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Therapeutic Effect of *Nigella sativa* on Alcohol-induced Liver Disease in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author MB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AM, HZ, HK and CM managed the analyses of the study. Author NKC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Alcohol-induced fatty liver disease is the earliest liver disorder associated with excessive consumption of alcohol. Various treatment regimens have been proposed for the treatment of alcohol-induced liver disorders including the use of medicinal plants such as *Nigella sativa* (NS), a miracle plant with a wide spectrum of activities.
Objective: The aim of the present study was to investigate the therapeutic property of NS total oil (TO) and neutral lipid fraction (NLF) on alcohol-induced liver injury in male albino rats.
Methodology: The TO was first extracted from NS seed, and then fractionated using a chromatography column to obtain the NLF. These two extracts were used separately as treatment regimen for alcohol-induced liver toxicity in rats. Some serum biochemical markers of hepatic

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function including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) and histopathological features of the liver section were assessed, and results were compared to those in the control group.
Results: Serum liver enzyme activities increased significantly (p<.05) in the ethanol-treated group compared with the control group. However, treatment with TO or NLF of NS seed caused a significant reduction in serum levels of liver enzymes (AST, ALP, ALT), cholesterol, triglyceride and glucose compare to the corresponding levels in the ethanol treated group. There were also improvement in histo-pathological features in the TO and NLF treated groups compared with alcohol-treated group.
Conclusion: Both extracts of NS seeds possess ameliorative potential against ethanol-induced hepatotoxicity in rats.

Keywords: Nigella sativa; extracts; alcohol-induced liver disorder; amelioration; rat; liver histopathology.

1. INTRODUCTION

Alcohol-induced liver disease is a global public health problem. Alcohol consumption causes various liver lesions including steatosis, acute alcoholic hepatitis, fibrosis and cirrhosis. However, the degree of severity of these diseases is linked to excessive and prolonged alcohol intake. The mechanisms involved in the development of alcohol liver diseases remain unclear. However, induction of oxidative stress has been hypothesized by many investigators [1,2].

To prevent liver damage induced by oxidative stress, human body has developed several endogenous antioxidant systems which are divided into two groups; enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and the non-enzymatic antioxidants are Lipid-soluble vitamin E (tocopherol), vitamin A (β-carotene), as well as vitamin C (L-ascorbic acid) and glutathione (GSH) [3].

Over the last few decades, human medical research has focused on finding new molecules, mainly from medicinal plants, that can be considered as a source of antioxidant molecules with diverse biological and therapeutic properties. Evidently, NS is one such plant with a large curative spectrum [4-6]; anti-inflammatory activity [7], diuretic, hypotensive [8] and antiviral [9] activities. However, despite the extensive literature on the beneficial effect of the different extracts of NS [10-12], to date little is known about the protective effect of TO and NLF extracts from NS seeds on alchohol-induced liver disorders.

In this study, we investigated the therapeutic effect of NS seed TO and NLF against ethanol-induced hepatotoxicity in rats fed with Lieber-DeCarli liquid diet. The use of this diet to investigate metabolic effects of ethanol in a rat model including those involving the fetal alcohol syndrome has been extensively acknowledged [13,14]. N-acetyl cysteine (NAC) was used as a positive control due to its therapuetic effect on xenobiotic-induced liver injuy [15].

2. MATERIALS AND METHODS

2.1 Vegetable Material

Nigella sativa dry seeds, *specimen* LINN No 700.4, were obtained in April, 2015, from an agricultural farm in Bechar, a city situated in the Algerian desert and botanically identified by Professor LAOUAR, director of the Plant Biology Laboratory (PBL) at Setif University, Algeria.

2.2 Extraction and Fractionation of the Total Oil

The extraction procedure was carried out using Soxhlet-Methanol to produce methanol extract. To the latter, n-hexane was added, mixed and, then a decanter was used to get two separating phases. Solvent was removed on a rotary evaporator at 40℃ and TO was obtained from the hexane phase.

To fractionate TO, we used a silica gel 60 G (70-230 mesh) column (30 cm x 2 cm) where neutral lipids were eluted by using chloroform (3 times, 100 ml) following the protocol reported by Ramadan and Mörsel [16].

