



## *Nigella sativa* oil protects against tartrazine toxicity in male rats

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### ABSTRACT

This study aimed to evaluate the protective role of *Nigella sativa* oil against the adverse effects of tartrazine on male rats. 18 albino rats were divided randomly into four groups (n = 6). The first (G1) is the negative control, the second group (G2) is the positive control received 10 mg/kg b.w. tartrazine in the diet and the third (G3) received the same dose of tartrazine as in G2 and co-treated with *Nigella sativa* oil for 8 weeks. Tartrazine decreased total protein, antioxidants and high density lipoproteins, whereas increased liver enzyme, kidney function parameters, total cholesterol, triglycerides, low density lipoproteins and lipid peroxidation in the positive control group. In addition, it caused pathological changes in the tissues of liver, kidney, testes and stomach. Treating tartrazine supplemented rats of G3 with *Nigella sativa* oil for 8 weeks significantly improved all biochemical parameters and restored the tissues of kidney, stomach, testes and liver to normal. It could be concluded that *N. sativa* oil succeeded in protecting male rats against the adverse conditions resulted from tartrazine administration.

### 1. Introduction

Tartrazine is a widely used additive in food products, drugs to improve appearance and taste [1]. The toxic effects of tartrazine come from the reductive biotransformation of the azo bond during its metabolism [2].

Black seed (*Nigella sativa*, *Ranunculaceae*) oil contains many substances such as thymoquinone, monoterpenes “*p*-cymene and apinene1 nigellidine2, nigellimine3 and a saponin” [3]. *Nigella sativa* oil has cytoprotective and antioxidant effect along with inhibitory effect on lipid peroxidation [4,5]. It is also decreases the total serum lipids and body weight [6] and decreases also fasting plasma glucose [7]. Recently, *N. sativa* was effective in protecting the liver against tetrachlorocarbon toxicity [5].

The present study aims to evaluate the antioxidant and protective potential of *Nigella sativa* oil against the adverse effects of tartrazine in male rats.

### 2. Materials and methods

#### 2.1. Materials

Tartrazine was purchased from Sigma, USA, whereas *Nigella sativa* oil was purchased from a spice shop in Jeddah, Saudi Arabia.

#### 2.2. Animals and housing conditions

Eighteen adult male albino rats (*Rattus norvegicus*) of East China Origin weighing 175–185 g were obtained from Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia. All experiments were carried out under protocols approved by the Institutional Animal House of the University of King Abdulaziz at Jeddah, Saudi Arabia.

#### 2.3. Test diet

The conventional animal basal diet was purchased from a grain mill

**Abbreviations:** ALP, serum alkaline phosphatase; ALT, serum alanine aminotransferase; AST, serum aspartate aminotransferase; B.W., body weight; BWG, body weight gain; FER, food efficiency ratio; G1, the first negative control untreated group fed basal diet containing 3.75 mg/kg b.w. sulfanilic acid; G2, the second positive control group fed diets containing 10 mg/kg b.w. tartrazine and 3.75 mg/kg b.w. sulfanilic acid; G3, the third group (G3) received 10 mg/kg b.w. tartrazine and 3.75 mg/kg b.w. sulfanilic acid and cotreated with 10 ml/kg body weight *Nigella sativa* oil for 8 weeks; LDL, low density lipoprotein; MDA, malondialdehyde; N.N cellulose, non-nutritive cellulose; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoproteins

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**Table 1**  
Effect of *Nigella sativa* oil on serum lipids in rats cosupplemented with tartrazine for 8 weeks.

Parameters	Statistics	G1 –ve Control	G2 Positive Control	G3 <i>Nigella sativa</i>
S.TC mg%	Mean ± SE	164.166 ± 2.25 <sup>c</sup>	261.50 ± 3.12 <sup>a</sup>	202.83 ± 4.66 <sup>b</sup>
	LSD 0.05 = 9.945			
	t-test	–	–61.42 <sup>***</sup>	12.11 <sup>***</sup>
S.T.G mg/dl	Mean ± SE	133.17 ± 2.71 <sup>c</sup>	224.67 ± 3.25 <sup>a</sup>	196.67 ± 2.55 <sup>b</sup>
	LSD 0.05 = 8.793			
	t-test	–	–51.28 <sup>***</sup>	4.89 <sup>***</sup>
S.HDLc mg/dl	Mean ± SE	46.50 ± 0.95 <sup>c</sup>	32.50 ± 0.76 <sup>a</sup>	38.00 ± 0.51 <sup>b</sup>
	LSD 0.05 = 2.246			
	t-test	–	8.79 <sup>***</sup>	–8.19 <sup>***</sup>
S.LDLc mg/dl	Mean ± SE	89.83 ± 1.95 <sup>c</sup>	183.83 ± 3.04 <sup>a</sup>	125.00 ± 4.46 <sup>b</sup>
	LSD 0.05 = 9.218			
	t-test	–	–19.05 <sup>***</sup>	12.29 <sup>***</sup>
V.LDLc mg/dl	Mean ± SE	26.63 ± 0.54 <sup>c</sup>	44.93 ± 0.65 <sup>a</sup>	39.33 ± 0.51 <sup>b</sup>
	LSD 0.05 = 1.750			
	t-test	–	–9.28 <sup>***</sup>	4.89 <sup>***</sup>

Data are represented as mean ± SE. t-test values; \*\*\*: significant at P < 0.001. ANOVA analysis: within each row, means with different superscript (a, b, c) are significantly different at P < 0.05, whereas means superscripts with the same letters mean that there is no significant difference at P > 0.05. LSD: least significant difference.

**Table 2**  
Effect of *Nigella sativa* oil on serum liver enzymes, total protein and total bilirubin in rats cosupplemented with tartrazine for 8 weeks

