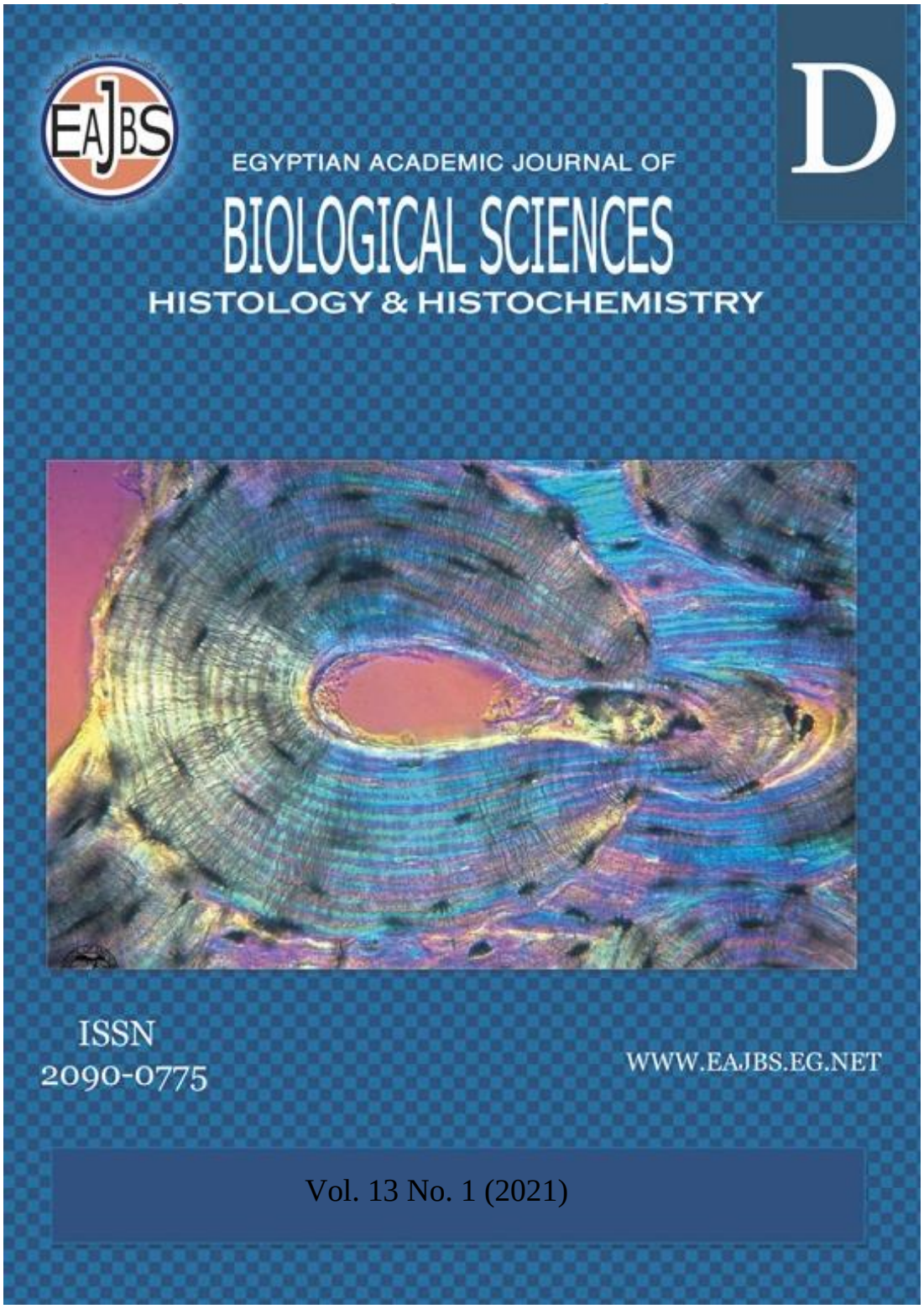

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Protective Effects of *Thymus vulgaris* Essential Oil Against Voliam Targo® Induced Kidney and Brain Toxicity in Male Rabbits

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ABSTRACT

The present study was designed to investigate the protective effects of *Thymus vulgaris* essential oil against kidney and brain toxicity induced by an abamectin-based insecticide Voliam targo® (VT) in male rabbits (*Oryctolagus cuniculus*). The extraction of the essential oil from *T. vulgaris* (TEO) by hydrodistillation allowed us to obtain an essential oil with a yield of 0.30 and the characterization of this essential oil by GC / MS indicates that the major component is carvacrol (86.25%). Twenty rabbits were randomly allocated to four equal groups and treated for 21 consecutive days: Control group, VT-group (4 mg ABA kg^{-1} body weight), TEO-group (0.5 mg kg^{-1} body weight), and VT + TEO-group (0.5 mg kg^{-1} body weight of TEO plus the same dose of VT). Our results revealed that the administration of VT resulted in a statistically significant ($p < 0.05$) increase in serum creatinine and uric acid levels as compared to the control group. Voliam targo® was found to induce histopathological alterations in the kidney, namely dilatation and congestion of blood vessels, dilatation of proximal and distal tubules and lymphocytes infiltration within the renal cortical interstitium. Furthermore, the subacute exposure to VT resulted in neurotoxic effects on the cerebral cortex, the hippocampus and the cerebellum of treated rabbits. However, co-administration of *Thymus vulgaris* essential oil significantly reversed renal function biomarkers to near normal levels and improved morphological changes of the kidney and brain tissues. The present results indicate that carvacrol-rich thyme essential oil exerts protective effects against VT-induced renal and neuro-toxicity.

INTRODUCTION

Over the last two decades, pesticides have become an important component of global agricultural systems, allowing for a substantial improvement in crop yields and reducing spoilage rates (Carvalho, 2017). Nevertheless, the extensive application of pesticides leads to their accumulations in different biological matrices which can result in serious health hazards, including cardiovascular diseases, liver and kidney damage, cancers, reproductive difficulties and neurological effects (Pereira *et al.*, 2015; Nicolopoulou-Stamati *et al.*, 2016). "Voliam Targo® 063SCe" (VT) is a broad-spectrum insecticide and acaricide recently marketed in Algeria. It is a combination of two insecticides: abamectin and chlorantraniliprole, belonging to two different chemical families (avermectins and anthranilic diamides respectively). This biopesticide is effective on several key pests of fruits and vegetables. The synergy of its two active ingredients offers better efficiency in insects due to its action on ryanodine receptors and chloride channels (Diaz-Fleischer *et al.*, 2016).

