Slov Vet Res 2021; 58 (Suppl 24): 209–21
DOI 10.26873/SVR-1441-2021

Original Research Article

THE PROTECTIVE ROLE OF THYMOL AGAINST METHOMYL- INDUCED TOXICITY IN MALE RATS: CLINICO-BIOCHEMICAL, HISTOPATHOLOGICAL AND IMMUNO-HISTOCHEMICAL STUDIES

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Abstract: This experiment was carried out to evaluate the presumptive protective effect of thymol (T) against methomyl (M) - induced toxicity in male albino rats. Therefore, forty adult healthy male albino rats were randomly divided into 4 equal groups as follow: control group, intoxicated group received methomyl (4 mg/kg b.wt./day), treated group received thymol (100 mg/kg b.wt.), and co-treated group received both methomyl and thymol with the previous doses. The treatment regimens were given orally three times a week, day after day, for eight weeks. Biochemically, the M- intoxicated rats revealed a significant increase in the serum aminotransferases (ALT& AST) and gamma glutamyle transferase (GGT) activities, total, direct and indirect bilirubin, total cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C), urea, malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity, in addition to a significant decrease in serum albumin and testosterone levels with a significant increase in the percentage of sperm abnormalities in comparison with the control rats. Regarding to the histopathological examination, M- intoxicated group showed degenerative and necrotic lesions in the hepatic, renal and testicular tissues. But, the co- treatment with thymol ameliorated the M- induced biochemical alterations, oxidative stress and apoptosis and improved tissues architecture. It could be concluded that co-treatment with thymol may provide a prolonged remedy against methomyl -induced toxicity.

Key words: Methomyl; Thymol; oxidative stress; caspase 3; rats; sperm

Introduction

Methomyl is one of the most used carbamates insecticides in agriculture. It is used for paper-backed treatment of fruits, vegetables, commercial ornamentals and excluding flies in poultry houses and dairies (1). Also, it is used for controlling more than 100 species of insects at different stages of its developments as ovicide, larvicide, adulticide, and an acaricide to control ticks, spiders and arthropods (2). Methomyl is rapidly absorbed, distributed and excreted within 24hr by urine and exhaled air. It has few hours half-life with low levels in tissue and blood (3). In human, methomyl enters the body through ingestion, inhalation and dermal infiltration. Consequently, its toxicity occurs through the ingestion of the contaminated food, fly spraying and occupational handling (4). So, it is restricted in use as an insecticide due to its dangerous toxicity to non-target species (5). The main mechanism of pesticides toxicity is overproduction of reactive oxygen species (ROS) inducing peroxidative damage that induce damage to lipids, proteins of cell membrane and nucleic acids (6). Methomyl is considered as an endocrine disruptor and a potent genotoxic as it capable of inducing structural and numerical chromosomal aberration in mammalian cells (7).

Received: 23 September 2021
Accepted for publication: October 2021

Many antioxidants have been used in the experimental trials to reduce the toxic effects of pesticides such as vitamin C, E, selenium and Zinc (8). Thymol is considered one of the phytochemicals that is plant-derived and present in many edible plants as *Lippia multiflora, Ocimum gratissimum, Nigella sativa, Satureja thymbra, Thymus spp as* (*T. vulgaris and T. ciliates*) *and Zataria multiflora.* Thymol has many powerful pharmacological properties including antioxidant (9) and anti-inflammatory (10) effects. Its antioxidant effects depend on ability to protect different body cells from oxidative damage by scavenging free radicals and preventing lipid peroxidation (11). Accordingly, in this study, we aimed to evaluate the possible protective effect of thymol against methomyl induced toxicity in male rats by evaluation of biochemical, oxidative stress biomarkers, and semen evaluation as well as histopathological and immunohistochemical examinations of different organs.

Materials and methods

Chemicals

Methomyl (Lannate®) was purchased from Central Laboratories of Agricultural Pesticides, Dokki, Egypt and thymol was purcased from Bio- Lab Company, Egypt.

Animals and experimental design

Forty apparently healthy adult male albino rats with 200 – 230g average body weight were purchased from a closed bred colony at the Medical Research Institute of Alexandria University, Egypt. Rats were saved in separated sterile metal cages and saved under constant environmental conditions. The rats received food and water *ad libitum.* All rats were adapted for two weeks before starting the experiment for accommodation. All the above mentioned care routines are strictly followed standard rules described by Institutional Animal Care and Use Committee (IACUC) with an ethical approval number 2021047.

The animals were divided into 4 equal groups. Group1 received distilled water and sunflower oil (0.5 ml/100g b.wt.) as vehicles and kept as control group. Group2 received methomyl (M) dissolved in distilled water at a dose of 4 mg/kg b.wt. (12). Group3 received thymol (T100) dissolved in sunflower oil as a vehicle at a dose of 100 mg/kg b.wt. (13). Group4 treated with combination of M and T100 at the same aforementioned doses. All treatments were given once per day 3 times/ week for 8 weeks via oral gavage.

Blood sampling

Twenty four hours after the last treatments administration, the blood samples were collected under light ether anesthesia (in jar with cotton piece soaked in diethyl ether alcohol) in plain tubes from the retro-orbital venous plexus of each rat before euthanasia for serum collection. After centrifugation of clotted samples at 3000 rpm for 15 minutes, the serum was separated and carefully collected into clean dry epindorffs and kept frozen at –20°C for biochemical analysis.

