JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

SHORT COMMUNICATION

J Adv Biotechnol Exp Ther. 2024 Sep; 7(3): 633-641
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2024.d54
Published by www.bsmiab.org

Effect of *Nigella sativa* extract on the elevation of serum MDA levels in aluminum-induced oxidative stress in rats

Ema Qurnianingsih[1], Citrawati Dyah Kencono Wungu[1, 2], Siti Khaerunnisa[1], Lina Lukitasari[1], Ira Humairah[1, *], Rimbun Rimbun[3], Gwenny Ichsan Prabowo[1], Arif Nur Muhammad Ansori[4, 5]

[1]Department of Physiology and Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Anatomy, Histology and Pharmacology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
[4]Postgraduate School, Universitas Airlangga, Surabaya, Indonesia
[5]Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India

***Corresponding author**
Ira Humairah
Department of Physiology and Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.
Email: ira-h@fk.unair.ac.id

Academic editor
Md. Atikur Rahman, PhD
ABEx Bio-Research Center, Dhaka, Bangladesh

Article info
Received: 13 January 2024
Accepted: 31 August 2024
Published: 28 September 2024

Keywords
Aluminium, Black cumin, Malondialdehyde, Oxidative stress, *Nigella sativa*

ABSTRACT

Aluminum (Al) constitutes approximately 8% of the Earth's surface. Al is extensively used in various industrial applications, including medicines, food additives, cosmetics and farming practices. However, Al poses a risk to human health when it enters the body, potentially leading to various pathological conditions. *Nigella sativa,* known for its active compound thymoquinone (TQ), has demonstrated anti-inflammatory and antioxidant properties. TQ is recognized for its potential in the prevention and treatment of numerous diseases. This study aimed to explore the effect of *N. sativa* extract (NSE) in preventing the elevation of malondialdehyde (MDA) levels in the blood serum of rats subjected to aluminum-induced oxidative stress. Twenty-four male Wistar rats were divided into four groups. Groups K1 and K2 were fed a standard diet, while groups K3 and K4 received NSE at doses 200 mg/kg/day and 400 mg/kg/day, respectively, administrated through oral gavage. After two weeks of NSE pretreatment, groups K2, K3, and K4 were exposed to AlCl3 (34 mg/kg, oral gavage) for an additional three weeks. There were no significant differences ($P > 0.05$) in body weight or hemoglobin levels among the groups. Interestingly, a significant reduction in MDA level ($P < 0.05$) was observed among groups. Notably, MDA levels were lower in the NSE-treated and control groups compared to the AlCl3-exposed group. In conclusion, NSE may have a positive effect against oxidative stress induced by Al exposure.

INTRODUCTION

The earth's surface contains 8% aluminum (Al), it appears in rocks such as bauxite, silicates, and cryolite [1]. Al is widely used in industrial setting, including medicines (such as antacids), food additives, cosmetics (such as deodorants), and various household goods. Additionally, it is present in common items salt, spices, maize, tea, and yellow-colored cheese. It is also frequently employed as a flocculant in drinking water treatment, making it easy for Al to enter the body through various routes [1,2]. Al can become toxic to the human body and various pathological conditions have been linked to Al toxicity. These include anemia, granulomatosis, fibrosis, interstitial pneumonia, toxic myocarditis, ischemic stroke, autism, Alzheimer's, hepatorenal disease, breast cancer, diabetes mellitus, inflammatory bowel disease, and many more [3].

Previous animal studies have implicated Al as an exogenous factor to the development of anemia [4]. Rats treated with Al showed a decreased red blood cell count during the first month and worsened in the following months. Al can accumulate in the bone marrow led to osteomalacia. Elevated levels of Al in the body have been associated

with a reduction in osteoblasts and osteoclasts, as well as impaired bone marrow remodeling. Additionally, Al has the potential to impede hemoglobin (Hb) synthesis in the bone marrow, likely due to its inhibition of protein synthesis. This impairment in Hb synthesis can result in microcytic hypochromic anemia [4].