Abamectin (ABA) is a macrocyclic lactone disaccharide; a mixture of avermectins containing more than 80% of avermectin B_{1a} and less than 20% of avermectin B_{1b}. Avermectins are widely used throughout the world in veterinary and human medicine to protect against a broad spectrum of parasitic infections and in agriculture for pest control. Avermectins are generated as fermentation products by the soil-dwelling actinomycete *Streptomyces avermitilis* (Fent, 2014). Abamectin exerts its effect through high-affinity binding to glutamate-gated chloride channels (Kolar *et al.*, 2008); it is considered very toxic to insects and fishes and may be highly toxic to mammals as well (Lankas and Gordon, 1989; Jenčič *et al.*, 2006). In addition, previous studies demonstrated that abamectin can harm kidney function (Eissa and Zidan, 2010; El-Shafey *et al.*, 2011; Magdy *et al.*, 2016; Khaldoun-Oularbi *et al.*, 2017). Besides, histological examination of the renal tissue from male albino rats exposed to abamectin orally revealed severe tubular cell necrosis, atrophy of the glomeruli with hemorrhage (Abd-Elhady and Abou-Elghar, 2013). Kidneys are the main dynamic organs responsible for maintaining the body's homeostasis; they play a key role in the filtration, biotransformation, and elimination of xenobiotics and their metabolites, and thus, are one of the most frequent sites for toxicity (Maliakel *et al.*, 2008). The P-glycoprotein (P-gp) efflux pump has been evidenced to affect abamectin biodistribution in the host while cytochromes P450 is responsible for its biotransformation (Albérich *et al.*, 2014). P-gp is known to limit the penetration of toxic compounds across the blood-brain barrier and thus prevents brain toxicity (Roulet *et al.*, 2003). Additionally, it mediates the intestinal excretion of abamectin (Ballent *et al.*, 2006).

Chlorantraniliprole is a novel anthranilic diamide insecticide, effective in controlling lepidopteran pests, and some species in Coleoptera, Diptera and Hemiptera orders (He *et al.*, 2019). This insecticide has a unique mode of action; it selectively binds to and activates the ryanodine receptors of insects which stimulate the release and depletion of internal calcium stores from the sarcoplasmic reticulum in muscles, causing an impaired regulation of muscle, paralysis and ultimately death of sensitive species (Lai and Su, 2011). Chlorantraniliprole is non-polluting and is considered a valuable alternative as compared to more toxic conventional insecticides because its toxicity to non-target animals is

relatively very low (Han *et al.,* 2012, Nawaz *et al.,* 2017).

It has long been recognized that the toxicity of pesticides is correlated with the increased generation of reactive oxygen species (ROS) (Bagchi *et al.,* 1995; Verma *et al.,* 2007). Moreover, the production of ROS has been postulated as one of the main mechanisms by which xenobiotics and pathological conditions may generate oxidative stress and cause diverse tissue damages (Yu *et al.,* 2008). In addition, oxidative stress has recently been shown to be a key factor in abamectin-induced cytotoxicity (Liang *et al.,* 2020). Therefore, the use of antioxidants to alleviate the toxic hazards of abamectin insecticides is a logical approach.

Recently, there has been a growing interest in using natural resources, which are culturally acceptable and economically viable. Thyme (*Thymus vulgaris L*.) is a perennial aromatic herb of the Lamiaceae family, native to the Mediterranean region (Domaracký *et al.,* 2007). The leafy parts of thyme and its essential oil are commonly utilized in foods as culinary herb spices, natural food preservatives and also in traditional medicines. Moreover, it has been reported that Thyme possesses numerous interesting bioactivities, including antimicrobial, antiseptic, antifungal, antioxidant properties and it has also been suggested as a natural replacement for synthetic antioxidants (Rasooli *et al.,* 2006). The therapeutic potential of thyme is based on its contents of flavonoids, thymol, carvacrol, eugenol, aliphatic phenols as well as saponins, luteolin and tetramethoxylated flavones (Dorman and Deans, 2000). It must be noted that the information about the impact of *Thymus vulgaris* essential oil on the toxicity of abamectin-based insecticides in literature is relatively rare.

Accordingly, the aim of the present work is to investigate the potentially toxic effects of Voliam targo® on kidney function biomarkers and histological changes in renal and brain tissues and to evaluate the protective role of *Thymus vulgaris* essential oil against Voliam targo® induced toxicity in male rabbits (*Oryctolagus cuniculus*).

MATERIALS AND METHODS

Chemicals:

The tested molecule "Voliam Targo® 063SC" (VT) in the current study is a new insecticide formulation containing abamectin 1.8% and chlorantraniliprole 4.5%. It is marketed by SYNGENTA Crop Protection Agrochemicals, Greensboro, USA. All other chemicals and biochemical reagents used in the present study were purchased from commercial sources (BIOLABO SA, France).

Plant Materials and Essential Oil Extraction:

T. vulgaris samples were collected in July 2019 during the flowering period, from the Blida region at Hammam Melouane (North-Algeria) (36°29' N, 2°50' E, Altitude: 200 m). The botanical identification of the species was performed at the Department of Botany, National Higher School of Agronomy, Algiers. The air-dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia 5.0. The extracted essential oil was dried over anhydrous sodium sulfate and stored in darkness at 4°C until analysis.

Gas Chromatography-Mass Spectrometry Identification:

The composition of thyme essential oil was identified by gas chromatography coupled to mass spectrometry (GC–MS) analysis on an HP6890 instrument coupled to a 5973A mass spectrometer, using two fused-silica-capillary columns with different stationary phases. The polar column was a Stabilwax™ consisting of Carbowax™–PEG (60 m × 0.2 mm i.d., 0.25 μm film thickness) and the non-polar one was an HP5MS™ (30 m × 0.25 mm i.d., 0.25 μm film thickness).

GC–MS spectra were acquired under the following conditions: carrier gas helium; flow rate 0.3 ml/min; mode split-less; injection volume 1 µl; injection temperature 250 °C; oven temperature program 60 °C for 8 min, then increased at 2 °C/min to 250 °C and held at 250 °C for 15 min. The ionization mode used was an electronic impact at 70 eV.

Constituents' identification was based on a comparison of their GC Kováts retention index (RI) determined with respect to a homologous series of n-alkanes (C5–C28) and with those of corresponding reference standards available in the authors' laboratory. Identification was confirmed by comparing their mass spectral fragmentation patterns with those reported in the literature data and stored in MS database [National Institute of Standards and Technology (NIST) and Wiley libraries] (Adams, 2007).

