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Research Article

Protective Effect of Black Seed and Lettuce Oils Against Paracetamol-Induced Hepatotoxicity in Rats

^{1,2}Elsayed Hamed Ali Bakr and ¹Areej Abdulhamid Hamdan Almuraee

¹Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, P.O. Box 7067, Makkah 24382, Saudi Arabia

²Department of Nutrition and Food Sciences, Faculty of Home Economics, Menoufia University, Shibin el Kom, Menofia Governorate 6131567, Egypt

Abstract

Background and Objective: The liver is one of the organs that play an essential role in the human body, including supporting metabolism, immune functions, digestive system, detoxification, storage of vitamins and other functions. This investigation aimed to study the protective effects of black seed and lettuce oil against hepatotoxicity as induced by paracetamol in experimental rats.

Materials and Methods: Twenty male Sprague-Dawley albino rats weighing 150 ± 5 g were divided randomly into four groups (5 rats each) and distributed as follows; 1st group was controlled negative (C -ve group), 2nd group controlled positive (orally administered with 500 mg/kg b.wt., paracetamol), 3rd and 4th groups were orally administered with black seed oil and lettuce oil at a dose of 1 mL/kg b.wt., each) as a preventive dose. All rats were sacrificed and blood was collected for biochemical analysis and then statistically analyzed.

Results: The rat administered with black seed and lettuce oils enhanced body weight gain, food intake and feed efficiency ratio. Moreover, exhibited a significant reduction in the liver enzymes AST, ALT, ALP and TBIL. Meanwhile, black seed and lettuce oils significantly improved kidney functions, lipid profiles and some immune biomarkers including creatine kinase (CK), Creatine Kinase-MB (CK-MB) and Lactate Dehydrogenase (LDH). **Conclusion:** This study revealed that the oils of black seed (*Nigella sativa*) and lettuce (*Lactuca sativa*) have a protective role in improving body weight gain, food intake, feed efficiency ratio, liver enzymes, kidney functions, lipid profiles and some immune biomarkers against paracetamol-induced hepatotoxicity in experimental rats.

Key words: Liver toxicity, *Nigella sativa*, *Lactuca sativa*, protective, paracetamol, rats

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Corresponding Author: Elsayed Hamed Ali Bakr, Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, P.O. Box 7067, Makkah 24382, Saudi Arabia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver is an essential organ in the human body that serves a vital function. It supports metabolism, immune function, the digestive system, detoxification and vitamin storage, among other things¹. Drug-induced liver injury (DILI) is the most common cause of liver impairment. The DILI can cause a wide range of symptoms, from mild nonspecific symptoms like cholestasis and asymptomatic hepatitis to liver failure. Numerous prescription drugs, herbal remedies and nutritional supplements could be to blame². Liver disease is a major cause of acute hepatitis and is responsible for approximately 2 million deaths worldwide. Drug-induced liver injury is also on the rise. Despite their alarming nature, these figures point to a significant opportunity to improve overall health because the majority of liver disease causes can be avoided³.

Analgesic and antipyretic properties can be found in paracetamol, which is an active phenacetin metabolite. As an over-the-counter treatment for fever and other pain conditions, it is well tolerated. One of the organs most affected by paracetamol is the liver. From 1998 to 2003, 131 out of the total 274 reported accidental poisoning cases in the United States were associated with paracetamol⁴. According to a population control study conducted in the United States, there are approximately 1,600 annual cases of acute liver failure (ALF), with paracetamol being the most common cause⁵.

Traditional herbs and micronutrients, such as lettuce oils and *Nigella*, have been shown in recent studies to protect the liver from hepatotoxicity. The black seed is a perennial herbaceous plant that grows in Pakistan, India, Iran and the countries that are near the Mediterranean Sea⁶. It is a member of the buttercup family. The majority of *Nigella sativa* oil is fixed black seed oil (NSO), with about 0.4 to 0.5% volatile. Ethyl ether is used to extract the oil, which is obtained by steam distillation of ground black seed pods. However, petroleum ether extraction of the seeds yields approximately 35% oil, which upon steam distillation yields 1.5% of the volatile oil⁷. Terpenic hydrocarbons (monoterpenoid hydrocarbons, monoterpenoid ketones and sesquiterpenoid hydrocarbons) make up the majority of the essential oil of *Nigella sativa*. Essential oils contain minor amounts of oxygenated sesquiterpenes, diterpenes, alkanes, alkenes and fatty acids⁸. *Nigella sativa* oil includes protecting the stomach and liver, treating diabetes, fighting bacteria and tumors and regulating the immune system^{7,9}. *Nigella sativa* seed's active ingredient, thymoquinone, is a pharmacologically active quinone that has analgesic and anti-inflammatory properties¹⁰. Thymoquinone