Toxic effects associated with Al exposure are diverse, in which one of the suspected contributors is oxidative stress [3]. *In vivo*, Al does not undergo redox reaction. However, reactive oxygen species (ROS), type of free radicals have been reported to increase during Al exposure [5]. Al induces cellular damage in multiple ways, such as excessive production of ROS and depletion of glutathione levels within the cell. These processes lead to mitochondrial dysfunction and activate caspases, ultimately resulting the cell death [6]. Free radicals are highly reactive and have deleterious effects on essential cell components such as proteins, DNA, and membrane lipids. The damage of cell-membrane polyunsaturated fatty acids (PUFA) caused by free radicals impairs cell structure and function [7]. Free radical-mediated lipid peroxidation of PUFA generates various bioactive aldehydes, such as malondialdehyde (MDA), which may form covalent adducts with cellular proteins. Many studies have reported that protein carbonylation resulting from bioactive aldehyde overproduction is associated with a broad spectrum of disorders [3, 8]. Therefore, these lipid peroxidation end products may serve as reliable markers for disease risk and progression [8]. Al can bind to a variety of amino acids or proteins firmly due to its substantial positive charges and short ionic radius, which induces conformational changes and inhibits protein degradation by protease, leading to apoptotic cell death [9].

Medicinal plants with antioxidant properties have been used by human to treat or prevent disorders caused by oxidative stress [10]. One such plant is *Nigella Sativa,* commonly relgarded as miracle herb, which belongs to the *Ranunculaceae* family. Numerous studies have revealed its broad spectrum of pharmacological potential [11].

N. sativa contains oils (mostly fixed oils and smaller amounts of volatile oils), carbohydrates, amino acids, peptides or proteins, vitamins, minerals, alkaloids such as saponins, and coarse fiber. The extract of *N. sativa* contains thymoquinone (TQ), nigellone, and α-hederin, which have demonstrated anti-histaminic, anti-immunoglobulin, anti-leukotrienes, anti-eosinophilic, and anti-inflammatory effects across various models [12]. TQ particularly exhibits anti-inflammatory and antioxidant effects [13], supporting immune function, cellular viability, and energy metabolism, which forms the basis for its broad range of health benefits. These benefits include safeguard against a variety of disorders, such as metabolic, cardiovascular, digestive, hepatic, renal, respiratory, reproductive, and neurological issues, and cancer [14]. TQ suppresses inflammatory factors such as nitric oxide (NO), nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and cyclooxygenase 2 (COX-2) by inhibiting IRAK-linked AP-1/NF-κB pathways [15]. The redox properties of TQ, along with its ability to cross biological barriers and enter intracellular spaces, suggest its potential role in preventing and/or treating a wide spectrum of diseases [16]. Thus, this study aimed to invistigate the role of *N. sativa* extract (NSE) in preventing the increase of serum MDA levels in rats subjected to AlCl3-induced oxidative stress.

MATERIALS AND METHODS

Sampling of *N. sativa*

Black cumin was considered as the study material that was obtained from the Batu Herbal Materia Medica Laboratory, Health Ministry of Batu, East Java, Indonesia, with letter number 074/478A/102.7/2019. At room temperature, two thousand grams of *N. sativa* seeds were extracted by maceration using 96% ethanol obtained from PT Graha Jaya Pratama Kinerja, Jakarta, Indonesia for 72 h. Subsequently, the filtrate and the residue were separated by filtration. The ethanol extract was evaporated using a rotary vacuum evaporator from PT. Indolab Utama, Cengkareng, Jakarta Barat, Indonesia to obtain a thick extract.

Animal study

This study was permitted by the Faculty of Veterinary Medicine's Institutional Review Board of the Ethics Committee, Universitas Airlangga, Surabaya, Indonesia, under ethical clearance number 2.KE.103.05.2019.

The study was involved with twenty-four Wistar male rats (*R. norvegicus*), aged 2–3 months, body weight (BW) ranging from 180 to 200 g. A randomized post-test-only control group was designed. The rats were provided pellet feed and mineral water *ad libitum*. NSE was dissolved in 0.5% Na-CMC obtained from PT Hab Chemical Indonesia (Semarang, Indonesia). The AlCl3 (Sigma Aldrich Co, St. Louis, MO, United States) was used for Al treatment.

Total four groups were considered in a randomization allocation such as K1 (control): supplied standard diet and water ad libitum for 36 days, K2: received AlCl3 100mg/kg BW daily for 21 days (days 16-36), with a standard diet and water ad libitum from day 1 to 36, K3: received NSE 200 mg/kg BW and AlCl3 100mg/kg BW daily for 21 days (day 16-36), with standard diet and water ad libitum from day 1 to 36, and K4: received the NSE 400 mg/kg BW for 15 days and day 16 – 36 (21 days) and AlCl3 100mg/kg BW daily for 21 days (day 16-36), with standard diet and water were given ad libitum since day 1 to 36. On day 36, the rats were euthanized using ketamine HCl from PT Dexa Medica, Palembang, Indonesia. Post euthanasia blood samples were collected from twenty-four Wistar rats.