2.3 Animals and Treatment

Male albino rats weighing between 120-180 g were obtained from the animal house of Mentouri University, Constantine, Algeria. Hepatotoxicity was induced in all rats by ethanol consumption with a specific diet (Lieber-DeCarli liquid diet) for six weeks. Rats were given the first dose of ethanol (12 mg/kg/day) during the first week and this dose was increased to 17 mg/kg/day during the five following weeks and the body weight was measured daily. The control group was fed with the control liquid diet. Six weeks after ethanol administration, rats received TO, NLF and NAC for four weeks. Ethanol and the different treatments TO, NLF and NAC were given daily, orally, by gavages.

Forty rats were randomly divided into five groups of eight rats per group:

Ethanol group : Treated with Ethanol mixed diet for 6 weeks,
TO group : Treated with ethanol mixed diet (6 weeks) + TO (400 mg/ml) for 4 weeks,
NLF group : Treated with ethanol mixed diet (6 weeks) + NLF (300 mg/ml) for 4 weeks,
NAC group : Treated by ethanol mixed diet (6 weeks) + NAC (1, 2 mg/kg) for 4 weeks,
Control group : Treated with control liquid diet for 6 weeks.

2.4 Sample Collection

The animals were fasted overnight, weighed and sacrificed by cervical dislocation. Animals in the ethanol group and control group were sacrificed, under diethyl ether anesthesia, after six weeks of treatment and the treated groups were sacrificed after ten weeks of treatment. Blood samples were collected into heparinized tubes for biochemical analyses.

2.5 Estimation of Serum Hepatic Enzyme Activities

Serum biochemical markers of liver function were used to assess the functional status of the liver specifically; activities of three liver enzymes ASP, ALT, and ALP were assessed using sprinreact diagnostic kits with a Bechaman auto analyzer at the biochemistry laboratory of Setif University Hospital, Algeria.

2.6 Histopathological Examination

The effect of ethanol and different treatments on liver tissue was evaluated by assessing the morphological changes in liver sections. Liver tissue was cut into small pieces and immersed in neutral formalin. The fixed tissues were processed and paraffin embedded sections were stained with hematoxylin and eosin (HE), using standard techniques as reported by Bancroft and Gamble [17]. Histopathological evaluation was carried out in the pathology laboratory of Setif University Hospital. Algeria.

2.7 Statistical Analysis

All experiments were performed three times and analysis for each experiment was carried out in triplicates. Results were expressed as means ± standard error (SEM). Statistical analysis was performed using one way analysis of variance (ANOVA), followed by multiple comparison post hoc tests (Tukey). $p \leq 0.05$ was considered statistically significant.

3. RESULTS

3.1 Effect on Hepatic Enzymatic Activities

The results of the present study show signifcant decreases in serum levels of AST and ALT in animals treated with TO, NLF and NAC compared to animals in the ethanol treated group (Table 1).

Treatment with TO fraction led to a significant ($p \leq .05$) decrease in cholesterol level, whereas rats from the group treated with NLF showed a significant ($p \leq .05$) decrease on triglycerides compared to the group of rats treated with ethanol. Equally, a significant ($p \leq .05$) reduction in blood glucose was observed in the two groups of rats treated either with TO or NLF (Table 1).

3.2 Histopathological Study

Microscopic observation of thin cut embedded slides from liver of ethanol treated rats showed features of severe hepatotoxicity including centrilobular necrosis, degeneration in hepatocytes, congestion in the central vein and sinusoids, proliferation of Kupffer cells and mononuclear leucocytes, inflammatory cells infiltration mainly surrounding the central vein (Figs. 1 A&B) compared to liver slides from rats of the control group (Fig. 1C). Also, treatment of rats with TO and NLF led to remodelling changes

in liver tissue with clear improvement of the lesions and a marked reduction in necrosis and infiltration compared to liver tissues from rats in ethanol-treated group (Table 2, Fig. 1).