may also prevent tissue lipid membrane peroxidation by acting as an antioxidant^{11,12}. The way it works is still mostly a mystery. Nevertheless, it may be related to the inhibition of membrane lipid peroxidation and the suppression by cyclooxygenase, moreover, 5-lipoxygenase¹³.

An important leafy vegetable that can be eaten fresh or in salads is lettuce (*Lactuca sativa*)¹⁴. It has been discovered that various lettuce varieties contain antioxidant-active phenolic compounds¹⁵. Lettuce has been shown to protect humans and rats from cardiovascular disease in studies^{16,17}. The phenolic content of the lettuce methanol extract is high, indicating significant radical scavenging activity. It was effective against some bacteria, both Gram-positive and Gram-negative; in addition, its methanol and aqueous extracts were effective against Cox-B3 viruses¹⁸. Quercetin and luteolin rhamnosyl-hexosides have been linked to the health benefits of lettuce¹⁵.

Accordingly, this investigation was conducted to determine whether lettuce (*Lactuca sativa*) and black seed (*Nigella sativa*) oils protect or not experimental rats from paracetamol-induced hepatotoxicity.

MATERIALS AND METHODS

Study area: The study was carried out from February 2023 to April 2023 in the Medical Biochemistry Department, Faculty of Medicine, Umm Al-Qura University, Makkah, Kingdom of Saudi Arabia.

Ingredients: Paracetamol as a drug was obtained from Trust Company, Jeddah, Saudi Arabia, moreover, 5 mL of black seed and lettuce oils each, were obtained from the herb market, Makkah, Kingdom of Saudi Arabia.

Rats: Twenty albino male rats 150 ± 5 g each were brought from the Department of Medical Biochemistry, Medicine College, Umm Al-Qura University, Holy Makkah, Kingdom of Saudi Arabia.

Basal diet: The basal diet components were 12% of casein protein as a source of nitrogen in the diet, 10% from corn oil as a source of fats in the diet, 4% mixture of various minerals, 1% mixture of various vitamins, 5% of cellulose as a source of fibers, in addition to 0.2% chlorine chloride and 0.3% methionine, finally to remained to complete 100% was corn starch (67.5%)¹⁹.

Liver toxicity induction: The induction of paracetamol liver toxicity for rats occurred by oral injection with paracetamol at a dose of 500 mg/kg b.wt.²⁰.

Experimental design: Under controlled conditions, twenty male Sprague-Dawley rats weighing 150, 5 g were housed in special cages. Before the experiment began, all rats were fed a basal diet for a week (seven consecutive days) to adapt. To prevent contamination and food loss, the rats' diets were served in specialized, non-scattering feeding cups. Rats received tap water from inverted bottles supported on one side of their cages through glass tubes that protruded through wire cages. There were four groups of five rats in each group. Over the course of the eight-day experimental period (C -ve group), Group I was fed a basal diet. Group II received 500 mg/kg b.wt., of paracetamol orally after being fed a basal diet for seven days. Group III received an oral injection of black seed oil at a dose of 1 mL/kg b.wt., on the eighth day of the experimental period, which served as the final day of feeding on a basal diet for 7 days, then taken orally with paracetamol at a dose of 500 mg/kg b.wt. Group IV received an oral injection of lettuce oil at a dose of 1 mL/kg b.wt., on the 8th day of the experimental period, which served as the final day of the experiment. The animals were fed a basal diet for 7 days, then taken orally with paracetamol at a dose of 500 mg/kg b.wt. The experiment ended on the 8th day of the experimental period.

