Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2016; 8(5); 320-325

ISSN: 0975-5160

Research Article

Thiamethoxam-Induced Biochemical, Hormonal and Histological Alterations in Rats

El Okle O S[1], Lebda M A[2*], Tohamy H G[3]

[1]*Department of Forensic Medicine and Toxicology, Faculty of Veterinary medicine, Alexandria University, Edfina, Egypt*
[2]*Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Edfina, Egypt*
[3]*Department of Pathology, Faculty of Veterinary medicine, Alexandria University, Edfina, Egypt*

Available Online: 25th October, 2016

ABSTRACT

Modulatory effect of the insecticide thiamethoxam (TMX) on biochemical and hormonal parameters, as well as histological structure was investigated. For this purpose twenty adult male Wistar rats were randomly and equally divided into two groups. The first group were treated orally with TMX by gavage at dose of 100 mg/kg bw, while the second group served as control. Treated animals received TMX once daily for 7 consecutive days. There is a significant elevation in the activities of AST, ALP, GGT, LDH, acid phosphatase and prostatic acid phosphatase in the serum of the TMX-intoxicated rats. On the contrary, 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-myocytes, and depletion in germinal epithelium of semineferous 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

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 nAChRs[1].Rats treated with the TMX at different doses showed an increase in the anxiety behavior and there was a significant decrease in both high-affinity choline uptake (HACU) and the acetylcholinesterase activity in different brain regions[2]. It has been reported that, TMX is a highly effective systemic and contact insecticide with relatively large oral LD_{50} in albino rats (1563 mg/kg bw) indicating low acute mammalian toxicity[3].

TMX is rapidly and almost completely absorbed following single oral doses in the rats. It is widely distributed in the body and the highest tissue residues are found in the liver. TMX is poorly metabolized in the rats at the highest dose level (100 mg/kg bw) and 70-80% of the dose was eliminated unchanged, which is in contrast to the complete metabolism at the lowest dose level. The major biotransformation is cleavage of the oxadiazine ring to form the corresponding nitroguanidine CGA322704 (clothianidin). The N-demethylated nitroguanidine metabolite CGA265307 is formed either directly to from the clothianidin or via the intermediate N-demethylated thiamethoxam metabolite (CGA330050)[4]. The generation of formaldehyde considered as an alternative mechanism for TMX-induced hepatotoxicity and hepatocarcinogenicity, as it can yield more formaldehyde than any other commercial neonicotinoid[5].

Despite the well defined low mammalian acute toxicity of TMX, to the author's knowledge, several aspects of the toxic effects of this compound are still not well investigated. Thus, this study was carried out in order to explore the short term toxic effect of TMX on some biochemical and hormonal parameters, as well as on the histological structure of several body organs in rats.

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-ylidene-N-nitroamine was obtained commercially from local market of pesticides. Paraxon, $CaCl_2$ 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 (T_4) obtained from Dima Gesell-schaft Fur Diagnostika [GmbH], Germany. Acetylcholinesterase

**Author for Correspondence: biochemistry232@yahoo.com.*

Table 1: Effect of TMX on biochemical parameters of rats

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

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

	Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)	T_4 (ng/ml)	Testosterone (ng/ml)	ACP (U/l)	PACP (U/l)
Treated	0.34±0.03*	0.11±0.01	0.94±0.02*	2.6±0.42*	1.4±0.14	13.4±0.36*	4.2±0.13*
Control	0.46±0.02	0.08±0.01	0.70±0.02	4.1±0.22	1.7±0.20	10.0±0.25	3.2±0.17

Data represented as *mean* ± *SEM* (*n=10*).
*p<0.05 compared with control group.