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 <i>Nigella sativa</i>
ALT U/L	Mean ± SE	27.83 ± 1.47 <sup>c</sup>	77.16 ± 1.68 <sup>a</sup>	50.33 ± 1.08 <sup>b</sup>
	LSD 0.05 = 4.054			
	t-test	–	–30.33 <sup>***</sup>	23.58 <sup>***</sup>
AST U/L	Mean ± SE	30.50 ± 1.11 <sup>c</sup>	79.16 ± 1.57 <sup>a</sup>	56.16 ± 1.13 <sup>b</sup>
	LSD 0.05 = 4.054			
	t-test	–	–30.71 <sup>***</sup>	9.77 <sup>***</sup>
ALP U/L	Mean ± SE	158.00 ± 3.89 <sup>c</sup>	275.83 ± 3.56 <sup>a</sup>	196.83 ± 4.20 <sup>b</sup>
	LSD 0.05 = 12.643			
	t-test	–	–24.62 <sup>***</sup>	10.66 <sup>***</sup>
Total protein g/dl	Mean ± SE	7.54 ± 0.05 <sup>a</sup>	5.39 ± 0.20 <sup>c</sup>	6.61 ± 0.06 <sup>b</sup>
	LSD 0.05 = 0.354			
	t-test	–	10.25 <sup>***</sup>	*** –5.05
Albumin g/dl	Mean ± SE	4.28 ± 0.09 <sup>a</sup>	2.65 ± 0.14 <sup>c</sup>	3.60 ± 0.09 <sup>b</sup>
	LSD 0.05 = 0.265			
	t-test	–	12.73 <sup>***</sup>	*** –7.55
Globulin g/l	Mean ± SE	3.25 ± 0.12 <sup>a</sup>	2.74 ± 0.19 <sup>a</sup>	3.18 ± 0.16 <sup>a</sup>
	LSD 0.05 = 0.582			
	t-test	–	2.33 <sup>°</sup>	–1.420 <sup>NS</sup>
A/G ratio g/L	Mean ± SE	1.32 ± 0.07 <sup>a</sup>	0.99 ± 0.11 <sup>b</sup>	0.96 ± 0.18 <sup>ab</sup>
	LSD 0.05 = 0.251			
	t-test	–	3.28 <sup>°</sup>	0.13 <sup>NS</sup>
Total Bilirubin mg/dl	Mean ± SE	0.48 ± 0.02 <sup>b</sup>	0.70 ± 0.04 <sup>a</sup>	0.51 ± 0.01 <sup>b</sup>
	LSD 0.05 = 0.084			
	t-test	–	–4.98 <sup>***</sup>	4.25 <sup>***</sup>

Data are represented as mean ± SE. t-test values; \*\*\*: significant at P < 0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P < 0.05, whereas means superscripts with the same letters mean that there is no significant difference at P > 0.05. LSD: least significant difference.

in Jeddah. Each 100 g consisted of the following: 12% protein (17.14 g 70% casein), 4 g corn oil, 0.3 g methionine, 0.2 g choline chloride, 4 g minerals, 1 g vitamin mixture, 4 g cellulose, and 69.36 g corn starch.

#### 2.4. Experiment design

The rats were housed 6/cages. Cages, bedding, and glass water bottles were replaced twice per week. Test diets, control diets, and tap water were available *ad libitum*. The animals were fed a standard basal diet and kept under observation before the start of the experiment for two weeks to exclude any undercurrent infection. The test animals were divided randomly into three groups, each consists of six rats as follows: the first group (G1) is untreated control group fed basal diet with 3.75 mg/kg b.w. sulfanilic acid, the second group (G2) fed diets containing 10 mg/kg b.w. tartrazine and 3.75 mg/kg b.w. sulfanilic acid and the third group (G3) received the same dose of tartrazine and sulfanilic acid as in the second group and cotreated with a daily dose of 10 ml/kg body weight *Nigella sativa* oil for 8 weeks.

#### 2.5. Physiological evaluation

The following biological parameters were estimated according to the method of Davies and Morris [8]:

- i Daily food Intake.
- ii Daily body weight gain (BWG)
- iii Percentage of Body weight gain (BWG%),
- iv Food efficiency ratio (FER).
- v Percentage of food efficiency ratio (FER%).

#### 2.6. Blood and sample collection

At the end of the experiment, animals were fasted for 14 h, and then blood samples were collected in plain tubes from the dorsal pedal vein for biochemical analyses. Blood serum was obtained by centrifugation at 1000 rpm for 10 min at room temperature, and then analyzed within 24 h.

**Table 3**Effect of *Nigella sativa* oil and honey on kidney functions and electrolytes in rats cosupplemented with tartrazine for 8 weeks.

Parameters	Statistics	G1 Negative control	G2 Positive control	G3 <i>Nigella sativa</i> oil
Uric acid mg/dl	Mean ± SE	4.18 ± 0.06 <sup>c</sup>	6.70 ± 0.09 <sup>a</sup>	5.91 ± 0.10 <sup>b</sup>
	LSD 0.05 = 0.2766			
	t-test	–	–27.66 <sup>***</sup>	4.45 <sup>***</sup>
Creatinine mg/dl	Mean ± SE	0.66 ± 0.03 <sup>c</sup>	2.91 ± 0.11 <sup>a</sup>	1.63 ± 0.08 <sup>b</sup>
	LSD 0.05 = 0.270			
	t-test	–	–19.13 <sup>***</sup>	7.00 <sup>***</sup>
Urea mg/dl	Mean ± SE	23.76 ± 0.88 <sup>c</sup>	65.66 ± 1.42 <sup>a</sup>	58.50 ± 1.43 <sup>b</sup>
	LSD 0.05 = 4.425			
	t-test	–	–19.84 <sup>***</sup>	5.61 <sup>***</sup>
Na <sup>+</sup> mmol/l	Mean ± SE	139.33 ± 1.52 <sup>a</sup>	140.83 ± 1.01 <sup>a</sup>	139.83 ± 1.57 <sup>a</sup>
	LSD 0.05 = 3.878			
	t-test	–	–1.01 <sup>NS</sup>	0.58 <sup>NS</sup>
K <sup>+</sup> mmol/l	Mean ± SE	4.73 ± 0.06 <sup>a</sup>	4.65 ± 0.07 <sup>a</sup>	4.70 ± 0.06 <sup>a</sup>
	LSD 0.05 = 0.211			
	t-test	–	0.75 <sup>NS</sup>	–0.54 <sup>NS</sup>

Data are represented as mean ± SE. t-test values; \*\*\*: significant at P < 0.001. ANOVA analysis: within each row, means with different superscript (a, b, c) are significantly different at P < 0.05, whereas means superscripts with the same letters mean that there is no significant difference at P > 0.05. LSD: least significant difference. NS: non significant.

**Table 4**Effect of *Nigella sativa* oil on antioxidants in serum, liver and kidney homogenate of male rats cosupplemented with tartrazine for 8 weeks.