Biological evaluation: Throughout the feeding period, body weight was measured weekly:

$$\text{BWG (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{FER} = \frac{\text{Gain in body weight (g)}}{\text{Feed intake (g)}}$$

Biochemical evaluation: At the end of the experimental period (28 days), blood samples were collected for analyzing the following parameters: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) according to Huang *et al.*²¹; Alkaline Phosphatase (ALP) according to Akcakaya *et al.*²²; total bilirubin (TBIL) was determined due to the method of Doumas and Wu²³. The blood urea nitrogen (BUN), uric acid (U. Acid) and creatinine (Creat) were measured in accordance with to Gochman and Young²⁴; total cholesterol (TC) was analyzed in accordance to Allen *et al.*²⁵; also, serum triglycerides (TG) was measured in accordance to Fossati and Prencipe²⁶. Lipoprotein fractions High Density Lipoprotein (HDL), Low-Density Lipoproteins (LDL) according to Borén, *et al.*²⁷; Very Low Density Lipoprotein (VLDL) was calculated by dividing triglycerides by 5 and atherogenic index (AI) evaluated by using the equation of Kikuchi-Hayakawa *et al.*²⁸ by dividing

HDL by the sum of LDL (LDH), creatine kinase (CK) and Creatine Kinase MB (CK-MB) were identified²⁹.

Statistical analysis: The computerized SPSS Statistic Program Sigma stat version-22 was used to conduct statistical analysis on all of the data. Using Duncan's multiple-range test and a One-way Analysis of Variance (ANOVA), the effects of various treatments were examined. A significance level of $p \leq 0.05$ was used to distinguish between the various groups.

Ethical consideration: Ethical approval was obtained from the Committee for the Care and Use of Laboratory Animals at the Deanship of Scientific Research, Umm Al-Qura University, Holy Makkah, Saudi Arabia.

RESULTS

This investigation aimed to estimate the protective effects of black seed (*Nigella sativa*) oil and lettuce (*Lactuca sativa*) oil against paracetamol-induced hepatotoxicity in experimental rats.

Hepato-protective effect of black seed and lettuce oils on BWG, FI and FER in paracetamol-intoxicated rats:

In paracetamol-intoxicated rats, the protective effects of lettuce and black seed oils on body weight gain for rats weights (BWG), food intake eaten by each rat group (FI) and feed efficiency ratio calculated for all rats (FER) were presented in Table 1.

Total body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) were 0.70 ± 0.04 , 18.80 ± 0.29 and 0.037 ± 0.005 g/7 days, respectively, in paracetamol-intoxicated rats (C +ve group), compared to 3.90 ± 0.01 , 26.40 ± 0.19 g/7 days and 0.148 ± 0.002 in (C -ve) normal rats ($p < 0.05$). The body weight gain, food intake and feed efficiency ratio of paracetamol-intoxicated rats are significantly lower than those of normal rats, as shown by these findings. Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention. The BWG, FI and FER were significantly lower in BWG-positive rats than in control-positive rats. When compared to the lettuce oil group and the positive control group, the rats receiving orally administered black seed oil showed the most significant increases in BWG and FER. In contrast to the black seed oil group, the oral preventable administered with lettuce oil exhibited the greatest significant increase in FI.

Table 1: Protective effect of black seed and lettuce oils on BWG, FI and FER in paracetamol-intoxicated rats

Parameter	BWG (g/8 days)	FI (g/8 days)	FER
Animal group	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*
Control (-)	3.90 $\pm$ 0.01 ^a	26.40 $\pm$ 0.19 ^a	0.148 $\pm$ 0.002 ^a
Control (+)	0.70 $\pm$ 0.04 ^d	18.80 $\pm$ 0.29 ^d	0.037 $\pm$ 0.005 ^d
Black seed oil	2.30 $\pm$ 0.03 ^b	23.60 $\pm$ 0.15 ^c	0.097 $\pm$ 0.001 ^b
Lettuce oil	1.10 $\pm$ 0.02 ^c	25.00 $\pm$ 0.23 ^b	0.044 $\pm$ 0.002 ^c
LSD	0.10	01.20	0.009

*SE is standard error and letters of (a, b, c, d) in similar columns differ significantly at $p \leq 0.05$

Table 2: Protective effect of black seed and lettuce oils on AST, ALT, ALP and TBIL in paracetamol-intoxicated rats