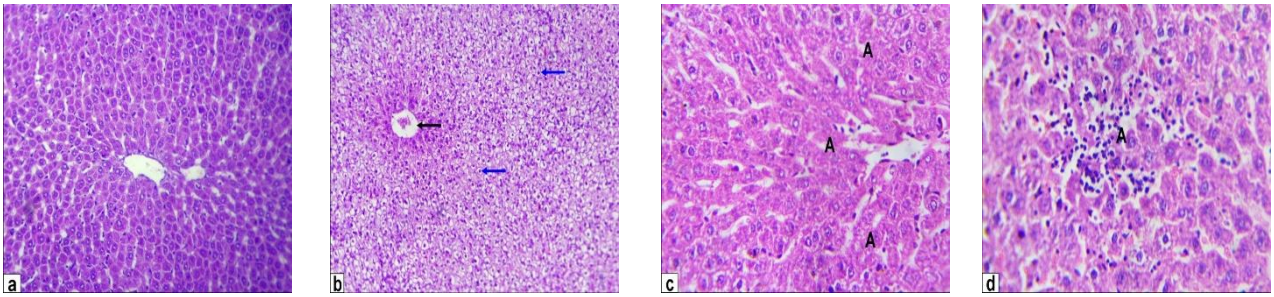

Figure 1: Photomicrograph of rat liver stained with hematoxylin and eosin. (a) The histoarchitecture of the liver is intact in controls. (b) Rats treated with thiamethoxam showed severe hydropic degeneration of the hepatocytes (blue arrows) and congestion of blood vessel (black arrow, X160). (c) lytic necrosis of the hepatocytes (A, X400). (d) necrotic hepatocytes with phagocytic cells infiltration (A, X400)

(AchE) kits were obtained from Biodiagnostic Co. Egypt. All the reagents used were of analytical grade.

Animals and Experimental Design: Twenty adult male Wistar rats, weighing 240±10 g were used in the present study. Animals were obtained from the Laboratory Animal Resource Section of the Faculty of Veterinary Medicine, Alexandria University, Egypt and kept under standard animal house condition in plastic cages for 7 days before experiment. After acclimatization period, animals were randomly and equally divided into two groups (*n=10*). Sub-acute toxicity study was conducted, as TMX was orally administered by intra-gastric gavage using normal saline as solvent to the first group (treated group) at dose of 100 mg/kg once daily for 7 consecutive days. The selected dose represents about 6.0 % of the LD_{50} in rats (1563 mg/kg b.wt). The second group served as control and received saline orally. At the end of one week exposure and consequently after 24 hr of the last dose, blood samples were collected via the orbital plexus into a standard test tubes under effect of diethyl ether anesthesia, then the animals were sacrificed. Blood samples were centrifuged to separate serum and stored at - 70°C till analysis. After sacrificing rats, specimens of the liver, kidney, heart, and testis were quickly collected for histopathological examination. All experimental procedures were carried out incompliance with the Egyptian law and regulations of the scientific research.

Hormonal and Biochemical Analysis: Serum activities of ALT, AST, CK, ALP, GGT, ACP, prostatic ACP, paraoxinase (PON-1), AchE, and LDH, and serum concentrations of urea, uric acid, and creatinine were determined using automated enzyme analyzers (Biochemical analyzer AE-600N, ERMA-INC-Japan) and commercial diagnostic kits. ELISA procedure was used for quantitative determination of serum total testosterone, and T_4 according to manufacturer's instructions.

Histopathological Examination: Specimen of liver, kidney, heart, testes, and epididymis were fixed rapidly in 10% neutral-buffered formalin for at least 24 hr. The fixed specimens were processed through the conventional paraffin-embedding technique, sectioned at 5 µm and stained with Mayer's haematoxylin and eosin (HE)[6].

Statistical Analysis: Data were expressed as mean ± SEM (*n=10*). The significance of the difference between treated and control parameters was analyzed by computerized CoStat software program version 6.4. Statistical significance was drawn at $p<0.05$.

