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ORIGINAL ARTICLE

Evaluation of the hematoprotective and hepato-renal protective effects of *Thymus vulgaris* aqueous extract on thermally oxidized oil-induced hematotoxicity and hepato-renal toxicity

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Abstract

The present study was intended to evaluate hematoprotective and hepato-renal protective effects of *Thymus vulgaris* against the toxicity induced by thermally oxidized oil. Rabbits were differed into four groups. Group 1, retained as normal control, groups 2–4 received *T. vulgaris* (2 g/kg ration, thermally oxidized oil (5% of ration), finally *T. vulgaris* together with thermally oxidized oil respectively. Erythrogram, showed non-significant changes at first month where, thermally oxidized oil group at third month revealed macrocytic hypochromic anemia. Also, a significant leucocytosis, monocytosis, heterophilia, and lymphopenia were perceived. There were a significant excess in malondialdehyde (MDA) level alongside, a significant subside in catalase (CAT) activity. Also the activities of liver enzymes (ALT, AST, and ALP), bilirubin, urea, creatinine, uric acid, triglycerides, cholesterol, and low density lipoprotein (LDL-c) were increased where, there were a significant reduction in serum total proteins, albumin, and high density lipoprotein (HDL-c).These alterations present in lesser level at the end of third month when *T. vulgaris* added together with the oxidized oil. These changes were correlated to the pathological vagaries. It could be established that using of thermally oxidized sunflower oil caused several toxicological effects on hepatic, renal, and oxidative status. Using of *T. vulgaris* alleviated the hematotoxicity and hepato-renal toxicity induced by addition of thermally oxidized sunflower oil.

Keywords Biochemistry · Hematology · Thermally oxidized oil · Antioxidant · Thyme · *T. vulgaris* · Lipid profile · Liver · Kidney

Introduction

Nowadays, we are obliged to use thermally oxidized oil as for food processing. It is an inexpensive, rapid and relatively produces uniquely colored and flavored products which are highly acceptable to consumers. Diets having repeatedly heated oil give rise to compound formation consequence products such as triglyceride dimers, oxidized fatty acids, diglycerides, triglyceride polymers, and/or oxidized triglycerides (Lopez-Varela et al. 1995). These toxic and harmful free radicals caused many pathological effects after consumption (Narasimhamurthy and Raina 1999).

Many studies have reported varied beneficial therapeutic properties of some medicinal plants as antianemic, antioxidant properties and renal protective of *Allium saralicum* (Goorani et al. 2019b), wound healing of *Falcaria vulgaris* (Goorani et al. 2019c), antihyperglycemic (Hagh-Nazari et al. 2017), and renal protective (Zangeneh et al. 2018b; Zangeneh et al. 2018e; Zangeneh et al. 2018f). Also some plants have hepatoprotective, hematoprotective, cytotoxicity, and nephroprotective effects against varied toxicant (Goorani et al. 2019a; Goorani et al. 2019d; Goorani et al. 2019e; Moradi et al. 2019; Zangeneh et al. 2018a; Zangeneh et al. 2019; Zangeneh et al. 2018c; Zangeneh et al. 2018d; Zangeneh et al. 2018g; Zangeneh et al. 2018h). The active ingredients of some plants such as enzymes, flavonoids, alkaloids, titerpenoids, polyphenols, and saponins are extensively used for anticipation and treatment of countless

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of diseases. Some plant-extracts and natural product are possessing good antioxidant effect (El-Murr et al. 2019; Neamat-Allah et al. 2019b; Sun et al. 2011). In view of detrimental side effects of synthetic medications, plants have been endorsed in different diseases. They have been proved to be minimal toxic, safe, and even exempt from critical side effects (Oliver 2013). About 50% of drugs used in modern medicine are of plant origin. Many natural antioxidant's origin are plants which found in every portion of the plant inclusive the seeds, leaves, or even fruits (Altemimi et al. 2017).

Herbs are an important source of natural antioxidants so they play an essential role in prevention of many sicknesses resulting from peroxidation of lipid (Nakatani 2000). *T. vulgaris* (thyme) is one of the extreme foremost medical herbs. The foremost essential constituent's oils are carvacrol and thymol. They own forceful antioxidant properties (Aeschbach et al. 1994). Today, there is an excessive attention to finding safe antioxidants from natural source to avert oxidative impairment of living cells of unlike animal spices or even fish (Neamat-Allah et al. 2019a; Neamat-Allah et al. 2019b).

The present study has been carried out to weigh consequence of using *T. vulgaris* as antioxidant against the oxidative damage encouraged by thermally oxidized oil through the valuation of hematology, blood chemistry, and associated pathological injuries.

Materials and methods

Trial animals

Forty New Zealand rabbits (10 weeks old age) have been partitioned into 4 even groups. They were administered for 90 days, as follows, Group 1: retained as normal control group. Groups 2–4 received *T. vulgaris* (2 g/kg ration, thermally oxidized oil (5% of ration) and finally *T. vulgaris* together with thermally oxidized oil respectively. They were fed a balanced diet containing (19%) fiber, (15%) protein in pellet form at all time of experiment.

Thymus vulgaris

Thyme was obtained from local market, then air dried, and ground. Thirty grams of the dried material were imparted in 60 ml of distilled water for a day before; samples were filtered using filter paper and the two filtrates were stored at − 20 °C for 3 days only (Shati and Elsaid 2009). Diets were assembled in pellets shape 4 mm in thickness at El- Sharkia Factory, Sharkia, Egypt. It was added by a dose of 2 g/kg ration (Tousson et al. 2011).