Parameter	AST (U/L)	ALT (U/L)	ALP (U/L)	TBIL (U/L)
Animal group	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*
Control (-)	107.50 $\pm$ 3.03 ^d	26.96 $\pm$ 0.98 ^d	71.00 $\pm$ 1.88 ^d	1.36 $\pm$ 0.01 ^{cd}
Control (+)	311.90 $\pm$ 7.11 ^a	318.00 $\pm$ 5.54 ^a	229.00 $\pm$ 5.37 ^a	2.40 $\pm$ 0.03 ^a
Black seeds oil	185.20 $\pm$ 2.62 ^c	83.00 $\pm$ 1.65 ^c	139.00 $\pm$ 3.91 ^c	1.40 $\pm$ 0.02 ^c
Lettuce oil	231.40 $\pm$ 3.72 ^b	107.60 $\pm$ 3.91 ^b	190.00 $\pm$ 2.65 ^b	2.50 $\pm$ 0.03 ^b
LSD	5.55	10.00	15.00	0.05

*SE is standard error and letters of (a, b, c, d) in similar columns differ significantly at $p \leq 0.05$

Hepato-protective effect of black seed and lettuce oils on AST, ALT and ALP in paracetamol-intoxicated rats:

Table 2 shows that paracetamol-intoxicated rats' Aspartate Aminotransferase (AST) in serum, serum Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) in serum and serum total bilirubin (TBIL) levels were protected by lettuce oil and black seed oil, respectively.

The AST, ALT, ALP and TBIL were found to be 107.50 ± 3.03 , 26.96 ± 0.98 , 71.00 ± 1.88 and 1.36 ± 0.01 U/L in paracetamol-intoxicated rats (C +ve group) versus 311.90 ± 7.11 , 318.00 ± 5.54 , 229.00 ± 5.37 and 2.40 ± 0.03 U/L in (C -ve) normal rats ($p < 0.05$). Paracetamol-intoxicated rats had significantly higher levels of the hepatic enzymes AST, ALT, ALP and TBIL than normal rats. Black seed oil and lettuce oil was administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention and exhibited a significant reduction in the liver enzymes AST, ALT, ALP and TBIL when compared to rats that were positive for control. The levels of AST, ALT, ALP and TBIL in the lettuce oil-treated rats were significantly higher than in the black seed oil group. When compared to the control-positive rats and the black seed oil group, the oral preventative that was given with lettuce oil showed the greatest significant increase in TBIL.

Hepato-protective effect of black seed and lettuce oils on Creat., BUN and U. Acid in paracetamol-intoxicated rats:

The effects of black seed oil and lettuce oil on the levels of creatinine, blood urea nitrogen (BUN) and Uric Acid (U. Acid) in paracetamol-intoxicated rats were shown in Table 3.

The creatinine (creat.), which was observed in the C +ve group of paracetamol-intoxicated rats, blood levels of urea nitrogen (BUN) and Uric Acid (U. acid) were 32.20 ± 0.16 , 5.90 ± 0.03 and 146.00 ± 3.81 μ mol/L, respectively, in contrast to 22.00 ± 0.05 , 4.38 ± 0.01 and 115.0 ± 1.30 μ mol/L, in (C -ve)

normal rats. Compared to normal rats, paracetamol-intoxicated rats had significantly higher levels of creatinine, blood urea nitrogen and uric acid. Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention. When compared to rats that were positive for the control, the levels of creatinine, blood urea nitrogen and uric acid all decreased significantly. When compared to the lettuce oil group and the positive control group, the rats receiving oral preventative treatment with black seed oil experienced a non-significant drop in creatinine and uric acid levels. In contrast to the black seed oil group, the oil administered orally showed the most significant decrease in BUN levels.

Hepato-protective effect of black seed and lettuce oils on TC and TG in paracetamol-intoxicated rats:

The effects of black seed oil and lettuce oil on total cholesterol and triglyceride paracetamol-intoxicated rats were shown in Table 4.

