



# Effect of the aqueous root extract of *Curcuma longa* L. (turmeric) against thermally oxidized oil-induced hematological, biochemical and histopathological alterations

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## Abstract

This study was performed to evaluate the role of using *Curcuma longa* L. (turmeric) against thermally oxidized oil-induced hematological, biochemical, and histopathological alterations. Eighty male rabbits were randomly divided into 4 groups (20 of each) and treated for 90 days as follows: The first one was kept as standard control group. Groups 2 and 3 received *Curcuma longa* (CL) 13 g/kg, 5% thermally oxidized oil to ration separately, where the last group was supplemented by CL together with the thermally oxidized oil. Blood samples were collected via ear veins from all rabbits for hematological and biochemical markers analysis. After 30 days, there were non-significant changes in hematological parameters in all groups. The third group exhibits a considerable increase of ALT, AST, ALP, bilirubin, urea, creatinine, uric acid, total cholesterol, triglycerides, LDL-c, and MDA with a considerable decrease in HDL-c and CAT, while the second and fourth groups display non-significant deviations in the previous parameters. Where at 90 days, the third group showed a significant diminution in the RBCs count, PCV, Hb concentration, and MCHC with increase in the MCV and MCH. The fourth group shows a significant increase in levels of total cholesterol, triglycerides, and LDL-c with a significant decrease in HDL-c. Microscopically at 30 days, liver, kidneys, and spleen showed severe degeneration and necrosis which were increased at 90 days that confirm hemato-biochemical findings. It was achieved that CL demonstrated protective effects of *C. longa* against thermally oxidized oil-induced toxicities. These ameliorative effects are attributed to the inherent phytochemical constituents of turmeric. Therefore, turmeric and its derivatives should be a regular constituent of our diets. Also, turmeric may be exploited as an adjuvant in the phytotherapeutic management of some toxicities.

**Keywords** Biochemistry · Hematology · Thermally oxidized oil · Antioxidant · Lipogram · *Curcuma longa*

## Introduction

Junk foods are a rapid-processing commercial food that contains huge sugar and fat content with little dietary fiber, minerals, and vitamins (Scott 2018). They have little nutritional value but contain plenty of calories (Duram 2008). In recent years, the consumption of junk foods has increased. They

contain huge amount of oxidized oils (Abdallah et al. 2019; Boone-Heinonen et al. 2011).

Great concentration of thermally oxidized oil prompts injury of unique parts of body (Burenjargal and Totani 2009). Numerous complexes in the thermally oxidized oils stimulate oxidation of polyunsaturated fatty acids resulting in oxidative stress (Olivero David et al. 2010). Oxidized oil generates lethal effects in the liver and hematological parameters (Zeb and Ullah 2015). The antioxidants properties of some herbs have the potential to inhibit the oxidative breakdown of the fat constituent of food and are also vital for protection of living tissue against oxidative stress (Dos Santos et al. 2019).

The active constituents of some plants such as flavonoids, polyphenols, alkaloids, terpenoids, saponins, enzymes, and tannins are widely used for prevention and treatment of many diseases. Nowadays, there is a great worldwide interest to

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verdict safe antioxidants from natural origin to prevent oxidative damage of living cells of different species (Mahmoud et al. 2020; Badr et al. 2011; Neamat-Allah et al. 2019a). *Curcuma longa* (CL) is a plant food including of several phytochemical constituent associated to several medicinal uses. Curcumin, the most important constituent of turmeric, it has a powerful antioxidant (Hewlings and Kalman 2017) by its ability to scavenge or neutralize free radicals. It has hepatoprotective and nephroprotective properties against toxic agents-induced hepato-renal failure (Farkhondeh et al. 2018; Karamalakova et al. 2019). Also, it has protective activity against cytotoxic, teratogenic, and neurodegenerative diseases (Bagheri et al. 2019; Barandeh et al. 2019). Consequently, this experiment was aimed to evaluate results of CL supplementation of on the adverse impact of thermally oxidized oil in a rabbit model.

## Materials and methods

### Experimental design

Eighty male rabbits were randomly divided into 4 groups (20 of each) and treated for 90 days as follows: The first one was kept as standard control group. Groups 2 and 3 received (CL 13 g/kg, 5% thermally oxidized oil of ration, and the last group was supplemented with CL together with thermally oxidized oil). At the end of 30 and 90 days of the experiment, blood samples were collected from the ear veins of all rabbits then ten were sacrificed for histopathological examination. This study was issued by the Institutional Animal Precaution and Usage Commission (IAPUC) (No.:IAPUC/3/F/107/2018).

### *Curcuma longa*

Turmeric rhizomes were purchased from the local shop (Indian in origin) and air dried then macerated and extracted in water using hot process later which the supernatant portion was concentrated under condensed pressure and the concentrate powder was done by spray drying (Kawasaki et al. 2015). The obtained powdered CL was orally fed at a dose of 13 g/kg ration according to (Elwakf 2011).