Preparation of thermally oxidized oil

Refined sunflower oil was undergoing a total of 75 operations deep-fat frying in a fryer. At 180 °C, the oil was heated for 20 min as mentioned (Lopez-Varela et al. 1995). This thermally oxidized oil was added to the ration at ratio 5%.

Blood samples

At the end of first and third month of the experiment: From the peripheral ear veins (Hashem et al. 2018b), blood was amassed and allocated into two sections. The first section was 0.5 ml blood collected in a washed out tubes having dipotassium salt of (EDTA) (Mahmoud 2015; Prasanna et al. 2017) exercised for hematological checking (Badr et al. 2011; Hashem et al. 2018a; Neamat-Allah and Mahmoud 2019). The 2nd section was 5 ml blood collected without anticoagulant in a test tube to harvest the serum (Salem et al. 2011) for biochemical analysis (Neamat-Allah 2015; Neamat-Allah and Damaty 2016).

Hematological studies

The sum of leucocytes and the erythrocyte counts, packed cell volume (PCV), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and differential leucocytic numbers were carried out using Hospitex Hema screen 18 (hematology analyzer).

Biochemical studies

Biochemical analysis include serum levels of total cholesterol, triacyleglycerols, high density lipoproteins (HDL-c),low density lipoproteins (LDL-c),alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase activity (ALP),total and direct bilirubin, indirect bilirubin, total proteins, albumin, malondialdehyde (MDA),creatinine, urea, and uric acid were estimated using test kits of Diamond Egypt, Vitro, Bio diagnostic and Monobind Inc.

Pathological examination

The tissue samples from the livers and kidneys were studied under microscope. In briefly, the samples were set in 10% neutral buffer formalin, and then dried using ethanol (70–100%), subsequent by xylene, and lastly fixed in paraffin and stained with (H&E) (Cordatos 2002).

Statistical analysis

By one-way analysis of variance (ANOVA), the data established were investigated using IBM SPSS, version 22.

Duncan's multiple variety test was run to equate variances within means, and a significance was defined as $p \leq 0.05$ (IBM 2013).

Results

Erythrogram results

At the end of the first month, (Table 1) illustrate that RBCs count, PCV, Hb concentration, MCV, MCH, and MCHC showed non-significant changes paralleled with control group.

At the end of the third month, erythrogram showed a significant decrease in the RBCs count, PCV, Hb concentration, and MCHC in thermally oxidized oil-treated group weighed with the control. Other groups show non-significant transformations in the erythrogram.

On the other hand, a significant increase in the MCV and MCH was documented in the same group with a significant leucocytosis, monocytosis, heterophilia, and lymphopenia weighed with the control.

Some liver and kidney biomarkers

At the end of the first month, Table 2, concerning the results of aminotransferases (ALT and AST), ALP, bilirubin (total, direct and indirect), urea, creatinine, and uric acid thermally oxidized oil administered group show a significant enlarge paralleled with the normal control group. While groups (2 and 4) show non-significant transformations in these parameters.

At the third group, it showed a significant acceleration in the serum activities of these factors in evaluation with the normal control group. The supreme values were recorded in the third group. While groups (2 and 4) demonstrate non-significant variations in these factors related with the normal control.

Protein profile

At the end of the first and third month, Table 3 illustrates that there was non-significant changes in all groups except the third group show a significant decline in serum levels of total proteins, albumin, and A/G ratio in divergence with all groups. Moreover, serum globulins proved non-significant modifies in all groups paralleled with control.

