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Research Article
Immunomodulatory Protective Effects of *Nigella sativa* and *Lactuca sativa* Oils on Liver Intoxication in Experimental Animals

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Abstract

Background and Objective: The liver plays an important role in transforming and clearing chemicals in human body. Hepatic injury is usually caused by numerous toxic chemicals such as carbon tetrachloride, thioacetamide, galactosamine and drugs including paracetamol as overdoses consumption. This investigation aimed to study the immunomodulatory protective effects of black seed (*Nigella sativa* L.) oil and lettuce (*Lactuca sativa* L.) oil against paracetamol liver intoxication in rats. **Materials and Methods:** Twenty-four male albino rats weighing 150±10 g each, were randomly divided into 4 equal groups (6 rats each) as follows: Control negative; control positive as paracetamol hepatotoxicity; *Nigella sativa* oil and *Lactuca sativa* oil at a dose of 1 mL kg^{-1} b.wt., as protective from hepatotoxicity, then serum analysis for all rats were conducted and the obtained data were analyzed using SPSS version-22. **Results:** All rats orally preventable injected with *Nigella sativa* and *Lactuca sativa* oils caused significant decrease in Unsaturated Iron Binding Capacity (UIBC), creatine kinase (CK), Creatine Kinase-MB (CKMB), magnesium (Mg), phosphor (Phos.), iron (Fe), sodium (Na), potassium (K), amylase (Amyl), tri-glycerides (TG), total cholesterol (TC), Low Density Lipoprotein (LDL), creatinine (Creat), Lactate Dehydrogenase (LDH) as compared to liver intoxicated rats. **Conclusion:** Black seed oils and *Lactuca sativa* oils could be used as natural immunomodulatory agents against paracetamol liver intoxication and enhance the body's immune functions with improving the health status of the liver.

Key words: Hepatotoxicity, immunomodulatory, black seed, lettuce, rats

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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 plays an important function in transforming and clearing chemicals in human body[1]. It has a major effective role in the synthesis of vital body molecules, including protein, fats and carbohydrates, in addition to eliminating toxic compounds resulting from the metabolism of alcohol and drugs[2]. Liver injury may result from many toxins that enter the body, including carbon tetrachloride, chloroform, galactosamine and many medications such as acetaminophen and diclofenac[3]. Meanwhile, more than 900 drugs had been involved in inducing liver injury. These toxic substances cause oxidative stress in the cells underlying factors for a large number of diseases[4].

Nigella sativa, a member of the Ranunculaceae family, is among the herbs that are called by different names, such as black cumin and black seed in Arab countries. Black seed oil has preventive influences to prevent diseases of the liver, digestive tract, renal, blood vessels and heart, in addition to its activity as an anti-inflammatory, antibacterial, antioxidant[5].

In the *Nigella sativa* essential oil, thymoquinone was identified as the main component (up to 50%). It has been shown that thymoquinone reduces the oxidative stress, inflammation and apoptosis in an experimental model[6]. At the same time, scientific studies conducted on black seed have confirmed that it has therapeutic effects on the liver, which has been adopted in many research projects[7]. The effective effects of black seed are due to the fact that it contains thymoquinone, which acts as an antioxidant in the body, in addition to reducing oxidative stress and increasing the body's defense by reducing malondialdehyde and vital indicators of oxidative stress while contributing to increasing thiol and glutathione with inhibition of the activity of hepatic CYP1A*A2 isozymes involved in the biotransformation of many xenobiotics into reactive genotoxic radical derivatives. Furthermore, inhibiting lipogenesis in the hepatocytes, as well as working as an anti-inflammatory by increasing the ratio of helper to suppressor T cells with enhancing natural killer cell activity, production of Interleukin-3 (IL-3), as soon as stimulatory effect on macrophages[8].

