplasm of the degenerated myocytes, as well as, mild to moderate epicardial lymphocytic infiltration, lytic necrosis and hemorrhage. Moreover, Zone 3 of the hepatic acinus has a higher concentration of AST, and damage to this zone, either by ischemia or toxic compounds may have resulted in greater alteration of AST activity indicating the possibility of regional toxic selectivity of TMX on the liver. Our results also could be explained in the light of the fact that AST occur in high concentration in the mitochondria, while ALT is located mainly in the cytosol. At the beginning of cellular damage, cytosolic content will be liberated first resulting in the elevation of serum ALT activity. Continuous exposure to TMX enhanced the mitochondrial damage and the liberation of AST into the blood. The half-life in the circulation is about 47 hours for ALT and 87 hours for mitochondrial AST and this may be reflected by the decrease in ALT activity and the continuous elevation in AST activity. Furthermore, the activity of CK was non-significantly elevated indicating that the main origin of elevated AST is hepatocytes rather than skeletal myocytes.

Moreover, the serum ALP activity may be elevated in both acute, chronic liver diseases, and marked elevation indicates cholestasis. Unlike serum AST and ALT, the elevations of ALP activity is not due to leakage of enzyme from the damaged cells, but may be the result of the decreased biliary excretion of the enzyme as in the case of the cholestatic liver disease, because the bile contains a great deal of ALP activity.

The possible explanation of elevation in the activity of LDH is that, this enzyme is of wide distribution in many organs. Because of its wide distribution, increases in total LDH activity can be very difficult to interpret. However, due to its large size and long half-life LDH activity remains raised for some time after the initial damage.

Analysis of the AchE activity revealed a significant decrease in its activity in the serum of treated animals when compared with control. Similar result was obtained by Rodrigues et al who found that TMX induced an increase in the anxiety behavior and a significant decrease in both high-affinity choline uptake (HACU) and AchE activity in brain of rats, indicating the presence of TMX affinity to both true and pseudo-cholinesterase. Also, the serum of TMX-treated rats showed decreased activity of PON-1 enzyme in comparing with control animals. It was established that the PON-1 enzyme was first discovered through its ability to hydrolyse and therefore detoxify organophosphorus compounds which are widely used as pesticides and nerve gases. After decades of research it is...

Figure 4: Photomicrograph of rat testis stained with HE. (a) Most seminiferous tubules are well-organized with intact interstitial tissue in control rats (b) Rats treated with thiamethoxam showed faint eosinophilic-albuminous material in the interstitial tissue of the testis (stars X160). (c) Depletion of germinal cells and hyalinization of the luminal contents of the seminiferous tubules (stars X 250). (d) Sloughing of the germinal epithelium (blue arrow) with giant cell formations (black arrow) beside the majority of seminiferous tubules had single or double cell layers and devoid of spermatids and spermatozoa (stars X160).
only now becoming clear that PON-1 protects humans from the acute and chronic harmful effects of these compounds. Therefore, low PON-1 activity may increase their susceptibility to organophosphates\textsuperscript{15,16}. In the same trend, Ferre’ et al\textsuperscript{17} reported a decrease in PON-1 activity in the serum of patients with chronic liver diseases which was related to the degree of liver damage. PON-1 activity was lower in patients with cirrhosis than in those with hepatitis and was correlated with the serum albumin and bilirubin concentrations. Two mechanisms may explain this relationship. In the first, a decrease in PON-1 enzymatic activity or gene expression could be the consequence of the hepatic dysfunction. Supporting this hypothesis is the observation of an inhibition of microsomal PON-1 activity in rats chronically administered CCl\textsubscript{4}\textsuperscript{18}. The second mechanism is that, the hepatic PON-1 concentration may be normal, while serum PON-1 activity could be decreased as a consequence of an altered synthesis and/or secretion of HDL secondary to impaired lecithin cholesterol acyl transferase (LCAT) activity. Alterations in HDL structure and concentration associated with decreases in hepatic LCAT activity are frequent in chronic liver diseases\textsuperscript{19} and a decrease of serum PON-1 activity in mice with LCAT deficiency resulting from LCAT gene targeted disruption\textsuperscript{20}.

Morphological examination of renal tissue revealed presence of hyaline cast in the lumen of renal tubules and this may be due to the toxic effect of TMX on glomeruli which become more permeable to plasma protein as albumin. Moreover, there were perivascular inflammatory cells infiltrations and mild to moderate multifocal interstitial nephritis. Obtained histological lesions are consistent with the observed significant elevation in the level of creatinine in serum of intoxicated rats. Unexpectedly, urea concentration was significantly decreased in the serum of TMX-intoxicated rats. This could be attributed to hepatic damage which led to the leakage of urea cycle enzymes resulting in decrease in the serum urea level. It has been reported that some of the urea cycle enzymes leak rapidly from hepatocytes when liver cells are damaged\textsuperscript{21}.

Also, TMX exposure can alter the morphological structure of the testicular tissue as evidenced by presence of the interstitial edema, coagulative necrosis, and depletion of the germinal epithelium with hyalinization of the luminal content of seminiferous tubules. Furthermore, some tubules had sloughed germinal epithelial cells within their lumina. These findings are in agreement with Breckenridge and Stevens\textsuperscript{22} who mentioned that high doses of TMX caused testicular effects in the multi-generation reproduction study. In the same context, serum level of testosterone hormone showed no significant alteration in TMX-exposed animals when compared to the control group, indicating that TMX couldn't able to suppress testosterone production despite the detected histological lesions.

The elevation in the activities of both acid TACP and PACP indicate prostatic damage as PACP has been used extensively as a serum marker for cancer of prostate\textsuperscript{23}. Concerning the lowering level of thyroxine hormone which was observed in the serum of TMX-intoxicated rat, we suggested that the thyroid system is considered as a major target for the so-called endocrine disrupting chemicals. Such disruption may have severe consequences as thyroid hormones play an important role in the maintenance of normal physiological status in vertebrates. Many other pesticides had been shown to have endocrine disruptive activity\textsuperscript{24}. The exposure to benzene hexachloride (organochlorine), malathion (organophosphate) and Talstar (pyrethroid) led to decrease in serum concentration of triiodothyronine (T3) and thyroxine hormone (T4), with concomitant stimulation of thyroid stimulating hormone (TSH) in rats\textsuperscript{25}. More recently, the sub-acute treatments of commercial formulations of thiacloprid (neonicotinoid insecticide) and mixture of deltamethrin (pyrethroid insecticide) with thiacloprid increased serum triiodothyroin and free thyroxine hormone levels in rats\textsuperscript{26}. These findings emphasize the need to further studies to distinguish the association between TMX and thyroid hormone level regarding dose-response and mechanism.

CONCLUSION
In conclusion, the findings suggested that TMX exposure was not only associated with pronounced deleterious effects on the hepatic, renal, cardiac and testicular functions, but also induced disruption in the activity of thyroid gland as evidenced biochemically, hormonally and histopathologically.

ETHICAL APPROVAL
All experimental protocol and handling of animals were in compliance with guidelines of the institute (Faculty of Veterinary Medicine, Alexandria University, Egypt). Extensive care was taken to decrease pain of animals during dosing and sampling.

CONFLICT OF INTERESTS
The authors declare that there is no conflict of interests.

ACKNOWLEDGMENT
This work not receives any grant from any funding agency in the public or commercial. This work was self-financed by authors. Research was conducted at the Faculty of
Vernay Veterinary Medicine, Alexandria University, Edfina, Egypt.

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