	Parameters Serum	Statistics	G1 Negative control	G2 Positive control	G3 <i>Nigella sativa</i>
Serum	Catalase (CAT) U/ml	Mean ± SE	0.96 ± 0.03 <sup>a</sup>	0.19 ± 0.01 <sup>c</sup>	0.59 ± 0.01 <sup>b</sup>
		LSD 0.05 = 0.059			
		t-test	–	25.30 <sup>***</sup>	–19.64 <sup>***</sup>
		Mean ± SE	544.32 ± 11.59 <sup>a</sup>	131.67 ± 1.24 <sup>c</sup>	395.05 ± 5.02 <sup>b</sup>
Superoxide dismutase(SOD) U/ml	Glutathione reduced(GSH) U/ml	LSD 0.05 = 22.014			
		t-test	–	32.68 <sup>***</sup>	–52.78 <sup>***</sup>
		Mean ± SE	4762.20 ± 25.60 <sup>a</sup>	2369.00 ± 15.09 <sup>c</sup>	3541.80 ± 52.90 <sup>b</sup>
		LSD 0.05 = 126.857			
Glutathione reductase (GR) U/ml	Catalase (CAT) U/g	t-test	–	75.84 <sup>***</sup>	–25.62 <sup>***</sup>
		Mean ± SE	5.80 ± 0.13 <sup>a</sup>	1.40 ± 0.06 <sup>c</sup>	4.01 ± 0.11 <sup>b</sup>
		LSD 0.05 = 0.342			
		t-test	–	34.31 <sup>***</sup>	–36.27 <sup>***</sup>
Liver	Superoxide dismutase(SOD) U/g	Mean ± SE	5.53 ± 0.09 <sup>a</sup>	1.88 ± 0.07 <sup>c</sup>	3.08 ± 0.14 <sup>b</sup>
		LSD 0.05 = 0.356			
		t-test	–	41.24 <sup>***</sup>	–7.25 <sup>***</sup>
		Mean ± SE	920.50 ± 5.20 <sup>a</sup>	340.67 ± 5.33 <sup>c</sup>	741.00 ± 6.14 <sup>b</sup>
Glutathione reduced(GSH) U/g	Glutathione reductase (GR) U/g	LSD 0.05 = 14.695			
		t-test	–	172.53 <sup>***</sup>	–63.21 <sup>***</sup>
		Mean ± SE	7599.70 ± 7.59 <sup>a</sup>	3196.20 ± 4.96 <sup>c</sup>	5354.08 ± 5.27 <sup>b</sup>
		LSD 0.05 = 22.157			
Kidney	Catalase (CAT) U/g	t-test	–	395.36 <sup>***</sup>	–321.16 <sup>***</sup>
		Mean ± SE	9.15 ± 0.20 <sup>a</sup>	2.30 ± 0.11 <sup>c</sup>	6.53 ± 0.08 <sup>b</sup>
		LSD 0.05 = 0.428			
		t-test	–	24.55 <sup>***</sup>	–27.84 <sup>***</sup>
Superoxide dismutase(SOD) U/g	Glutathione reduced(GSH) U/g	Mean ± SE	4.60 ± 0.09 <sup>a</sup>	1.36 ± 0.08 <sup>c</sup>	2.53 ± 0.09 <sup>b</sup>
		LSD 0.05 = 0.317			
		t-test	–	4.71 <sup>***</sup>	–8.43 <sup>***</sup>
		Mean ± SE	762.83 ± 4.96 <sup>a</sup>	261.17 ± 1.92 <sup>c</sup>	529.67 ± 3.17 <sup>b</sup>
Glutathione reductase (GR) U/g	Catalase (CAT) U/g	LSD 0.05 = 10.867			
		t-test	–	96.23 <sup>***</sup>	–82.53 <sup>***</sup>
		Mean ± SE	7198.80 ± 7.40 <sup>a</sup>	2749.30 ± 14.51 <sup>c</sup>	4624.00 ± 8.43 <sup>b</sup>
		LSD 0.05 = 31.468			
Superoxide dismutase(SOD) U/g	Glutathione reductase (GR) U/g	t-test	–	353.33 <sup>***</sup>	116.20 <sup>***</sup>
		Mean ± SE	7.28 ± 0.11 <sup>a</sup>	1.95 ± 0.11 <sup>c</sup>	5.36 ± 0.10 <sup>b</sup>
		LSD 0.05 = 0.317			
		t-test	–	27.44 <sup>***</sup>	–39.16 <sup>***</sup>

Data are represented as mean ± SE. t-test values; \*\*\*: significant at P < 0.001. ANOVA analysis: within each row, means with different superscript (a, b, c) are significantly different at P < 0.05, whereas means superscripts with the same letters mean that there is no significant difference at P > 0.05. LSD: least significant difference.

The animals were dissected and the target organs were rapidly dissected out. A kidney, a testis, a piece of liver and stomach were washed in sterile saline and fixed in 10% buffered formalin for histopathological studies. The other kidney and a piece of liver were saved in ice-cold for determining antioxidant and lipid peroxidation values.

## 2.7. Tissue homogenate

A piece of kidney and liver were per fused with phosphate buffered saline solution (pH 7.4) containing 0.16 mg/ml heparin to remove any red blood cells and clots. One gram of tissue was homogenized in 10 ml cold buffer i.e., 100 mM potassium phosphate, pH 7.4 containing 2 mM EDTA, and then was cut into small pieces in cold condition. After that, the tissue was grinded by homogenizer instrument 6 times at 1400

**Table 5**  
Effect of *Nigella sativa* oil and honey on lipid peroxide (MDA) in rats cosupplemented with tartrazine for 8 weeks.

Parameters	Statistics	G1 Positive control	G2 Negative control	G3 <i>Nigella sativa</i>
MDA nmol/ml Serum	Mean ± SE LSD 0.05 = 0.394 <i>t</i> -test	1.50 ± 0.09 <sup>c</sup> –	3.50 ± 0.10 <sup>a</sup> –10.64 <sup>***</sup>	2.63 ± 0.12 <sup>b</sup> 6.50 <sup>***</sup>
MDA nmol/g. Liver tissue	Mean ± SE LSD 0.05 = 0.535 <i>t</i> -test	1.81 ± 0.12 <sup>c</sup> –	11.06 ± 0.23 <sup>a</sup> –33.44 <sup>***</sup>	6.83 ± 0.13 <sup>b</sup> 14.28 <sup>***</sup>
MDA nmol/g. Kidney tissue	Mean ± SE LSD 0.05 = 0.402 <i>t</i> -test	1.08 ± 0.08 <sup>c</sup> –	7.83 ± 0.16 <sup>a</sup> –28.25 <sup>***</sup>	4.31 ± 0.13 <sup>b</sup> 15.38 <sup>***</sup>
In kidney tissue nmol/g. Kidney tissue	<i>t</i> -test	–	–	–

Data are represented as mean ± SE. *t*-test values; <sup>\*\*\*</sup>: significant at P < 0.001. ANOVA analysis: within each row, means with different superscript (a, b, c) are significantly different at P < 0.05, whereas means superscripts with the same letters mean that there is no significant difference at P > 0.05. LSD: least significant difference.

**Table 6**  
Effect of *Nigella sativa* oil on body weight gain (BWG) and food efficiency ratio (FER) in rats co-supplemented with tartrazine for 8 weeks.