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

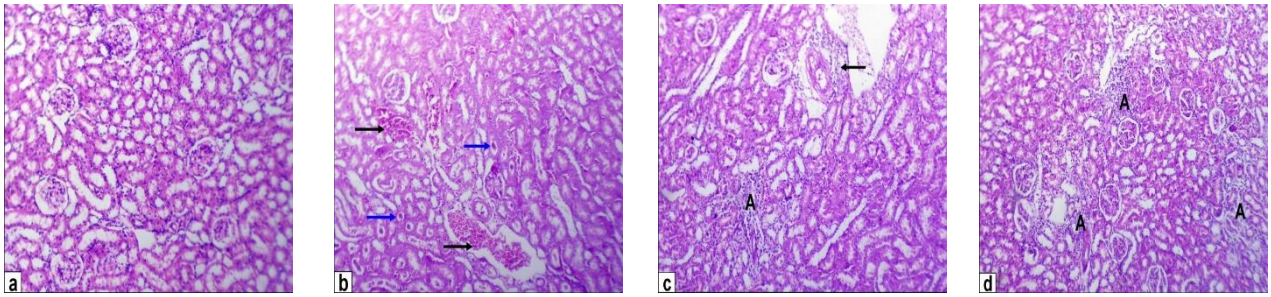

Figure 2: Photomicrograph of rat kidney stained with HE (X160). (a) The histoarchitecture of the kidney is intact in controls (b) Kidney of rats treated with thiamethoxam showed congestion of blood vessel (black arrows) and hyaline casts in the lumen of renal tubules (blue arrows). (c) Pervascular inflammatory cell infiltration (arrow) and mild interstitial nephritis (A). (d) Multifocal interstitial nephritis (A).

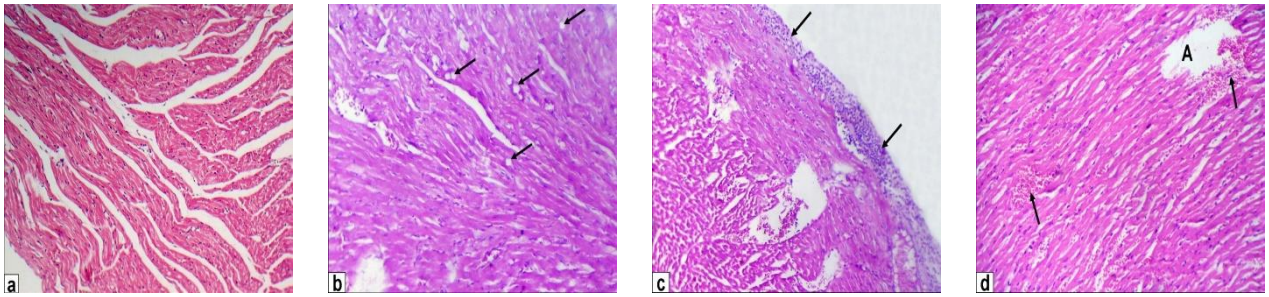

Figure 3: Photomicrograph of rat myocardium stained with HE (X160). (a) Normal histological structure of myocardium of control rats. (b) Rats treated with thiamethoxam showed sharp edge outlines vacuoles in the sarcoplasm of degenerated myocytes (arrows). (c) mild to moderate epicardial lymphocytic infiltration (d) segmental lytic necrosis (A) and hemorrhage (arrows)

rats when compared with control. Moreover, the level of serum creatinine was significantly increased, while the concentration of serum urea was decreased in the TMX-treated rats compared with control group. The level of serum uric acid and CK activity were also elevated after the administration of TMX but not statistically significant. TMX also induced a significant decrease in the level of thyroxine hormone (T_4) and a non-significant decrease in the concentration of testosterone hormone.

Histopathological Examination: Hepatic tissue of control rats showed normal histology of intact portal areas, central vein, blood sinusoid and normal hepatocytes with abundant eosinophilic cytoplasm and most have a single, round and centrally placed nucleus (Figure 1-a). Liver of treated rats revealed remarkable hydropic degeneration and congestion of blood vessel (Figure1-b). On the other hand, sections of liver of some treated rats showed lytic necrosis where the necrotic cells have brightly acidophilic cytoplasm and no nuclei, but they are still arranged in chords (Figure 1-c) as well as focal hepatic necrosis and phagocytic cells infiltration (Figure1-d).

Kidneys of control rats exhibited normal renal tissue, where normal glomeruli, tubular epithelium and interstitial tissue were observed (Figure 2-a). The detectable lesions in the kidney of treated rat were congestion of blood vessel and the hyaline cast in the lumen of renal tubules (Figure 2-b). Moreover, perivascular inflammatory cells infiltrations (Figure 2-c) and mild to moderate multifocal interstitial nephritis as the inflammatory cell infiltrations in the interstitial space (Figure 2-d) were also noticed.