The total cholesterol (TC) and triglyceride (TG) levels in the paracetamol-intoxicated rats (the C +ve group) were 1.76 ± 0.03 and 3.80 ± 0.05 mmol/L respectively, compared to 1.35 ± 0.02 and 1.35 ± 0.02 mmol/L in (C -ve group) normal rats $p \leq 0.05$. Paracetamol-intoxicated rats have significantly higher levels of TC and TG than normal rats, as shown by these findings. Black seed oil and lettuce oil were given orally to all of the rats at a rate of 1 mL/kg b.wt., showed significantly lower levels of TC and TG than control-positive rats. When compared to the black seed oil group and control-positive rats, rats receiving orally preventable treatment with lettuce oil demonstrated the greatest significant decrease in TG. On the other hand, rats receiving orally preventable treatment with black seed oil demonstrated the least significant change in TC and TG levels.

Table 3: Protective effect of black seed and lettuce oils on Creat., BUN and U. Acid in paracetamol-intoxicated rats

Parameter	Creat. (umol/L)	BUN (mmol/L)	U. Acid (umol/L)
Animal group	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*
Control (-)	22.00 $\pm$ 0.05 ^{cd}	4.38 $\pm$ 0.01 ^{cd}	115.00 $\pm$ 1.30 ^d
Control (+)	32.20 $\pm$ 0.16 ^a	5.90 $\pm$ 0.03 ^a	146.00 $\pm$ 3.81 ^a
Black seeds oil	22.60 $\pm$ 0.07 ^c	5.30 $\pm$ 0.03 ^b	122.00 $\pm$ 2.54 ^c
Lettuce oil	24.80 $\pm$ 0.44 ^b	4.70 $\pm$ 0.02 ^c	136.00 $\pm$ 2.98 ^b
LSD	0.70	0.50	5.00

*SE is standard error and letters of (a, b, c, d) in similar columns differ significantly at $p \leq 0.05$

Table 4: Protective effect of black seed and lettuce oils on TC and TG in paracetamol intoxicated rats

Parameter	TC (mmol/L)	TG (mmol/L)
Rats group	Mean $\pm$ SE*	Mean $\pm$ SE*
Control (-ve)	1.35 $\pm$ 0.02 ^d	1.35 $\pm$ 0.02 ^d
Control (+ve)	1.76 $\pm$ 0.03 ^a	3.80 $\pm$ 0.05 ^a
Black seeds oil	1.42 $\pm$ 0.02 ^{bc}	1.95 $\pm$ 0.01 ^{bc}
Lettuce oil	1.47 $\pm$ 0.01 ^b	2.05 $\pm$ 0.03 ^b
LSD	0.05	0.11

*SE is standard error and letters of (a, b, c, d) in similar columns differ significantly at $p \leq 0.05$

Table 5: Protective effect of black seed and lettuce oils on HDL, LDL, VLDL and AI in paracetamol-intoxicated rats

Parameter	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)	AI
Rats group	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*
Control (-ve)	0.84 $\pm$ 0.01 ^a	0.24 $\pm$ 0.03 ^d	0.27 $\pm$ 0.02 ^d	0.61 $\pm$ 0.02 ^d
Control (+ve)	0.49 $\pm$ 0.02 ^d	0.51 $\pm$ 0.04 ^a	0.76 $\pm$ 0.03 ^a	2.59 $\pm$ 0.05 ^a
Black seeds oil	0.70 $\pm$ 0.02 ^b	0.33 $\pm$ 0.02 ^c	0.39 $\pm$ 0.01 ^{bc}	1.03 $\pm$ 0.03 ^c
Lettuce oil	0.60 $\pm$ 0.03 ^c	0.46 $\pm$ 0.03 ^b	0.41 $\pm$ 0.02 ^b	1.45 $\pm$ 0.01 ^b
LSD	0.09	0.03	0.03	0.35

*SE is standard error and letters of (a, b, c, d) in similar columns differ significantly at $p \leq 0.05$

Hepato-protective of black seed and lettuce oils effect on HDL, LDL, VLDL and AI in paracetamol-intoxicated rats:

The effects of black seed oil and lettuce oil on the levels of atherogenic index (AI), serum High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL) in serum and serum Very Low-Density Lipoprotein (VLDL) in paracetamol-intoxicated rats were shown in Table 5.

