Article
JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)
Vol. 33 ; No.2 | 2025

Effects of Aqueous Extract of Black Cumin on Cholesterol and Total Protein Levels (albumin and globulin) in Male Healthy Rabbits

Ekhlas Hatem AL-Asadi[1], Ban Jabber Edan[2], Hawraa Hamid Hussein[3]

[1]Department of physiology,College of Medicine,University of Babylon
ekhlasasa@uobabylon.edu.iq
[2]Department of physiology,College of Medicine,University of Babylon
Med.ban.edan@uobabylon.edu.iq
[3]Department of physiology,College of Medicine,University of Babylon
Med278.hawraa.hamid@uobabylon.edu.iq

*Corresponding Author email: ekhlasasa@uobabylon.edu.iq

Accepted: 24/6/2025 **Published:** 30/6/2025

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ABSTRACT

Background: Black cumin (Nigella sativa L.) is a medicinal and aromatic herb from the Ranunculaceae family, traditionally used for its health benefits. It supports immune function, enhances brain activity, and offers protection against various chronic diseases. This study aimed to investigate the effects of black cumin on cholesterol and total protein levels (albumin and globulin) in healthy male rabbits.

Materials and Methods: Twenty-four healthy male New Zealand rabbits (5–6 months old, weighing 1200–1500 g) were divided into six groups. One group served as the control, while the other five received increasing doses of aqueous black cumin extract: 80, 130, 180, 230, and 280 mg/kg body weight per day. Blood samples were collected to assess serum cholesterol, total protein, albumin, and globulin levels after dosing.

Results: Cholesterol levels significantly decreased in the 80 and 130 mg/kg groups, while higher doses (180–280 mg/kg) caused a significant increase. Total protein levels were significantly elevated in groups C, D, and E, with the highest in group E (90.4 ± 1.4) compared to the control (70.6 ± 0.85). Globulin also increased in group E (280 mg/kg), while albumin levels showed no significant changes.

Conclusion: Low doses of black cumin reduced cholesterol levels, whereas high doses increased .them Total protein and globulin levels increased, while albumin remained unchanged.

Keywords: Black cumin seed; cholesterol; Total protein; Albumin; Globulin.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

INTRODUCTION

The herbaceous plant known as black cumin (*Nigella sativa L.*) is a member of the Ranunculaceae family and has fragrant and therapeutic qualities. It can reach a 20–30 cm height and is grown yearly [1]. Due to its phenolic components, black cumin is cultivated in several climates and geographical regions, with its seeds used across various industries, including food, medicine, and cosmetics.

Although black cumin seed (BCS) has a very faint scent, it aids in digestion and the removal of intestinal and stomach gas[2].

Black seeds, sometimes known as black cumin, are a medicinal plant used in traditional medicine. Its benefit to the immune system, its goodness for brain function, and its importance for protecting the body from many chronic diseases, like type 2 diabetes and diseases of the heart. It contains minimal levels of vitamins C, A, E, and B [3]. Black seeds are made up of around 20% carbs, 45% fat, and20% protein. They also appear to provide some phosphorus, potassium, ,magnesium and calcium [4]. The ripe seeds are the used part of Nigella sativa. They are used in treating coughs, chest ;diseases, and rheumatism as an intestinal ;remedy as a repellent of infectious ;gases and as a diuretic and menstrual diuretic. Laboratory studies of their medical importance and pharmacological properties have found that they have a synergistic effect with antibiotics along with gentamicin and erythromycin [5]. Nigella sativa also has an effect on the natural immunity of humans, as giving it at a dose of 1 gram daily for five weeks is a strong stimulus to the human immune system.