### Preparation of thermally oxidized oil

Thermally oxidized oil was obtained by cultivated sunflower oil at 180 °C. Oil was heated for at least 20 min as cited (Lopez-Varela et al. 1995).

### Blood samples

At the end of 30 and 90 days, blood samples were collected from the ear vein (Hashem et al. 2018a), and each into two portions. The 1<sup>st</sup> portions were placed in rinsed-out tubes

containing dipotassium salt of EDTA which were used for hematological analysis (Hashem et al. 2018b; Neamat-Allah 2015; Neamat-Allah and Damaty 2016; Neamat-Allah et al. 2019a; Neamat-Allah et al. 2020b). The 2<sup>nd</sup> portions were placed without anticoagulant in a test tube for serum yield for biochemical evaluation (El-Murr et al. 2019; Hashem et al. 2019; Neamat-Allah et al. 2020a; Neamat-Allah et al. 2019b). Erythrocyte number, Hb concentration, mean corpuscular volume (MCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), TLC, and differential leucocytic number were analyzed using hematology auto-analyzer (Hospitex Hema Screen 18) (Neamat-Allah and Mahmoud 2019; Salem et al. 2011), where ALT, AST, ALP, bilirubin, urea, creatinine, uric acid, total cholesterol, triglycerides, LDL-c, and HDL-c assessments were assessed in full automated spectrophotometer (chemray 240® Rayto, Shenzhen, China) using Biodiagnostic Kits, Egypt and where MDA and CAT were estimated in (Photometer 5010® Riele, Germany) using Diamond-diagnostic Kits, Egypt.

### Histopathology examination

Small specimens from livers, kidneys and spleen have been examined for histopathology. In briefly, the processing of the selected tissues for histopathology examination by set in 10% neutral buffer formalin then dry using ethanol (70–100%), consequent by xylene, and lastly fixed in paraffin (Cordatos 2002). Pieces of six micron were ready to cut and then usually stained with (H & E stain).

### Statistical analysis

By one-way analysis of variance (ANOVA), the generated data were analyzed using IBM SPSS, version 22. Duncan's multiple variety test was run to compare variances within means. A significance was outlined as  $p < 0.01$  (IBM 2013).

## Results

### Hematological results

At the end of the 30 days, Table 1 illustrates that erythrogram and leucogram displayed non-significant deviations compared with the normal control group. Where the third group showed a significant heterophilia.

Where at the 90 days, there was non-significant deviations in erythrocytes in all treated groups, but the third group showed a significant diminution in the RBCs count, PCV, Hb concentration, and MCHC compared with the normal control where a significant increase in the MCV and MCH was recorded. The 3<sup>rd</sup> and 5<sup>th</sup> groups show a significant leucocytosis, monocytosis, and heterophilia compared with the control.

**Table 1** Effect of *C. longa* and thermally oxidized oil on hematological indices (mean values  $\pm$  SE) of treated rabbits