Table 1 Changes in hematological parameters (mean values ± SE) in all groups

Parameters		Groups			
		Control	*T. vulgaris*	Thermally oxidized oil	*T. vulgaris* + thermally oxidized oil
First month of experiment	RBCs ($\times 10^6$/UL)	$5.85^{a} \pm 0.18$	$5.90^{a} \pm 0.21$	$5.68^{a} \pm 0.16$	$5.70^{a} \pm 0.15$
	PCV (%)	$36.48^{a} \pm 0.28$	$36.68^{a} \pm 0.34$	$35.60^{a} \pm 0.95$	$35.70^{a} \pm 0.85$
	Hb (g/dl)	$10.57^{a} \pm 0.30$	$10.71^{a} \pm 0.36$	$10.47^{a} \pm 0.29$	$10.50^{a} \pm 0.28$
	MCV (fl)	$62.36^{a} \pm 1.21$	$62.17^{a} \pm 1.09$	$62.68^{a} \pm 1.46$	$62.63^{a} \pm 1.42$
	MCH (pg/cell)	$18.07^{a} \pm 0.52$	$18.15^{a} \pm 0.45$	$18.43^{a} \pm 0.30$	$18.42^{a} \pm 0.30$
	MCHC (g/dl)	$28.97^{a} \pm 0.55$	$29.20^{a} \pm 0.45$	$29.41^{a} \pm 0.95$	$29.41^{a} \pm 0.95$
	TLC ($\times 10^3$/μl)	$9.96^{a} \pm 0.28$	$9.61^{a} \pm 0.55$	$10.29^{a} \pm 0.42$	$9.66^{a} \pm 0.83$
	Lymphocytes ($\times 10^3$/μl)	$5.40^{a} \pm 0.57$	$5.32^{a} \pm 0.72$	$5.06^{a} \pm 1.08$	$5.13^{a} \pm 0.65$
	Monocytes ($\times 10^3$/μl)	$2.08^{a} \pm 0.76$	$2.10^{a} \pm 0.28$	$2.15^{a} \pm 0.39$	$2.20^{a} \pm 0.46$
	GRA ($\times 10^3$/μl)	$2.48^{b} \pm 0.13$	$2.19^{b} \pm 0.49$	$3.08^{a} \pm 0.39$	$2.33^{b} \pm 0.23$
Third month of experiment	RBCs ($\times 10^6$/UL)	$5.79^{a} \pm 0.21$	$5.93^{a} \pm 0.18$	$4.75^{b} \pm 0.20$	$5.54^{a} \pm 0.41$
	PCV (%)	$36.32^{a} \pm 0.83$	$36.35a \pm 0.75$	$34.20^{b} \pm 0.23$	$35.40^{ab} \pm 0.95$
	Hb (g/dl)	$10.33^{a} \pm 0.19$	$10.22^{a} \pm 0.16$	$9.12^{b} \pm 0.36$	$9.97^{a} \pm 0.25$
	MCV (fl)	$62.73^{b} \pm 1.82$	$61.30^{b} \pm 1.12$	$72.00^{a} \pm 0.21$	$63.90^{b} \pm 1.35$
	MCH (pg/cell)	$17.84^{b} \pm 0.61$	$17.23^{b} \pm 0.44$	$19.20^{a} \pm 0.28$	$18.00^{b} \pm 0.50$
	MCHC (g/dl)	$28.44^{a} \pm 0.29$	$28.12^{a} \pm 0.22$	$26.67^{b} \pm 0.31$	$28.16^{a} \pm 0.16$
	TLC ($\times 10^3$/μl)	$10.05^{b} \pm 0.26$	$9.70^{b} \pm 0.63$	$12.05^{a} \pm 0.38$	$11.73^{a} \pm 0.40$
	Lymphocytes ($\times 10^3$/μl)	$5.42^{a} \pm 0.12$	$5.54^{a} \pm 0.24$	$3.00^{c} \pm 0.05$	$4.00^{b} \pm 0.17$
	Monocytes ($\times 10^3$/μl)	$2.29^{c} \pm 0.21$	$2.07^{c} \pm 0.06$	$4.73^{a} \pm 0.09$	$3.80^{b} \pm 0.04$
	GRA ($\times 10^3$/μl)	$2.34^{b} \pm 0.70$	$2.09^{b} \pm 0.26$	$4.32^{a} \pm 0.33$	$3.93^{a} \pm 0.19$

Means within the same column carrying different letters are significantly different at $p \leq 0.05$

RBC, red blood corpuscles; *Hb*, hemoglobin; *PCV*, packed cell volume; *MCH*, mean corpuscular hemoglobin; *MCV*, mean corpuscular volume; *MCHC*, mean corpuscular hemoglobin concentration; *MCHC*, mean corpuscular hemoglobin concentration, *TLC*, total count of leucocytes; *GRA*, granulocytes

Table 2 Changes in some liver and kidney function parameters (mean values ± SE) in all groups

Parameters		Groups			
		Control	*T. vulgaris*	Thermally oxidized oil	*T. vulgaris* + thermally oxidized oil
First month of experiment	ALT U/l	$35.00^{b} \pm 1.22$	$34.87^{b} \pm 2.00$	$57.20^{a} \pm 3.40$	$36.00^{b} \pm 3.16$
	AST U/l	$24.35^{b} \pm 1.69$	$24.50^{b} \pm 2.00$	$36.40^{a} \pm 1.54$	$25.60^{b} \pm 1.16$
	ALP U/l	$23.65^{b} \pm 1.88$	$22.77^{b} \pm 1.26$	$67.33^{a} \pm 3.00$	$24.53^{b} \pm 2.44$
	Total bilirubin mg/dl	$0.86^{b} \pm 0.08$	$0.82b \pm 0.06$	$1.36^{a} \pm 0.03$	$0.93^{b} \pm 0.09$
	Direct bilirubin mg/dl	$0.49^{a} \pm 0.02$	$0.46^{a} \pm 0.04$	$0.50^{a} \pm 0.02$	$0.54^{a} \pm 0.03$
	Indirect bilirubin mg/dl	$0.37^{b} \pm 0.01$	$0.36^{b} \pm 0.04$	$0.56^{a} \pm 0.02$	$0.39^{b} \pm 0.02$
	Urea mg/dl	$27.94^{b} \pm 0.71$	$27.76^{b} \pm 0.66$	$52.55^{a} \pm 0.93$	$28.22^{b} \pm 1.00$
	Creatinine mg/dl	$1.04^{b} \pm 0.11$	$1.02^{b} \pm 0.06$	$1.73^{a} \pm 0.05$	$1.10^{b} \pm 0.08$
	Uric acid mg/dl	$4.51^{b} \pm 0.25$	$4.66^{b} \pm 0.20$	$7.32^{a} \pm 0.43$	$4.87^{b} \pm 0.50$
Third month of experiment	ALT U/l	$35.20^{c} \pm 1.18$	$34.39^{c} \pm 1.26$	$67.40^{a} \pm 3.75$	$52.20^{b} \pm 4.51$
	AST U/l	$25.20^{c} \pm 1.65$	$24.40^{c} \pm 1.57$	$69.42^{a} \pm 1.49$	$40.10^{b} \pm 3.54$
	ALP U/l	$23.50^{c} \pm 1.22$	$23.15^{c} \pm 2.90$	$96.58^{a} \pm 8.10$	$54.22^{b} \pm 7.26$
	Total bilirubin mg/dl	$0.88^{c} \pm 0.13$	$0.72^{c} \pm 0.04$	$2.29^{a} \pm 0.03$	$1.88^{b} \pm 0.04$
	Direct bilirubin mg/dl	$0.50^{c} \pm 0.06$	$0.40^{c} \pm 0.03$	$1.49^{a} \pm 0.05$	$1.15^{b} \pm 0.04$
	Indirect bilirubin mg/dl	$0.38^{b} \pm 0.02$	$0.32^{b} \pm 0.04$	$0.80^{a} \pm 0.03$	$0.73^{a} \pm 0.01$
	Urea mg/dl	$27.90^{c} \pm 0.95$	$28.44^{c} \pm 1.28$	$59.64^{a} \pm 1.13$	$36.65^{b} \pm 0.57$
	Creatinine mg/dl	$1.11^{c} \pm 0.14$	$1.08^{c} \pm 0.13$	$2.41^{a} \pm 0.10$	$1.88^{b} \pm 0.09$
	Uric acid mg/dl	$4.78^{c} \pm 0.40$	$4.85^{c} \pm 0.29$	$9.93^{a} \pm 0.2$	$6.03^{b} \pm 0.22$