According to some earlier research, the antioxidant elements in aromatic and medicinal plants' structures have the ability to scavenge free radicals and stop the negative effects of oxidative stress. [3]

Antioxidants are plant components that protect your cells from harm and illness. Nigella sativa seems to be particularly high in antioxidants from the polyphenol, tocopherol, terpenoid, and terpene families. Thymoquinone, an antioxidant from the terpene and terpenoid families, is by far the most prevalent [5]. Experts believe that this active component is responsible for the majority of Nigella sativa's reported health advantages [6].Nigella sativa seems to include alkaloids and phytosterols, two kinds of helpful plant compounds. Phytosterols have nutritional properties such as the capability of lowering cholesterol, antitumor, anticancer, and antioxidant properties besides their role in the detection of vegetable oil adulteration [7].

Studies in the poultry industry have largely concentrated on performance and serum parameters, despite the fact that research examining the effects of BCS on various parameters has gained prominence recently [8],[9].

The current study aims to show the affects of black cumin on cholesterol and total protein levels (albumin and globulin) in male rabbits.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

MATERIALS AND METHODS

Twenty-four healthy male New Zealand rabbits were used, with ages ranging between (6-5) months and weights ranging between (1200-1500). Using galvanized wire cages with automated feeders and nipple drinkers, the rabbits were housed and fed for four weeks, split evenly between an introductory period of two weeks and an experimental period of two weeks. Feed and water were provided ad, libitum and then they were randomly divided into groups that were dosed using the gastric tube as follows: a control group was dosed with the physiological solution (0-9 % NaCl), while the remaining five groups were dosed with aqueous extract of black cumin. The dosage continued for 2 weeks, blood samples were drawn after a 12-hour fast, and cholesterol levels, total protein levels, albumin, and globulin were measured for the samples.

Blood samples were individually collected from each rabbit in gel tubes to estimate blood parameters. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at 18 °C until analysis. Blood serum was used for the determination of some biochemical ,parameters including ,cholesterol according to Mei et al. [10], total ,protein according to Sempos et a [11]. albumin according to [12]. and globulin according to according to Kumar et al [13]. by subtracting the albumin value from the total protein value for all samples.

Statistical analysis

Data was processed using a one-way analysis of variance (generalized linear model; GLM) software program (SAS, 2010). Significant differences were defined at $p < 0.05$ applying the Duncan multiple range test [14].

RESULTS AND DISCUSSION

1. The effect of black seed on blood serum cholesterol levels

Figure (1) indicates that there are significant differences ($p>0.05$) in the level of serum cholesterol between the control group and the rest of the groups, it was found that the best dose to lower cholesterol the Aand B group. While an increase in cholesterol levels was found with increasing dose, it is possible that increasing the intake of black seed may cause an increase in cholesterol and be harmful.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