Determination of MDA and Hb

The MDA was determined by considering of thiobarbituric acid reactive substance (TBARS) with a single beam UV-Vis spectrophotometer (Merck Bosco, Darmstadt, Germany) according to the manufacturer's instructions. The reagent of MDA was divided into 4 types: 2-thiobarbituric acid from Merck Manufacture, East Jakarta, Indonesia with catalog number 108180; Trichloroacetic acid from Milipore Manufacture, East Jakarta, Indonesia with catalog number: 100807; 1,1,3,3-tetraethoxypropane from Aldrich Manufacture, Jakarta, Indonesia with catalog number: 122-31-6; and hidroclorat acid 37% from Milipore Manufacture, East Jakarta, Indonesia with catalog number: 100317.

Hb measurements were obtained using the STARTUP cycle. During the whole blood cell (WBC) analysis, 0.52 mL of Lyse reagent was added to 2.05 mL of diluted blood in the WBC chamber. The lyse reagent, containing potassium ferricyanide and potassium cyanide (PT. Smart Lab Indonesia, Tangerang Selatan, Indonesia) that broke down the

red blood cell (RBC) membrane and released Hb within the RBC. The Hb level was measured using the ABX Micros 60 (Horiba Indonesia, Tangerang, Banten, Indonesia).

Data analysis

The data were first subjected to a normality analysis using the Shapiro-Wilks test and homogeneity analysis using the Levene Test. The data for BW, Hb, and MDA data were analyzed using an analysis of variance (ANOVA) to compare groups' means, followed by a least significant difference (LSD) test with a significant level $p < 0.05$. The Wilcoxon test was used to compare BW before and after treatment. Data analysis was performed using SPSS version 24.0.

RESULTS

Effect of *N. sativa* extract on body weight in AlCl3-treated rats

Table 1 shows that the K2 group had the lowest BW (171.5 ± 23.03 g) before treatment, while the K1 group had the highest BW (181.13 ± 28.75 g) before treatment. After treatment, the K1 group had the lowest (181.13 ± 28.75 g), and the K3 group had the highest BW (289.43 ± 53.06 g) Overall, we can conclude that the BW increased after treatment in rats.

ANOVA and LSD analysis showed no significant difference in BW among groups before treatment (p = 0.876). Additionally, the Kruskal-Wallis test revealed no significant difference in BW among groups after treatment (p = 0.078). However, using the Wilcoxon test, a significant difference (p = 0.0001) in rat BW before and after treatment was found (Table 1).

Table 1. Effect of NSE on BW in rats before and after treatments

BW (g)	Groups (Mean ± SD)				ANOVA (p value)	Wilcoxon (p value)
	K1	K2	K3	K4		
Before treatment	181.13 ± 28.75	171.5 ± 23.03	178.71 ± 24.89	179.56 ± 13.14	0.876	0.0001*
After treatment	242.5 ± 24.78	269.33 ± 48.29	289.43 ± 53.06	232.67 ± 17.79	0.078	

K1 (control): supplied standard diet and water, K2: received AlCl3 (100 mg/kg BW) only, K3: received NSE 200 mg/kg BW and AlCl3, and K4: received the NSE 400 mg/kg and AlCl3. *Significant, BW: Body weight; g: gram, NSE: *N. sativa* extract.

Effect of *N. sativa* extract on hemoglobin levels in AlCl3-treated rats

The value of Hb levels is summarized in Table 2. The Hb levels of all rats in groups K1, K2, K3 and K4 were 12.85 ± 0.26, 13.08 ± 0.33, 13.07 ± 0.25, and 12.92 ± 0.33, respectively. Statistical analysis using ANOVA and LSD revealed no significant differences among the control, AlCl3 -treated, and NSE-treated groups.

Table 2. Effect of NSE on Hb levels in rats

Parameter	Groups (Mean ± SD)				(p value)
	K1	K2	K3	K4	
Hb (g/dL)	12.85 ± 0.26	13.08 ± 0.33	13.07 ± 0.25	12.92 ± 0.33	0.934

K1 (control): supplied standard diet and water, K2: received AlCl3 (100 mg/kg BW) only, K3: received NSE 200 mg/kg BW and AlCl3, and K4: received the NSE 400 mg/kg and AlCl3. Hb, hemoglobin, NSE: *N. sativa* extract.