Means within the same column carrying different letters are significantly different at $p \leq 0.05$

ALT, alanine aminotransferase; *AST*, aspartate aminotransferase, *ALP*, alkaline phosphatase

Oxidative status

Oxidative stress markers (Table 3) at the end of the first month, show a significant diminish in the serum malondialdehyde level with a significant increase in the catalase activity in the second group. On the other hand, the thermally oxidized oil group shows a significant increase in the serum malondialdehyde level with a significant diminish in the activity of catalase enzyme. Moreover, these parameters show non-significant modifications in the fourth group compared with the normal control.

At the third month, group (2) demonstrates a significant diminish in serum MDA level with a significant amplifications in catalase enzyme activity compared with normal control. While the third group shows a significant amplification in in serum MDA level with a significant diminish in catalase activity in comparison with the normal control group.

Lipid profile

At the end of the first month, Table 4 shows a significant diminution in serum levels of triglycerides, LDL-c and cholesterol, with a significant expansion in HDL-c in group (2). On the other hand, group (4) demonstrates non-significant modifications in serum lipid profile. Otherwise, group (3) demonstrates a significant upturn in serum levels of total cholesterol, triglycerides, and LDL-c with a significant drop in HDL-c.

At the third month, group (2) had a significant decrease in serum levels of total cholesterol, triglycerides, and LDL-c with a significant increase in HDL-c. Otherwise, group (4) demonstrates a significant upturn in serum levels of total cholesterol, triglycerides, and LDL-c with a significant decline in HDL-c. Group (4) revealed the greatest values in serum levels of total cholesterol, triglycerides, and LDL-c and the lowest value in HDL-c.

Pathological results

1. Thermally oxidized oil group:

(a) Liver

At the end of the first month, hepatic cells showed hydropic degeneration and vacuolization of cytoplasm. Thickening in the wall of congested hepatic blood vessels infiltrated with numerous lymphocytes (Fig. 1).

At the end of the third month, congestion of portal vein and fibroblastic proliferation in portal area with hyperplasia and desquamation of epithelium lining bile duct

Table 3 Changes in proteins profile and some antioxidant parameters (mean values ± SE) in all groups

Parameters		Groups			
		Control	*T. vulgaris*	Thermally oxidized oil	*T. vulgaris* + thermally oxidized oil
First month of experiment	Total proteins g/dl	$6.65^{a} \pm 0.24$	$6.81^{a} \pm 0.16$	$5.09^{b} \pm 0.17$	$6.50^{a} \pm 0.38$
	Albumin g/dl	$4.50^{a} \pm 0.11$	$4.64^{a} \pm 0.19$	$3.04^{b} \pm 0.12$	$4.36^{a} \pm 0.14$
	Globulins g/dl	$2.15^{a} \pm 0.13$	$2.17^{a} \pm 0.10$	$2.05^{a} \pm 0.08$	$2.14^{a} \pm 0.06$
	A/G ratio	$2.09^{a} \pm 0.09$	$2.14^{a} \pm 0.03$	$1.48^{b} \pm 0.08$	$2.04^{a} \pm 0.11$
	MDA nmol/ml	$11.50^{b} \pm 0.18$	$9.00^{c} \pm 0.71$	$14.00^{a} \pm 0.33$	$12.16^{b} \pm 0.55$
	Catalase U/l	$390.81^{b} \pm 6.77$	$420.60^{a} \pm 6.00$	$330.59^{c} \pm 7.00$	$379.00^{b} \pm 5.22$
Third month of experiment	Total proteins g/dl	$6.69^{a} \pm 0.30$	$6.74^{a} \pm 0.06$	$4.40^{c} \pm 0.09$	$4.90^{b} \pm 0.08$
	Albumin g/dl	$4.46^{a} \pm 0.14$	$4.55^{a} \pm 0.28$	$2.00^{c} \pm 0.05$	$2.55^{b} \pm 0.09$
	Globulins g/dl	$2.23^{a} \pm 0.15$	$2.19^{a} \pm 0.07$	$2.40^{a} \pm 0.16$	$2.35^{a} \pm 0.11$
	A/G ratio	$2.00^{a} \pm 0.15$	$2.08^{a} \pm 0.12$	$0.83^{b} \pm 0.04$	$1.09^{b} \pm 0.06$
	MDA nmol/ml	$11.70^{c} \pm 0.70$	$8.72^{d} \pm 0.11$	$26.94^{a} \pm 1.00$	$21.00^{b} \pm 0.60$
	Catalase U/l	$391.13^{b} \pm 6.09$	$423.58^{a} \pm 5.29$	$155.78^{d} \pm 7.59$	$232.16^{c} \pm 5.54$