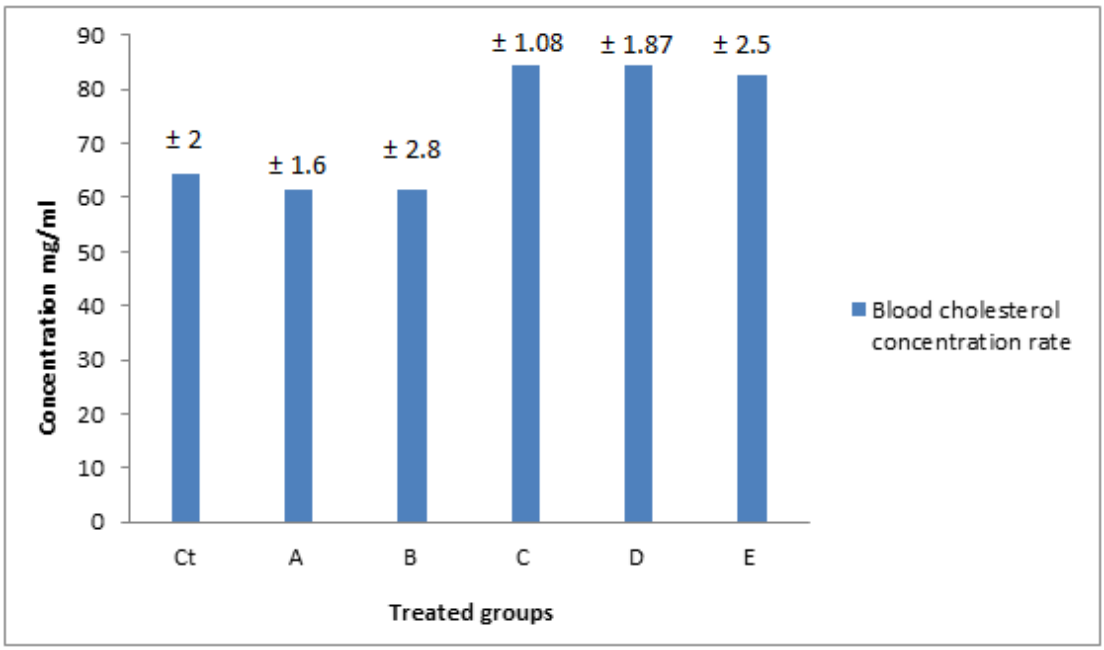

Figure (1) The effect of giving increasing doses of aqueous black seed extract (mg/kg) on serum cholesterol levels (mg/100 mL of blood). Ct (control), [A (80) B (130), C (180), D (230) and E (280)]mg/kg body weight in sequences.

This might be due to cholesterol being susceptible to the feedback mechanism. Another research determined that using black cumin supplements may enhance lipid profiles and prevent CVDs in both healthy persons and hyperlipidemic patients. The specific mechanisms of NS's lipid-modifying actions are unknown, they may be connected with suppression of intestinal cholesterol absorption, reduced hepatic cholesterol production, and up-regulation of LDL receptors [15].

According to Tousson et al [16]. the feeding meal supplemented with Nigella sativa lowers total lipids, cholesterol, and triglycerides in rabbit. Also, The results of Shewita et al. [17] found that serum cholesterol, triglyceride and visible fat were significantly decreased with Nigella sativa supplementation.as research has proven that black seed contains cholesterol in its chemical composition. When taken through the digestive system, it leads to an increase in cholesterol in the blood, which in turn inhibits the enzymes responsible for making cholesterol, so the concentration of cholesterol in the blood remains almost constant [18].

2. The effect of black seed on total serum protein level

Figure (2) indicates a significant ($p>0.05$) increase in the level of total protein in the treated groups compared with the control group. It was noted that there was an increase in the concentration of total proteins when the given dose was increased.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