Effect of *N. sativa* extract on serum MDA levels in AlCl3-treated rats

The mean and standard deviation of MDA serum levels of all groups are summarized in Table 3. Notably, the NSE-treated groups exhibited a significant reduction in MDA levels compared to AlCl3 -treated group, highlighting the potential effectiveness of NSE in mitigating oxidative stress.

A comparison between K1 (the control group) with K2 revealed that significantly higher MDA levels were observed in K2, attributed to its exclusive exposure to AlCl3. These results suggest increased oxidative stress or lipid peroxidation in this group. Further comparisons between K2 and K3 (which received AlCl3 and NSE at a dosage of 200 mg/kg) showed that a reduction in MDA levels in K3 (Table 3). Similarly, comparing K2 with K4 (where AlCl3 and NSE were administered at a dosage of 400 mg/kg), also revealed a reduction in MDA levels in K4 (Table 3). Between K3 and K4, K4 exhibits the lowest MDA levels (Table 3), indicating that a higher dose of NSE may further reduce oxidative stress.

Table 3. Effect of NSE on serum MDA levels in rats

Parameter	Groups (Mean ± SD)				(*p* value)
	K1	K2	K3	K4	
MDA (nmol/mL)	19.84 ± 2.75	31.79 ± 5.37	20.85 ± 1.64	17.26 ± 3.26	0.01*

K1 (control): supplied standard diet and water, K2: received AlCl3 (100 mg/kg BW) only, K3: received NSE 200 mg/kg BW and AlCl3, and K4: received the NSE 400 mg/kg and AlCl3. *Significant, MDA: malondialdehyde; NSE: *N. sativa* extract. *Significant.

DISCUSSION

Al is present in the soil, various foods, household products, and drinking water, posing human health risks [1,3]. Al exposure is believed to cause detrimental effects on the human body [3,5,8,17,18]. Numerous studies have discussed the connection between AlCl3 exposure and reduced weight in rats. In one study, rats given 900 mg/kg of AlCl3 via oral intubation for twenty-eight days showed significant weight loss from day 22 to day 28, a result not observed in rats given 100 and 300 mg/kg of AlCl3 [19]. Another study by Parasuraman *et al*. (2020) demonstrated that intraperitoneal injection of AlCl3 (10 mg/kg BW) for twenty-eight days caused weight reduction in rats [20]. Interestingly, our present study showed the opposite result, with no significant difference observed between groups after treatment, despite the fact that mean BWs in all groups were higher than before treatment. Thus, the current study suspected that different combinations of AlCl3 doses, application techniques, duration of exposure, and *ad libitum* feeding contributed to the inconsistent result compared to previous studies.

Our study on Hb concentration showed no significant difference among groups. This finding is consistent with a prior study by Aftab *et al*. (2018), where Albino rats-treated with AlCl3 80 mg/kg of BW for 16 days via oral administration showed no effect on blood count parameters, including Hb levels [18]. Similar outcome was observed in another study where three weeks of AlCl3 administration (dose of 34 mg/kg BW) combined with food did not alter Hb levels [2, 21]. On the contrary, a significant decrease in Hb concentration was reported in male rats fed a diet supplemented with 726 mg/kg BW of AlCl3 for six weeks [22]. A corresponding result was found in a study where 100 mg/kg BW of AlCl3 was given orally to female rats for twenty-eight days caused a significant reduction in RBCs count and Hb levels($P<0.05$) [23].

Another study demonstrated a similar effect, showing the chronic exposure to Al (as aluminum hydroxide) in rats from weaning with a dose of 80 mg/kg BW administered solution (0.3-0.5 ml) intraperitoneally three times a week induced microcytic anemia. Lower RBC counts and hematocrit (Hct) were found in the first month of treatment and mean corpuscular Hb (MCH) levels decreased starting in the second month, indicating microcytosis. Hct and Hb levels also decreased during the third and fourth months. Variations between study outcomes could be due to differences in the experimental animals (including their age at the time of the exposure), the molecular form of Al, dosage, and duration of application [2, 21, 23].