Means within the same column carrying different letters are significantly different at $p \leq 0.05$

A/G, albumin globulin, *MDA*, malondialdehyde

farther revealed incidence of eosinophilic mass in its lumen and existence of hemosiderin in portal vein, presence of fibrosis with leucocytes infiltration, and hemosiderosis. There was plain degenerative vagaries in hepatocytes including fatty changes in which hepatocytes showing signet ring appearance (Fig. 2).

(b) Kidneys

At the end of the first month, renal sections revealed presence of pre-tubular and pre-glomerular fibroblastic proliferation and congested glomerulus and coagulative necrosis upsetting renal epithelia infiltrating with leucocytes (Fig. 3).

At the end of the third month, hyperplasia in the wall of congested blood vessels with vacuolization in endothelial cells intruded with leucocytes and fibroblast cells with the presence of hemosidrosis, plain deteriorating vagaries in lining epithelium of renal tubules containing hydropic degeneration, coagulative necrosis of epithelial cells lining renal tubules with thickening in the wall of congested blood vessels, and vacuolization of its endothelial cells (Fig. 4).

2. ***T.*** *vulgaris* together with thermally oxidized oil group:

(a) Liver

At the end of the first month, there was no microscopically alterations.

At the end of the third month, the liver indicated focal area of necrosis with mild degenerative changes of hepatocytes (Fig. 5).

(b) Kidneys

At the end of the first month, there was no microscopically alterations.

At the end of the third month, the kidneys indicated coagulative necrosis of epithelial cells lining, nearly renal tubules with lobulization of glomerular truss and pre-glomerular and pre-tubular leucocytes infiltration (Fig. 6).

Discussion

Fried products absorb a large quantity of its frying oil thus finally accumulating degradation products to become part of the diet (Ghidurus et al. 2010). Toxic and hurtful free radicals of thermal oxidized oil stimulate oxidative stress which implicated in the progress of many hurtful and pathological effects in many tissues after consumption (Perumalla Venkata and Subramanyam 2016).

Concerning with the results of erythrogram, group (2) exposed non-significant fluctuations paralleled with the control along the experimental periods. This indicates that using *T. vulgaris* did not have negative impact on the erythrogram. On the other hand, at the end of the third month, group that received thermally oxidized oil showed a significant decrease in the RBCs count, Hb concentration, and PCV with specter of macrocytic hypochromic anemia. This recorded anemia may due to release of free radicals from ingestion of thermally

Table 4 Changes in lipid profile (mean values ± SE) in all groups

Parameters		Groups			
		Control	*T. vulgaris*	Thermally oxidized oil	*T. vulgaris* + thermally oxidized oil
First month of experiment	Cholesterol mg/dl	$90.08^{b} \pm 1.15$	$79.43^{c} \pm 2.07$	$110.24^{a} \pm 1.10$	$91.44^{b} \pm 1.12$
	Triglycerides mg/dl	$120.38^{b} \pm 2.31$	$107.45^{c} \pm 1.91$	$180.49^{a} \pm 1.18$	$130.52^{b} \pm 1.15$
	HDL-c mg/dl	$30.04^{b} \pm 0.21$	$35.50^{a} \pm 0.14$	$15.03^{c} \pm 0.24$	$28.18^{b} \pm 0.15$
	LDL-c mg/dl	$35.96^{b} \pm 0.43$	$22.44^{c} \pm 0.14$	$59.11^{a} \pm 0.18$	$37.16^{b} \pm 0.19$
Third month of experiment	Cholesterol mg/dl	$89.37^{c} \pm 1.20$	$74.64^{d} \pm 1.12$	$133.72^{a} \pm 1.19$	$110.19^{b} \pm 1.13$
	Triglycerides mg/dl	$117.98^{c} \pm 1.34$	$83.29^{d} \pm 1.25$	$238.31^{a} \pm 1.45$	$175.44^{b} \pm 1.13$
	HDL- c mg/dl	$31.93^{b} \pm 0.26$	$40.47^{a} \pm 0.21$	$13.95^{d} \pm 0.11$	$29.89^{c} \pm 0.07$
	LDL-c mg/dl	$33.84^{c} \pm 0.40$	$17.51^{d} \pm 0.21$	$72.11^{a} \pm 0.41$	$45.21^{b} \pm 0.12$

Means within the same column carrying different letters are significantly different at $p \leq 0.05$

HDL-c, high density lipoprotein; *LDL-c*, low density lipoprotein

oxidized oil on RBCs which led to a decrease in their life span (Mesembe et al. 2005). The increase in MCH designates incidence of hemolysis. Moreover, the picture of macrocytic hypochromic anemia is due to the release of reticulocytes from bone marrow as a response to anemia (Hostetter and Andreasen 2004). Fourth group revealed non-significant changes in the RBCs count, Hb concentration, and PCV along the experimental periods compared with the normal control. This designates the success of the antioxidant activity of *T. vulgaris* against the free radicals produced from administration of thermally oxidized oil.