Gas Chromatography-FID Quantification:

The percent composition of the identified compounds was electronically calculated from GC–FID peak areas. Gas chromatography analysis was carried out using a Hewlett-Packard 6890 GC–FID system, fitted with a fused-silica-capillary column with a non-polar stationary phase HP5MS™ (30 m × 0.25 mm i.d., 0.25 µm film thickness). The column temperature program was 60 °C for 8 min, then increased at 2 °C/min to 250 °C and kept at 250 °C for 15 min. The injection was performed at 250 °C in the split-less mode with an injection volume of 1 µl. The carrier gas was nitrogen at a flow of 0.3 ml/min with flame ionization detection at 320°C.

Animals and Experimental Procedure:

A total of 20 male rabbits (*Oryctolagus cuniculus*), aged 3 to 4 months and weighing between 2.5 kg to 2.6 kg each, were used in this experiment. They were procured from the Technical Breeding Institute (ITELV, Baba-Ali) and kept for experimentation in the CRD Saidal Algeria. The rabbits were acclimatized for 3 weeks prior to the experiment in standard cages at 25 ± 3 °C under a 12h/12h light/dark cycle and received a standard commercial pellet diet and water *ad libitum.* The experimental procedure followed the National Guidelines on the care and use of animals in laboratory research (National Research Council, 2010).

Animals were randomly divided into four groups (n=5): (1) control group; (2) VT-treated group received Voliam targo® alone (4 mg kg^{-1} ABA body weight); (3) TEO-treated group, rabbits were treated with thyme essential oil alone (0.5 mg kg^{-1} body weight); (4) VT + TEO-treated group, rabbits received 0.5 mg kg^{-1} body weight of TEO plus the same dose of VT as in VT-treated group. The treatments were administered once daily orally by gavage for 21 consecutive days. Voliam targo® was dissolved in distilled water. The rabbits were weighed daily early in the morning before feeding throughout the acclimation (3 weeks) and experimental (3 weeks) periods. Feed and water intakes were recorded daily.

Blood Sampling:

At the end of the experimental period, the animals of all groups were sacrificed and blood samples were collected from the rabbit ear vein into dry clean tubes containing EDTA as an anticoagulant. Then, plasma was obtained by centrifugation at 3000 r/min for 20 min and kept at −20 °C for further biochemical analysis.

Biochemical Analysis:

Plasma levels of uric acid and creatinine were determined by standardized enzymatic procedures using commercial diagnostic kits (Biolabo, Maizy, France) on an auto-analyzer (Hitachi 912) instrument (Roche Diagnostics, Mannheim, Germany).

Histological Examination:

The effects of VT on the histopathology of the kidney and brain

were investigated. At the time of sacrifice, kidney and brain tissues were removed, trimmed from excess fat, fixed in a 10% neutral buffer formalin solution and then dehydrated with different ethanol solutions and embedded in paraffin. The paraffin blocks were cut into serial histological sections of 2 µm thickness using Leica rotary microtome. The sections were stained with Hematoxylin-Eosin **(**H&E) and Masson's trichrome and then examined using an Olympus microscope (Zeiss, Axiostar plus, Oberkochen, Germany).

Statistical Analysis:

Data analyses were carried out by one-way ANOVA and Duncan's multiple range tests using Statistica version 10.0 (Stat Soft Inc., Tulsa, Oklahoma, USA).

Results were expressed as means ± SD. P-value < 0.05 was considered to be statistically significant.

RESULTS

Analytical Study of *T. vulgaris* Essential Oil:

The extraction of the essential oil (EO) from *T. vulgaris* by hydrodistillation allowed us to obtain an EO with a yield of 0.30% v/w. GC-MS analysis of the TEO identified 13 volatile compounds representing 99.73% of the total detected constituents. The chemical composition of TEO is given in Table 1. Carvacrol (86.25%) was identified as the major constituent. The amount of the other components varies between (0.03 - 1.70%) except for linalool with (3.00%), and alpha-Humelene with (3.90%). Thereby, based on the analysis results, our *Thymus vulgaris EO* can be considered as a carvacrol chemotype.

Table 1: Chemical composition of *T. vulgaris* essential oil.

N°	Compound	Area %	Retention time (min)
	Monoterpenes		
1	Alpha Terpinene	0,28	1.019
2	*para*-Cymene	0,67	1.028
3	*trans*-Ocimene	0,40	1.052
4	*gamma*-Terpinene	0,13	1.065
	Oxygenated monoterpenes		
5	Linalool	3,00	1.123
6	Terpin-4-ol	0,05	1.179
7	Thymol	0,54	1.302
8	Carvacrol	86,25	1.318
	Sesquiterpenes		
9	Aromadendrene	1,15	1.439
10	*alpha*-Humelene	3,90	1.454
11	*gamma*-Cadinene	1,70	1.513
12	*delta*-Cadinene	0,03	1.542
	Other oxygenates		
13	Carvacrol methyl ether	1,63	1.282

Results of the Toxicological Study:

Effects of Treatments on Body and Kidney weights, Food Intake and Water Consumption:

No mortality occurred during the experimental period, some clinical signs of toxicity were observed in animals treated with VT, namely decreased activity and tremors. The mean body weight, absolute and relative kidney weights, the average feed and water consumption are summarized in Table 2.

There was a homogeneous weight gain in the control rabbits, those treated with TEO and those treated with VT + TEO during the experimental period (21 days). However, a significant decrease ($p < 0.05$) in the average body weight of rabbits treated with VT

compared with all other groups was observed. Also, there was a significant reduction in food consumption and water intake in the VT group during the experimental period compared to the other three groups**.** In contrast, the sub-acute exposure to VT resulted in a significant increase in absolute and relative kidney weights in VT-treated rabbits compared to control rabbits. While co-administration of thyme essential oil to VT-intoxicated rabbits caused a significant improvement of the altered weights.