Serum biochemical parameters

The serum activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were detected according to previous method (14), while gamma glutamyle transferase (GGT) was measured according to Szasz (15). Also, serum levels of total proteins (TP), albumin, and bilirubin (total, direct and indirect) were detected as recorded by Doumas et al. (16), Doumas et al. (17) and Walter and Gerade (18), respectively. In addition, the serum levels of total cholesterol, triglyceride and high density lipoprotein cholesterol (HDL-C) were estimated according to Allain et al. (19), Fassati and Prencipe (20) and Lopez - Virella et al. (21) respectively, while LDL-C (low density lipoprotein cholesterol) and VLDL-C (very low density lipoprotein cholesterol) were calculated according to Friedewald et al. (22). Serum urea and creatinine levels were estimated according to Rock *et al.* (23) and Fabiny and Ertingshausen (24) respectively. All of the previous parameters were detected using commercially available diagnostic kits (Bio-diagnostic Co. Egypt).

Oxidative stress assays and antioxidant status in blood

Serum Lipid peroxidation (LPO) was measured as malondialdehyde (MDA) (25). Furthermore, spectrophotometrical assessment of serum reduced glutathione (GSH) level and superoxide dismutase (SOD) activity was done according to aforementioned methods (26, 27) respectively using kits supplied by Bio-diagnostic co., Egypt.

Testosterone level and semen analysis

The serum testosterone level was estimated using indirect enzyme immunoassay using Monobind (Lake Forest, CA 92630 USA) (ELISA) kits as outlined by Tietz (28). Mass motility, epididymal sperm count and percentage of sperm abnormalities were detected by the methods reported by Bearden and Fuquay (29).

Histopathological examination

After necropsy, we collected small specimens from liver, kidney and testis from all groups then fixed rapidly in 10% neutral formalin for at least 24 hrs. After fixation, tissue specimens were processed through the conventional paraffin embedding technique. Five µm thick sections were obtained from paraffin blocks, stained with hematoxylin and eosin (30) and examined under light microscope.

Immunohistochemical Caspase- 3 examination

The standard horseradish peroxidase (HRP) immunohistochemistry technique was applied according to Ramos- Vera (31). Rabbit anti-rat caspase-3 was used as described in the guidelines of manufacturer. Five mm-thick sections of liver, kidneys and testis were dewaxed, rehydrated and pretreated with 3% H_2O_2. for block endogenous peroxidase activity then placing slides in a microwave for 10 min in 10 µm sodium citrate buffer (pH 6.0) for antigen retrieval. Slides were incubated with the primary antibody then rinsed with Tris buffer saline and the secondary antibody applied. Slides were incubated with 3, 3' -Diaminobenzidine (DAB) substrate chromogen solution was then counterstained with Mayer's hematoxylin.

Statistical analysis

The Data were analyzed statistically using the Statistical Analysis System software (32). ANOVA was used to detect the differences among groups. Duncan's Multiple Range test was used to clarify the differences among means at a significance level of $P < 0.05$. Values are represented as means $\pm$ standard errors.

Results

Serum biochemical results:

As demonstrated in Table (1), The M- intoxicated group showed a significant increase in the serum ALT, AST and GGT activities as compared to the control group. While, the co-treatment of M- intoxicated rats with T100 displayed a significant decrease in these liver enzymes in comparison with the toxic group and normalized the ALT and GGT serum activities. The serum total proteins and albumin levels showed a significant decrease in the M- intoxicated rats without any changes in other treated groups (T100 and MT100) in comparison with the control rats. Administration of M insecticide evoked a significant increase in the serum levels of total, direct and indirect bilirubin as matched with control group. The total and direct bilirubin showed insignificant decrease in MT100 co- treated group and this was returned towards the values of control. But, the indirect bilirubin level did not show any changes if compared with M- intoxicated group. Whereas, rats treated with T100 did not show any changes in serum total, direct and indirect bilirubin if compared with the control rats.

Regarding to the lipid profile, the total cholesterol, triglyceride and LDL-C levels revealed a significant increase in M- intoxicated rats without any marked changes in the other treated groups (T100 and MT100) where the co- treatment with T100 returned their levels to normal if compared with the control.

Concerning to the renal function tests, the M-intoxicated rats showed a significant increase in serum urea and creatinine levels if compared with the control group. While the co-treatment with T100 insignificantly decreased the urea and significantly decreased creatinine levels matched with M- intoxicated group and the creatinine value was reverted to normal if compared with the control.

Oxidative stress assays and Antioxidant status in blood:

As recorded in Table (2), the serum concentration of MDA exhibited a marked increase in M- intoxicated group and a significant decrease in T100-treated group as compared to control group. Also, the SOD activity showed a significant increase only in M- intoxicated rats when compared to control

one. While, the co- treatment of M- intoxicated rats with T100 returned MDA level and SOD activity to their normal levels as the control. The GSH level did not show any marked changes in all of the treated groups if compared with the control.

Testosterone level and semen analysis:

As shown in Table (3), there was a significant decrease in the serum testosterone level with a significant increase in the percentage of sperm abnormalities in M- intoxicated rats as compared with the control rats. While, the co- treatment with T100 returned the sperm abnormalities to normal percentage and numerically increased the testosterone level in comparison with the M-intoxicated rats. While, the sperm count and motility did not show any marked changes in all of the treated rats (M, T100 and MT100) in comparison with the control ones.

Histopathological results:

As presented in Fig. (1), the liver of control (Fig. a) and T100- treated rats (Fig. b) showed normal hepatic architecture, hepatocytes were arranged around the central vein in radiating manner with normal appearance of blood vessels and portal triads all over the experimental period.