Although this study's findings suggest that Al had no effect on the Hb level, numerous studies have shown *N. sativa* improves the hematological profile in anemia caused by environmental agents. A study by Mohamed and Awad (2008) showed a significant increase in Hb levels in both Al and NSE groups, with a notable reduction in Hb levels in the *N. sativa* group compared to control group [24]. Similar findings have been reported in studies of other environmental agents. Elshamy *et al*. (2019) found that treatment with *N. sativa* oil significantly improved all the tartrazine-induced hematological disturbances in rats [25]. Afghani *et al*. (2020) demonstrated that Wistar rats exposed to cigarette smoke given 400 mg/kg of NSE showed a significant improvement in erythrocytes and Hb levels [26].

Al exposure has also been linked to oxidative stress. The mechanisms are diverse, with one hypothesis suggesting that Al generates an Al-superoxide complex that promotes the Fenton reaction, potentially increasing oxidative stress in biological systems[24]. Another study stated Al does not undergo redox reactions *in vivo*, yet free radical level still increased [5]. Several studies have also reported that Al inactivates antioxidant defense enzymes, such as superoxide dismutase (SOD) as well as catalase (CAT) [2,25].

Lipid peroxidation is a key process of in oxidative stress, and one of the major and end products is MDA, which is commonly measured to estimate oxidative stress. Measuring circulating MDA levels is a standard method to estimate oxidative stress status [8]. Examining MDA levels is also known to be easier than other biomarkers for lipid peroxidation. This is based on the fact that a significant portion of the circulating MDA is attached to plasma proteins; and acid or alkaline treatments can hydrolyze this fraction [8, 26].

The outcome of our research revealed significant differences in MDA levels between control, AlCl3-treated, and NSE-treated groups. The highest MDA levels were observed in the AlCl3-treated group. Significantly high MDA levels found in this group could be linked to the biological membrane damage by lipid peroxidation. This finding aligns with previous studies showing that AlCl3 treatment induces oxidative stress and increases MDA level [25, 27, 28].

The lowest MDA levels were found in group K4, which received the highest dose of NSE (400 mg/kg BW). This finding is consistent with prior studies showing that *N. sativa* can reduce MDA levels and lipid peroxidation end products. Research by Bouasla *et al.* (2014) found that NS oil improved the MDA level in rats undergoing oxidative stress induced by AlCl3 [2]. Other studies have shown that NS oil reduces MDA levels in streptozotocin (STZ)-induced diabetic rats and dyslipidemic rats treated with NS seed extract [29, 30]. Similarly, NSE has been shown to prevent the increase in MDA levels in piroxicam-induced rats in a dose-dependent manner [31].

Numerous investigations have been highlighted the protective benefit of *N. sativa* against oxidative stress. *N. sativa* acts through various mechanisms, such as scavenging free radicals , inhibiting lipid peroxidation, and enhancing antioxidant enzyme activity,

such as glutathione peroxidase, glutathione transferase, superoxide dismutase, and catalase [32, 33].

The effect of *N. sativa* in blocking lipid peroxidation seems to be due to its phenolic compounds such as TQ, flavonoids and vitamins like ascorbic acid, which act as antioxidants. It functions as a scavenger of several reactive oxygen species, such as hydroxyl radicals, superoxide anion, and chelate metal ions [34, 35]. Of all the phenolic compounds contained in *N. sativa*, TQ has been proven in numerous studies to exhibit various potentials, including as an antioxidant. Therefore, TQ is considered as active component of *N. sativa* [16, 33, 35, 36].

The *N. sativa* and its active component TQ as antioxidants linked to their efficiency to scavenge free radicals and increase antioxidant enzyme activity [32, 36, 37]. The redox property of TQ quinine structure and its ability to cross morphological barriers unrestrictedly allows TQ to access subcellular compartments easily [16, 34].

This antioxidant potential of *N. sativa* and TQ also provides more comprehensive benefits, including reducing nephropathy-related toxicity in nephrotic syndrome and presenting pancreatic β-cells in STZ-induced diabetes rats [29, 34]. *N. sativa* has also been shown to enhance T cell responses in rats infected with cytomegalovirus through antioxidant mechanisms [38]. Furthermore, TQ has been explored frequently for its potential in malignancy treatment. For instance, in an experiment study by Sayed-Ahmed et al., TQ supplementation was found to prevent liver cancer initiation by sustaining antioxidant enzyme expression and activity, thus lowering oxidative stress [39].