Physiological evaluation parameters	Statistics	G1 Negative control	G2 Positive control	G3 <i>Nigella sativa</i>
BWG g/8 week	Mean ± SE LSD 0.05 = 4.816 <i>t</i> -test	46.00 ± 1.064 <sup>a</sup> –	–39.50 ± 1.727 <sup>d</sup> 44.75 <sup>***</sup>	30.66 ± 1.429 <sup>b</sup> –23.79 <sup>***</sup>
BWG g/day	Mean ± SE LSD 0.05 = 0.078 <i>t</i> -test	0.763 ± 0.017 <sup>a</sup> –	0.655 ± 0.029 <sup>a</sup> 2.92 <sup>**</sup>	0.508 ± 0.024 <sup>b</sup> 6.95 <sup>***</sup>
BWG%	Mean ± SE LSD 0.05 = 2.400 <i>t</i> -test	25.29 ± 0.606 <sup>a</sup> –	13.57 ± 6.977 <sup>d</sup> 5.94 <sup>***</sup>	15.27 ± 0.733 <sup>b</sup> –4.20 <sup>***</sup>
FER g/day	Mean ± SE LSD 0.05 = 0.084 <i>t</i> -test	0.041 ± 0.000 <sup>a</sup> –	–0.011 ± 0.015 <sup>b</sup> 3.58 <sup>**</sup>	0.027 ± 0.001 <sup>a</sup> –2.66 <sup>*</sup>
FER%	Mean ± SE LSD 0.05 = 0.458 <i>t</i> -test	4.116 ± 0.094 <sup>a</sup> –	3.585 ± 0.152 <sup>d</sup> 45.12 <sup>***</sup>	2.766 ± 0.142 <sup>b</sup> –23.01 <sup>***</sup>

Data are represented as mean ± SE. *t*-test values; <sup>\*</sup>: significant at P < 0.05, <sup>\*\*</sup>: significant at P < 0.01, <sup>\*\*\*</sup>: significant at P < 0.001. ANOVA analysis: within each row, means with different superscript (a, b, c or d) are significantly different at P < 0.05, whereas means superscripts with the same letters mean that there is no significant difference at P > 0.05. LSD: least significant difference. NS: Non significant.

round for 30 s, and then, the resulted solution was separated under cooling by cold centrifuge at 4000 rpm for 15 min at 4 °C. The supernatant was removed and stored on ice for assay. For glutathione-S-Transferase (GST) assay, an additional step was needed. Homogenate solution was taken and separated on cold centrifuge at 10,000 × g for 60 min at 4 °C and then, the pellet was taken and dissolved in 1 ml of phosphate buffered saline (PBS). After that, it was stored in ice for assay.

## 2.8. Serum lipids

Serum total cholesterol (TC) and serum triglyceride (TG) were estimated according to the method of Schettler and Nussel [9] using Human kit (Germany) according to the instruction of the supplier. Serum high density lipoprotein (HDL) was estimated according to the method of Gordon et al. [10] using Human kit (Germany) according to the instruction of the supplier. Serum low density lipoprotein (LDL) was estimated according to the method of [11] using Human kit (Germany) according to the instruction of the supplier.

## 2.9. Liver enzymes

Activities of alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were estimated according to the method of Thefeld et al. [12] using Human kit (Germany) according to the instruction of the supplier. Alkaline phosphatase (ALP) was estimated according to the method of Schlebusch et al. [13] using Human kit (Germany) according to the instruction of the supplier.

## 2.10. Estimation of bilirubin

Total bilirubin was estimated according to the method of Balistreri and Shaw [14] using spectrum kit (Germany) according to the instruction of the supplier.

## 2.11. Estimation of total protein

Total protein was quantified according to the method of Weissman et al. [15] using Human Kit (Germany) according to the instruction of the supplier.

## 2.12. Estimation of albumins

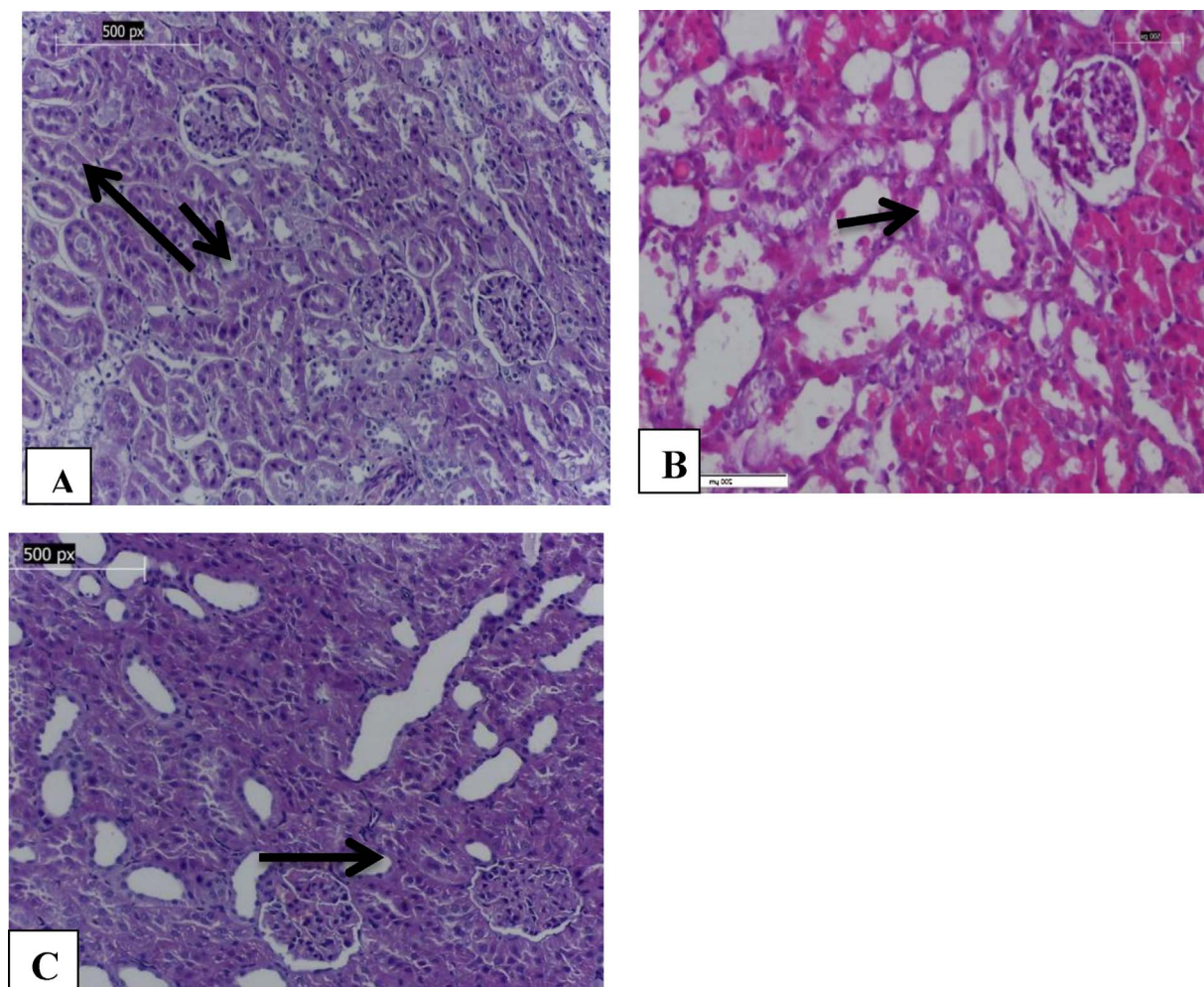
Albumins were estimated according to the method of Rebecca [16] using Sigma-Aldrich (USA) according to the instruction of the supplier.