Lettuce (*Lactuca sativa* L.) is one of the most consumed vegetables in many parts of the world, despite the reduction in its nutritional value. Lettuce is low in calories and low in fat and sodium, but it is a rich source of many compounds that biologically activate the body and promote health[9]. It possesses high quantities of antioxidants, especially vitamin C and polyphenols as chlorogenic acids and other derivatives of caffeic acid, as well as fiber which had health-promoting properties[10]. *Lactuca sativa* also possess beneficial health effects due to its content of various phytochemicals including flavonoids, betalains and carotenoids[11-15]. Caffeic and chlorogenic acids found in lettuce have been reported to have antibacterial, antioxidant, anticancer and anti-mutagenic properties[16-19].

Accordingly, this investigation aimed to study the immunomodulatory protective effects of black seed (*Nigella sativa* L.) oil and lettuce (*Lactuca sativa* L.) oil against paracetamol liver intoxication in rats.

MATERIALS AND METHODS

The study was carried out from February, 2022 to April, 2022 in the Medical Biochemistry Department, Faculty of Medicine, Umm Al-Qura University in Makkah, Kingdom of Saudi Arabia.

Ingredients: Paracetamol as a drug was obtained from Alnahdi Pharmacy and 5 mL of black seed and lettuce oils each, were obtained from the local market, Makkah, Saudi Arabia.

Rats: Twenty-four mature male albino rats weighing 150±10 g, obtained from the Department of Medical Biochemistry, Faculty of Medicine, Umm Al-Qura University, Holy Makkah, Saudi Arabia.

Basal diet: Basal diet consisted of source of protein, fats, carbohydrate, fibers, vitamins and minerals to achieve rat's nutritional requirements including the following ingredients: Casein (12.5%), corn oil (10%), choline chloride (0.2%), vitamins mixture (1%), cellulose (5%), salt mixture (4%), sucrose (22%) and corn stash (up to 100%).

Induction of liver intoxication: The induction of paracetamol toxicity in rats' liver occurred by oral injection with paracetamol at a single dose of 500 mg kg^{-1} b.wt.[20].

Experimental design: Rats were randomly divided into 4 equal groups (6 rats each) as follows: The first group was and the fourth group, fed on a basal diet at all experimental periods concurrently with orally administered with *Lactuca sativa* oil at a dose of 1 mL kg^{-1} b.wt., at all experimental period then induced by liver intoxication by paracetamol once a dose as following:

- **Group 1:** Control negative group that fed on basal all-over diet experimental period (C-ve) for 28 days
- **Group 2:** Control positive that fed on basal diet at all experimental period then induced by liver intoxication by paracetamol once a dose (C+ve) for 28 days
- **Group 3:** Preventable group No. I which fed on basal diet at all experimental period (28 days) concurrently with orally administered with *Nigella sativa* oil at a dose of 1 mL kg^{-1} b.wt., then induced by liver intoxication by a single dose of 500 mg kg^{-1} b.wt., of paracetamol
- **Group 4:** Preventable group No. II which fed on basal diet at all experimental period (28 days) concurrently with orally administered with *Lactuca sativa* oil at a dose of 1 mL kg^{-1} b.wt., then induced by liver intoxication by a single dose of 500 mg kg^{-1} b.wt., of paracetamol

Biochemical evaluation: At the end of the experimental period (28 days), after 12 hrs of fasting, rats were sacrificed after anesthetization by diethyl ether. Blood samples were centrifuged through anesthetization then analyzed, 5 mL serum separation were collected for to determine the following parameters: Unsaturated Iron Binding Capacity (UIBC), creatine kinase (CK), Creatine Kinase-MB (CKMB), magnesium (Mg), phosphor (Phos.), iron, sodium (Na), potassium (K), amylase (Amyl), triglycerides (TG), total cholesterol (TC), Low Density Lipoprotein (LDL), creatinine (Creat), Lactate Dehydrogenase (LDH) using colorimetric methods.

Statistical analysis: The obtained data were analyzed by using computer program Statistical Package for Social Sciences (SPSS) version-22. Data was expressed as Means±Standard Error of the means and the significant between groups calculated using one-way ANOVA test $p \leq 0.05$.