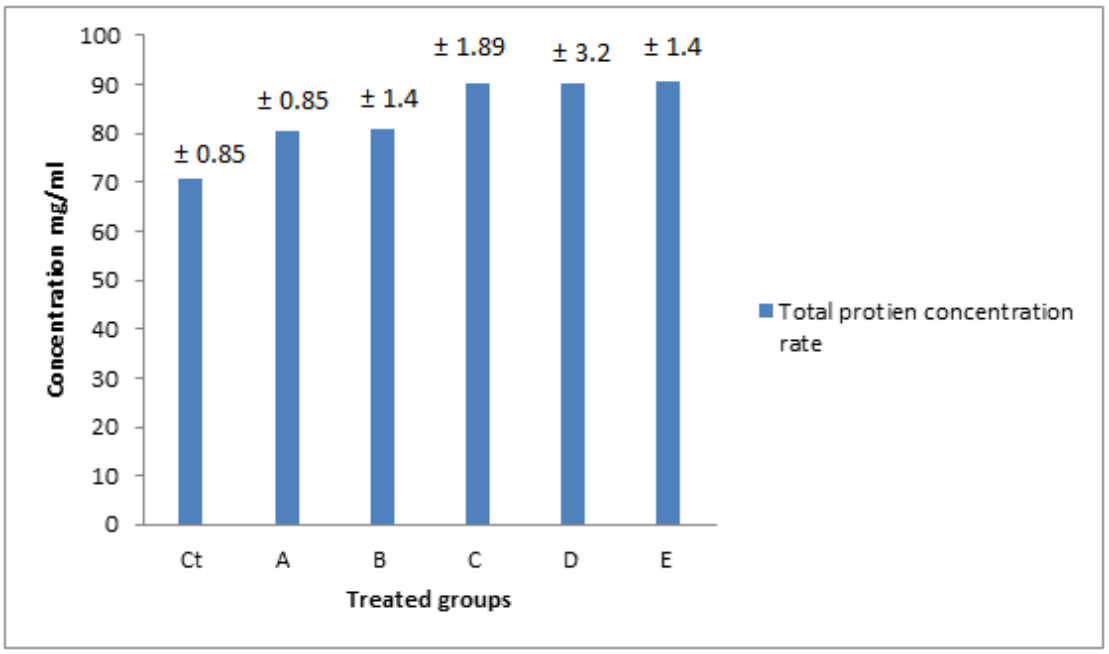

Figure (2) The effect of giving increasing doses of the aqueous black seed extract on blood total protein levels, mg/100 ml of blood. Ct (control), [A (80) B (130), C (180), D (230) and E (280)]mg/kg body weight in sequences .

The reason is attributed to the fact that black seed contains essential amino acids in its chemical composition, which are considered the basic nucleus for building total proteins in the body. It was noted that the increase in the level of total protein was increasing with the increase in the dose due to the increase in amino acids when the dose increased [19],[20].

Another study found that the decrease in total protein was consistent with an overall mean decrease of albumin concentration [21]. Serum total protein levels in the group receiving[22] found that black seed oil supplements dropped significantly and treatment-related mean total protein levels showed a reduction of roughly 12.55%, albumin levels had a mean reduction of 25.98% overall, which was consistent with a decrease in total protein that was also considerable, black seed oil treatment improved the Gama-globulin levels, resulting in a 23.68% rise in the overall mean compared to the control.

According to [16] the feeding meal supplemented with Nigella sativa raised plasma total proteins, albumin, and globulin in the rabbit. In a study conducted by [23] male rabbits were given black seed oil orally at 5ml/kg body weight/day for 60 days and found that substantial rise in plasma total proteins, albumin, and globulin. From this perspective, adding black seed oil to the diet enhanced total protein [24] The protein in black seeds is composed of fifteen amino acids, including eight of the nine necessary amino acids. The majority of trace elements, including calcium, iron, sodium, and potassium, are also found in black seeds and serve as vital cofactors for a variety of enzyme functions [25] Due to its low cost compared to other protein sources, nigella seeds are a good source of protein for farm animals to consume (30% or more). Therefore, due to its high basic protein and fat content, black seed may be used as an excellent energy and protein source for animal nutrition [26].