Table 2: Effects of treatments on body weight, absolute and relative kidney weights, average Feed and Water consumption in rabbits at the acclimation (21 days) and experimental (21 days) periods**.**

	Period		Control	TEO	VT	VT + TEO
Body weight (kg)	**Acclimation**	**Week 1**	2.49 ± 0.02	2.50 ± 0.03	2.60 ± 0.04	2.57 ± 0.03
		Week 2	2.53 ± 0.03	2.67 ± 0.03	2.66 ± 0.05	2.62 ± 0.05
		Week 3	2.71 ± 0.03	2.84 ± 0.07	2.81 ± 0.06	2.76 ± 0.09
	Expérimentation	**Week 1**	2.88 ± 0.02	2.93 ± 0.03	2.84 ± 0.07	2.91 ± 0.09
		Week 2	2.93 ± 0.03	3.05 ± 0.04	**2.82± 0.08**$^{b*, c*}$	3.08 ± 0.06
		Week 3	3.12 ± 0.01	3.11 ± 0.03	**2.85±0.11**$^{a*, b*, c*}$	3.12 ± 0.08
Average feed intake (g / rabbit)	**Acclimation**	**Week 1**	91.91 ± 5.62	**120.11±12.46**^{a}	**84.88 ± 9.72**^{b*}	107.94±9.37
		Week 2	130.51±1.11	**171.34±6.50**^{a**}	**156.56 ± 10.55**^{a}	**145.43±6.51**^{b}
		Week 3	159.56±5.28	183.33 ± 6.88	177.90 ± 9.17	176.46± 6.23
	Expérimentation	**Week 1**	138.60±4.69	141.70 ± 4.17	127.75 ± 6.71	135.05± 4.66
		Week 2	163.00±5.93	155.10 ± 3.04	**113.73±6.97**$^{a**, b}$	**136.63±4.76**^{a*}
		Week 3	188.80±5.02	163.00 ± 2.10	**86.55±9.21**^{a**,b**,c**}	**143.75±3.19**^{a**}
Average water consumption (ml / rabbit)	**Acclimation**	**Week 1**	32.06 ± 0.55	**73.28 ± 5.45**^{a*}	47.74 ± 3.74	**36.68 ± 1.25**^{b*}
		Week 2	75.18±14.13	101.77 ± 6.05	102.77 ± 7.58	97.17 ± 7.69
		Week 3	129.50±4.09	141.20 ± 5.80	118.56 ± 5.98	128.33 ± 9.55
	Expérimentation	**Week 1**	138.05±7.57	148.30 ± 19.72	147.75 ± 17.85	135.10 ± 14.70
		Week 2	133.10±04.28	141.83 ± 12.24	117.77 ± 8.80	143.67 ± 13.59
		Week 3	135.60± 2.75	139.55 ± 12.37	**107.46 ± 8.00**$^{a, b, c}$	127.50 ± 15.64
Kidney weight (g)	**Right Kidney**	**Absolute**	7.01 ± 0.18	7.15 ± 0.52	**7.78 ± 0.18**^{a}	6.95 ± 0.18
		Relative	0.23 ± 0.005	0.24 ± 0.004	**0.25 ± 0.002**^{a}	0.24 ± 0.007
	Left Kidney	**Absolute**	6.65 ± 0.34	7.41 ± 0.40	**7.86 ± 0.18**^{a}	7.06 ± 0.08
		Relative	0.22 ± 0.01	0.24 ± 0.01	**0.26 ± 0.002**^{a*}	0.23± 0.01

Results are given as a mean ± SD for five rabbits in each group. **a** indicates significantly different from control group ($p < 0.05$), **a*** indicates highly significantly different from control group ($p < 0.01$), **a**** indicates very highly significantly different from control group ($p < 0.001$).**b** indicates significantly different from TEO group ($p < 0.05$), **b*** indicates highly significantly different from TEO group ($p < 0.01$), **b**** indicates very highly significantly different from TEO group ($p < 0.001$).**c** indicates significantly different from (VT + TEO) group ($p < 0.05$), **c*** indicates highly significantly different from (VT + TEO) group ($p < 0.01$), **c**** indicates very highly significantly different from (VT + TEO) group ($p < 0.001$).

Biochemical Parameters:

The effect of VT treatment on kidney function indicators in rabbits is displayed in Table 3. The administration of Voliam targo® caused kidney dysfunction in the treated rabbits as evidenced by the significant increase ($p < 0.05$) in plasmatic uric acid and creatinine levels relative to the control. Whereas, co-administration of thyme essential oil with VT restored the elevated levels of kidney function biomarkers towards the normal range.

Table 3: Effects of treatments on kidney function biomarkers in experimental rabbits**.**

Parameters/Groups	Control	TEO	VT	VT + TEO
Uric acid (mg/dl)	1.57 ± 0.37^{a}	1.70 ± 0.20^{a}	**2.70 ± 0.62**^{b}	1.86 ± 0.48^{a}
Creatinine (mg/dl)	1.15 ± 0.07^{a}	1.28 ± 0.13^{a}	**3.58 ± 0.82**^{b}	1.36 ± 0.17^{a}

Results are given as a mean ± SD for five rabbits in each group. VT: Voliam targo ; TEO**:** Thyme essential oil; VT + TEO: Voliam targo + Thyme essential oil. Means within the same row with different superscripts are significant at $p < 0.05$

Histological Results:

Histological examination of kidney and brain tissues of male rabbits is illustrated in Figures 1-6.

The histological analysis of kidney sections of the control and TEO-treated rabbits revealed normal morphological structures of the glomeruli as well as proximal and distal

convoluted tubules (Figure 1 A-H). However, the treatment with Voliam targo® caused histopathological changes in renal tissues when compared to control, namely dilatation and congestion of blood vessels, dilatation of proximal and distal tubules and lymphocytes infiltration within the renal cortical interstitium. Intertubular and glomerular congestion were also observed. While co-administration of *Thymus vulgaris* essential oil revealed significant improvement in morphological alterations of the kidney of (VT + TEO)-treated animals (Figure 2 A-I).

Light microscopic observation of sections of the cerebral cortex from the control group and TEO group were similar and revealed the well-known normal histological structure of the cerebral cortex, and most neurons appear intact (Figure 3 A-B, Figure 4 A-B). Sections of the cerebral cortex of rabbits treated with VT exhibited structural alterations in the form of pericellular edema and vacuolation. Dilatation and congestion of some blood vessels were also noticed (Figure 5 A-C). Cerebral cortex sections of (VT + TEO) treated-rabbits showed less histopathological changes (Figure 6A).

Sections of the hippocampus of control rabbits and those treated with TEO showed normal architecture of the hippocampal tissue with normal small pyramidal cells, arranged nerve fibers and small glial cells forming the molecular layer (Figure 3 C, Figure 4 C). Histological sections of the hippocampus after administration of VT revealed marked structural changes. The hippocampal tissue was the site of significant edema and disorganization of the architecture of nerve cells; the latter appear retracted and present clear pericellular halots and picnotic nuclei (Figure 5 D-E). Thyme essential oil co-administration with VT treatment showed a prominent decrease in histomorphological damage of hippocampus tissue; CA1 region appeared nearly similar to that of the control group (Figure 6 B-C).