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, Kingdom of Saudi Arabia.

RESULTS

Protective effect of black seed and lettuce oils on UIBC, CK and CKMB in paracetamol intoxicated rats: Data show the protective effect of black seed and lettuce oils on Unsaturated Iron Binding Capacity (UIBC), creatine kinase (CK) and Creatine Kinase MB (CK-MB) in paracetamol intoxicated rats as shown in Table 1.

It could be observed for paracetamol-intoxicated rats (C+ve group) that Unsaturated Iron Binding Capacity (UIBC), creatine kinase (CK) and Creatine Kinase MB (CK-MB) were 68.68±4.40 umol L^{-1}, 3464±9.02 and 881±15.56 U L^{-1} compared to 64.38±3.34 umol L^{-1}, 2436±4.40 and 1912±10.02 U L^{-1} in (C-ve) rats. These results denote that there was a significant increase in UIBC and CK in paracetamol-intoxicated rats compared to normal rats and a decrease in CKMB. All rats were orally preventable and administered with black seed and lettuce oils at a dose of 1 mL kg^{-1} b.wt., showing a significant decrease in UIBC and CK and a significant increase in CKMB when compared to control-positive rats. Rats orally preventable administered with black seed oil reflected the highest significant decrease in UIBC and CK compared to not only the control positive group but also the lettuce oil group.

Protective effect of black seed and lettuce oils on Mg, Phos. and iron in paracetamol intoxicated rats: Data show the protective effect of black seed oil and lettuce oil on magnesium (Mg), phosphate (Phos.) and iron in paracetamol intoxicated rats as shown in Table 2.

It could be observed for paracetamol intoxicated rats (C+ve group) that magnesium, phosphate and iron were 1.00±0.04, 1.79±0.03 mmol L^{-1} and 19.35±1.08 µmol L^{-1} compared to 0.85±0.01, 2.52±0.05 mmol L^{-1} and 28.22±1.21 µmol L^{-1} in (C-ve) rats. These results denote that there was significant increase in magnesium, as soon as significant decrease in phosphate and iron in paracetamol intoxicated rats compared to normal rats. All rats were orally preventable and administered with black seed and lettuce oils at a dose of 1 mL kg^{-1} b.wt., showed a significant decrease in Mg and increase in Phos. and iron when compared to control positive rats. Rats orally preventable administered with black seed oil reflected the highest significant increase in iron compared to not only the positive control group but also the lettuce oil group. Meanwhile, orally preventable administered with lettuce oil reflected the highest significant increase in Phos. compared black seed oil group.

Protective effect of black seed oil and lettuce oil on Na, K and Amyl in paracetamol intoxicated rats: Table 3 show the protective effect of black seed oil and lettuce oil on sodium (Na), potassium (K) and amylase (Amyl) in paracetamol intoxicated rats.

Table 1: Effect of black seeds and lettuce oils on UIBC, CK and CKMB in paracetamol intoxicated rats

Parameter	UIBC (umol L^{-1})	CK (U L^{-1})	CKMB (U L^{-1})
Animal group	Mean±SE	Mean±SE	Mean±SE
Control (-)	64.38±3.34[b]	2436±4.40[c]	1912±10.02[a]
Control (+)	68.68±4.40[a]	3464±9.02[a]	881±15.56[d]
Black seed oil	53.08±2.29[d]	2044±5.71[d]	1304±12.41[b]
Lettuce oil	59.50±1.01[c]	2652±3.45[b]	1209±9.29[c]
LSD	3.00	50.00	30.00

[a,b,c,d]Significantly differ at $p \leq 0.05$, SE: Standard error, UIBC: Unsaturated iron binding capacity, CK: Creatine kinase and CKMB: Creatine kinase-myocardial band

Table 2: Protective effect of black seeds and lettuce oil on Mg, Phos. and iron in paracetamol intoxicated rats