Table 1: Effects of methomyl and thymol administration alone or in combination for eight weeks on some serum biochemical parameters in male albino rats

	Groups				
Parameters	control	M	T100	MT100	P values
ALT (U/L)	53.43 ± 5.79 ^{b}	**74.53 ± 10.62 ^{a}**	52.93 ± 4.71 ^{b}	60.07 ± 7.12 ^{ab}	0.0152
AST (U/L)	73.14 ± 5.55 ^{c}	107.04 ± 5.60 ^{a}	79.97 ± 0.84 ^{c}	90.22 ± 14.68 ^{b}	0.0037
GGT (U/L)	1.94 ± 0.34 ^{b}	2.98 ± 0.29 ^{a}	1.87 ± 0.44 ^{b}	1.58 ± 0.22 ^{b}	0.0052
Total protein (g/dl)	5.94 ± 0.14 ^{b}	5.30 ± 0.23 ^{c}	6.97 ± 0.47 ^{a}	6.07 ± 0.08 ^{ab}	0.0094
Albumin (g/dl)	4.27 ± 0.41 ^{a}	3.38 ± 0.22 ^{b}	3.69 ± 0.02 ^{ab}	3.83 ± 0.17 ^{ab}	0.0371
Total bilirubin (mg/dl)	0.11 ± 0.01^{c}	0.19 ± 0.02 ^{a}	0.11 ± 0.01 ^{c}	0.17 ± 0.02 ^{ab}	0.0038
Direct bilirubin (mg/dl)	0.02± 0.01 ^{b}	0.05 ± 0.01 ^{a}	0.03 ± 0.01 ^{ab}	0.03 ± 0.01 ^{ab}	0.0356
Indirect bilirubin (mg/dl)	0.09 ± 0.00 ^{b}	0.14 ± 0.02 ^{a}	0.08± 0.01 ^{b}	0.14 ± 0.02 ^{a}	0.042
Total cholesterol (mg/dl)	80.81 ± 6.76 ^{b}	101.78 ± 7.07 ^{a}	85.58 ± 6.68 ^{ab}	78.43 ± 3.20 ^{b}	0.0425
Triglyceride (mg/dl)	99.73 ± 2.73 ^{bc}	105.68 ± 4.64 ^{a}	97.67 ± 1.17 ^{c}	103.96 ± 3.55 ^{ab}	0.0487
LDL- C (mg/dl)	20.40 ± 3.85 ^{b}	44.08 ± 5.55 ^{a}	29.50± 1.37 ^{b}	21.37 ± 1.46 ^{b}	0.0023
HDL- C (mg/dl)	40.44 ± 3.50 ^{a}	36.51 ± 2.05 ^{a}	36.55 ± 6.52 ^{a}	36.12 ± 3.67 ^{a}	0.1380
VLDL- C (mg/dl)	19.96 ± 0.55 ^{a}	21.19 ± 0.94 ^{a}	19.53 ±0.23 ^{a}	20.79 ± 0.71 ^{a}	0.0978
Urea (mg/dl)	29.66 ± 1.91^{b}	40.12 ± 1.25 ^{a}	29.02 ± 2.14 ^{b}	38.30 ± 1.31 ^{a}	0.0056
Creatinine (mg/dl)	0.62 ± 0.09 ^{b}	0.74 ± 0.05 ^{a}	0.66 ± 0.04 ^{b}	0.64 ± 0.06 ^{b}	0.0024

M= methomyl; T100= thymol; ALT= alanine aminotransferase; AST= aspartate aminotransferase; GGT= Gamma glutamyle transferase; LDL-C= low density lipoprotein cholesterol; HDL-C= high density lipoprotein cholesterol; VLDL-C= very low density lipoprotein cholesterol.
Values are means ± standard errors. Means with different letter within the same row differ significantly at P≤0.05.

Table 2: Effects of methomyl and thymol administration alone or in combination for eight weeks on lipid peroxidation and antioxidant biomarkers in male albino rats

	Groups				
Parameters	control	M	T100	MT100	P values
MDA (nmol/ml)	7.20 ± 0.23 ^{b}	13.74 ± 0.39 ^{a}	4.67± 2.09 ^{c}	8.03 ± 0.63 ^{b}	0.0027
GSH (mmol/L)	0.47 ± 0.02 ^{a}	0.44 ± 0.05 ^{a}	0.45 ± 0.03 ^{a}	0.42 ± 0.05 ^{a}	0.3681
SOD (U/ml)	30.80 ± 1.07^{b}	47.24 ± 2.43 ^{a}	25.07 ± 10.18 ^{b}	33.30 ± 2.11^{b}	0.0219

M= methomyl; T100= thymol; MDA= Malondialdhyde; GSH=Reduced glutathione and SOD= Superoxide dismutase.
Values are means ± standard errors. Means with different letter within the same row differ significantly at P≤0.05.

Table 3: Effects of methomyl and thymol administration alone or in combination for eight weeks on serum testosterone level and semen analysis in male albino rats

	Groups				
Parameters	control	M	T100	MT100	P Values
Testosterone (ng/ml)	5.34 ± 0.57 [a]	1.84 ± 0.24 [b]	5.22± 0.97 [a]	2.36 ± 0.26 [b]	0.0025
Mass motility (%)	45.00 ± 9.35 [ab]	20.00 ± 9.35 [b]	50.00 ± 0.25 [ab]	31.25± 18.75 [b]	0.0219
Sperm count (10^6/ml)	8.55 ±1.37 [a]	4.86 ± 1.12 [a]	5.70 ± 0.30 [a]	5.18 ± 2.23 [a]	0.4360
Sperm abnormalities (%)	11.14 ± 0.89 [b]	16.39 ± 1.37 [a]	15.17 ± 0.83 [ab]	14.67 ± 2.36 [ab]	0.0126

M= methomyl; T100= thymol. Values are means ± standard errors. Means with different letter within the same row differ significantly at P≤ 0.05.