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

3. The effect of black seed on the level of albumin in blood serum

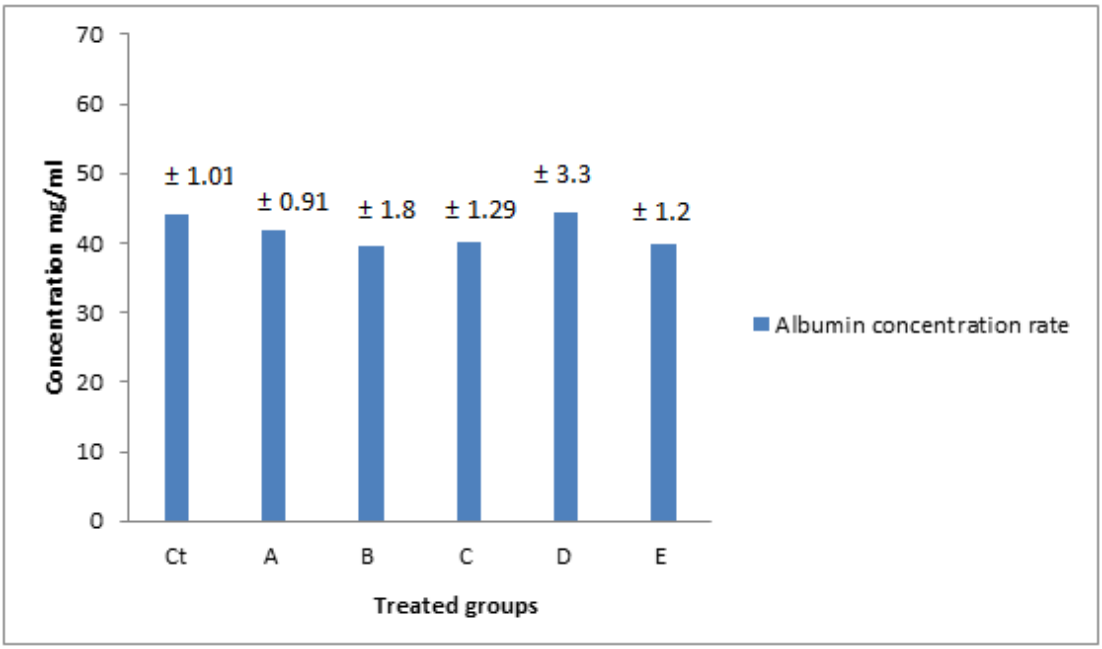

Figure (3) The effect of giving increasing doses of the aqueous extract of black seed extract on serum albumin levels, mg/100 ml of blood. Ct (control), [A (80) B (130), C (180), D (230) and E (280)]mg/kg body weight in sequences ..

As for figure (3), there were no significant differences($p>0.05$) between the control group and treated groups, albumin maintained its normal level in the body because it only changes in pathological conditions such as dehydration [27]. This explains the absence of a significant difference in the level of albumin in the blood serum.

Another research study found that blood total protein, albumin, and globulin levels increased significantly in rabbit groups given diets containing Nigella sativa or garlic as assessed to the control treatment [24]. [25] observed similar results, including a rise in serum albumin. A common mechanism seems to influence albumin, perhaps resulting in enzyme induction by Nigella sativa [28]

4. The effect of black seed on the level of blood serum globulin

An increase in the level of globulin is noted in figure (4) for the groups dosed with black seed versus the control group a significant difference ($p>0.05$) increase in the level of globulin in the treated groups compared with control group.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