In group (2), leucogram showed non-significant changes in the counts of WBCs, lymphocytes, monocytes, and heterophils throughout the experimental periods. These findings are in stratification with several studies referred to the safety of *T. vulgaris* (Dehghani et al. 2018; Toghyani et al. 2010). The leucocytosis, observed in the third group at the end of the third month, is due to monocytosis and heterophilia. Such increase in the cells of phagocytosis (heterophils and monocytes) could be due to tissue destruction. A significant lymphopenia was also recorded in the third group. This may be due the oxidative stress resulted from free radicals present in thermally oxidized oil. Our findings supported the previously obtained results (El-bialy et al. 2015). Group (4) showed an improvement in the leucogram towards the normal level.

Regarding to the results of antioxidants, administration of *T. vulgaris* elicited a significant amplification in the activity of catalase enzyme alongside a significant diminish in MDA level thru the experimental periods. Our results are in harmony with that obtained by (El-Nekeety et al. 2011). Otherwise, group (3) bared a significant subside in CAT activity with a significant surge in MDA level along the experimental phases. This may be attributed to the formation of diglycerides, oxidized triglycerides, triglyceride dimers, oxidized fatty acids, and triglyceride polymers during heating process which leads to increase of lipid peroxidation (MDA) and utilization of antioxidant enzymes (Kaffashi Elahi 2012). Also, vitamins E and C which present in sunflower oils are destroyed by hydroperoxides generated during repeat frying (Srivastava et al. 2010). Our outcome are in accordance with that of Rouaki et al. (Rouaki et al. 2013) who found a significant decrease in CAT enzyme activity following ingestion of diet containing thermally oxidized sunflower oil group. The serum MDA level and CAT enzyme activity were improved significantly in group (4) compared with group fed heated oil only. This may be attributed to the antioxidant activity of *T. vulgaris* (Ložienė et al. 2007) or its constituents carvacrol (Mohseni et al. 2019).

Among the experimental stages in proteinogram, AST, ALT, ALP, and bilirubin levels in group (2) revealed obvious

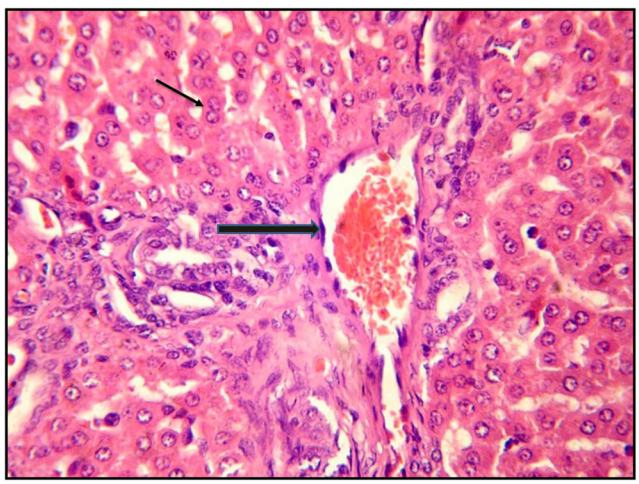

Fig. 1 Hepatocytes (H&E. × 1200) of the third group presenting vacuolization of cytoplasm (thin arrow) with thickening in the wall of congested hepatic blood vessels (thick arrow) infiltrated with numerous lymphocytes (arrow head) at the end of first month

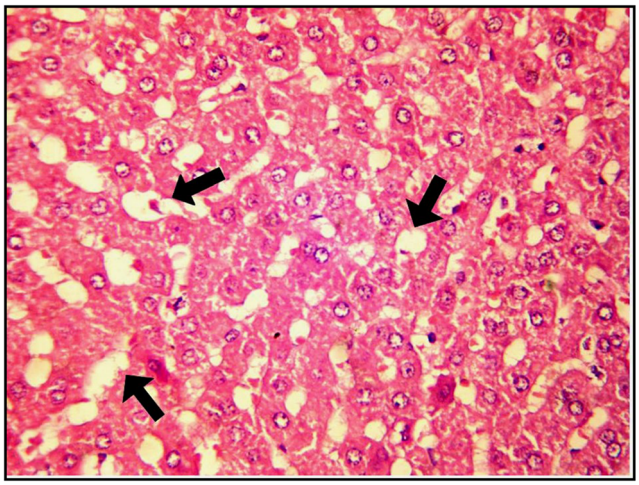

Fig. 2 Liver section (H&E. × 1200) of the third group presenting severe degenerative changes in hepatocytes including fatty changes in which hepatocytes showing signet ring appearance (arrows) at the end of the third month