Table 4: The score of detected lesions in various examined organs of methomyl-intoxicated, thymol 100-treated and co- treated male albino rats at the end of 8th weeks post-treatment

	Groups			
Organ / Lesion	control	M	T100	MT100
Liver				
Cytoplasmic vacuolation	-	+++	-	++
Hepatocytic necrosis	-	+++	-	+
Mononuclear cell infiltration	-	+++	-	+
Congested blood vessels	-	++	-	+
Kidneys				
Tubular Epithelial vacuolation	-	+++	-	+
Tubular necrosis	-	+++	-	+
Perivascular edema	-	++	-	-
Testes				
Buckled basement membrane	-	+++	-	-
Interstitial congestion and edema	-	++	-	-
Sloughed germinal epithelium	-	++	-	-

M= methomyl; T100= thymol; (-): No lesions; (+): mild lesion = 5-25%; (++): Moderate =26-50%; (+++) = Severe = >50% of examined tissue sections.

While, the liver of M- intoxicated rats showed an increase in the thickness of portal area with inflammatory cells; newly formed bile ductules and congested blood vessels (Fig. c), in addition to severe hydropic cytoplasmic vacuolation and multifocal areas of midzonal and periportal necrosis associated with severe inflammatory cells infiltration. On the other hand, the MT100 co-treated rats exhibited small areas of hepatic necrosis associated with mild inflammatory cells infiltrates associated with mild congestion (Fig. d).

In Fig. (2), the kidneys of control (Fig. a) and T100- treated rats (Fig. b) exhibited normal structure of both renal tubules and the glomeruli. But, the M- intoxicated rats showed severe tubular epithelial vacuolation in some renal tubules and others showed tubular necrosis associated with mononuclear inflammatory cells infiltration as well as inter-tubular edema (Fig. c). Whereas, the MT100 co- treated kidneys showed mild renal epithelial vacuolation, mild tubular necrosis with mononuclear cells infiltrates around focal necrotic tubules (Fig. d).

As shown in Fig. (3), the testicular tissue showed normal histological structure with functioning seminiferous tubules associated with complete spermatogenesis in control (Fig. a) and T100- treated (Fig. b) groups. But, the M- intoxicated rats revealed disorganization of seminiferous tubules with corrugation of cell membrane, incomplete spermatogenesis, vacuolation and sloughing of the germinal epithelium (Fig. c). On

the other hand, the co- treatment with MT100 appeared nearly normal testicular tissue (Fig. d).

Immunohistochemical Caspase- 3 examination

As shown in Fig. (4), the liver of control rats (Fig. a) showed negative caspase-3 immune- reactivity in hepatocytes. However, the liver of M- intoxicated rats showed diffuse cytoplasmic caspase-3 immune reactive areas (Fig. b). On the other hand, the MT100 co- treated rats revealed mild caspase-3 immune reactivity in hepatocytic tissue (Fig. c) in comparison with M- intoxicated rats.

Also, the kidney of control rats (Fig. d) revealed negative caspase-3immune- reactivity. In contrast, the administration of M showed strong caspase-3 immune reactivity in tubular and glomerular tissues (Fig. e). While, the MT100 co-treated rats revealed renal mild caspase-3 immune reactivity (Fig. f) as compared with M- intoxicated rats.

In addition to, the testicular tissue of control rats (Fig. g) exhibited negative immune- reactivity of caspase-3. Meanwhile, the testes of M- intoxicated rats showed strong caspase-3 immune reactivity in germinal epithelium and spermatogonial cells (Fig. h). The testes of MT100 co- treated rats showed mild caspase-3 immune reactivity (Fig. i) if compared with M- intoxicated rats.