CONCLUSIONS

The MDA level was found to be lower in the NSE-treated group compared to the AlCl3-treated group, while no significant difference was found between the NSE-treated and control groups. Therefore, NSE may have a beneficial effect in preventing oxidative stress and may consequently inhibit disease development and progression. It is believed that further studies are required to fully understand the potentials and mechanisms of NSE in preventing oxidative stress triggered by Al exposure, particularly regarding its effects on the antioxidant defense system and other markers related to oxidative stress.

ACKNOWLEDGMENTS

Our study has several limitations, one of which is that the parameters examined in this research need to be expanded and deepened to confirm the results more clearly. This study was supported by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (Grant number: 133/UN3.1.1/KD/2019).

AUTHOR CONTRIBUTIONS

Conceptualization and design of the research, EQ and CKDW; methodology, SK, LL; experimental investigation, EQ, LL and IH; sample resources, SK, R; writing–original draft preparation, EQ and CDKW; writing–review and editing, CDKW, SK, GIP, and ANMA; supervision, EQ, IH, and GIP. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] Krewski D, Yokel RA, et al. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. Journal of Toxicology and Environmental Health, Part B. 2007;10:1-269.

[2] Bouasla I, Bouasla A, et al. *Nigella sativa* oil reduces aluminium chloride-induced oxidative injury in liver and erythrocytes of rats. Biol Trace Elem Res. 2014;162:252-61.

[3] Igbokwe IO, Igwenagu E, et al. Aluminium toxicosis: A review of toxic actions and effects. Interdiscip Toxicol. 2019;12:45-70.

[4] Rahimzadeh MR, Rahimzadeh MR, et al. Aluminum poisoning with emphasis on its mechanism and treatment of intoxication. Emerg Med Int. 2022;2022:1480553.

[5] Flora SJS, Mehta A, et al. Aluminum-induced oxidative stress in rat brain: Response to combined administration of citric acid and hedta. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2003;134:319-28.

[6] Laabbar W, Abbaoui A, et al. Aluminum induced oxidative stress, astrogliosis and cell death in rat astrocytes, is prevented by curcumin. J Chem Neuroanat. 2021;112:101915.

[7] Ayala A, Muñoz MF, et al. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438.

[8] Ito F, Sono Y, et al. Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation. Antioxidants. 2019;8.

[9] Kawahara M, Kato-Negishi M. Link between aluminum and the pathogenesis of alzheimer's disease: The integration of the aluminum and amyloid cascade hypotheses. Int J Alzheimers Dis. 2011;2011:276393.

[10] Sharifi-Rad M, Anil Kumar NV, et al. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. Frontiers in Physiology. 2020;11.

[11] Majumdar M, Samanta A, et al. Study of wound healing activity of different formulations of *Nigella sativa* seed extract. Research J Pharm and Tech. 2016;9:2097-105.

[12] Koshak A, Koshak E, et al. Medicinal benefits of *Nigella sativa* in bronchial asthma: A literature review. Saudi Pharm J. 2017;25:1130-6.

[13] Sutrisna E, azizah T, et al. Potency of nigella sativa linn. Seed as antidiabetic (preclinical study). Research Journal of Pharmacy and Technology. 2022;15:381-4.

[14] Hannan MA, Rahman MA, et al. Black cumin (*Nigella sativa* l.): A comprehensive review on phytochemistry, health benefits, molecular pharmacology, and safety. Nutrients. 2021;13:1784.

[15] Hossen MJ, Yang WS, et al. Thymoquinone: An irak1 inhibitor with in vivo and in vitro anti-inflammatory activities. Sci Rep. 2017;7:42995.

[16] Kurkin V, Mubinov A, et al. Comparative research of fatty acid composition and volatile components of fatty oils from seeds of nigella sativa and argania spinosa. Research Journal of Pharmacy and Technology. 2021;14:1586-90.

[17] Klotz K, Weistenhöfer W, et al. The health effects of aluminum exposure. Dtsch Arztebl Int. 2017;114:653-9.

[18] Aftab MN, Ali A, et al. Effect of AlCl3 mediated toxicity on the hemato-biochemical profile of adult male albino mice. Pakistan Journal of Zoology. 2018;50:1549-52.

[19] Lim JO, Jung TY, et al. Evaluation of 28-day repeated oral dose toxicity of aluminum chloride in rats. Drug Chem Toxicol. 2022;45:1088-97.