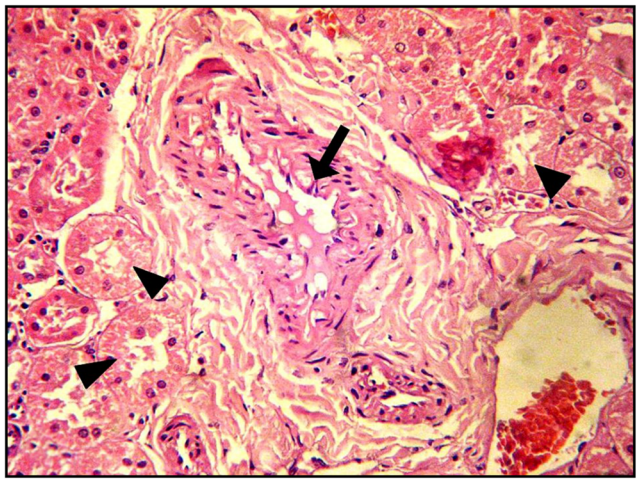

Fig. 4 Kidney section (H&E. × 1200) of the third group presenting coagulative necrosis of epithelial cells lining renal tubules (arrow heads) with thickening in wall of blood vessels and vacculization of its endothelial cells (arrow) at the end of the third month

non-significant modifications compared with the normal control. This was previously recorded by (El-Nekeety et al. 2011). Heating oil has a harmful effect on the hepatic cells through production of toxic products which causing damage. These effects were reflected in group (3) thru a significant intensification in the serum ALT, AST, ALP, and total direct and indirect bilirubin levels. The increase in aminotransferases could be due to the unwholesome effect of free radicals on hepatocytes membranes resulted in structural and functional changes and thus leakage of enzymes (Chacko 2011). The same results were previously obtained (Burenjargal and Totani 2009; Janakat and Al-Khateeb 2011; Totani and Ojiri 2007). The uplifting level of total bilirubin is due to the surge in the levels of direct and indirect bilirubin. This surge may be due to hepatocellular destruction. Also, the increase in the level of indirect bilirubin may be due to the hemolysis of RBCs, while the increase in the direct bilirubin level and ALP activity may be due to cholestasis (Tennant and Center 2008). Our results were confirmed by the presence of hydropic degeneration and vacuolization of cytoplasm of hepatocytes. Wall thickening and congested hepatic blood vessels infiltrated with numerous lymphocytes besides presence of newly formed bile ductile, hyperplasia and desquamation of epithelium lining bile duct, and severe degenerative changes in hepatocytes. These outcomes agree with Ammouche et al. 2002; El-bialy et al. 2015; and Rouaki et al. 2013. Group (4) showed an improvement in the serum ALT, AST, ALP, and total, direct, and indirect bilirubin towards the normal values. Our outcomes partially agree with El-Nekeety et al. 2011 and Mohseni et al. 2019 as amelioration oxidative effect in the liver.

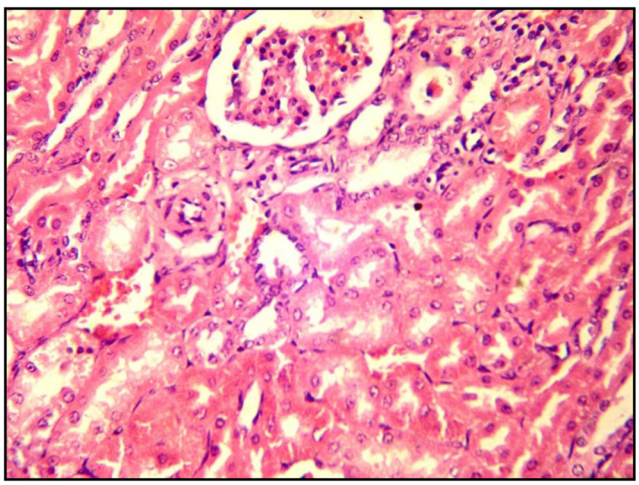

Fig. 3 Kidney section (H&E. ×1200) of the third group presenting pretubular and pre-glomerular fibroblastic proliferation with congested glomerulus (arrows) at the end of the first month

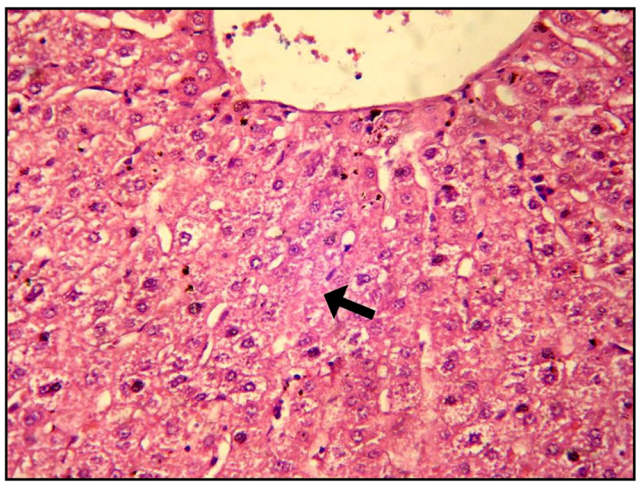

Fig. 5 Liver section (H&E. ×1200) of fourth group presenting focal area of necrosis with mild degenerative changes of hepatocytes (arrow) at the end of the third month