4. DISCUSSION

In this study, we investigated the effect of both TO and NLF of NS seeds on ethanol induced liver toxicity in rats fed with a specific diet (Lieber-DeCarli liquid diet). By allowing efficient consumption of ethanol, this diet is suitable to investigate metabolic effects of chronic-ethanol feeding, including fetal alcohol syndrome in rats [18,19].

This model is the most useful one for causing typical liver tissue damage and inflammation, In addition, this ethanol diet leads to high levels of alcohol in the blood. Exposure to ethanol can lead to the peroxidation of polyunsaturated fatty acids and degradation of phospholipids that cause cellular damage. The monitoring of alterations is made through markers biochemical assessment and histopathological study of liver tissue [20,21].

Our results showed a significant increase in plasma liver enzyme activities (AST, ALT and ALP) in rats fed with ethanol, High serum levels of ALT and ALP is indicative of cellular dysfunction and loss of functional integrity of liver cell membrane. After treatment with TO and NLF extracts from *NS* seeds, there were significant decreases in activities of the liver enzymes to levels approaching the normal values; 28.0 ± 11.9 and 66.1 ± 12.1 respectively [22,23].

The administration of NS seed TO and NLF lowered significantly plasma glucose and lipid (triglycerides and Cholesterol). These findings, added to the decrease in body weight, suggest that the action of these extracts is likely *via* regulating fat metabolism. Our data are in agreement with previous findings; Le and his research team [24] using either petroleum extract or volatile oil of NS seeds. The mechanism of action of Ns seeds extracts on decreasing blood glucose and lipid levels is still unclear; although, it is likely that the hypoglycaemic effect is by remodeling hepatic sugar and lipid metabolisms [24,25].

Oral administration of TO and NLF significantly reduced blood glucose, triglycerides and cholesterol levels after four weeks of treatment. Our results are in agreement with what has been reported by previous studies. It was found that the oil fraction lowered significantly blood glucose and hepatic glucose production, from gluconeogenesis [26]. Recently, it has been reported that the significant reduction of plasma glucose level. In a recent study, oral administration of ethanol extract of NS seeds to STZ-induced diabetic rats, for 30 days, reduced significantly the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation and antioxidant enzymes [27].

Table 1. Serum levels of liver enzymes following different treatments

Biochemical parameters	Control	Ethanol	NAC	TO	NLF
AST	113,38 ±16,36	133,33 ± 63,73	76,33 ± 8,45*	78,66 ±12,48*	71,61 ± 18,49*
ALT	31 ±5,47	38,63 ± 5,97	28,83 ± 5,45*	26 ± 5,72**	24,90 ± 2,40***
APL	154,83 ± 28,43	180,83 ± 57,92	89 ± 17,23	117,66 ± 38,74*	120,17 ±7,98*
Glucose	0,83 ± 0,12***	1,54 ± 0,33	1,11 ± 0,11**	1,10 ± 0,16**	0,99 ± 0,20***
Cholesterol	0,67 ± 0,06	0,66 ± 0,08	0,56 ± 0,09	0,49 ± 0,07*	0,62 ± 0,14
Triglyceride	0,45 ± 0,09**	0,90 ± 24	0,83 ± 0,05	0,81 ± 0,09	0,49 ± 0,09*

Values are expressed as mean ± SEM, (N = 8).

** Significant difference (*: p≤.05, **: p≤.01, ***: p≤.001) against ethanol treated group. Experiments were performed three times and analysis for each experiment was carried out in triplicates*

Table 2. Effect of TO and NLF from *NS* seeds on histopathological changes of rat hepatic tissues (n = 8)

Groups	Hepatic tissue
Control	Normal
Ethanol	Severe congestion in the central vein and sinusoids, steatosis, infiltration of inflammatory Cells, severe, necrosis
Ethanol + NAC	Mild congestion and mild necrosis
Ethanol + TO (400 mg/ml)	Normal
Ethanol + NLF (300 mg/ml)	Normal