Parameters	Groups			
	Control	CL	Thermally oxidized oil	CL + thermallt oxidized oil
At 30 days				
RBCS ( $X10^6/\mu\text{L}$ )	5.84 <sup>a</sup> $\pm$ 0.19	6.04 <sup>a</sup> $\pm$ 0.32	5.62 <sup>a</sup> $\pm$ 0.15	5.75 <sup>a</sup> $\pm$ 0.14
PCV (%)	36.48 <sup>a</sup> $\pm$ 0.23	36.53 <sup>a</sup> $\pm$ 0.29	35.63 <sup>a</sup> $\pm$ 0.81	36.07 <sup>a</sup> $\pm$ 0.52
Hb (g/dl)	10.57 <sup>a</sup> $\pm$ 0.27	11.07 <sup>a</sup> $\pm$ 0.64	10.41 <sup>a</sup> $\pm$ 0.13	10.65 <sup>a</sup> $\pm$ 0.33
MCV (fl)	62.36 <sup>a</sup> $\pm$ 1.01	60.48 <sup>a</sup> $\pm$ 1.34	63.39 <sup>a</sup> $\pm$ 2.06	62.73 <sup>a</sup> $\pm$ 1.50
MCH (pg/cell)	18.07 <sup>a</sup> $\pm$ 0.61	18.33 <sup>a</sup> $\pm$ 0.34	18.52 <sup>a</sup> $\pm$ 0.24	18.52 <sup>a</sup> $\pm$ 0.29
MCHC (%)	28.97 <sup>a</sup> $\pm$ 0.49	30.30 <sup>a</sup> $\pm$ 1.82	29.21 <sup>a</sup> $\pm$ 0.95	29.53 <sup>a</sup> $\pm$ 1.07
TLC ( $X10^3/\mu\text{l}$ )	9.84 <sup>a</sup> $\pm$ 0.28	9.78 <sup>a</sup> $\pm$ 0.49	10.17 <sup>a</sup> $\pm$ 0.42	9.62 <sup>a</sup> $\pm$ 0.66
Lymphocytes ( $X10^3/\mu\text{l}$ )	5.39 <sup>a</sup> $\pm$ 0.43	5.55 <sup>a</sup> $\pm$ 0.77	5.05 <sup>a</sup> $\pm$ 1.08	5.24 <sup>a</sup> $\pm$ 0.43
Monocytes ( $X10^3/\mu\text{l}$ )	2.07 <sup>a</sup> $\pm$ 0.85	1.92 <sup>a</sup> $\pm$ 0.59	2.14 <sup>a</sup> $\pm$ 0.39	2.13 <sup>a</sup> $\pm$ 0.34
GRA ( $X10^3/\mu\text{l}$ )	2.38 <sup>b</sup> $\pm$ 0.24	2.31 <sup>b</sup> $\pm$ 0.30	2.98 <sup>a</sup> $\pm$ 0.39	2.25 <sup>b</sup> $\pm$ 0.17
At 90 days				
RBCS ( $X10^6/\mu\text{L}$ )	5.79 <sup>a</sup> $\pm$ 0.21	6.09 <sup>a</sup> $\pm$ 0.26	4.75 <sup>b</sup> $\pm$ 0.20	5.65 <sup>a</sup> $\pm$ 0.32
PCV (%)	36.32 <sup>a</sup> $\pm$ 0.83	36.87 <sup>a</sup> $\pm$ 1.00	34.20 <sup>b</sup> $\pm$ 0.23	35.70 <sup>a</sup> $\pm$ 0.61
Hb (g/dl)	10.33 <sup>a</sup> $\pm$ 0.19	10.48 <sup>a</sup> $\pm$ 0.16	9.12 <sup>b</sup> $\pm$ 0.36	10.10 <sup>a</sup> $\pm$ 0.32
MCV (fl)	62.73 <sup>b</sup> $\pm$ 1.82	60.54 <sup>b</sup> $\pm$ 2.00	72.00 <sup>a</sup> $\pm$ 0.21	63.19 <sup>b</sup> $\pm$ 1.63
MCH (pg/cell)	17.84 <sup>b</sup> $\pm$ 0.61	17.21 <sup>b</sup> $\pm$ 0.35	19.20 <sup>a</sup> $\pm$ 0.28	17.88 <sup>b</sup> $\pm$ 0.22
MCHC (%)	28.44 <sup>a</sup> $\pm$ 0.29	28.42 <sup>a</sup> $\pm$ 0.30	26.67 <sup>b</sup> $\pm$ 0.31	28.29 <sup>a</sup> $\pm$ 0.20
TLC ( $X10^3/\mu\text{l}$ )	9.64 <sup>c</sup> $\pm$ 0.41	9.78 <sup>c</sup> $\pm$ 0.54	12.76 <sup>a</sup> $\pm$ 0.69	11.02 <sup>b</sup> $\pm$ 0.67
Lymphocytes ( $X10^3/\mu\text{l}$ )	4.81 <sup>a</sup> $\pm$ 0.14	5.40 <sup>a</sup> $\pm$ 0.14	3.02 <sup>c</sup> $\pm$ 0.12	5.00 <sup>a</sup> $\pm$ 0.14
Monocytes ( $X10^3/\mu\text{l}$ )	2.49 <sup>c</sup> $\pm$ 0.33	2.18 <sup>c</sup> $\pm$ 0.14	4.94 <sup>a</sup> $\pm$ 0.07	2.90 <sup>b</sup> $\pm$ 0.03
GRA ( $X10^3/\mu\text{l}$ )	2.34 <sup>c</sup> $\pm$ 0.56	2.20 <sup>c</sup> $\pm$ 0.21	4.80 <sup>a</sup> $\pm$ 0.24	3.12 <sup>b</sup> $\pm$ 0.09

Means within the same raw carrying a different superscript letters are significantly different at  $p \leq 0.01$

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 leucocytic count, CL *Curcuma longa*, GRA granulocyte

## Antioxidant results

At the end of the 30 days, paralleling with the control, (Table 2), there was a significant decrease in the serum MDA level with a significant increase in the CAT in group 2. On the other hand, third group showed a significant increase in the serum MDA with a substantial decrease in the activity of CAT. Moreover, these parameters showed non-significant changes in the 4<sup>th</sup> group.

At the end of 90 days, the results of group 2 showed a significant diminution in MDA with a significant rise in CAT compared with control. While groups 3 and 4 showed a significant rise in in serum MDA level with a significant diminution in catalase activity in matched with the control group.

## Lipogram and some biochemical results

Lipid parameters (Table 3) showed significant decrease in serum levels of total cholesterol, triglycerides, and LDL-c with a significant increase in HDL-c in group 2. On the other hand, group 4 shows non-significant changes in serum lipid profile. Otherwise, group 3 showed a significant increase in

serum levels of total cholesterol, triglycerides, and LDL-c with a significant decrease in HDL-c. Where at the 90 days, data of 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 showed a significant increase in serum levels of total cholesterol, triglycerides, and LDL-c with a significant decrease in HDL-c. The third group after 30 days exhibited a substantial increase of ALT, AST, ALP, bilirubin, urea, creatinine, and uric acid (Table 3) compared to the control group, while groups 2 and 4 displayed non-significant deviations in these parameters along the experimental intervals and were more pronounced at 90 days.