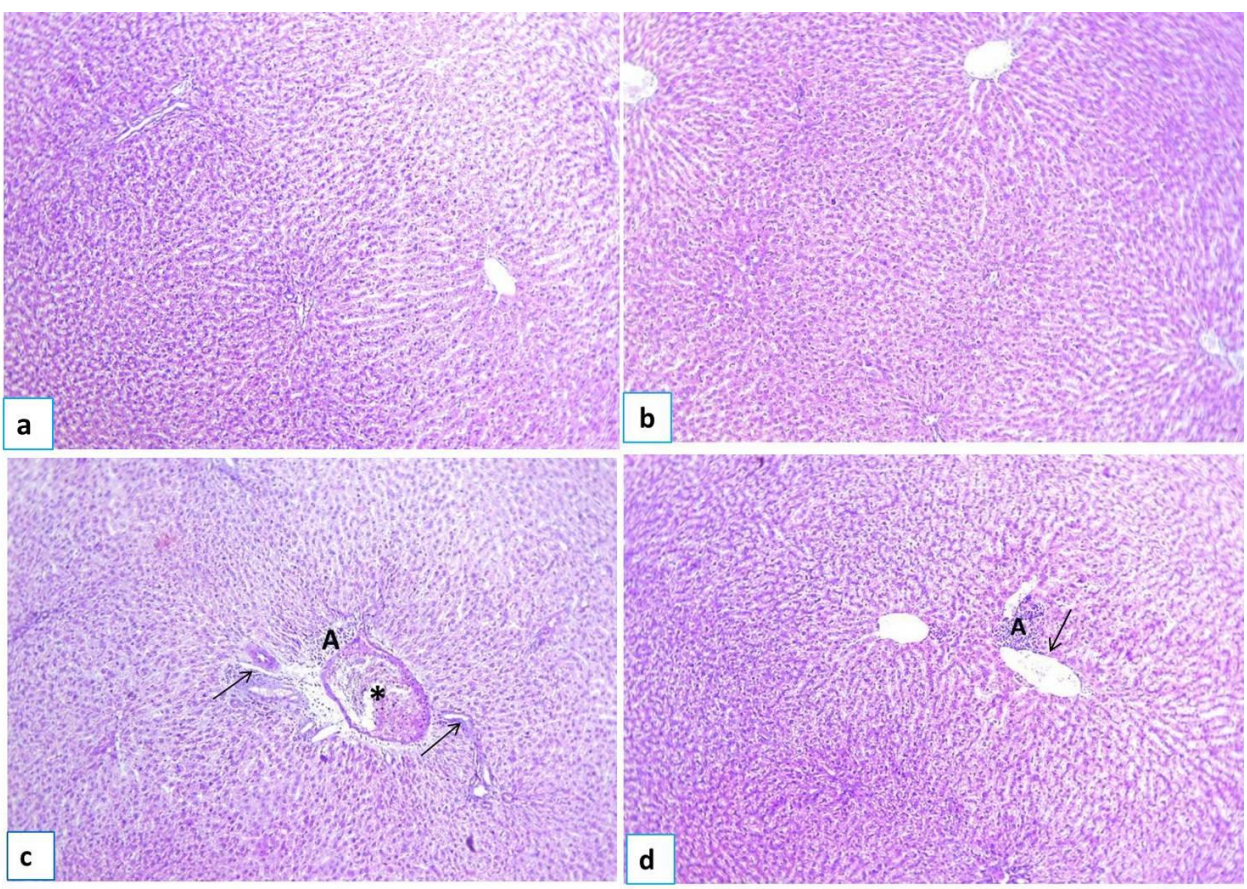

Figure 1: Photomicrograph of rat liver stained with H&E(X100): (a) Control rats and (b) T100- treated rats showing normal histological hepatic architecture, (c) M- intoxicated rats showing hepatic necrosis associated with mononuclear cell infiltrates (A), severe congestion (asterisk) and increased the thickness of portal area with newly formed bile ductules (arrows) and (d) MT100 co- treated rats showing mild mononuclear cell infiltration (A) associated with mild congestion (arrow).

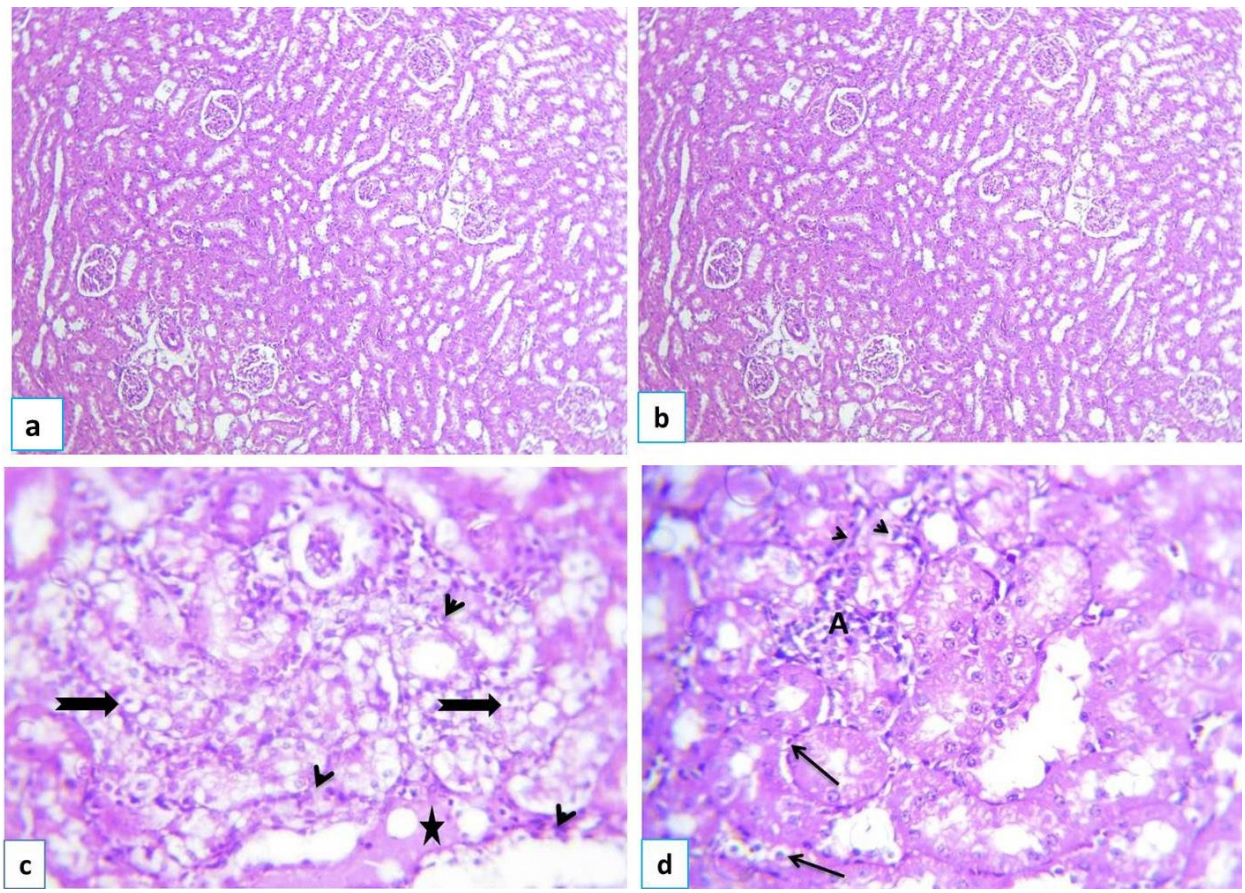

Figure 2: Photomicrograph of rat kidney stained with H&E: (a) Control rats and (b) T100- treated rats showing normal histological structure of renal tubules and glomeruli (X100), (c) M- intoxicated rats showing epithelial vacuolation (notched arrows) and severe necrosis of renal tubules(arrow heads) associated with mononuclear inflammatory cells infiltration as well as intertubular edema (star) (X400) and (d) MT100 co- treated rats showing tubular epithelial vacuolation (arrows) and mild tubular necrosis (arrow heads) with mononuclear cell infiltration (A) (X400)

Discussion

Methomyl is a systemic broad-spectrum N-methyl carbamate insecticide, which is widely used in agriculture. Higher application rates may improve the control of insects but also increase the risk of phytotoxicity and threaten human health through direct contact and long-term bioaccumulation in food or water (33).