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

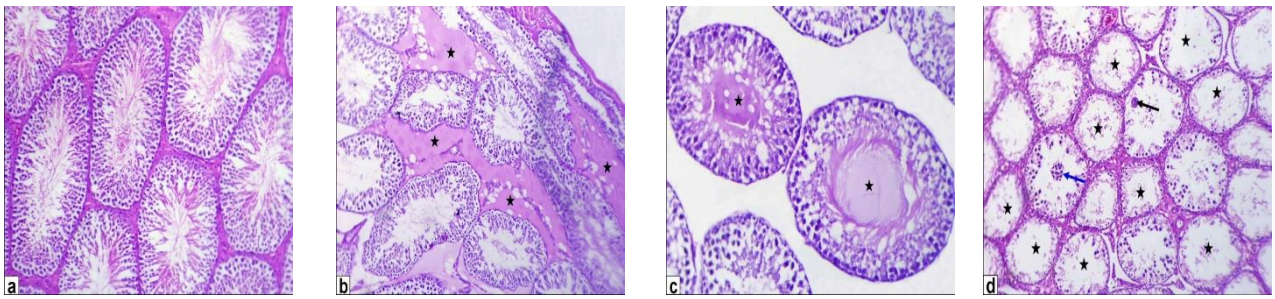

Figure 4: Photomicrograph of rat testis stained with HE. (a) Most seminiferous tubules are well-organized with intact interstitial tissue in control rats (b) Rats treated with thiamethoxam showed faint esinophilicalbuminous material in the interstitial tissue of the testis (stars X160). (c) Depletion of germinal cells and hyalinization of the luminal contents of the semineferous 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).

selectively as agonists of the insect nicotinic acetylcholine receptors (AChRs)[7]. 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 rat's 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[3] 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[8]. We suggested that TMX perhaps decrease PLP which contributed to decreased ALT activity. This explanation is consistent with Diehl et al[9] 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[10] 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[11]. 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[12]. The half-life in the circulation is about 47 hours for ALT and 87 hours for mitochondrial AST[8] 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[13].

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[14].

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[2] 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

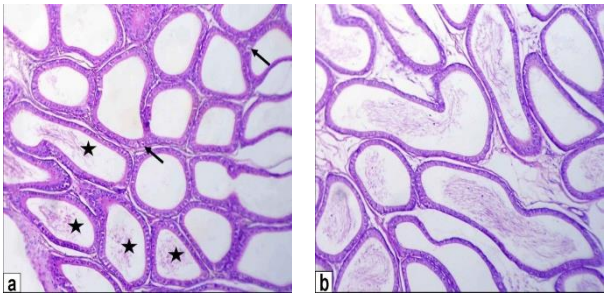

Figure 5: Photomicrograph of rat epididymis stained with HE (X160). (a) Epididymalductules of rats treated with thiamethoxam had very low sperm density (stars) and the majority free from sperm beside vacuolation of few epithelial cells (arrows). (b) Caudaepididymalductules had very low sperm density.

only now becoming clear that PON-1 protects human from the acute and chronic harmful effects of these compounds. Therefore, low PON-1 activity may increase their susceptibility to organophosphates[15,16]. In the same trend, Ferre´ et al[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_4[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[19] and a decrease of serum PON-1 activity in mice with LCAT deficiency resulting from LCAT gene targeted disruption[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[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[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[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 distrupting 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[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[25]. More recently, the sub-acute treatments of commercial formulations of thiacloprid (neonicotinoid insecticide) and mixture of deltamethrin (pyrethroid insecticide) with thiacloprid increased serum triiodothyronine and free thyroxine hormone levels in rats[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

Veterinary Medicine, Alexandria University, Edfina, Egypt.