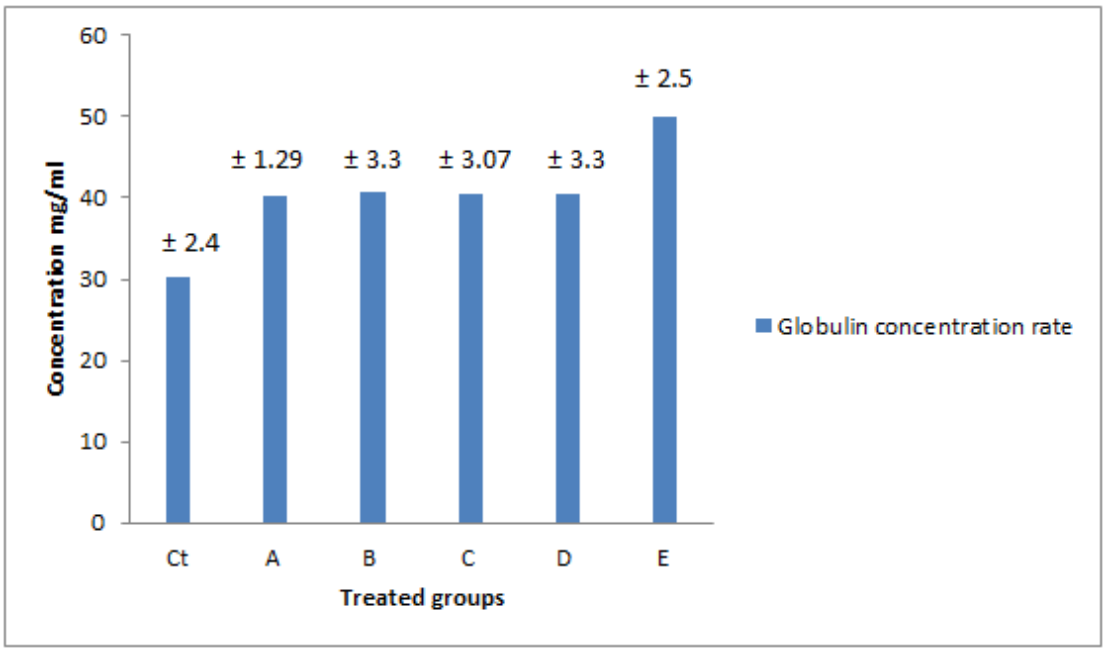

Figure (4) The effect of giving increasing doses of the aqueous extract of black seed on serum globulin levels, mg/100 mL of blood. Ct (control), [A (80) B (130), C (180), D (230) and E (280)]mg/kg body weight in sequences ..

This rise might be attributed to the fact that an increase in serum globulin coincides with an increase in total proteins [29] ,or the reason may be an increase in the two types (Beta & Alpha) responsible for transporting proteins within the blood circulation. This is consistent with what was found by Kadim,et.al. [30] where it was found that giving black seed leads to an increase in serum gamma-glutamate.Mahmoud,et.al.[30] demonstrate that the antioxidant activity, stimulation of cytokine production, increase in CD8 expression, and decrease in splenic apoptosis of Nigella sativa seeds lead to immunostimulant effects. In their study, Gandhi ,et.al. [31] found that certain bioactive components of N. sativa affect inflammatory and immunomodulatory mediators, including TNF-α, IFN-γ, NF-kB, COX, LOX, TGF-β, interleukins, and immunoglobulin levels. N. sativa is also known to impact cyclooxygenase (COX), lipoxygenase (LOX), and transforming growth factor beta (TGF-β). Also, Habeeb,et.al. [21] found that black seed oil treatment improved the Gama-globulin levels, resulting in a 23.68% rise in the overall mean compared to the control. According to Tousson,et.al. [16], the feeding meal supplemented with Nigella sativa raised in serum globulin in the rabbit. In a study conducted by Umar et al. (2018), male rabbits were given black seed oil orally at 5ml/kg body weight/day for 60 days and found a substantial rise in plasma total proteins, albumin, and globulin.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

CONCLUSION

Administration of high doses of black seed extract led to a highly significant elevation in cholesterol levels in rabbits, whereas lower doses were associated with a reduction in cholesterol. Additionally, total protein and globulin concentrations showed an increase, while albumin levels remained unchanged.

Conflict of interests.

There are non-conflicts of interest.

References

[**1**] A. A. Ibrahim, E. S. A. Razik, S. Abd-Ellatif, and S. Ramadan, "Nigella sativa and stressful conditions," in Medicinal Plant Responses to Stressful Conditions, CRC Press, 2023, pp. 223–242.

[2] M. Haque et al., "Nigella sativa: A promise for industrial and agricultural economic growth," in Black Seeds (Nigella sativa), 2022, pp. 439–460.

[3] N. M. P. Maideen et al., "An overview of conventional and black cumin seeds (Nigella sativa) therapy in the management of Nipah viral infection," Infect. Disord. Drug Targets, vol. 24, no. 2, pp. 77–86, 2024.

[4] T. Capcanari, E. Boaghe, and C. Negoiţa, "Hemp (Cannabis sativa L.) seeds nutritional aspects and food production perspectives: A review," Food Systems, pp. 52–58, 2024.

[5]G. A. E. Elmowalid et al., "Nigella sativa extract potentially inhibited methicillin resistant Staphylococcus aureus induced infection in rabbits: potential immunomodulatory and growth promoting properties," Animals, vol. 12, no. 19, p. 2635, 2022.

[6]Z. Zhu et al., "Use of integrative, complementary, and alternative medicine in children with epilepsy: A global scoping review," Children, vol. 10, no. 4, p. 713, 2023.