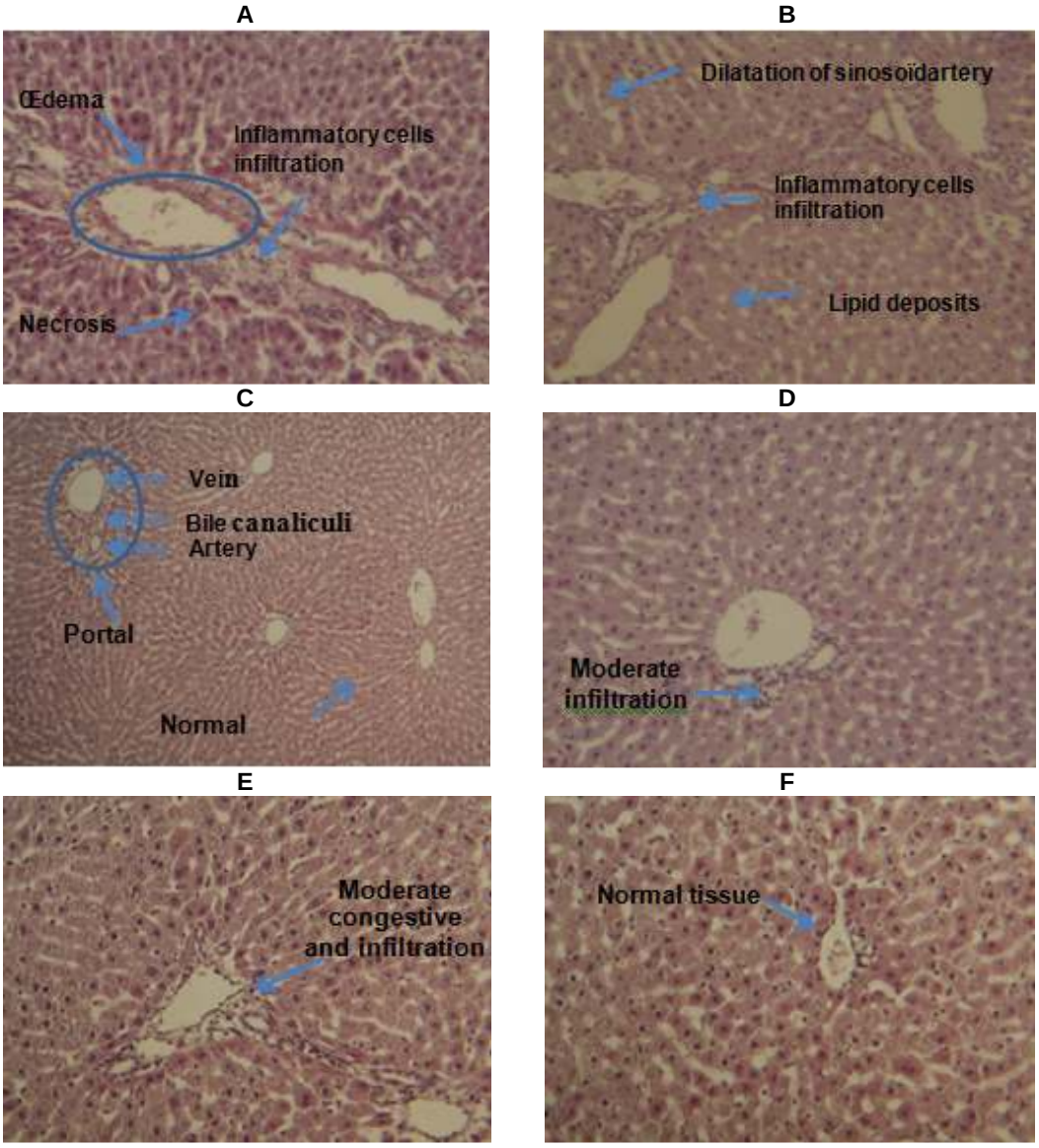

Fig. 1. An impressive improvement in the histology of the liver tissue from rats treated with TO and NLF from *NS* seeds. Severe histopathological changes were observed in the liver tissue from rats with ethanol-induced liver toxicity

A and B: Histopathological changes in the liver tissue of ethanol induced hepatotoxicity; C: Normal structure from the control group; D: ethanol + NAC treatment; E. ethanol + TO treatment and F: ethanol +NLF treatment

Histopathological assessment revealed significant histological changes in liver tissue slides of rats with ethanol-induced liver lesions. Our findings are consistent with previous reports on ethanol induced hepatotoxicity [28,29]. However, histological section of the liver tissue of rats treated with TO or NLF extract showed marked improvement of the liver lesions compared to those of the ethanol treated group suggesting hepato-protective effect of NG seed.