## Histopathological pictures of liver, kidneys and spleen after supplementation with thermally oxidized oil (third group):

At 30 days, the hepatic cells showed hydropic degeneration and vacuolization of cytoplasm. Thickening in the wall of congested hepatic blood vessels infiltrated with numerous lymphocytes (Fig. 1). Renal sections revealed presence of

**Table 2** Effect of *C. longa* and thermally oxidized oil on biochemical parameters (mean values  $\pm$  SE) of treated rabbits

Parameters	Groups				
	At 30 days	Control	CL	Thermally oxidized oil	CL + thermally oxidized oil
ALT U/l		35.02 <sup>b</sup> $\pm$ 1.02	34.80 <sup>b</sup> $\pm$ 2.14	61.24 <sup>a</sup> $\pm$ 2.98	35.77 <sup>b</sup> $\pm$ 1.73
AST U/l		24.31 <sup>b</sup> $\pm$ 1.51	23.90 <sup>b</sup> $\pm$ 1.37	43.32 <sup>a</sup> $\pm$ 1.04	25.00 <sup>b</sup> $\pm$ 1.00
ALP U/l		23.60 <sup>b</sup> $\pm$ 1.76	23.90 <sup>b</sup> $\pm$ 1.37	69.09 <sup>a</sup> $\pm$ 2.10	24.15 <sup>b</sup> $\pm$ 1.35
Total bilirubin mg%		0.87 <sup>b</sup> $\pm$ 0.09	0.81 <sup>b</sup> $\pm$ 0.07	1.26 <sup>a</sup> $\pm$ 0.04	0.90 <sup>b</sup> $\pm$ 0.06
Direct bilirubin mg%		0.50 <sup>b</sup> $\pm$ 0.03	0.47 <sup>b</sup> $\pm$ 0.06	0.51 <sup>b</sup> $\pm$ 0.01	0.50 <sup>b</sup> $\pm$ 0.02
Indirect bilirubin mg%		0.37 <sup>b</sup> $\pm$ 0.02	0.34 <sup>b</sup> $\pm$ 0.03	0.75 <sup>a</sup> $\pm$ 0.01	0.40 <sup>b</sup> $\pm$ 0.02
MDA nmol/ml		11.61 <sup>c</sup> $\pm$ 0.14	8.11 <sup>d</sup> $\pm$ 1.45	15.08 <sup>a</sup> $\pm$ 0.24	12.00 <sup>b</sup> $\pm$ 0.61
Catalase kU/l		390.81 <sup>b</sup> $\pm$ 7.97	425.00 <sup>a</sup> $\pm$ 5.36	330.59 <sup>c</sup> $\pm$ 6.12	383.95 <sup>b</sup> $\pm$ 7.00
At 90 days					
ALT U/l		34.79 <sup>d</sup> $\pm$ 1.16	36.26 <sup>c</sup> $\pm$ 1.35	77.43 <sup>a</sup> $\pm$ 3.035	41.45 <sup>b</sup> $\pm$ 1.24
AST U/l		24.23 <sup>c</sup> $\pm$ 1.33	22.80 <sup>d</sup> $\pm$ 1.36	79.95 <sup>a</sup> $\pm$ 2.02	31.80 <sup>b</sup> $\pm$ 1.38
ALP U/l		23.51 <sup>c</sup> $\pm$ 1.20	22.10 <sup>c</sup> $\pm$ 1.3	95.98 <sup>a</sup> $\pm$ 6.43	37.00 <sup>b</sup> $\pm$ 3.41
Total bilirubin mg%		0.88 <sup>c</sup> $\pm$ 0.13	0.79 <sup>c</sup> $\pm$ 0.05	2.29 <sup>a</sup> $\pm$ 0.03	1.25 <sup>b</sup> $\pm$ 0.02
Direct bilirubin mg%		0.50 <sup>c</sup> $\pm$ 0.06	0.48 <sup>c</sup> $\pm$ 0.04	1.49 <sup>a</sup> $\pm$ 0.05	0.75 <sup>b</sup> $\pm$ 0.02
Indirect bilirubin mg%		0.38 <sup>c</sup> $\pm$ 0.02	0.31 <sup>c</sup> $\pm$ 0.05	0.80 <sup>a</sup> $\pm$ 0.03	0.50 <sup>b</sup> $\pm$ 0.02
MDA nmol/ml		11.66 <sup>c</sup> $\pm$ 0.64	6.00 <sup>d</sup> $\pm$ 0.56	27.87 <sup>a</sup> $\pm$ 1.04	15.51 <sup>b</sup> $\pm$ 0.81
Catalase kU/l		391.99 <sup>b</sup> $\pm$ 6.15	457.00 <sup>a</sup> $\pm$ 4.75	155.88 <sup>d</sup> $\pm$ 7.07	339.79 <sup>c</sup> $\pm$ 4.40