Serum high-density lipoprotein, serum low density lipoprotein, serum very low-density lipoprotein and atherosclerosis index levels were 0.49 ± 0.02 , 0.51 ± 0.04 , 0.76 ± 0.03 and 2.59 ± 0.05 mmol/L, respectively, in paracetamol-intoxicated rats (C +ve group), as opposed to 0.84 ± 0.01 , 0.24 ± 0.03 , 0.27 ± 0.02 and 0.61 ± 0.02 mmol/L in (C -ve) normal rats ($p < 0.05$). When compared to rats that were not intoxicated with paracetamol, these findings indicate that paracetamol-intoxicated rats had significantly higher levels of LDL, VLDL and AI. Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention. when compared to control-positive rats, showed an increase significantly in HDL with a decrease significantly in LDL, VLDL and AI levels. When compared to the lettuce oil group, rats administered orally preventable black seed oil showed a significant increase in HDL and a significant decrease in LDL and AI.

Hepato-protective effect of black seed and lettuce oils on CK, CK-MB and LDH in paracetamol-intoxicated rats:

The effects of black seed oil and lettuce oil on paracetamol-intoxicated rats' creatine kinase (CK), Creatine Kinase-MB (CK-MB) and Lactate Dehydrogenase (LDH) were shown in Table 6.

Creatine kinase (CK), Creatine Kinase-MB (CK-MB) and lactate dehydrogenase (LDH) levels were 3464.00 ± 20.00 umol/L, 1849.30 ± 19.00 and 2007.00 ± 23.00 U/L, respectively, in the paracetamol-intoxicated rats (C +ve group), compared to 2155.00 ± 15.00 umol/L, 1376.00 ± 15.00 and 1604.00 ± 12.00 U/L in (C -ve group) normal rats $p \leq 0.05$. Paracetamol-intoxicated rats had significantly higher levels of CK, CK-MB and LDH than normal rats. Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention, when compared to rats that were positive for the control, showed significantly lower levels of the parameters. The levels of CK, CK-MB and LDH in the lettuce oil-treated rats were significantly higher than those in the black seed oil group. When compared to the lettuce oil group and control-positive rats, the orally preventable rats given black seed oil showed the most significant reductions in CK and LDH levels. In parallel, rats administered lettuce oil for oral prevention showed a significant decrease in CK-MB compared to the black seed oil group.

Table 6: Protective effect of black seed and lettuce oils on CK, CK-MB and LDH in paracetamol-intoxicated rats

Parameter	CK (umol/L)	CK-MB (U/L)	LDH (U/L)
Rats group	Mean $\pm$ SE*	Mean $\pm$ SE*	Mean $\pm$ SE*
Control (-)	2155.00 $\pm$ 15.00 ^d	1376.00 $\pm$ 15.00 ^b	1604.00 $\pm$ 12.00 ^b
Control (+)	3464.00 $\pm$ 20.00 ^a	1849.30 $\pm$ 19.00 ^a	2007.00 $\pm$ 23.00 ^a
Black seeds oil	2244.00 $\pm$ 10.00 ^c	1304.80 $\pm$ 11.00 ^c	1107.80 $\pm$ 13.00 ^d
Lettuce oil	2652.00 $\pm$ 13.00 ^b	1209.30 $\pm$ 17.00 ^d	1214.60 $\pm$ 17.00 ^c
LSD	50.00	75.00	100.00

*SE is standard error and letters of (a, b, c, d) in similar columns differ significantly at $p \leq 0.05$

DISCUSSION

The black seed oil contains high phytosterols, fatty acids and toluenes. Its main fatty acids are linoleic and oleic. The composition of fatty acids includes Polyunsaturated Fatty Acids (PUFA, 60%), Monounsaturated Fatty Acids (MUFA, 24%) and saturated fatty acids (SFA, 16%). The black seed oil contains 1200 mg/kg tocotrienol and 208 mg/kg beta-tocopherol. The β -sitosterol is the main phytosterol compound and phenols (1-1.4 mg gallic acid equivalents/g oil). Moreover, Black seed oil contains 3-8 mg/g of thymoquinone with health-promoting effects³⁰. Lettuce oil contains oleic acid (61%) and stearic acid (20%). The lettuce seed oil contains α -tocopherol (134 mg/kg oil) and β -sitosterol (134 mg/kg oil)³¹. Moreover, lettuce oil exhibited sedative analgesic with anti-inflammatory activities^{32,33}.

Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention. The BWG, FI and FER were significantly lower in BWG-positive rats than in control-positive rats. When compared to the lettuce oil group and the positive control group, the rats receiving orally administered black seed oil showed the most significant increases in BWG and FER. In contrast to the black seed oil group, the oral preventable administered with lettuce oil exhibited the greatest significant increase in FI. These results are confirmed by the finding of Al-Seeni *et al.*³⁴, who concluded that the treatment of CCl₄-induced hepatotoxicity in male rats with black seed oil and the BWG % and FER % were higher than the treatment of the positive control group. In the study, researchers believe that increasing the dose of *Nigella sativa* oil to 1.5 mL/kg b.wt., of leads to better results and improves liver tissue and restores it to normal, as *Nigella sativa* contains antioxidants such as flavonoids and phenolic compounds that help get this result.

Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention, exhibited a significant reduction in the liver enzymes AST, ALT, ALP and TBIL when compared to rats that were positive for control. The results confirmed the findings of Mansourian *et al.*³⁵, who concluded that an overdose of acetaminophen not only in mice but also in rats causes

necrosis severely in liver cells. High serum ALT/AST levels in rats, also acetaminophen toxicity causes hepatocyte necrosis within the centers of liver lobules, sometimes extending throughout them. Moreover, Nagi *et al.*³⁶ revealed that thymoquinone (TQ) is the main active ingredient in *Nigella sativa* the volatile oil. Oral administration of thymoquinone (TQ) as a single dose (100 mg/kg) effect on Carbon Tetrachloride (CCl₄)-induced hepatotoxicity was investigated in swiss male albino rats. The data approved the *in vivo* protective action of thymoquinone against carbon tetrachloride-induced hepatotoxicity relating to antioxidant properties. However, thymoquinone has an effective role in ischemia in all body parts such as the human brain, hepatic, renal lungs, heart and ovary³⁷. Furthermore, Bourguou *et al.*³⁸ found that thymoquinone acts as an anti-inflammatory due to its ability to block cellular nitric oxide synthesis and a free radical formed in various tissues from L-arginine.

Also, Al-Ghamdi³⁹ studied *Nigella sativa* protective effect against carbon tetra chloride-induced damage in the liver. Moreover, experimental animals treated with CCl₄ showed significant changes in the central lipids with the reduction in animals treated with *N. sativa*⁴⁰. Meanwhile, Ghadlinge *et al.*⁴¹ confirmed that *N. sativa* oil composes a hepatoprotective effect that prevents paracetamol-induced hepatotoxicity. Furthermore, thymoquinone possesses a positive protective effect in a lot of conditions where cellular damage results from nitrosative and/or oxidative stress⁴².

Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention. When compared to rats that were positive for the control, the levels of creatinine, blood urea nitrogen and uric acid all decreased significantly. These data were supported by Hefnawy and Ramadan⁴³ who studied the protective effects of lettuce leaves ethanolic extract against hepatic intoxication by carbon tetrachloride in the rats' reproductive system indicating enhancing properties of lettuce extract against carbon tetrachloride toxicity with a therapeutic role in free radical-mediated diseases. Meanwhile, Al-Attar *et al.*⁴⁴ evaluated the impact of olive, also sesame and black seed oils on diazinon-exposed rats for physiological parameters. The results concluded that the supplement with olive, also sesame

and black seed oils revealed lowering remarkably influences of physiological alterations with possessing antioxidative effects against diazinon toxicity.

Rats receiving orally preventable treatment with black seed oil demonstrated the least significant change in TC and TG levels. These results were confirmed by the findings of Ali *et al.*⁴⁵, who studied administering (30 mg/kg b.wt.) of black seed to albino mice for 12 weeks, the study showed that TG and TC levels were significantly lower in the treatment groups compared to the control group. In addition, another study done by Moghadam *et al.*⁴⁶ showed that the effect of lettuce seed capsules was demonstrated in patients with high blood lipids at a dose of 1000 mg of lettuce seed capsules once daily for 12 weeks. Moreover, Ibrahim *et al.*⁴⁷ said that the hypolipidemic effects of *N. sativa* were due to the component of thymoquinone, soluble fiber (mucilage), sterols, flavonoids and poly-unsaturated fatty acids. The mechanism of *N. sativa* in regulating cholesterol synthesis is by regulating HMG-Co-A reductase, Apo-A1, Apo-B100 and LDL receptor genes; TQ and other combinations of *N. sativa* oil mediate these actions. Also, Morikawa *et al.*⁴⁸ found that nigellamines, which exist in black seed, have the equivalent activity of clofibrate which is considered a hypolipidemic agent, which explains the reduction of TG level.