In addition, we have assessed the histological changes in liver tissue of rats after the treatment with TO and NLF where a marked structural improvement was observed [6,17]. It has been suggested that such protective effect of TO fraction of NS seeds against histological damage induced by ethanol is mainly due to its antioxidant and anti-inflammatory potentials [30,31]. According to these findings, exposure to ethanol may result in peroxidation of

polyunsaturated fatty acids. This may lead to the degradation of phospholipids and hence causing cellular lesions as shown in this study. Such damage could be assessed by changes in biochemical parameters and severity of histological alterations as done in the present study.

5. CONCLUSION

The results of this study demonstrate that NS seeds have ameliorative effects on ethanol-induced liver toxicity in rats, probably due to its numerous bioactive constituents and their anti-oxidative activities. These findings confirmed the results of our previous study [32].

CONSENT

It is not applicable.

ETHICAL STATEMENT

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Public Health of Algeria (INSP). The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Ferhat Abbes–Setif.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cederbaum AI. Introduction serial review: Alcohol, oxidative stress and cell injury. Free Radical Biology and Medicine. 2001;31:1524-1526.
2. Molina, McClain C, Villa D. Molecular pathology and clinical aspects of alcohol-induced tissue injury. Alcoholism: Clinical and Experimental Research. 2003;26:120-128.
3. Halim AB, El-Ahmady O, Hassab-Allah S, Abdel-Galil F, Hafez Y, Darwish A. Biochemical effect of antioxidants on lipids and liver function in experimentally-induced liver damage. Annals of Clinical Biochemisty. 1997;34:656-663.
4. Haq A, Lobo PI, Al-Tufail M, Rama NR, Al-Sedairy ST. Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography. International Journal of Immunopharmacology. 1999;21: 283-295.
5. Hmza AJ, Osman MT, Adnan A, Omar E. Immunomodulatory effect of *Nigella sativa* oil in the disease process of type 1 diabetic rats. Research Journal of Pharmaceutical, Biological and Chemical sciences. 2013;4:980-988.
6. Ahmad S, Beg ZH. Mitigating role of thymoquinone rich fractions from *Nigella sativa* oil and its constituents, thymoquinone and limonene on lipidemic oxidative injury in rats. Springer Plus. 2014;3:316.
7. Chakrabarty A, Emerson MR, LeVine SM. Heme oxygenase-1 in SJL mice with experimental allergic encephalomyelitis. Multiple Sclerosis. 2003;9:372-381.
8. Khalife KH, Lupidi G, Nagi MN, Alam K, Badary OA, Al-Shabanah OA, Al-Sawaf HA, Al-Bekairi AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice *via* an antioxidant mechanism. International Journal of Biochemistry and Molecular Biology. 1999;47:153-159.
9. Detering N, Reed WD, Ozand PT, Karahasan A. Effects of maternal ethanol consumption in the rat on the development of the iroffspring. Journal of Nutrition. 1979;709:999-1009.
10. Ghishan FK, Patwardhan R, Greene HL. Fetal alcohol syndrome: Inhibition of Placenta !zinc transportasa potential mechanism For fetal growth retardation in the rat. Journal of Laboratory and Clinical Medecine. 1982;700:45-52.
11. Martin JJR, Leah H, Ben S, Alexei GB, Eugene OA, Emanuele A, Thomas MB, Dennis RP. Effects of long-term ethanol administration in a rat total enteral nutrition model of alcoholic liver disease. American Journal of Physiology Gastrointestinal and Liver Physiology. 2011;300:109-119.
12. Ramadan MF, Mörsel JL. Characterization of phospholipids composition of black cumin (*Nigella sativa L*.) seed oil. Nahrung. 2002a;46:40-244.
13. Bancroft JD, Gamble M. Theory and practice of histological techniques. 5. Ed. Edinburgh. Churchill Livingstone Pub. 2002;172-5,593-620.
14. Lieber CS, DeCarliL M. The feeding of alcohol in liquid diets: Two decades of applications and 1982 up date. Alcoholism: Clinical and Experimental Research. 1982;6:523-531.