Means within the same raw carrying different superscript letters are significantly different at  $p \leq 0.01$

CL *Curcuma longa*, ALP alkaline phosphatase, MDA malondialdehyde

ALT and AST, transaminases

**Table 3** Effect of *C. longa* and thermally oxidized oil on lipid profile and some renal function parameters (mean values  $\pm$  SE) of treated rabbits

Parameters	Groups				
	At 30 days	Control	CL	Thermally oxidized oil	CL + thermally oxidized oil
Cholesterol mg/dl		90.04 <sup>b</sup> $\pm$ 1.23	84.00 <sup>c</sup> $\pm$ 1.00	113.08 <sup>a</sup> $\pm$ 1.14	89.27 <sup>b</sup> $\pm$ 1.07
Triglycerides mg/dl		121.38 <sup>b</sup> $\pm$ 2.09	110.44 <sup>c</sup> $\pm$ 1.50	176.38 <sup>a</sup> $\pm$ 1.15	128.46 <sup>b</sup> $\pm$ 1.09
HDL-c mg/dl		29.87 <sup>c</sup> $\pm$ 0.29	32.49 <sup>b</sup> $\pm$ 0.18	16.13 <sup>d</sup> $\pm$ 0.35	29.95 <sup>c</sup> $\pm$ 0.25
LDL-c mg/dl		5.95 <sup>b</sup> $\pm$ 0.51	29.42 <sup>c</sup> $\pm$ 0.15	58.22 <sup>a</sup> $\pm$ 0.16	33.63 <sup>b</sup> $\pm$ 0.14
Urea mg/dl		27.76 <sup>b</sup> $\pm$ 0.63	26.90 <sup>b</sup> $\pm$ 0.74	52.44 <sup>a</sup> $\pm$ 0.97	27.02 <sup>b</sup> $\pm$ 0.68
Creatinine mg/dl		1.05 <sup>b</sup> $\pm$ 0.13	0.99 <sup>b</sup> $\pm$ 0.08	1.90 <sup>a</sup> $\pm$ 0.04	1.03 <sup>b</sup> $\pm$ 0.05
Uric acid mg/dl		4.55 <sup>b</sup> $\pm$ 0.65	4.44 <sup>b</sup> $\pm$ 0.37	7.39 <sup>a</sup> $\pm$ 0.35	4.78 <sup>b</sup> $\pm$ 0.32
At 90 days					
Cholesterol mg/dl		89.37 <sup>d</sup> $\pm$ 1.20	78.87 <sup>c</sup> $\pm$ 1.37	133.72 <sup>a</sup> $\pm$ 1.19	96.20 <sup>c</sup> $\pm$ 1.34
Triglycerides mg/dl		117.98 <sup>d</sup> $\pm$ 1.34	96.39 <sup>c</sup> $\pm$ 1.19	238.31 <sup>a</sup> $\pm$ 1.45	156.74 <sup>c</sup> $\pm$ 1.26
HDL-c mg/dl		31.93 <sup>b</sup> $\pm$ 0.26	35.45 <sup>a</sup> $\pm$ 0.13	13.81 <sup>d</sup> $\pm$ 0.13	25.99 <sup>c</sup> $\pm$ 0.31
LDL-c mg/dl		33.84 <sup>c</sup> $\pm$ 0.40	24.14 <sup>d</sup> $\pm$ 0.37	75.14 <sup>a</sup> $\pm$ 0.41	38.86 <sup>b</sup> $\pm$ 0.27
Urea mg/dl		28.74 <sup>c</sup> $\pm$ 0.95	27.92 <sup>c</sup> $\pm$ 1.04	59.21 <sup>a</sup> $\pm$ 1.09	29.02 <sup>b</sup> $\pm$ 0.56
Creatinine mg/dl		1.16 <sup>c</sup> $\pm$ 0.20	1.05 <sup>c</sup> $\pm$ 0.17	2.42 <sup>a</sup> $\pm$ 0.13	1.25 <sup>c</sup> $\pm$ 0.42
Uric acid mg/dl		4.69 <sup>c</sup> $\pm$ 0.41	4.65 <sup>c</sup> $\pm$ 0.58	9.93 <sup>a</sup> $\pm$ 0.6	4.99 <sup>b</sup> $\pm$ 0.24

Means within the same raw carrying different superscript letters are significantly different at  $p \leq 0.01$

CL *Curcuma longa*, HDL high-density lipoprotein, LDL low-density lipoprotein

pretubular and preglomerular fibroblastic proliferation and congested glomerulus. Coagulative necrosis affects renal epithelia infiltrating with leucocytes (Fig. 2). The spleen showed mild depletion in lymphocytes of white pulp (Fig. 3).