Parameter	Mg (mmol L^{-1})	Phos. (mmol L^{-1})	Iron (µmol L^{-1})
Animal group	Mean±SE	Mean±SE	Mean±SE
Control (-)	0.85±0.01[d]	2.52±0.05[a]	28.22±1.21[a]
Control (+)	1.00±0.04[a]	1.79±0.03[d]	19.35±1.08[d]
Black seeds oil	0.91±0.03[c]	1.90±0.05[c]	25.80±0.99[b]
Lettuce oil	0.99±0.02[ab]	2.20±0.02[b]	21.15±1.03[c]
LSD	0.02	0.10	1.03

[a,b,c,d]Significantly differ at $p \leq 0.05$, SE: Standard error, Mg: Magnesium and Phos.: Phosphate

Table 3: Effect of black seeds and lettuce oil on Na, K and Amyl, in paracetamol intoxicated rats

Parameter	Na (mmol L^{-1})	K (mmol L^{-1})	Amyl (U L^{-1})
Animal group	Mean±SE	Mean±SE	Mean±SE
Control (-)	135.00±1.03[d]	8.23±0.06[a]	1481±9.11[a]
Control (+)	144.20±3.01[a]	5.56±0.09[d]	968±5.32[d]
Black seeds oil	138.60±2.08[c]	5.98±0.03[c]	1014±3.62[c]
Lettuce oil	140.00±2.20[b]	6.96±0.02[b]	1272±2.51[b]
LSD	1.05	0.30	40.00

[a,b,c,d]Significantly differ at $p \leq 0.05$, SE: Standard error, Na: Sodium, K: Potassium and Amyl: Amylase

Table 4: Protective effect of black seeds and lettuce oil on TG, TC and LDL, in paracetamol intoxicated rats

Parameter	TG (mmol L^{-1})	TC (mmol L^{-1})	LDL (mmol L^{-1})
Rats group	Mean ±SE*	Mean ±SE*	Mean ±SE*
Control (-ve)	0.58±0.01[d]	1.09±0.02[d]	0.24±0.05[d]
Control (+ve)	1.40±0.03[a]	1.44±0.04[a]	0.51±0.08[a]
Black seeds oil	1.04±0.02[b]	1.39±0.03[b]	0.37±0.04[b]
Lettuce oil	1.01±0.02[bc]	1.25±0.02[c]	0.33±0.03[bc]
LSD	0.05	0.02	0.06

[a,b,c,d]Significantly differ at $p \leq 0.05$, SE: Standard error, TG: Triglycerides, TC: Total cholesterol and LDL: Low density lipoprotein

It could be observed for paracetamol intoxicated rats (C+ve group) that sodium (Na), potassium (K) and amylase (Amyl) were 144.20±3.01, 5.56±0.09 mmol L^{-1} and 968±5.32 U L^{-1} compared to 135.00±1.03, 8.23±0.06 mmol L^{-1} and 1481±9.11 U L^{-1} in (C-ve). These results denote that there was a significant decrease in K and Amyl and a significant Na increase in paracetamol-intoxicated rats compared to normal rats. All preventable groups that were orally administered with black seed and lettuce oils at a dose of 1 mL kg^{-1} b.wt., showed a significant increase in K and Amyl and at the same time a decrease in Na when compared to control-positive rats. Rats orally preventable administered with black seed oil reflected the highest significant decrease in Na compared to the lettuce oil group. Meanwhile, orally preventable administered with lettuce oil reflected the highest significant increase in Na, K and Amyl compared black seed oil group.

Protective effect of black seed oil and lettuce oil on TG, TC and LDL in paracetamol intoxicated rats: Table 4 show the protective effect of black seed oil and lettuce oil on triglycerides (TG), total cholesterol (TC) and Low Density Lipoprotein (LDL) in paracetamol intoxicated rats.