Histological examination of cerebellum sections of control rabbits and those treated with TEO showed normal histoarchitecture of the cerebellar cortex with three normal layers; a molecular layer, Purkinje cell layer, and a granular layer with a homogeneous cell distribution (Figure 3 D-E, Figure 4D). Whereas, microscopic examination of the cerebellar sections of rabbits treated with VT showed marked histopathological changes, namely neuronal degenerative changes and extensive neuropil vacuolation. Purkinje cells layer showed atrophy and degeneration of Purkinje cells (Figure 5 F-H). The co-administration of *Thymus vulgaris* essential oil to the VT-treated rabbits showed improvement in morphological alterations of the cerebellum with the presence of few neuropil vacuolations (Figure 6 D-E).

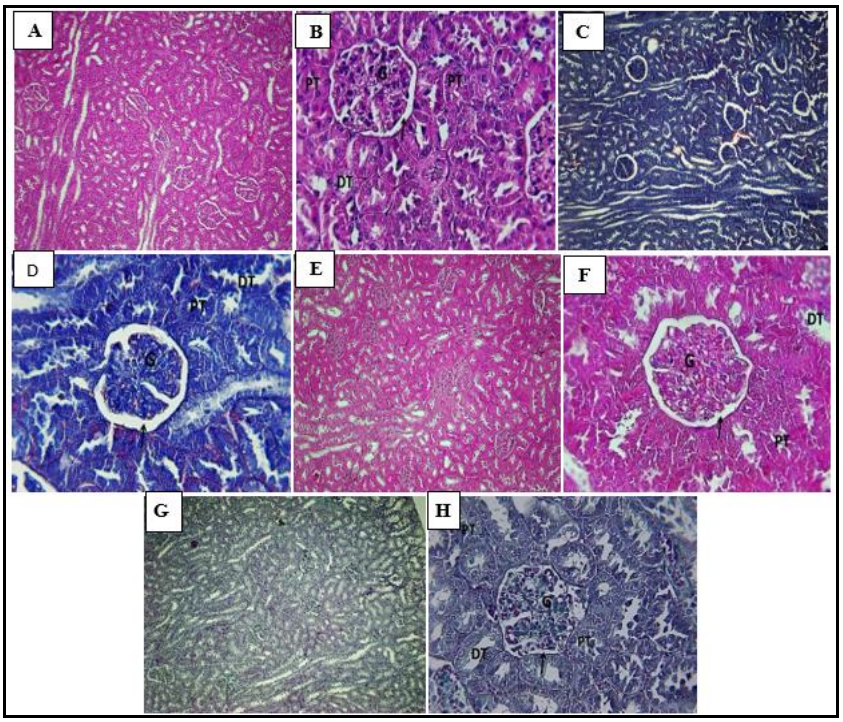

Fig. 1: Photomicrographs of rabbit kidney sections of control and TEO groups. A, B, C and D: Control group; E, F, G and H: TEO group. Kidney sections from control and TEO-treated rabbits showed normal morphology of renal tissue, proximal and distal tubules and glomeruler capsule are intact and no tissue damages were observed. G: Glomerulus. PT: Proximal convoluted tuble. DT: Distal convoluted tubule. Black arrow: Bowman's space. H&E staining and Masson's Trichrome staining: × 100 ; × 400.

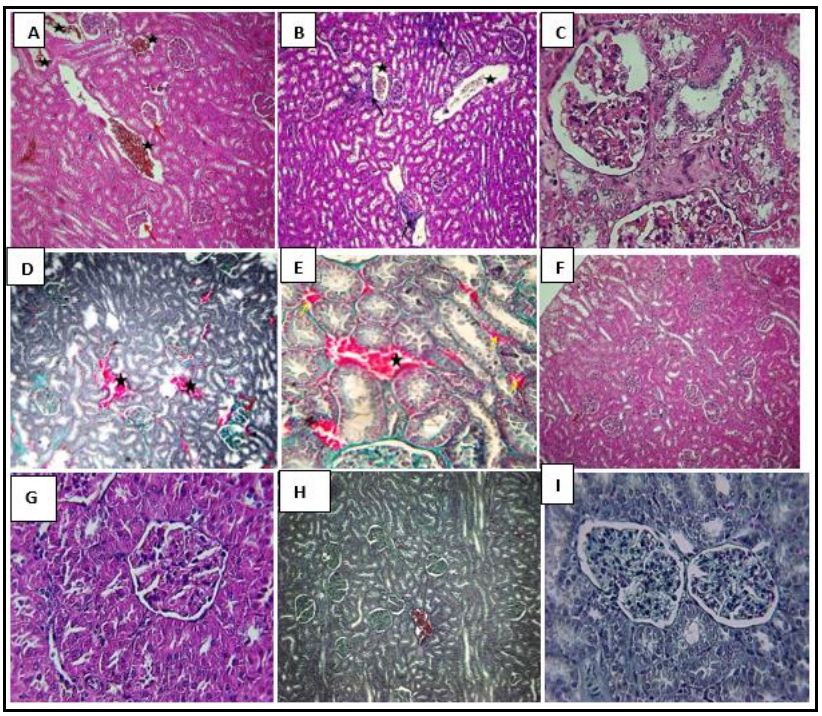

Fig. 2: Photomicrographs of kidney sections of VT-treated group and VT+ TEO group. A, B, C, D and E: VT group; F, G, H and I: (VT+ TEO) group. VT group sections showed dilatation and congestion of blood vessels (asterisk), leucocytes infiltration (black arrow), dilation of proximal and distal tubules, shrinkage and fragmentation of some golmeruli (red arrow), and intertubular and glomerular congestion (yellow arrow). Kidney sections from (VT+ TEO) group showed protective changes in most renal tubules and glomeruli structures. H&E staining and Masson's Trichrome staining: × 100 ; × 400.