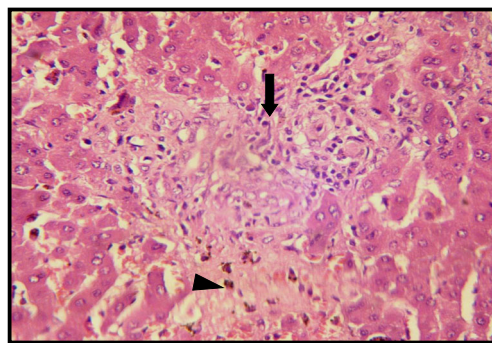
At 90 days, severe degenerative changes in hepatocytes including fatty changes in which hepatocytes showed signet ring appearance were also recorded (Fig. 4): Coagulative necrosis of epithelial cells lining renal tubules with thickening in the wall of congested blood vessels and vacuolization of its endothelial cells (Fig. 5). Spleen showed focal area of necrosis characterized by absence of lymphocytes of lymphoid follicle with massive hemosiderosis in the red pulp (Fig. 6).

### Histopathological pictures of liver, kidneys and spleen after supplementation with *C. longa* (fourth group):

At 30 days, normal tissue of the examined organs was displayed. Where at 90 days, mild thickening in the wall of bile ductule with proliferation of numerous lymphocytes was displayed (Fig. 7). Kidneys showed mild dilatation of some renal tubules without any characteristic lesion which could be detected (Fig. 8). Spleen showed mild depletion in white pulp, and spleen appeared normal with no characteristic lesion which could be detected (Fig. 9).

## Discussion

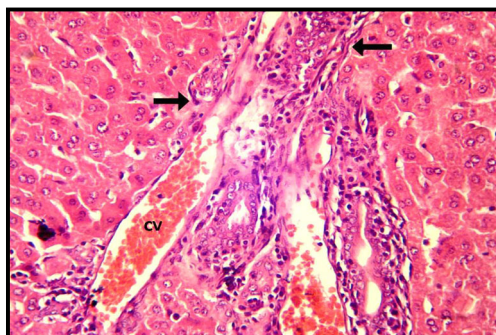
With regard to the findings of erythrocyte at the end of the 90 day, rabbits fed thermally oxidized oil had significant reduction in the RBCs number, Hb concentration, and PCV with expansion of macrocytic hypochromic anemia. The verified anemia can be due to a reduction in the life span of the RBCs as a direct influence of free radicals from consumption of thermally oxidized oil (Mesembe et al. 2005). The rise in MCH designates occurrence of hemolysis. Additionally, this model of anemia is due to liberate of free reticulocytes from bone marrow as a reaction to anemia (Hostetter and Andreasen 2004). Existence of hemosiderin in the liver with



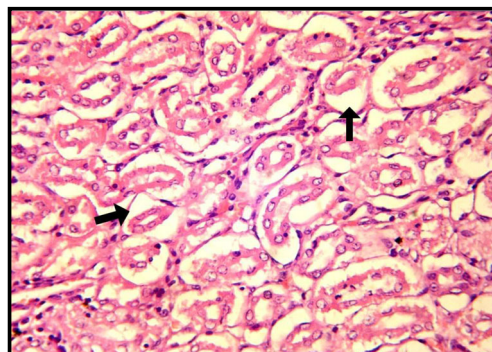
**Fig. 2** Group 3, liver section showing severe degenerative changes with leucocytic infiltration (arrow) and hemosiderosis (arrow head) at the end of the 90 days (H&E  $\times$  1200)

enormous hemosiderosis in the red pulp established these results. Supplementation with CL shows non-significant modifications in these parameters alongside the experimental intervals weighed with the control. This designates the efficiency of the antioxidant action of CL against the free radicals generated from administration of thermally oxidized oil (Hewlings and Kalman 2017). By evaluation of the leucocytes, group 2 showed non-significant variations in the numbers of WBCs, lymphocytes, heterophils, and monocytes during the experimental intervals. At the end of the 90 days, leucocytosis, heterophilia, and monocytosis were monitored in the 3rd group that could be due to tissue destruction. Also, a significant lymphopenia was documented. This can be owed to the oxidative stress occasioned from free radicals existent in thermally oxidized oil. Our results stood the heretofore attained by (Saleh et al. 2015). The occurrence of lymphoid depletion and necrosis of white pulp of spleen established our effect. The fourth group showed an improvement in the leucogram towards the normal level. This may be due to the powerful antioxidant activity of CL (Hewlings and Kalman 2017).