## 2.13. Kidney function

Kidney function enzymes (serum urea, creatinine and uric acid) were estimated according to the method of Fawcett and Scott [17] using Human kit (Germany) according to the instruction of the supplier.

## 2.14. Serum electrolytes

Serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>) were estimated according to the method of Berry et al. [18] using Human kit (Germany) according to the instruction of the supplier.



**Fig. 1.** A; Renal tissues of control group (G1) showing normal renal structure with regulated nuclear arrangement of uriniferous tubules (small arrow) and collecting tubules (long arrow) with glomerulus (G) and blood vessel (BV), B; Renal tissues of tartrazine supplemented group (G2) showed marked tubular degeneration with atrophic lining and congested glomeruli. C; renal tissues of black seeds oil treated group (G3) showed regenerating the tubular lining with mild congested glomeruli. (X 200, H&E stains).

### 2.15. Lipid peroxide and antioxidant enzyme estimation

GSH, Catalase, GR and SOD were measured according to colorimetric method of Aebi [19] using Biodiagnostic kit according to the instruction of the supplier.

### 2.16. Histopathological investigations

Tissue samples of liver, stomach, kidney and testis were allowed to remain in the fixative (10% neutral buffered formalin) for 24 h. The fixed samples were washed in running water for overnight. Organs were then dehydrated in gradual ethanol (50–99%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H&S) dye for microscopic investigation [20]. The stained sections were examined and photographed under a light microscope.

### 2.17. Statistical analysis

Values were analyzed using SPSS program to calculate the *t*-test and the mean  $\pm$  SD and then analyzed using one way analysis of variance (ANOVA,  $p < 0.05$ ) using a protected least significant difference (LSD) test of SAS program.

## 3. Results

### 3.1. Lipid profile

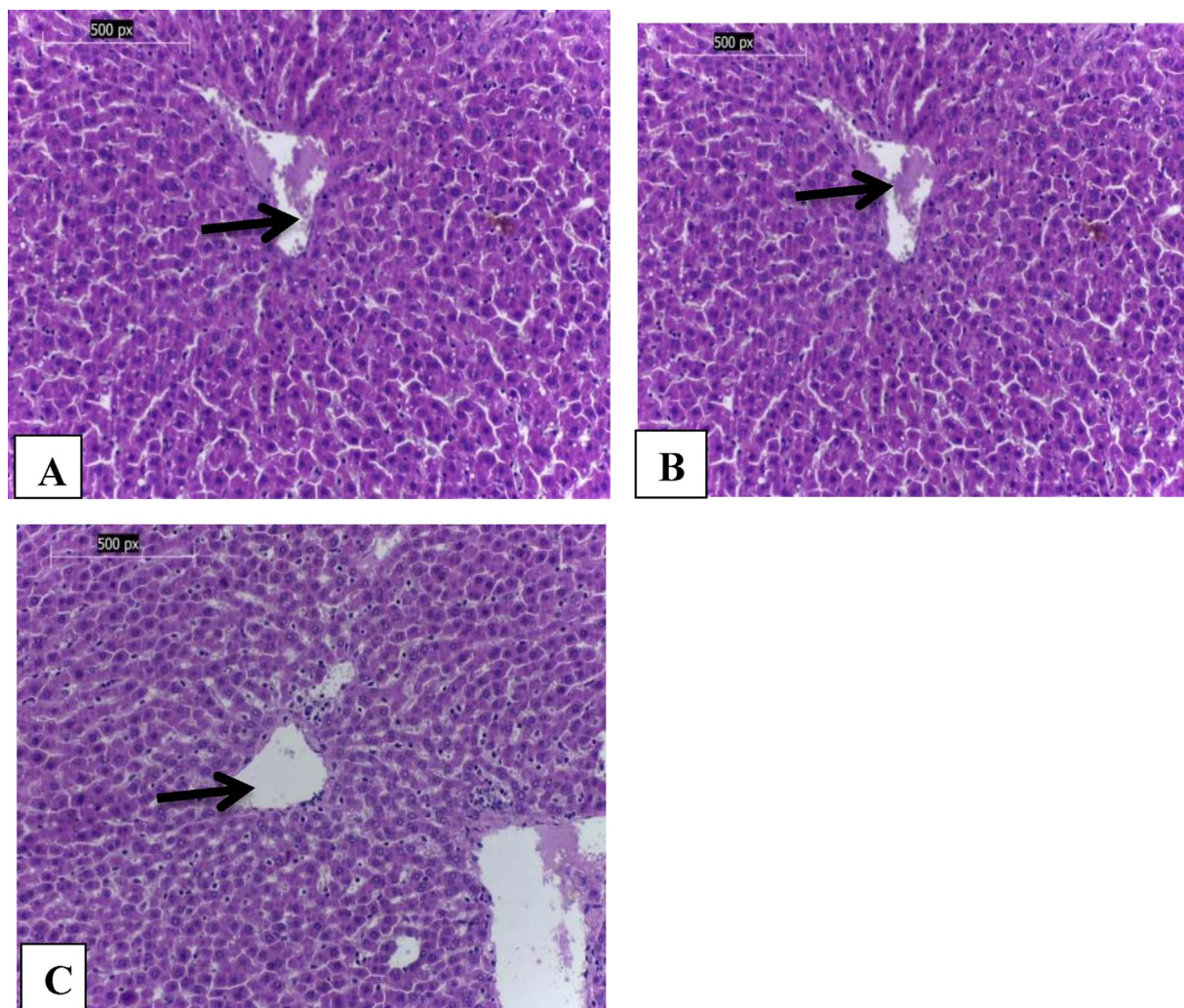
Table 1 shows the effect of *N. sativa* oil on serum lipids in male rats co-supplemented with tartrazine for 8 weeks. Administration of tartrazine to G2 rats (the positive control group) significantly increased total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) and decreased the high density lipoproteins (HDL) compared with the negative control group (G1). The co-treatment of the tartrazine administered rats with *N. sativa* in the 3rd group (G3) significantly decreased TC, TG, LDL and VLDL and increased HDL compared with the positive control group (G2).