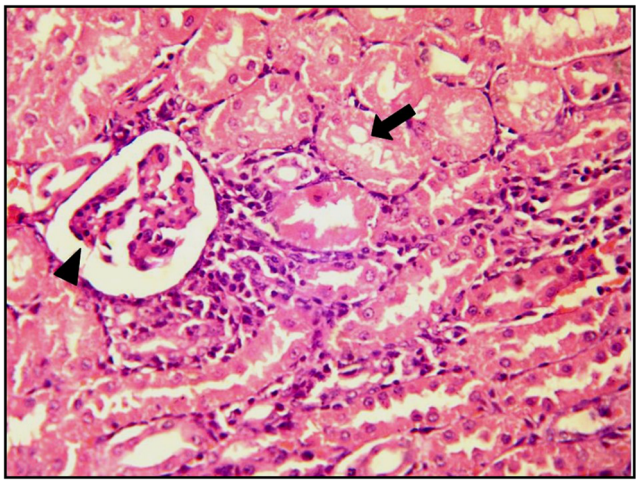

Fig. 6 Kidney section (H&E. × 1200) of fourth group presenting coagulated necrosis of epithelial cells lining some renal tubules (arrow) with glomerular tuft lobulization and leucocytes infiltration at pre-glomerular and pre-tubular (arrow head) at the end of the third month

Concerning to the protein profile results, group (3) showed a significant decrease in serum levels of total proteins, albumin, and A/G ratio. The reduction in the total proteins and A/G ratio is due to hypoalbuminemia. The chronicled hypoproteinemia may be due to the dwindle in feed intake and or digestibility and absorption of proteins (Shastry et al. 2011). Also, hypoalbuminaemia may be due to the decrease in synthesis of albumin by damaged liver and/or increase the excretion of albumin from damaged kidneys (Stockham and Scott 2013). These results are in harmony with that obtained (El-bialy et al. 2015) who proved that rabbits fed on basal diets mixed with repeatedly boiled oil for 3 months showed a gradual significant subside in serum total proteins and albumin. On the other side, group (4) revealed an recuperation in serum total proteins, albumin, and A/G ratio paralleled with group (3). This may be due to the antioxidant effect of *T. vulgaris* (Saleh et al. 2014).

In viewing to the results of lipid profile, group (2) showed a significant decrease in serum levels of triglycerides, cholesterol, and LDL-c with a significant raise in HDL-c level compared with the normal control group. These results may be due to *T. vulgaris* which is reliable for structure of intricate saponin-cholesterol multiplexes in the gastrointestinal tract, the later foil integration of cholesterol. Also, thymol or carvacol inhibiting the HMG-CoA (hepatic 3 hydroxy 3 methyl glutaryl coenzyme A) which is reliable for cholesterol biosynthesis (Lee et al. 2003). Our outcomes approve with Hosseini et al. (Hosseini et al. 2013) and incompletely agreed with Toghyani et al. 2010. From the other side, group (3) discover a significant increase in serum levels of cholesterol, triglycerides, and LDL-c, conversely a significant decrease in HDL-c level compared with the normal control. This may be due to oil intake which is correlated with the increase in serum cholesterol and LDL values (Leong et al. 2008). After oil absorption, triglycerides level was amplified that may be due to the increase in the convenience of substrate free fatty acids for esterification (Shastry et al. 2011). Also, hydroxy fatty acids and other secondary lipid oxidation products of oil cause an increase in blood lipids and cholesterol (Oarada et al. 1986). The fourth group showed a significant decrease in serum levels of triglycerides, cholesterol, and LDL-c with a significant increase in HDL-c comparing to group (4) along the experimental periods. This may be due to thymol or carvacol inhibiting the HMG-CoA, and *T. vulgaris* is accountable for the formation of insoluble saponin cholesterol complexes within gastrointestinal tract which prevents absorption of cholesterol (Khodarahmi and Azadbakht 2014).

Relating the renal function, group (2) showed non-significant changes in serum levels of urea, creatinine, and uric acid compared with the normal control along the experimental periods. This indicates that *T. vulgaris* did not have negative impact on renal function. The present work showed that heated oil damaged the renal tissue. The renal injury was epitomized by a substantial upturn in the serum levels of urea, creatinine, and uric acid. These results are in stratification with those stated by Totani and Ojiri 2007 who reported that rabbits preserved on repetitively boiled oil-based diet showed higher serum urea and creatinine levels. Such biochemical modifications in the present work are the outcome of nephropathy which is manifested by pre-tubular and pre-glomerular fibroblastic proliferation, alongside spots of coagulative necrosis with the end of the first month. However, vacuolization in endothelial cells of blood vessels which infiltrated with leucocytes and fibroblast cells with the presence of hemosidrosis were reported at the end of the third month. Our findings agreed with El-bialy et al. 2015. Furthermore, forth group showed a significant increase in serum levels of urea, creatinine, and uric acid at the end of the third month. Nearly results were obtained by El-Nekeety et al. 2011. Pathological modifications of the liver and kidneys of the fourth group confirm these results.

Conclusion

It can be established after this study that *T. vulgaris* aqueous extract has hematoprotective properties and shelter the liver and kidneys against thermally oxidized sunflower oil which caused several hematotoxicity hepato-renal injuries and anti-oxidant imbalance.

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Compliance with ethical standards

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

Informed consent None.

Ethical approval Analysis was managed in accordance with the standards set by Animal Health Research Ethics Training Initiative, Egypt, and experimental protocols were approved by the official animal ethics agency.

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