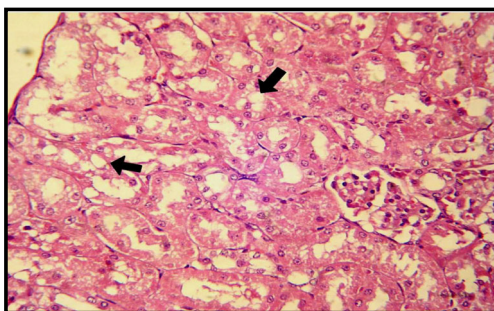
Between the different experimental intervals, the liver function of group 2 revealed non-significant deviations. These conclusions coincided with previous studies (Elwakf 2011; Haghghi Rabbani et al. 2013). Roasting oil has a



**Fig. 1** Group 3, liver section showing fibroblastic proliferation in portal area (thick arrows) with leucocytic infiltration (thin arrow) and congested blood vessels at the end of the 30 days (H&E  $\times$  1200)



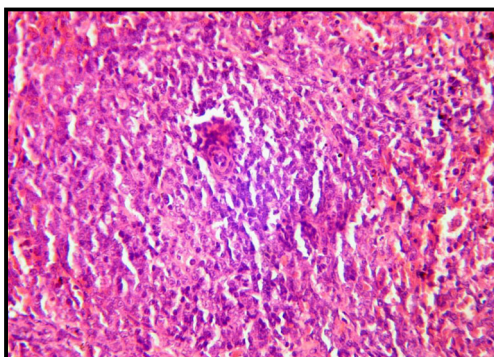
**Fig. 3** Group 3, kidney section showing pretubular edema with pressure atrophy of some renal tubules (arrows) at the end of the 30 days (H&E  $\times$  1200)



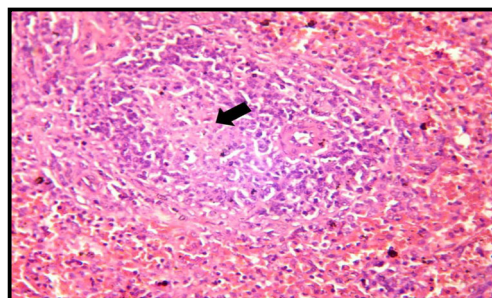
**Fig. 4** Group 3, kidney section showing severe degenerative changes in lining epithelium of renal tubules including hydropic degeneration (arrows) at the end of the 90 days (H&E × 1200)

harmful effect on liver tissues across manufacture of toxic consequences which instigating injury. These effects were mirrored in the third group by a significant elevation in the serum of ALT, AST, ALP, and levels of total, direct, and indirect bilirubin. The increase in aminotransferases could be due to the destructive action of free radicals on hepatocytes caused in structural and later functional transformations in layers and thus drip those (Chacko and T. 2011). The raising level of total bilirubin is due to amplification in the levels of direct and indirect bilirubin. This raising can be due to hepatocellular impairment. Likewise, the rise of indirect bilirubin can be owing to the hemolysis of erythrocytes, while the rise of the direct bilirubin level and ALP activity can be due to cholestasis (Tennant and Center 2008). Our consequences were established by the occurrence of hydropic deterioration and vacuolization of cytoplasm of hepatocytes. Thickening in the wall of congested hepatic blood vessels penetrated with several lymphocytes further existence of newly formed bile ductile: Hyperplasia and desquamation of epithelium lining bile duct. Harsh deteriorating alterations in hepatocytes were obtained (Saleh et al. 2015). Group 4 showed an improvement in ALT, AST, ALP, and bilirubin near the normal rates. These consequences are in accordance with that told by El-Nekeety et al. 2011.

Lipid parameters, group 2 exhibited a substantial diminution in serum levels of triglycerides, LDL-c, and cholesterol,



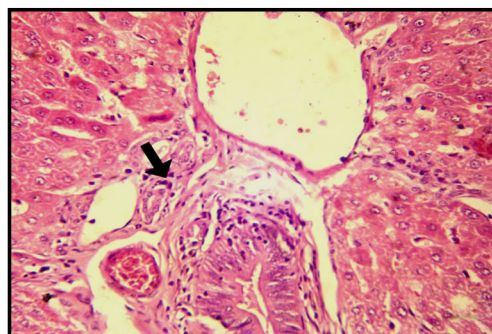
**Fig. 5** Group 3, spleen section showing mild depletion in lymphocytes of white pulp (arrow) at the end of the 30 days (H&E × 500)



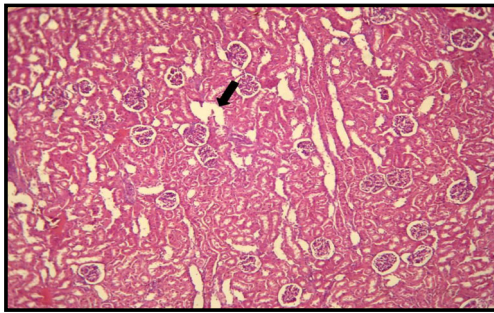
**Fig. 6** Group 3, spleen section showing focal area of necrosis characterized by absence of lymphocytes of lymphoid follicle (arrows) at the end of the 90 days (H&E × 500)