### 3.2. Liver enzymes

Table 2 shows the effect of *N. sativa* oil on serum liver enzymes in rats co-supplemented with tartrazine for 8 weeks. Rats of the positive control group showed significant increase in the mean values of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) compared with the negative control group as a result of tartrazine administration. The co-treatment with *N. sativa* oil in G3 decreased the liver enzymes (ALT, AST, ALP) compared with the positive control group and restored them nearly to their normal levels.

Table 2 shows that the mean values of total protein (albumin, globulin and their ratio) in the positive control were significantly





**Fig. 2.** A; Normal hepatic tissues (G1) showing hepatic strands of cells around the central vein (CV) leaving blood sinusoids (arrow), B; Hepatic tissues co-supplemented with tartrazine (G2) showing mild fatty changes with mild inflammatory infiltrate (arrow).C; Hepatic tissue of rats treated with black seed oil (G3) showing improvement of fatty degeneration (arrow). (X 200, H&E stains).

decreased as a result of tartrazine administration compared with the negative control group. While, treating the tartrazine administrated rats with *N. sativa* oil in G3 significantly increased the total protein compared with the positive control group.

Tartrazine supplementation also increased the total bilirubin in the positive control group compared with the negative control group. Treating the tartrazine administrated rats with *N. sativa* oil and honey in G3 for 8 weeks significantly decreased the total bilirubin compared with the positive control group.

### 3.3. Kidney functions

Table 3 shows the effect of *N. sativa* on kidney functions and electrolytes in rats co-supplemented with tartrazine for 8 weeks. Rats of the positive control group showed significant increase in the mean values of creatinine, urea and uric acid compared with the negative control group as a result of tartrazine administration. The co-treatment with *N. sativa* oil in G3 significantly decreased the kidney parameters compared with the positive control group and restored them nearly to their normal levels as in G1.

The mean value of sodium ion in the positive control (G2) was significantly increased, whereas the mean value of potassium ions was significantly decreased compared with that of the negative control as a result of tartrazine administration. Treating the tartrazine administered

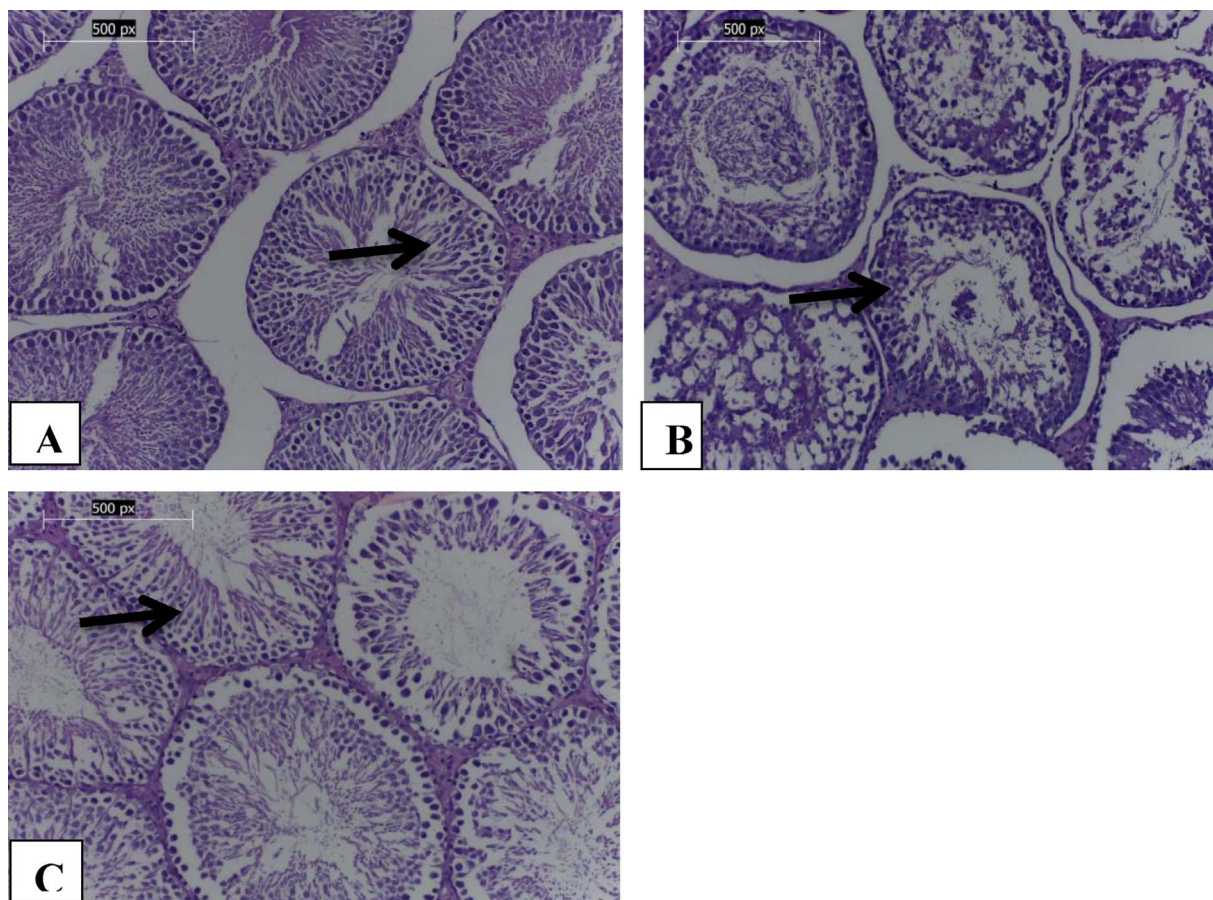
rats with *N. sativa* oil in G3 adjusted the electrolytes level and restored them nearly to the normal levels as in G1.

### 3.4. Antioxidant enzyme

Table 4 show that the mean values of catalase (CAT), superoxide dismutase (SOD), glutathione reduced (GSH) and glutathione reductase (GR) in serum, liver and kidney tissue homogenate of the positive control group were lower than that of the negative control, whereas the mean values of these enzymes in the serum, liver and kidney tissue homogenate in G3 was increased compared with the positive control as a result of treating the tartrazine administered rats with *N. sativa* oil.