About the biochemical results in the existing study, methomyl administration resulted in a significant increase in the serum activities of ALT, AST and GGT, this may be correlated to the ability of methomyl to induce oxidative stress causing production of free radicals exhibiting hepatotoxicity, damage and necrosis leading to the leakage of these enzymes from the hepatocytic cytoplasm into the blood stream (34). This increment may be indicative of initial cell injury occurring associated with methomyl toxicity with an enhancement of permeability, hepatocytic damage or necrosis. An increase in the activity of these enzymes is termed as the early recognition of toxic hepatitis (35). These results accords with Mansour et al. (36) and Chabane et al. (37) who reported that oral intake of methomyl induced significant increase in both serum and plasma activities of AST and ALT in male rats. However, the co-treatment with T100 ameliorated the increase in the activities of these hepatic enzymes and this is the primary evidence of hepatoprotective activity of thymol (38).

Also, our results revealed a significant hypoproteinemia and hypoalbuminemia in M-intoxicated rats which may be due to decrease the synthesis of albumin in the liver due to liver damage caused by methomyl administration (39). Likewise, this may be due to the usage of amino acids for antibodies production to overcome the methomyl toxicity (40).

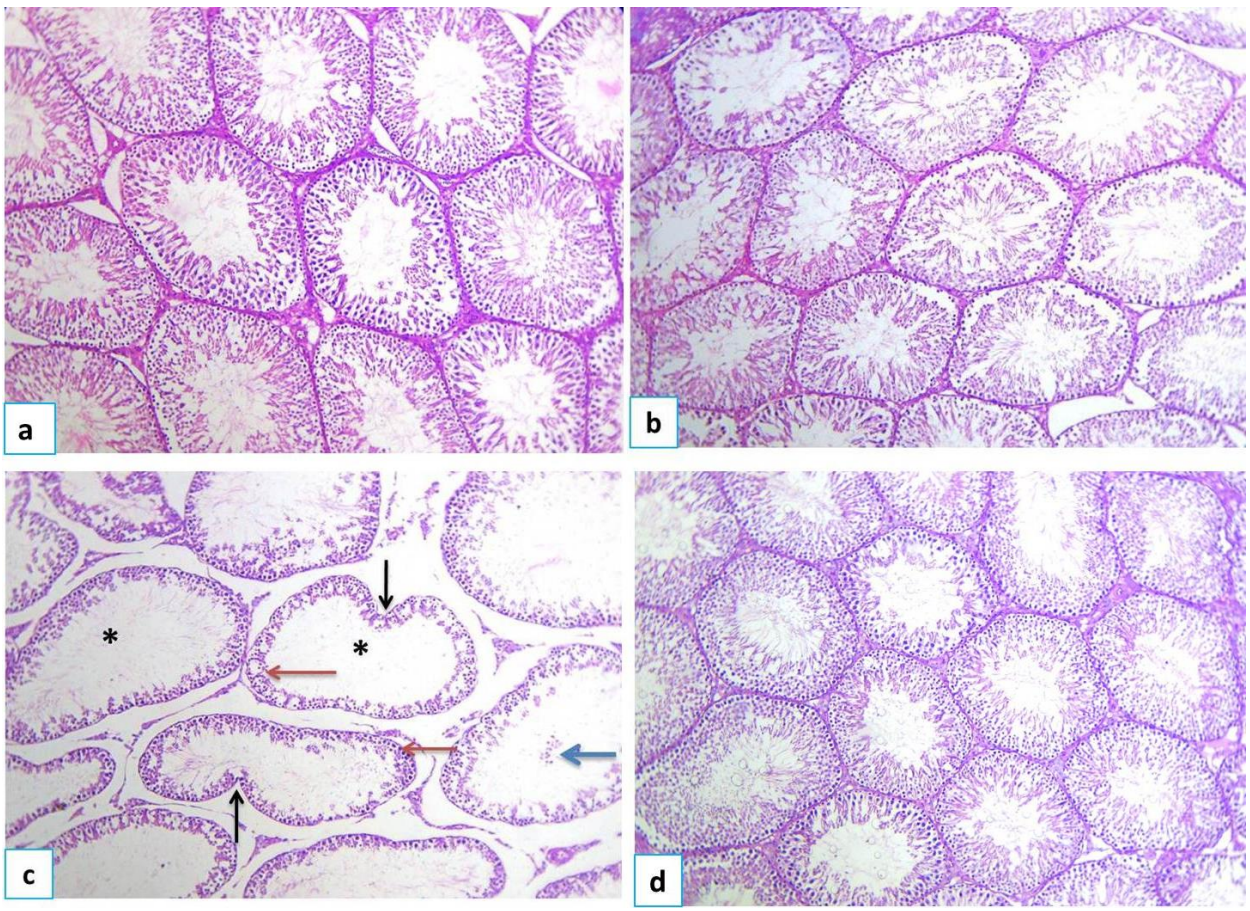

Figure 3: Photomicrograph of rat testes stained with H&E(X100): (a) Control rats and (b) T100- treated rats showing normal histological structure of seminiferous tubular structure with complete spermatogenesis, (c) M-intoxicated rats showing tubular epithelial vacuolation (red arrows), corrugation of tubular basement membrane (black arrows) with epithelial desquamation in tubular lumen (blue arrow) and complete absence of spermatogenesis (asterisks) and (d) MT100 co- treated rats showing nearly normal seminiferous tubular structure