REFERENCES

1. Tomizawa M, Casida JE. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annual Review of Entomology 2003; 48 (1): 339-364.
2. Rodrigues KJA, Santana MB, Do-Nascimento JLM. Behavioral and biochemical effects of neonicotinoid thiamethoxam on the cholinergic system in rats. Ecotoxicology and Environmental Safety 2010; 73 (1): 101-107.
3. Shalaby SEM, Farrag AH, El-Saed GSM. Toxicological potential of thiamethoxam insecticide on albino rats and its residues in some organs Journal Arab Society Medical Research 2010; 5 (2): 165-172.
4. Rose PH. Nicotine and the neonicotinoids, In:T.C.Marrs editor. *Mammalian Toxicology of Insecticides*. Published by the Royal Society of Chemistry, Cambridge,pp. 184, 2012.
5. Swenson TL, Casida JE. Neonicotinoid formaldehyde generators: possible mechanism of mouse-specific hepatotoxicity/hepatocarcinogenicity of thiamethoxam. Toxicology Letter 2013; 216 (2-3) 139-145.
6. Culling CF. Handbook of Histological and Histochemical Techniques," 3rd ed. London, Boston: Butterworth, 1983.
7. Matsuda K, Buckingham SD, Kleiner D. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends Pharmacological Science 2001*;* 22 (11): 573-580.
8. Dufour DR, Lott JA, Nolte FS. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clinical Chemistry 2000; 46 (12): 2027-2049.
9. Diehl AM, Potter J, Boitnott J. Relationship between pyridoxal 5'-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. Gastroenterology 1984; 86 (4): 632-636.
10. Hoffmann WE, Solter PF. Diagnostic enzymology of domestic animals. In:J.J.Kaneko, J.W.Harvey andM.L.Bruss, editors. *Clinical biochemistry of domestic animals.*6th ed. Elsevier Inc.pp. 351, 2008.
11. Rej R. Aminotransferases in disease. Clinical Laboratory Medicine 1989; 9 (4): 667-687.
12. Kamimoto Y, Horiuchi S, Tanase S. Plasma clearance of intravenously injected aspartate aminotransferases Isozymes: evidence for preferential uptake by sinusoidal liver cells. Hepatology 1985; 5 (3): 367-375.
13. Tennant BC, Center SA. Hepatic function," In:J.J. Kaneko, J.W.Harvey andM.L. Bruss, editors. *Clinical biochemistry of domestic animal*.6th ed. Elsevier Inc. pp. 397, 2008.
14. Kerr MG. Veterinary laboratory medicine,"2nd Edition. Published by Blackwell Science Ltd; 2002.
15. Costa LG, Giordano G, Cole TB. Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. Toxicology 2013; 307: 115-122.
16. Mackness M, Mackness B. Current aspects of paraoxonase-1 research," In: T. Komodo, editor. *The HDL handbook: biological functions and clinical implications,* 2nd edition. Amsterdam, pp.273-291, 2014.
17. Ferre´N, Camps J, Prats E. Serum Paraoxonase Activity: A New Additional Test for the Improved Evaluation of Chronic Liver Damage. Clinical Chemistry 2002; 48 (2): 2261–2268.
18. Ferre´ N, Camps J, Cabre´ M. Hepatic paraoxinase activity alterations and free radical production in rats with experimental cirrhosis. Metabolism 2001; 50 (9): 997–1000.
19. Sabesin SM, Hawkins HL, Kuiken L. Abnormal plasma lipoproteins and lecithin-cholesterol acyltransferase deficiency in alcoholic liver disease. Gastroenterology 1977; 72 (3): 510–518.
20. Forte TM, Oda MN, Knoff L. Targeted disruption of the murine lecithin:cholesterol acyl transferase gene is associated with reductions in plasma paraoxinase and platelet-activating factor acetyl hydrolase activities but not in apolipoprotein J concentration. Journal of Lipid Research 1999; 40 (7):1276–1283.
21. Ikemoto M, Tsunekawa S, Tanaka K. Liver-type arginase in serum during and after liver transplantation: a novel index in monitoring conditions of the liver graft and its clinical significance. Clinica Chimica Acta 1998; 271 (1): 11-23.
22. Breckenridge CB, Stevens JT. Crop protection chemicals: mechanism of action and hazard profiles,"In: A.W.Hayes,editor.Principles and methods of toxicology, 5th ed. Informa Healthcare USA,2008.
23. Bull H, Murray PG, Thomas D. Acid phosphatases. Molecular Pathology 2002; 55 (2): 65-72.
24. Villanger GD, Jenssen BM, Fjeldberg RR. Exposure to mixtures of organohalogen contaminants and associative interactions with thyroid hormones in East Green land polar bears (Ursusmaritimus). Environment International 2011; 37 (4): 694-708.
25. Akhtar N, Kayani S, Ahmad MM. Insecticide-induced changes in secretory activity of the thyroid gland in rats. Journal of Applied Toxicology 1996; 16 (5): 397-400.
26. Şekeroğlu V, Şekeroğlu ZA, Demirhan ES. Effects of commercial formulations of deltamethrin and/or thiacloprid on thyroid hormone levels in rat serum. Toxicology and Industrial Health 2014. 30 (1): 40-46