15. Lieber CS, DeCarliL M, Sorrell MF. Experimental methods of ethanol administration. Hepatology. 1989;10:501-510.
16. Seval D, Betül E, Esra BK, Necla KT, Gül Özdemirler E. Protective effect of *Nigella sativa* oil against binge ethanol induced oxidative stress and liver injury in rats. Chinese Journal of Natural Medicines. 2014;12:495-499.
17. Gani MS, John AS. Evalution of hepatoprotective effect of *Nigella sativa* L. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5:428-430.
18. Pal A, Banerjee B, Banerjee T, Masih M, Pal AK. Hepatoprotective activity of *Chenopodium album* L. plant against paracetamol-induced hepatic injury in rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3:55-57.
19. Hamed P, Elahe T, Khalil A, Alireza TH, Sayed Masoud H, Hossein H. Effect of *Nigella sativa* fixed oil on ethanol toxicity in rats. Iranian Journal of Basic Medical Sciences. 2014;17:1020-1031.
20. Fang ZH, Cui JW, Cao JM, Peng JH, Feng Q, Hu YY. Preparation of Lieber-DeCarli liquid diet induced alcoholic hepatic injury model in rats. Zhong Xi Yi Jie He Xue Bao. 2006;4:596-600.
21. Lieber CS, DeCarliL M, Sorrell MF. Experimental methods of ethanol administration. Hepatology. 1989;10:501-510.
22. Lieber CS. Alcoholic fatty liver: Its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol. 2004;34:9-19.
23. Adeline B, Stephanie M, Sung HK, Hua Wang BG. Mouse model of chronic and binge ethanol feeding (the NIAAA model). Nature Protocols. 2013;8:627-637.
24. Le PM, Benhaddou-Andaloussi A, Elimadi A, Settat A, Cherrah Y, Haddad PS. The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. Journal of Ethnopharmacology. 2004;94:251-259.
25. Houcher Z, Boudiaf K, Benboubetra M, Houcher B. Effects of methanolic extract and commercial oil of *Nigella sativa L*. on blood glucose and antioxidant capacity in alloxan-induced diabetic rats. Pteridins. 2007;18:8-18.
26. Fararh KM, Shimizu Y, Shiina T, Nikami H, Ghanem MM, Takewaki T. Thymoquinone reduces hepatic glucose production in diabetic hamsters. Research Veterinary Science. 2005;79:219-223.
27. Kaleem M, Kirmani D, Asif M, Ahmed Q, Bano B. Biochemical effects of *Nigella sativa L* seeds in diabetic rats. Indian Journal of Experimental Biology. 2006;44: 745-748.
28. Darwish HA, Abd Raboh NR, Mahdy A. Camel's milk alleviates alcohol-induced liver injury in rats. Food and Chemical Toxicology. 2012;50:1377-1383.
29. Woo C, Kumar A, Sethi G, Tan K. Thymoquinone: Potential cure for inflammatory disorders and cancer. Biochemical Pharmacology. 2012;83:443-451.
30. Al-Ghamdi M. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. Journal of Ethnopharmacology. 2001;76:45-48.
31. Shirpoor A, Minassian S, Salami S, Khadem-Ansari MH, Ghaderi-Pakdel F, Yeghiazaryan M. Vitamin E protects developing rat hippocampus and cerebellum against ethanol-induced oxidative stress and apoptosis. Food Chemistry. 2009;113:115-120.
32. Sobhi W, Stevigny C, Duez P, Claderon BD, Atmani D, Benboubetra M. Effects of lipid extract from *Nigella sativa* seeds on the liver ATP and alpha glucosidase reduction. Pakistani Journal of Pharmaceutical Sciences. 2016;29(1):111-117.

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