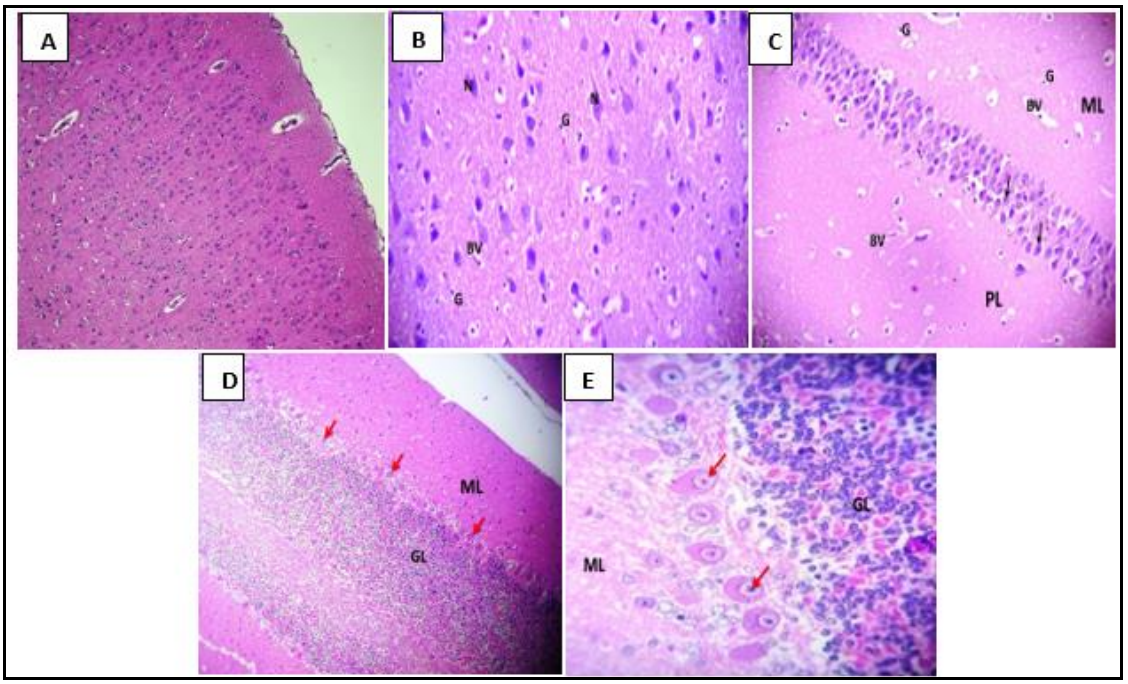

Fig. 3: Photomicrographs of brain sections of control rabbits stained with H&E. Sections of cerebral cortex (A and B), Hippocampus (C) and cerebellum (D and E) from Control rabbits showing normal histological structure. N: neurons; G: glial cells; BV: blood vessel. ML: molecular layer; PL: polymorphic layer; Black arrows: pyramidal cells; GL: granular layer; Red arrows: Purkinje cells. x 100 , x400.

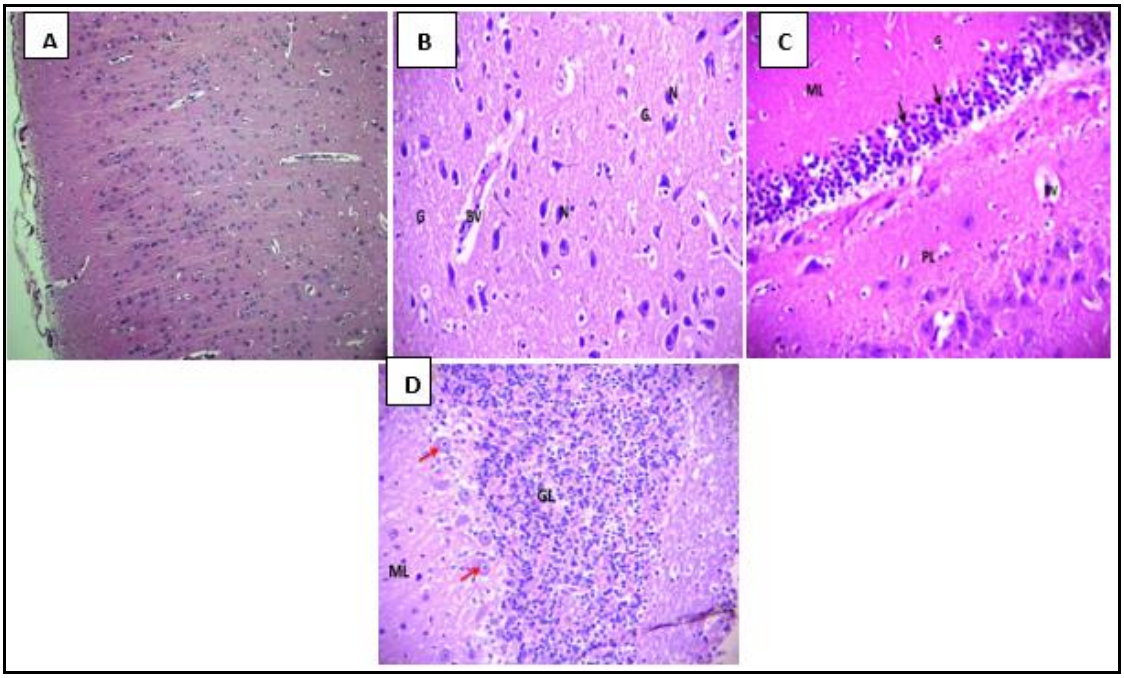

Fig. 4: Photomicrographs of brain sections of rabbit treated with TEO alone, stained with H&E. TEO group showing nearly normal histological architecture of the cerebral cortex (A and B), the hippocampus (C) and the cerebellum (D). N: neurons; G: glial cells; BV: blood vessel. ML: molecular layer; PL: polymorphic layer; Black arrows: pyramidal cells; GL: granular layer; Red arrows: Purkinje cells. x 100 and x 400.