Prolonged exposure to the methomyl was found to cause a significant decrease in total protein and albumin levels in rats which might be due to catabolism of protein and/ or malfunction of liver (41, 42). On the other hand, a significant increase in the serum TP level in T100- treated rats was observed. This may be due the ability of thymol to improve the digestion and the absorption of protein in the intestine (43) in accordance with the previous investigation of Elshopakey et al. (44). Also, the co- treatment of M–intoxicated rats with T100 showed a significant increase in albumin level in comparison with the methomyl and nearly restored the proteinogram to its normal values as the control. This indicated the ability of thymol to maintain the hepatocytic integrity and capacity to produce albumin due to its antioxidant properties (45).

Administration of methomyl caused a significant increase in the serum bilirubin (total, direct and indirect), total cholesterol, triglyceride and LDL-C levels. The proved methomyl- related hepatotoxicity may be the cause of the significant increase in the serum bilirubin in M- intoxicated rats. These results come in the harmony with the previous studies (37, 42). The total bilirubin was increased due to the significant increase in direct and indirect bilirubin, denoting that hepatocellular injury may be occurred. Also, the increased serum levels of total cholesterol, triglyceride and LDL-C in M- intoxicated rats may be due to the increased catecholamine concentration that may enhance the lipolysis and formation of fatty acids that directly affect the lipoproteins metabolism (46, 47) or due to the methomyl induced hepatic damage that resulted in disturbance in lipid metabolism as reported by Glaser and Mager (48). The combination of M with T100 (gp. 4) was significantly restored the serum total and direct bilirubin levels and lipid profile towards the normal

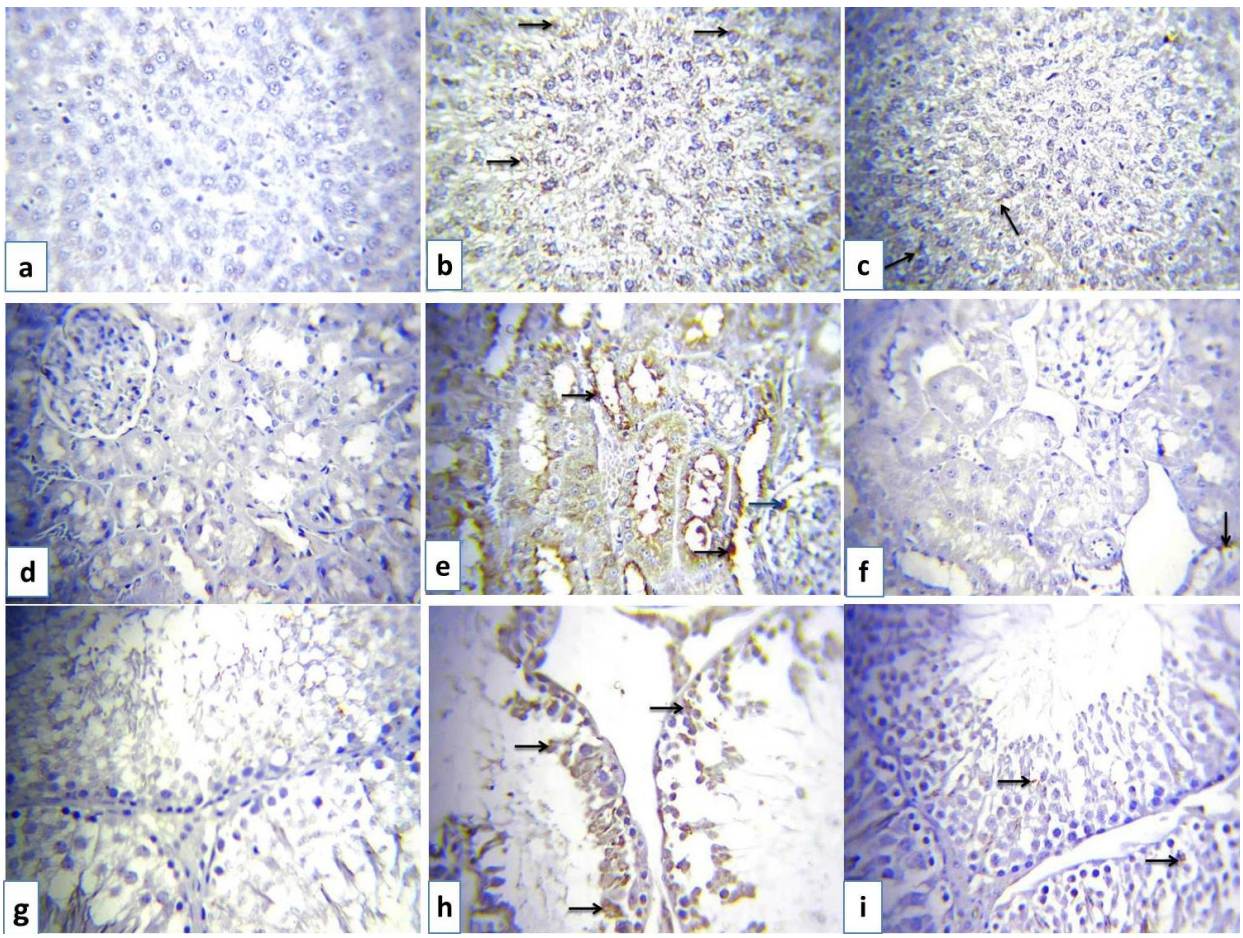

Figure 4: Photomicrograph of rat (a-c) liver, (d-f) kidneys and (g-i) testes immunohistochemically stained with caspase-3(X400): (a, d and g) control rats showing negative caspase-3 immune reaction, (b, e and h) M- intoxicated rats showing strong caspase-3 immune reaction that appeared as brown granules (arrows) and (c, f and i) MT100 co- treated rats showing mild caspase-3 immune reaction

value of control in accordance with Rašković et al. (38). This may be due to the antioxidant effect of thymol as previously mentioned.