Black seed oil and lettuce oil were administered at a concentration of 1 mL/kg b.wt., to all rats for oral prevention. When compared to control-positive rats, showed an increase significantly in HDL with a decrease significantly in LDL, VLDL and AI levels. When compared to the lettuce oil group, rats administered orally preventable black seed oil showed a significant increase in HDL and a significant decrease in LDL and AI. Buriro and Tayyab⁴⁹ confirmed these results and concluded that when albino rats were fed a low-fat diet containing 3% sunflower oil supplemented with black seed, they showed significant lipid profile effects by raising HDL levels and lowering LDL. The hypolipidemic effects of *N. sativa* have been applied via various mechanisms. The mechanism is still unknown, but it is likely due to Polyunsaturated Fatty Acid (PUFA), one of the bioactive compounds in *N. sativa*, which inhibits VLDL secretion.

Thymoquinone has been mentioned for its role in ischemia. According to the Oskouei *et al.*³⁷, thymoquinone effectively treats ischemia in the human brain, kidneys, liver, intestinal, skeletal muscles, heart and ovaries. The anti-cancer effect of essential oil of black seed has been detected in human lung cancer A-549 in addition to colon adenocarcinoma DLD-1³⁸. Meanwhile, Hajhashemi *et al.*⁵⁰ reported that the essential oil found in black seeds possesses analgesic with anti-inflammatory effects using animal models of rats and mice. Moreover, Tavakkoli *et al.*⁵¹ mentioned the

thymoquinone antidote and antitoxin characteristics referring that thymoquinone affected natural toxins such as verrucarins, lipopolysaccharides and D-galactosamine in addition to chemical toxins.

When compared to the lettuce oil group and control-positive rats, the orally preventable rats given black seed oil showed the most significant reductions in CK and LDH levels. In parallel, rats administered lettuce oil for oral prevention showed a significant decrease in CK-MB compared to the black seed oil group. Ebuehi *et al.*⁵² studied the effect of the oils of *Nigella sativa* on carbon tetrachloride induced liver intoxication and mediating levels of neurotransmitters in rats. About 2 and 4 mL/kg of *Nigella sativa* oil were taken orally for 28 days. The results showed a significant dose-dependent decrease in LDH compared to the CCl₄ group. However, more studies are needed to clarify the mechanisms of black seed and lettuce oil in reducing CK, CK-MB and LDH. The metabolites of *N. sativa* oil can reduce DNA damage and prevent toxic agents in colon tissues. Thymoquinone inhibits 5-lipoxygenase and 5-hydroxeicosa-tetraenoic acids production which are essential causes of colon cancer cells. In addition, thymoquinone was found to influence the cells of HCT-116 colon cancer with no effect on HT-29 colon cancer cells⁵³.

CONCLUSION

Black seed and lettuce oils possess a protective role against paracetamol-induced hepatotoxicity in experimental animals by improving body weight gain, food intake, feed efficiency ratio, liver enzymes, kidney functions, lipid profiles and some immune biomarkers against paracetamol-induced hepatotoxicity in experimental rats. Black seed and lettuce oils could be used to improve liver enzymes using different doses and combinations for the investigation of potential long-term effects. Moreover, more data is required relating to human clinical trials to ensure safety and efficacy.

SIGNIFICANCE STATEMENT

The importance of this study is attributed to reduce the negative effect of paracetamol on hepatotoxicity in case of excessive use. Therefore, this study uses some oils to reduce the potential effects of hepatotoxicity which focuses on the protective effects of black seed and lettuce oil against hepatotoxicity as induced by paracetamol in experimental rats. The results indicated that black seed and lettuce oils significantly improved body weight gain, food intake, feed efficiency ratio, liver enzymes, kidney functions, lipid profiles and some immune biomarkers.

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