[20] Parasuraman S, Qin B, et al. Effect of epigallocatechin gallate on aluminum chloride-induced changes in behavior, biochemical parameters, and spermatogenesis of sprague-dawley rats. Beni-Suef University Journal of Basic and Applied Sciences. 2020;9:1-10.

[21] Tiwari P, Jena S, et al. *Nigella sativa*: Phytochemistry, pharmacology and its therapeutic potential. Research Journal of Pharmacy and Technology. 2019;12:3111-6.

[22] Mahdy K, Farrag AR. Amelioration of aluminum toxicity with black seed supplement on rats. Toxicological & Environmental Chemistry. 2009;91:567-76.

[23] Ige SF, Aiyeola AW. Effect of aqueous *Allium cepa* extract from red onion on aluminum chloride-induced anemia in female rats. Journal of Applied Sciences and Environmental Management. 2018;21:1286-90.

[24] Rakhmawatie MD, Afghani NMA, et al. Effect of essential oil of black cumin (*Nigella sativa*) on hematological profiles and total cholesterol levels of Wistar rats exposed by cigarette smoke. Indonesian Journal of Pharmacology and Therapy. 2020;1(1):7-14.

[25] Elshamy RBMM, Amer AI. Study of the protective effect of *Nigella sativa* oil on tartrazine-induced hematological disorders in rats. The Medical Journal of Cairo University/the Medical Journal of Cairo University. 2019;87(12):4661–4670.
[26] Mohamed MA, Awad SM. Effect of *Nigella sativa* oil on some hematological values in aluminum-treated rats. Australian Journal of Basic and Applied Sciences. 2008;2(4):1157-1164.
[27] Benyettou I, Kharoubi O, et al. Aluminium-induced behavioral changes and oxidative stress in developing rat brain and the possible ameliorating role of omega-6/omega-3 ratio. Journal of Biological Sciences. 2017;17:106-17.
[28] Rui D, Yongjian Y. Aluminum chloride induced oxidative damage on cells derived from hippocampus and cortex of icr mice. Brain Res. 2010;1324:96-102.
[29] Kanter M, Coskun O, et al. Effects of *Nigella sativa* on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats. Anat Rec A Discov Mol Cell Evol Biol. 2004;279:685-91.
[30] Susilowati R, Ainuzzakki V, et al. The efficacy of *Nigella sativa* L. extracts to reduce cardiovascular disease risk in diabetic dyslipidemia. AIP Conference Proceedings. 2019;2120.
[31] Kurniasari S. Efek pemberian ekstrak jintan hitam (*Nigella sativa*) terhadap kadar malondialdehyde dan superoksida dismutase pada hepar tikus (*Rattus norvegicus*) yang diinduksi piroxicam. Jurnal Fisika Flux Jurnal Ilmiah Fisika FMIPA Universitas Lambung Mangkurat. 2021;18:133-8.
[32] Gholamnezhad Z, Keyhanmanesh R, et al. Anti-inflammatory, antioxidant, and immunomodulatory aspects of *Nigella sativa* for its preventive and bronchodilatory effects on obstructive respiratory diseases: A review of basic and clinical evidence. Journal of Functional Foods. 2015;17:910-27.
[33] Forouzanfar F, Hosseinzadeh H. Chapter 11 - protective role of nigella sativa and thymoquinone in oxidative stress: A review. In: Preedy VR, Watson RR, editors. Nuts and seeds in health and disease prevention (second edition): Academic Press; 2020. p. 127-46.
[34] Badary OA, Taha RA, et al. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol. 2003;26:87-98.
[35] Ahmad MF, Ahmad FA, et al. An updated knowledge of black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. J Herb Med. 2021;25:100404.
[36] Alenzi F, Altamimi A, et al. Antioxidant properties of nigella sativa. Journal of Molecular and Genetic Medicine. 2013;7.
[37] Ramadan MF, Kroh LW, et al. Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. J Agric Food Chem. 2003;51:6961-9.
[38] Salem ML. Immunomodulatory and therapeutic properties of the nigella sativa l. Seed. Int Immunopharmacol. 2005;5:1749-70.
[39] Sayed-Ahmed MM, Aleisa AM, et al. Thymoquinone attenuates diethylnitrosamine induction of hepatic carcinogenesis through antioxidant signaling. Oxid Med Cell Longev. 2010;3:254-61.