It could be observed for paracetamol intoxicated rats (C+ve group) that triglycerides (TG), total cholesterol (TC) and Low-Density Lipoprotein (LDL) were 1.40±0.03, 1.44±0.04 and 0.51±0.08 mmol L^{-1} compared to 0.58±0.01, 1.09±0.02 and 0.24±0.05 mmol L^{-1} in (C-ve) normal rats. These results denote that there was a significant increase in triglycerides, cholesterol and low-density lipoprotein in paracetamol intoxicated rats compared to normal rats. All rats orally preventable administered with black seed oil and lettuce oil at a dose of 1 mL kg^{-1} b.wt., showed significant decreases in TG, TC and LDL when compared to control positive rats. Rats orally preventable administered with lettuce oil reflected the

Table 5: Effect of black seeds and lettuce oil on Creat and LDH in paracetamol intoxicated rats

Parameter	Creat (μmol L^{-1})	LDH (U L^{-1})
Rats group	Mean±SE*	Mean ±SE*
Control (-ve)	22.00±1.02^{cd}	1315±5.00^{d}
Control (+ve)	30.20±2.08^{a}	2631±8.00^{a}
Black seeds oil	22.60±1.71^{bc}	2169±3.00^{b}
Lettuce oil	24.80±1.11^{b}	2083±2.00^{c}
LSD	3.00	50.00

^{a,b,c,d}Significantly differ at $p \leq 0.05$, SE: Standard error, Creat: Creatinine and LDH: Lactate dehydrogenase

highest significant decrease in TC compared to the black seed oil group. Meanwhile, orally preventable administered with black seed oil reflected no significant difference in TG and LDL compared to the lettuce oil group.

Protective effect of black seed oil and lettuce oil on Creat and LDH in paracetamol intoxicated rats: Table 5 shows the protective effect of black seed oil and lettuce oil on creatinine (Creat) and Lactate Dehydrogenase (LDH) in paracetamol intoxicated rats.

It could be observed for paracetamol intoxicated rats (C+ve group) that creatinine (Creat) and lactate dehydrogenase (LDH), were 30.20±2.08 μmol L^{-1} and 2631±8.00 U L^{-1} compared to 22.00±1.02 μmol L^{-1} and 1315±5.00 U L^{-1} in (C-ve) normal rats. These results denote that there was a significant increase in creatinine and lactate dehydrogenase in paracetamol intoxicated rats compared to normal rats. All rats were orally preventable administered with black seed and lettuce oils at a dose of 1 mL kg^{-1} b.wt., showed a significant decrease in Creat and LDH when compared to control positive rats. Rats orally preventable administered with lettuce oil had the highest significant decrease in LDH compared to the black seed oil group. Meanwhile, orally preventable administered with black seed oil and lettuce oil reflected the same significant decrease in Creat., compared to the control positive group.