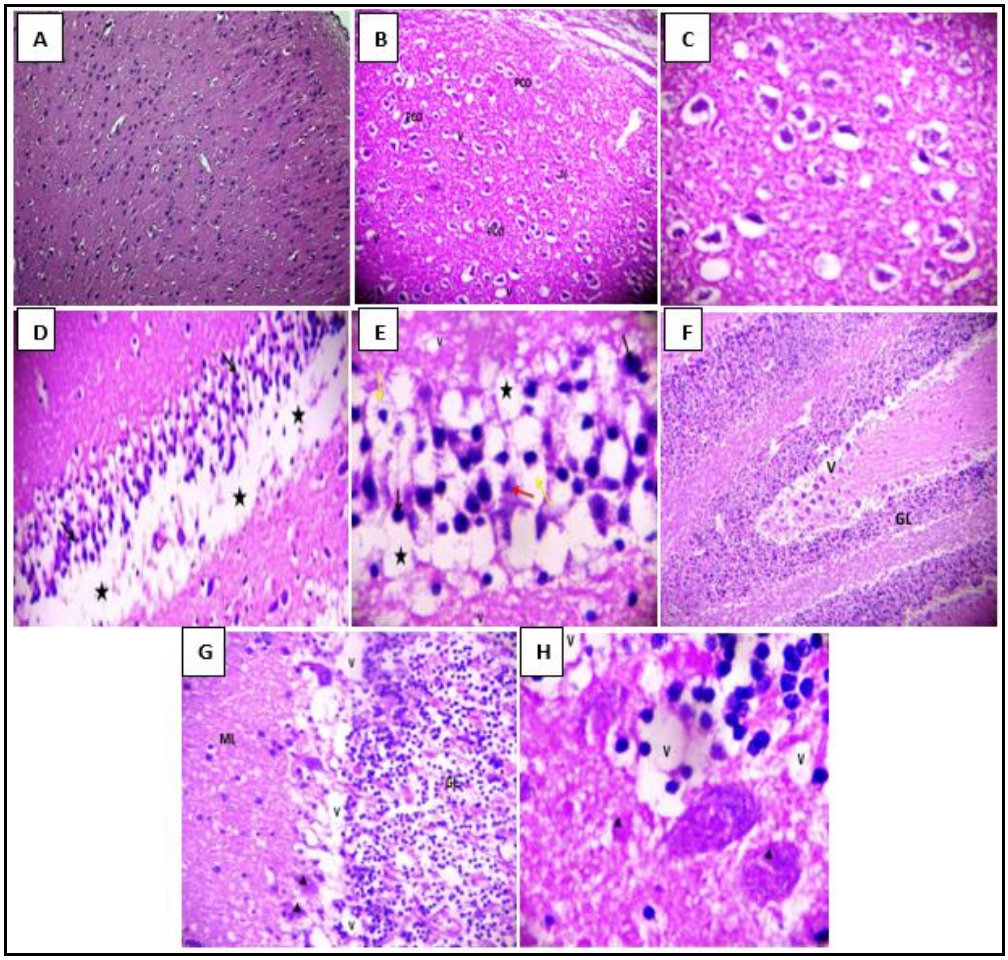

Fig. 5: Photomicrographs of brain sections of VT-treated rabbits stained with H&E. VT-treated group showing neuronal degenerative changes; pericellular oedema (PCO) and neuropil vacuolation (V) in the cerebral cortex (A, B and C). The hippocampal tissue was the site of significant oedema (asterisk) and disorganization of the architecture of nerve cells; shrunken pyramidal cells (red arrow), clear pericellular halots (yellow arrows) and picnotic nuclei (Black arrows) were observed (D and E). Purkinje cell layer showed atrophy and degeneration of Purkinje cells (arrowheads) and vacuolation (V) (F, G and H). x 100, x 400

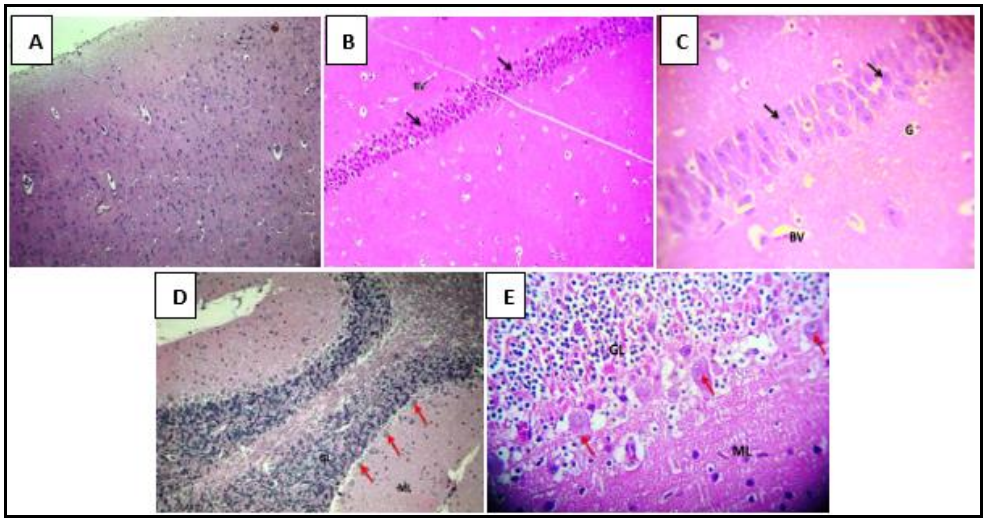

Fig. 6: Photomicrographs of brain sections of rabbit treated with (VT+TEO) stained with H&E. (VT+TEO)-treated rabbits showing a more preserved architecture of the cerebral cortex (A), the hippocampus (B and C) and the cerebellum (D and E). G: glial cells; BV: blood vessel; Black arrows: pyramidal cells; GL: granular layer; ML: molecular layer; Red arrows: Purkinje cells. x 100 , x 400.

DISCUSSION

In recent years, pesticides have been an essential part of our environment due to their increased use in agriculture and in public health programs. Nevertheless, widespread exposure to pesticides can cause serious toxicological hazards to humans and threaten ecosystems. Indeed, numerous studies have shown the role of pesticides in the occurrence of various diseases (Pereira *et al.,* 2015). Therefore, it's important to search for new strategies to reduce or even prevent the impact of these xenobiotics.

In our study, the focus of attention was on Voliam Targo® which is a broad-spectrum insecticide and acaricide. Our work aimed to evaluate the toxic effects of Voliam Targo® on kidney function markers, renal and brain tissues, and to investigate the protective effect of a co-administration of the *Thymus vulgaris* essential oil in male rabbits.

Thyme essential oil was obtained in 0.30% v/w yield. This result is agreed with those of Atti-Santos *et al.,* (2004) (0.25%); Abbassy and Marei, (2013) (0.30%) and Alsaraf *et al.,* (2020) (0.44%). On the other hand, this yield is lower than that obtained in a study previously done in Algeria (1.58%) (Bouguerra *et al.,* 2017) and that in Morocco (1%) (Imelouane *et al.,* 2009). This difference may be due to various factors including climate and geographical conditions, period of collection and cultivation practices (Hudaib and Aburjai, 2007).