### 3.5. Lipid peroxidation

Table 5 shows that tartrazine administration in the positive control group significantly increased lipid peroxidation as revealed by the increase of malondialdehyde (MDA) levels in the serum, liver and kidney tissue homogenate compared with the negative control group. Whereas the mean values of MDA in the serum, liver and kidney tissue homogenate of G3 were significantly decreased compared with the positive control as a result of treating the tartrazine administered rats with *N. sativa* oil.



**Fig. 3.** A; Normal testis tissues (G1) showing normal seminiferous tubules and spermatogenesis (arrow), B; Testis tissues co-supplemented with tartrazine (G2) showing arrest of spermatogenesis at different level (arrow), C; Testis tissue of rats treated with black seed oil (G3) showing regeneration of spermatogenesis in some seminiferous tubules restoring normal. (X 200, H&E stains).

### 3.6. Physiological evaluation

Table 6 shows that the mean value of body weight gain (BWG g/day, and 8 weeks) and BWG% was significantly lower than that of the negative control. Treating the tartrazine supplemented rats in G3 with *N. sativa* oil significantly reduced BWG compared with the negative and positive controls. The mean values of food efficiency (FER) and food efficiency ratio (FER%) in the positive control were significantly lower than that of the negative control. Treating the tartrazine supplemented rats in G3 with *N. sativa* oil significantly increased FER and reduced FER% compared with the negative and positive controls.

### 3.7. Histopathological investigations

#### 3.7.1. Kidney

The histological examination of kidney tissues of the negative control animals (Fig. 1A) shows normal renal tissues and normal uriniferous tubules and glomeruli in the untreated rat group. Kidney tissue of the tartrazine administered group showed marked atrophic tubular degeneration with lining and congested glomeruli (Fig. 2B). The kidney tissue of the third group represents the renal tissues of *N. sativa* oil treated group showing nearly restored normal tissues (Fig. 1C).

#### 3.7.2. Liver

The liver tissue of control group shows normal hepatic tissues with hepatic strands of cells around the central vein (Fig. 2A). The hepatic tissues of the tartrazine administered group showed mild fatty changes and broad infiltration of the lymphocytes comparable to those of the

control group. (Fig. 2B). On the other hand, the hepatic tissue of *N. sativa* oil treated group (G3) showed improvement of fatty degeneration with residual inflammation. (Fig. 2C).

#### 3.7.3. Testes

The testicular tissue of control group shows normal seminiferous tubules and normal spermatogenesis (Fig. 3A). The testicular tissue of the tartrazine administered group showed arrest of spermatogenesis at different level. (Fig. 3B). Also the testicular tissue of the *N. sativa* treated group (G3) showed improvement with regeneration of spermatogenesis restoring to normal (Fig. 3C).

#### 3.7.4. Stomach

The gastric tissue of the control group showed normal gastric lining. (Fig. 4A). The gastric tissue of the tartrazine treated group showed atrophy of the gastric glands with areas of ulceration. (Fig. 4B). The co-treatment of tartrazine administered group (G3) with *N. sativa* showed regeneration of gastric glands and elongation of velli restoring the normal histology (Fig. 4C).

### 3.8. Discussion

The present study shows the effect of food additive; tartrazine (azo dye) on kidney functions, liver functions, antioxidant, lipid peroxidation and serum electrolytes as revealed by biochemical test and histological changes in four organs (kidney, liver, testes and stomach).

In the present study, rat treated with tartrazine showed a significant increase in total cholesterol after 8 weeks compared to the control. These results agree with that recorded by Aboel-Zahab et al. [21]. The