DISCUSSION

This investigation aimed to study the immunomodulatory protective effects of black seed (*Nigella sativa* L.) oil and lettuce (*Lactuca sativa* L.) oil against paracetamol liver intoxication in rats. All rats orally preventable injected with *Nigella sativa* and *Lactuca sativa* oils caused significant decrease in Unsaturated Iron Binding Capacity (UIBC), creatine kinase (CK), Creatine Kinase-MB (CKMB), magnesium (Mg), phosphor (Phos.), iron (Fe), sodium (Na), potassium (K), amylase (Amyl), tri-glycerides (TG), total cholesterol (T.C), Low Density Lipoprotein (LDL), creatinine (Creat), Lactate Dehydrogenase (LDH) as compared liver intoxicated rats. The protective effect of black seed and lettuce oils on Unsaturated Iron Binding Capacity (UIBC), creatine kinase (CK) and Creatine Kinase-MB (CKMB) in paracetamol intoxicated rats as listed in Table 1 confirmed by the findings of Heshmati *et al.*[21], who concluded that creatine kinase increases with muscle, heart, or brain damage, which can be caused by many diseases include hormonal disorders including hypothyroidism and diabetes, indicating that *Nigella sativa* oil has an important role in thyroid diseases by showing a significant decrease in CK in diabetic and hypothyroid mice treated with NS oil. Moreover, Meanwhile, Al-Attar and Al-Taisan[22] showed in their experimental study that the group of rats treated with *Nigella sativa* seed extract showed a significant increase in CK activity, while the significant changes in hematological and biochemical parameters caused by diazinon intoxication were significantly reduced by treatment with NS seed extract. As soon as, Uchendu *et al.*[23] showed an elevated level of CK-MB as a result of the treatment with NS seed extract is potentially cardioprotective against harmful chemical toxins such as cadmium. Also, Xiao *et al.*[24] found that CK-MB significantly increased and NS has an anti-inflammatory and cardioprotective effects that may control several associated CVD risk factors. Moreover, Ahmed and Hassanein[25] indicated that the treatment of NS oil has significantly reversed the elevated CK-MB levels which could serve as a true functional food and may positively affect health promotion via reducing cardiovascular risk. In this respect, Sultan *et al.*[26] found that the level of CK-MB increased and the treatment with NS were effectiveness in attenuating the elevated levels of cardiac and liver enzymes owing to oxidative stress induced by potassium bromate. Also, Murugesan *et al.*[27] found that CK-MB serum were significantly increased and the pretreatment with NS offered a protective effect against isoproterenol induced myocardial infarction.

The effect of black seed oil and lettuce oil on magnesium (Mg), phosphate (Phos.) and iron in paracetamol intoxicated rats as listed in Table 2 confirmed by the findings of Tam *et al.*[28] who, recorded that magnesium has an effective role in immunity in the body through a specific or non-specific immune response. In biological systems, magnesium is the second largest cation present in cellular systems. It has many biological functions through the complexation of negatively charged groups, such as phosphates in nucleic acids, as well as controlling enzyme activation and inhibition and regulatory roles by modifying cell proliferation. Meanwhile, Vallabhapurapu and Karin[29] revealed that iron has been shown to influence the activation of Nuclear Factor Kappa B (NF-κB), a transcription factor that is required for the expression of a number of genes involved in innate immunity and inflammation.

Table 3 that showed the protective effect of black seed oil and lettuce oil on sodium (Mg), potassium (K) and amylase (Amyl), there were agreement with the findings of Benhelima *et al.*[30], who found a decrease in sodium and potassium levels in the positive control group for cisplatin-induced nephrotoxicity, which may be due to inhibition of water and electrolytes reabsorption through the renal tubule into the bloodstream.

Thymoquinone is the active substance in black seed, which works to synergize the kidney-protective effect against kidney injury induced by cisplatin and methotrexate toxins in laboratory rats. The percentage of thymoquinone may reach 30-48%, in addition to the black seed containing flavonoids, glycosides and saponins, all of which have antioxidant and anti-inflammatory properties[31,32].

Treatment with black seed resulted in an increase in the production of electrolytes, which leads to a natriuretic effect greater than the thermogenic effect, as well as a similar effect of uric acid in the control group in the experiment. The Na^+/K^+ ratio is a prediction in diuretic mechanism as higher of kaliuretic effect[33]. This may be due to the activity of the extracts as antioxidants due to the presence of active substances, including polyphenols and flavonoids, which have great effectiveness in reducing oxidative stress resulting from cisplatin, which helps restore kidney functions in the body[34].

Table 4 showed the protective effect of black seed oil and lettuce oil on triglycerides (TG), total cholesterol (TC) and Low Density Lipoprotein (LDL), there were accordance with the findings of Ismail *et al.*[35], who reported that in hypercholesterolemic rats treated with *Nigella sativa* there were lowering in total cholesterol in rats fed on high cholesterol diet. They attributed the hypercholesterolemic effect of *Nigella sativa* as the presence of thymoquinone, a phytochemical compound which regulates genes associated with cholesterol metabolism. A similar effect in type 2 diabetic mice treated with *Nigella sativa* extracts with lowering TC, LDL, triglyceride. The role of *Nigella sativa* in reducing blood pressure can be explained by inhibiting cholesterol synthesis by reducing HMG-CoA hepatic activity of reductase excretion of bile acids stimulation, resulting in serum cholesterol levels reduction[36].