The biochemical results related to liver were confirmed histopathologically wherever, the liver of M- intoxicated rats showed an increase in the thickness of portal area with inflammatory cells; newly formed bile ductules and congested blood vessels, in addition to severe hydropic cytoplasmic vacuolation and multifocal areas of midzonal and periportal necrosis associated with severe inflammatory cells infiltration. These data are consistent with previous observation in hepatic tissue of rats treated with different insecticides including methomyl that induce hepatic damage (36). While, the co- treatment of M- intoxicated rats with T100 improved the hepatic tissue architecture as there were mild hepatocytic necrosis and congestion if compared with the intoxicated group. These protective effects might be due to the antioxidant effect of thymol and its ability to scavenge the free radicals produced by methomyl (11) and this was supported by Elshopakey et al. (44).

Renal biomarkers (urea and creatinine) were also estimated in the serum of experimental rats to confirm the renal damage. The intoxicated rats exhibited a significant increase in urea and creatinine levels compared to control, this was related to oxidative stress which may explain the renal affection (36). The elevation of serum urea and creatinine are considered as significant markers of renal dysfunction (42). These results were confirmed by the renal damage evidenced pathologically. While, the co- treatment with T100 declined both of urea and creatinine levels in comparison with the toxic group. This decrement may be due to the reno- protective effect of thymol (49, 50) and its ability to decrease the lipid perox-

idation due to its potent antioxidant and anti-inflammatory properties (10). The biochemical results related to kidney (urea and creatinine) were supported hisopathologically where, the kidney of M- intoxicated rats exhibited severe tubular epithelial vacuolation, tubular necrosis associated with mononuclear cell infiltration and inter- tubular edema similarly with the previous study of Sakr et al. (51) who reported the role of methomyl to induce renal oxidative damage. While, the co- treatment with T100 ameliorated the renal lesions in comparison with the intoxicated group due to its antioxidant effect as previously mentioned and these observations was supported by the findings of Kokhdan et al. (50).

Our results showed that administration of methomyl provoked a significant increase in serum MDA level which proves the oxidative stress evoked by methomyl (51, 52). In addition, administration of methomyl resulted in an increment in SOD activity which may be due to an over production of ROS that lead to SOD production to overcome the induced oxidative stress (53). Also, the increment may be a physiological response of the animal body to justify the toxic effect of methomyl on the body due to its role in scavenging ROS as mentioned by Moeen et al. (54). The increment in SOD activity comes in agreement with Patil et al. (35). While, the results of Mansour et al. (8) and Mansour et al. (36) were different. The difference may be attributed to the use of dissimilar dose and/ or duration of treatment. On contrary, the co- treatment of M- intoxicated group with T100 revealed a significant decrease in MDA level and SOD activity returning their values toward control due to the antioxidant effect of thymol (44).

Regarding to serum testosterone level and semen parameters evaluation, our results showed a significant decrease in serum testosterone level in M- intoxicated rats in accordance with Sakr et al. (51) which may be explained by the ability of methomyl to induce oxidative damage (55). Also, the administration of methomyl led to a significant increase in the percentage of sperm abnormalities with non- significant decrease in sperm count and motility in comparison with the control rats. These abnormalities could be caused by sperm containing higher levels of polyunsaturated fatty acids, making them more susceptible to oxidative damage and LPO (56). On the other hand, the co-administration of T100 caused an improvement in the serum level of testosterone and percentage of sperm abnormalities in close to the control. These results come in the same consistence with El-Gindy (57). Histopathologically, these results were confirmed where, the testes of M- intoxicated rats revealed disorganization of seminiferous tubules with corrugation of cell membrane, incomplete spermatogenesis, vacuolation and sloughing of the germinal epithelium. The recorded M- induced testicular lesions induced by methomyl may be returned to the ability of methomyl to induce testicular oxidative damage in accordance with Meng et al. (58).While, the co- treatment with MT100 improved the testicular tissue and returned as control if compared with the M- intoxicated rats in the same harmony with Jafari et al. (59).

Regarding to immunohistochemical examination, the M- administration displayed a strong caspase-3 immune reaction in the hepatic, renal and testicular tissues. These findings indicate the role of methomyl to induce apoptosis. During apoptosis, caspase- 3 plays an important role in the damage of many biological cellular components for repairing and regulation of DNA and oxidative stress (60, 61). The M- induced toxic effects and hepato- renal and testicular damage could be explained by its direct cytotoxic effect and/ or indirectly via the increased level of ROS and apoptosis-mediated genes in rats and mice (62, 63). Similar findings were previously obtained (50). Mixed treatment (M with T100) ameliorated caspase- 3 immunoreactivity in the hepatic, renal and testicular tissues comparatively with the toxic group. These findings may be attributed to the potent anti-apoptotic (64) and antioxidant effects of thymol and its ability to scavenge ROS as previously mentioned (44) in accordance with Karimi et al. (65).

Conclusion

The current study showed that methomyl causes hepatotoxicity, nephrotoxicity and male reproductive toxicity in addition to oxidative damage in male rats. The co-treatment with thymol may provide a prolonged remedy against methomyl- induced oxidative damage.

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