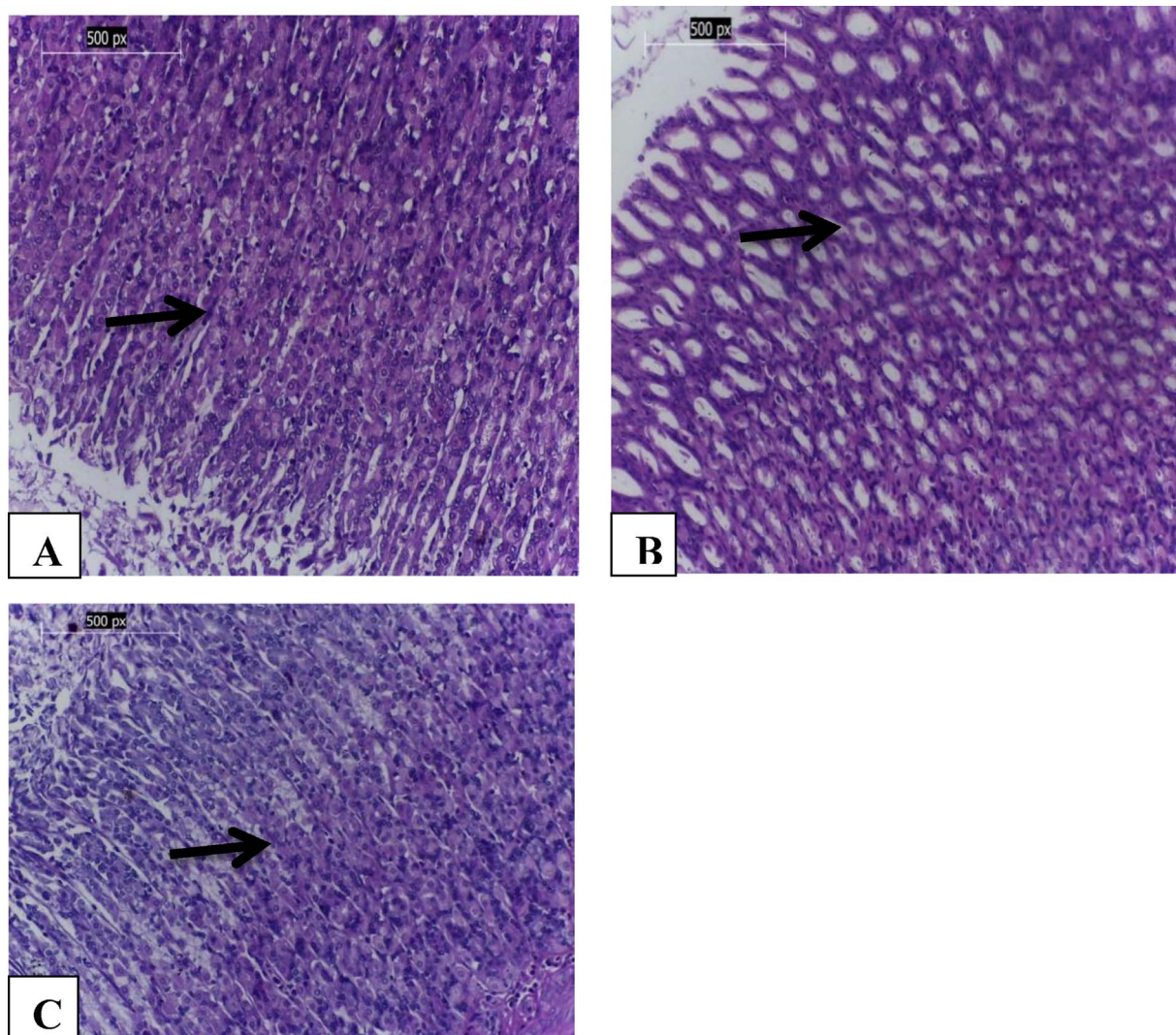


Fig. 4. A; Stomach of rat from negative control group (G1) showing normal gastric lining (arrow), B; Stomach of rat from positive control group (G2) showing atrophy of the gastric glands with areas of ulceration (arrow), C; Stomach tissue of rats treated with black seed oil (G3) showing regeneration of gastric glands restoring normal histology (arrow). (X 200, H&E stains).

results of the present study showed that the cotreatment of *N. sativa* for 8 weeks decreased lipid profile (TG, TC, LDL and VLDL) cholesterol and increased HDL cholesterol compared with the positive control. These findings are consistent with those reported by Al-Seeni et al. [5].

In spite of increasing the activity of ALT, AST and ALP as a result of tartrazine administration in the positive control [22]. The co-treatment with *Nigella sativa* oil for 8 weeks decreased the activity of these liver enzymes compared with that of the positive control. This result agrees with that of Essawy et al. [23] and Farooqui et al. [24].

In addition, serum concentration of total protein, globulin, A/G ratio and albumin decreased after the consumption of tartrazine in diet of rats for 8 weeks. [25]. The pronounced increase in serum contents of total protein and albumin were detected after the co-treatment with *Nigella Sativa* oil in diet of male rats [26].

In contrast, serum concentration of serum bilirubin increased after administration of tartrazine [27]. The decrease in serum contents of bilirubin was detected after oral administration of *N. Sativa* oil [28] as a result of the antioxidant activity of *N. sativa*.

Serum urea, uric acid and creatinine levels were significantly increased after treatment with tartrazine for 8 weeks [29] who reported elevation in serum creatinine and urea level of rats that ingested either low or high dose of tartrazine. The co-treatment with *N. sativa* for 8 weeks in G3 showed significant decrease in urea, creatinine and uric

acid compared to the positive control [30] and [31].

The increase of serum sodium levels in the tartrazine fed rats for 8 weeks compared to that of the negative control is consistent with Ibekwe et al. [32], whereas serum  $K^+$  was decreased that could be correlated to cell membrane damage leading to disturbances in  $Na^+$  and  $K^+$  pumping and disorders in membrane permeability. However, the current study showed a decrease in sodium and an increase in potassium ions when *N. Sativa* supplemented for 8 weeks to rats co-treated with tartrazine.

Administration of tartrazine decreased the activity of antioxidant enzymes under study (catalase, superoxide dismutase, and glutathione reductase) and the concentration of glutathione reduced compared with the negative control [33]. The co-treatment with *N. sativa* increased all antioxidant enzymes. Glutathione reduced which is non enzymatic antioxidant was also increased [34] due to the antioxidant activity of the constituents of *N. sativa*.

In contrast, the concentration of lipid peroxide increased as a result of tartrazine supplementation compared with the negative control, whereas decreased as a result of co-treatment with *N. sativa* which may attributed to the combined action of phyto-constituents present in *N. sativa* oil [5].

Moreover, tartrazine administration for 8 weeks in the current study decreased food intake compared with that of the negative control. This



result is in agreement with that of Mehedi et al. [35]. A pronounced increase in food intake was detected after oral treatment with *N. sativa* oil. In the present study, a decrease in the body weight after oral administration of tartrazine was noticed. This result agrees with Al-Shinnawy and Elkattan [36] who observed a decrease in body weight of rats treated with high dose of tartrazine. Our result showed an increase in body weight after treatment with *N. sativa* oil. This result is in agreement with that of Elshama et al. [37].

In the present study, the decrease in body weight gain and the percentage of body weight gain as a result of tartrazine treatment for 8 weeks is consistent with Helal et al. and Al-Shinnawy [22]. In our result, we noticed an increase in body weight gain after oral treatment of honey and *N. sativa* oil.

In the present study, food efficiency ratio was decreased in tartrazine treated group compared with the negative control. This result is in agreement with Mehedi et al. [35] who observed decrease in FER after administration of the rats with tartrazine. In contrast, FER was elevated after administration of honey and *Nigella sativa* oil.

In the present study, treatment with *N. sativa* significantly attenuated the detrimental effect of tartrazine on different organs of rat [35]. Liver section of the positive control group that received tartrazine showed degenerated hepatic tissues, mild change in fatty and inflammatory infiltrate of the lymphocytes and increased the release of the liver enzyme in the blood stream [38]. Moreover, the protective activity of *Nigella sativa* oil in this study is consistent with [5]. Also, Mehedi et al. [39] observed reduction in epidermal and testicular sperm counts including morphological abnormalities. In the present study, administration of honey or *Nigella sativa* oil caused regeneration of spermatogenesis in some seminiferous tubules. These results are consistent with those reported by Abu-Zinadah et al. [40]. Also, these results agree with Elshama et al. [37].

The stomach is the part of the digestive system that contains a gastric gland that produces various types of secretions. In the current study, stomach tissue of rats supplemented with tartrazine showed atrophy of the gastric glands and ulceration. This result is in agreement with Sarkar and Ghosh [41]. Moreover, administration of *N. sativa* oil to tartrazine supplemented rats caused regeneration of gastric glands and elongated villi. This result is in agreement with El-Dakhkhny et al. [42].

#### 4. Conclusion

This work showed that tartrazine caused kidney and liver dysfunction as revealed by the elevation in kidney and liver function parameters, and the histopathological signs of the examined sections. While, the concurrent treatment by *N. sativa* oil had protective effects on the kidney, liver, tests and stomach structure, and protected against tartrazine toxicity. This protective effect of *N. sativa* oil can be used to protect against the adverse health effects of tartrazine toxicity because it contains many pharmacological active constituents such as nigelline, nigellidine, dithymoquinone, thymohydroquinone, nigellone, steryl-glucoside, flavonoids, essential fatty acids, essential amino acids, ascorbic acid, iron and calcium.

#### Conflicts of interest

The authors of this paper have no conflict of interests.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.toxrep.2017.12.022>.

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