GC–MS analysis of *Thymus vulgaris* essential oil revealed the presence of 13 volatile compounds. The phenolic component carvacrol (86.25%) was found to be the major constituent, which suggests that the studied EO belongs to the carvacrol chemotype. The predominance of carvacrol also was reported by Alsaraf *et al.,* (2013) and El-Nekeety *et al.,* (2011). On the other hand, other studies reported different major compounds and diverse chemical compositions for the same species in different geographical regions (Atti-Santos *et al.,* 2004; Benabed *et al.,* 2017; Bouguerra *et al.,* 2017; Wesolowska and Jadczak, 2019; Alsaraf *et al.,* 2020). Antioxidants play an important role in maintaining an optimum person's health and well-being. There is a crucial defense against the harmful free radicals that cause several age-related diseases. It is considered that oxygenated monoterpenes and/or sesquiterpenes are the components responsible for the antioxidant potential of essential oils**.** Additionally, it has been found that the phenolic chemotype possesses a potent free-radicals-scavenging activity (Schelz *et al.,* 2006).

Analyses of body and organ weights are important criteria for toxicity evaluation in toxicological studies. In the present study, the mean body weight of rabbits treated with VT was significantly ($p< 0.05$) lower than that in the other three groups after 21 days of treatment. Besides, it has been found that the average feed intake and the average water consumption of VT-treated animals were significantly decreased during the experimental period compared to other groups**.** Therefore, the decrease in body weight of rabbits treated with VT seems to be due to the less food intake as a result of anorexia or food avoidance and decreased water consumption due to treatment-related toxicity (Mansour and Mossa, 2010). Our findings are in agreement with our previous studies in rats, showing that exposure to avermectin insecticide (abamectin or Emamectin benzoate), significantly reduced body weight gain (Khaldoun-Oularbi *et al.,* 2015; Khaldoun-Oularbi *et al.,* 2017). Results revealed that VT caused a significant increase in the kidney relative weight as compared to control. The increase in the relative weight of kidneys in rabbits exposed to VT may be attributed to pesticide toxic potential or to the bodyweight reduction of experimental animals.

Supplementation of thyme essential oil to VT-treated male rabbits restored the body weight gain and the relative and absolute weights of the kidney to normal weights.

The current study demonstrated that VT significantly increased the plasma levels of uric acid and creatinine. This rise of kidney biomarkers may be attributed to the decline in the renal glomerular filtration and demonstrated impaired kidney function. These results were confirmed by the renal morphological analysis which showed marked histopathological alterations in kidney tissue caused by VT including dilation of proximal and distal tubules, congestion of blood vessels and lymphocytes infiltration within the renal cortical interstitium. These findings concur with earlier studies that have documented similar results in male albino rats exposed to avermectin insecticides (Eissa and Zidan, 2010; Khaldoun-Oularbi *et al.,* 2015; Magdy *et al.,* 2016; Nasr *et al.,* 2016).

Our results indicated that subacute exposure to VT resulted in neurotoxic effects on the cerebral cortex, the hippocampus and the cerebellum of treated rabbits. Oxidative stress is considered a primary mechanism for neuronal cell damage involved in pesticide-induced neurotoxicity (Qiao *et al.,* 2005). Insecticides act as pro-oxidants and trigger oxidative damage in brain tissue (Limón-Pacheco and Gonsebatt, 2009). Avermectins are among the most widely used compounds for insect control today. Due to their physicochemical properties of lipophilicity and water insolubility, avermectins can cross the blood-brain barrier and elicit harmful effects to brain tissue (Tišler and Eržen, 2006). In addition, brain tissue is especially vulnerable to oxidative damage due to its high content of polyunsaturated fatty acids and its high oxygen consumption (Dringen, 2000; Lukaszewicz-Hussain, 2010).

The leaves and flowers of aromatic plants are rich sources of bioactive compounds, mainly phenols. Phenolic compounds are thought to have potential health beneficial effects via their antioxidant and free radicals scavenging capacities thereby protecting cell components against oxidative damage. Nevertheless, they are likely to possess different antioxidant activities because of their various chemical structures (Ferguson, 2001; Dapkevicius et al., 2002). Our study revealed that *Thymus vulgaris* essential oil is rich in phenolic constituent carvacrol. In addition, combined treatment of Voliam targo® and *Thymus vulgaris* EO in the current study evidenced a significant improvement in body weight, food intake, water consumption, kidney function biomarkers and histological alterations of the kidney and the brain. Thus, *Thymus vulgaris* essential oil may have a protective effect against VT-mediated kidney injury and neurotoxicity probably via its antioxidant and free radical scavenging capacities**.** The antioxidant activity of thyme oil has been documented by previous works applying different methods. The antioxidant properties of thyme extracts are mainly due to the presence of phenolic constituents, particularly thymol and carvacrol (Lee and Shibamoto, 2002; Miura *et al.,* 2002). Besides, Ünde_ger *et al.,* (2009) have proved that carvacrol revealed a potent antioxidant activity compared to that of the standard antioxidant Trolox® in the cell-free assay.

It should be noted that rabbits treated with 0.5 mg/kg per day of *Thymus vulgaris* essential oil alone did not reveal any significant changes in body weight, relative kidney weight, biochemical parameters, or histological structure of the kidney and the brain as compared to control. Furthermore, Domaracky *et al.,* (2007) reported that thyme essential oil did not affect mouse embryo development when was added to a commercial diet at a concentration

of 0.25% which suggested the safety of this oil. Recently, a repeated dose 28-day oral toxicity study of *Thymus vulgaris* essential oil showed no significant changes in the kidney relative weights and the levels of creatinine and urea in the treated groups with doses of 100, 250 and 500 mg/kg/day in rats. While thyme essential oil decreased significantly rat body weights but only with the 500 mg/kg dose. Furthermore, histopathological examination of the kidney and the brain from rats sacrificed after the 28-day treatment period revealed no alteration for all three dose levels. It has been suggested that the no-observed-adverse-effect level (NOAEL) of *Thymus vulgaris* essential oil is greater than 250 mg/kg/day in rats (Rojas-Armas *et al.*, 2019). Although there are few studies on the toxic effects of thyme active components, our results reinforce the view that the use of thyme does not cause negative influences.

CONCLUSION

In conclusion, the present data demonstrate the adverse effects of Voliam targo® on the kidney and brain. Interestingly, the co-administration of carvacrol-rich *T. vulgaris* essential oil improves these harmful effects and may have healing and protective properties. However, further studies are required to elucidate the molecular mechanism by which TEO may exert its protective action.

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Conflict of interest: The authors declare that they have no conflict of interest.

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