Nigella sativa oil extracts reduced levels of TC, LDL and triglycerides in type 2 diabetes patients[37]. *Nigella sativa* antioxidant activity resulting from reducing dyslipidemia in association to diabetes through insulin resistance decreasing[38]. Moreover, *Nigella sativa* influence for dyslipidemia is associated to hepatic HMG-CoA reductase activity substantial decrease as a regulatory effect on genes that affect on cholesterol metabolism[39].

The results in Table 5 were confirmed by the findings of Soliman *et al.*[40], who found a highly significant increase in *N. sativa* supplemented group in creatinine. Meanwhile, the liver showed improvement of histoarchitecture without fibrosis, improved the immune response and subsequent resistance of broilers against diseases. Moreover, El Boghdady and Darwish[41] observed that Fe-NTA treatment led to a significant increase by almost twofold in serum urea and creatinine levels, the normalization of creatinine level and was attained following *N. sativa* pretreatments, prevention the increase occurred in nephrotoxicity indices and histopathological lesions appreciably in pretreatment rats with *N. sativa* before Fe-NTA. Furthermore, Murugesan *et al.*[27] observed that black cumin treatment reduced LDH levels clearly indicating that black cumin could be extremely cardioprotective against myocardial infarction due to its significant antioxidant properties.

The study's recommendation includes examining the immunomodulatory preventable effect against paracetamol liver-intoxicated rats with enhancing body immune functions including unsaturated iron binding capacity, creatine kinase, creatine kinase-MB, magnesium, phosphorus, iron, sodium, potassium, amylase, triglycerides, total cholesterol, low-density lipoprotein, creatinine, lactate dehydrogenase as revealing better health status.

The strengths of this study include the indication of the immunomodulatory preventable effect against paracetamol liver-intoxicated rats by examining the liver and kidney function markers. However, the present study includes some limitations. Firstly, the protective dose of this study was not enough to reach the highest dose with the liver intoxication impact of tested oils before it led to a toxic dose. Secondly, the synergetic protective effect of *Nigella sativa* and *Lactuca sativa* oils was not studied according to the low number of rats available. Finally, this study was not funded for practical work. Therefore, the available resources for the laboratory rats were limited, which clarifies the variation in the rat's serum analysis.

CONCLUSION

Nigella sativa and *Lactuca sativa* oils possess an immunomodulatory preventable effect against paracetamol liver-intoxicated rats with enhancing body immune functions resulting in a significant decrease in unsaturated iron binding capacity, creatine kinase, creatine kinase-MB, magnesium, phosphorus, iron, sodium, potassium, amylase, triglycerides, total cholesterol, low-density lipoprotein, creatinine, lactate dehydrogenase revealing better health status. Black seed oils and *Lactuca sativa* oils could be used as natural immunomodulatory agents against paracetamol liver intoxication and enhance the body's immune functions with improving the health status of the liver.

SIGNIFICANCE STATEMENT

Hepatotoxicity occurs due to many factors, including the use of excessive doses of medications such as paracetamol. Therefore, some natural materials could be used to reduce the risk of these types of liver intoxications such as some natural herbs and plant oils, including black seed oil and lettuce oil. Accordingly, this investigation aimed to study the immunomodulatory protective effects of black seed (*Nigella sativa* L.) oil and lettuce (*Lactuca sativa* L.) oil against paracetamol liver intoxication in rats and the study revealed that paracetamol liver toxicity could be orally preventable injected by *Nigella sativa* and *Lactuca sativa* oils, in addition to the possibility of using them to improve the overall liver health status.

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