with a significant rise in HDL-c level paralleled to the control group. These findings sustained that heretofore gotten by (Akinyemi et al. 2016). In contrast, group 3 exposed a significant rise in serum levels of cholesterol, triglycerides, and LDL-c with a substantial diminution in HDL-c level paralleled to the control. This could be due to oil consumption which is allied with exaggerate in cholesterol and LDL rates (Leong et al. 2008). The intensification in the amount of triglycerides after oil consumption can be due to the elaborate in the accessibility of substrate free fatty acids for esterification (Perumalla Venkata and Subramanyam 2016). Also, hydroxy fatty acids and other lesser lipid oxidation upshots of oil instigate an expansion in blood cholesterol (Saleh et al. 2015). The 4th group displayed a significant diminution in serum levels of cholesterol, triglycerides, and LDL-c with a significant raise in HDL-c compared to group 3. As diminishing of cholesterol production by depressed setting of HMG-Co reductase which conscientious for cholesterol production, rising cholesterol elimination in bile and rising of LDLr enzyme expression on the surface of hepatocytes ease the deletion of LDL from blood (Akinyemi et al. 2016).

Renal function of group 2 displayed non-significant variations in the serum levels of creatinine, uric acid, and urea, paralleled to the control lengthways the experimental intervals. This designates that CL did not have deleterious influence on renal role. Heating oil initiates impairment of the renal



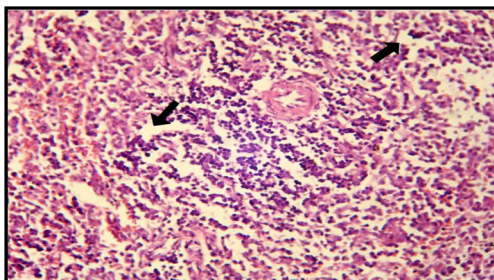
**Fig. 7** Group 4, liver section showing mild changes in portal area represented by mild edema with few leucocytic infiltration (arrow) at the end of the 90 days (H&E × 1200)



**Fig. 8** Group 4, kidney section showing coagulative necrosis of epithelial cells lining some renal tubules (arrow) with lobulization of glomerular tuft and preglomerular and pretubular leucocytic infiltration at the end of the 90 days (H&E × 1200)

tissue. The renal impairment was denoted by a substantial increase in the serum levels of creatinine, urea, and uric acid. These outcomes are in harmony with those reported by Burenjargal and Totani 2009. These alterations are the consequence of nephropathy which is demonstrated by pretubular and preglomerular fibroblastic production, alongside areas of coagulative necrosis at the end of the 30 days. Likewise, cloudy swelling of renal tubules and vacuolization in endothelial cells of blood vessels which penetrated with leucocytes and fibroblast cells with occurrence of hemosidrosis were reported at the end of the 90 days, but group 4 showed that levels of serum urea, creatinine, and uric acid were near the ranges of the controls. The nephroprotective means of CL could be endorsed to reserve of lipid peroxidation and improvement of antioxidant enzymes action (Mošovská et al. 2016). Histopathology of the kidney displayed mild dilatation of some renal tubules without any lesion at the end of the 90 days.

Viewing to the effects of supplementation with CL on antioxidants, group 2 prompted a significant intensification in the catalase activity, in addition to a significant diminution in MDA along the experimental intervals. This can be due to the antioxidant action of CL (Mošovská et al. 2016) through sifting the superoxide anions and nitric oxide radical (Song et al. 2011). This antioxidant action was verified by many researchers (Salama et al. 2013). On contrary, group 3 displayed a significant diminution in catalase activity with a significant



**Fig. 9** Group 4, spleen section showing mild depletion in white pulp (arrows). Spleen appeared normal with no characteristic lesion could be detected at the end of the 90 days (H&E × 1200)

intensify in MDA level along the experimental intervals. This can be endorsed to the development of triglyceride, oxidized triglycerides, diglycerides, and oxidized fatty acids in heating course that directs to lipid peroxidation (MDA) proliferation and consumption of antioxidant enzymes (Kaffashi Elahi 2012). Correspondingly, the vitamins which extant in sunflower oils are devastated by hydroperoxides engendered in repetition of frying (Srivastava et al. 2010). Our outcomes are in unity with that of Rouaki et al. 2013 who established a significant diminution in catalase of rats subsequent to intake of diet including thermally oxidized oil. The level of MDA and catalase was enriched significantly in group 4; this may be due to the capability of CL to recover the antioxidant mechanism (Mošovská et al. 2016). This enhancement of this mechanism arisen over the chelation of metal ions which motivate lipid peroxidation, sifting of free radicals, and reticence of cytochrome P450 and glutathione S-transferase actions (Song et al. 2011).

## Conclusion

The findings of the present study have demonstrated protective and antidotal effects of *C. longa* against thermally oxidized oil-induced toxicities. These ameliorative effects are attributed to the inherent phytochemical constituents of turmeric. Therefore, turmeric and its derivatives should be a regular constituent of our diets. Also, turmeric may be exploited as an adjuvant in the phytotherapeutic management of some toxicities.

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## Compliance with ethical standards

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

**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. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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