[7]E. Alves, A. Simoes, and M. R. Domingues, "Fruit seeds and their oils as promising sources of value-added lipids from agro-industrial byproducts," Crit. Rev. Food Sci. Nutr., vol. 61, no. 8, pp. 1305–1339, 2021.

[8]B. Sevim et al., "The effect of adding different levels of black cumin (Nigella sativa L.) seed to the feed on performance, serum parameters and reproductive hormones in male Japanese quails," Osmaniye Korkut Ata Univ. J. Sci., vol. 4, no. 3, pp. 441–451, 2021.

[9]S. Behlul et al., "The effect of adding different levels of black cumin (Nigella sativa L.) seed to the feed on performance, serum parameters and reproductive hormones in male Japanese quails," Osmaniye Korkut Ata Univ. J. Sci. (Online), vol. 4, no. 3, 2021.

[10]L. Mei et al., "Structural changes and cholesterol-lowering in denatured whey protein isolate: Malic acid combined enzymolysis," Food Hydrocoll., vol. 127, p. 107502, 2022.

[11]C. T. Sempos et al., "Measurement and quality assessment of total and free 25-hydroxyvitamin D," in Feldman and Pike's Vitamin D, Academic Press, 2024, pp. 1085–1113.

[12]J. F. Xu, Y. S. Yang, A. Q. Jiang, and H. L. Zhu, "Detection methods and research progress of human serum albumin," Crit. Rev. Anal. Chem., vol. 52, no. 1, pp. 72–92, 2022.

[13] V. Kumar and K. D. Gill, "To estimate the amount of total protein and albumin in serum and to find A/G ratio," 2018.

[14]L. Mei et al., "Structural changes and cholesterol-lowering in denatured whey protein isolate," Food Hydrocoll., vol. 127, p. 107502, 2022.

[15]A. Tavakkoli, V. Mahdian, B. M. Razavi, and H. Hosseinzadeh, "Review on clinical trials of black seed (Nigella sativa) and its active constituent, thymoquinone," J. Pharmacopuncture, vol. 20, no. 3, p. 179, 2017.

[16]E. Tousson, M. El-Moghazy, and E. El-Atrsh, "The possible effect of diets containing Nigella sativa and Thymus vulgaris on blood parameters and some organs structure in rabbit," Toxicol. Ind. Health, vol. 27, no. 2, pp. 107–116, 2011.

[17]R. S. Shewita and A. E. Taha, "Effect of dietary supplementation of different levels of black seed (Nigella sativa L.) on growth performance, immunological, hematological and carcass parameters of broiler chicks," World Acad. Sci. Eng. Technol., vol. 77, pp. 788–794, 2011.

[18]A. R. Lauridsen et al., "Why make it if you can take it: review on extracellular cholesterol uptake and its importance in breast and ovarian cancers," J. Exp. Clin. Cancer Res., vol. 43, no. 1, p. 254, 2024.

[19]J. Tomaszewska-Gras, "Chemical composition and structure of foods," in Chemical and Functional Properties of Food Components, CRC Press, 2023, pp. 13–38.

[20]R. Tagawa et al., "Dose–response relationship between protein intake and muscle mass increase: a systematic review and meta-analysis," Nutr. Rev., vol. 79, no. 1, pp. 66–75, 2021.

[21]A. A. Habeeb et al" ,.Benefits of adding black seed oil as a natural antioxidant in the diet of male adult rabbits," Ind. J. Agric. Life Sci., vol. 3, no. 6, pp. 9–22, 2023.

[22]M. J. Murtala, "Hepato-renal protective effects of Nigella sativa oil in cyclophosphamide-induced toxicity," 2024.

[23]Z. Umar et al., "Effects of oral administration of black seed (Nigella sativa) oil on histomorphometric dynamics of testes and testosterone profile in rabbits," Pak. J. Pharm. Sci., vol. 30, no. 2, pp. 531–536, 2017.

[24]A. J. Mohammad, "Role of black seed oil (Nigella sativa) and olive oil combination on serum rabbit treated with cholesterol," Int. J. Sci. Res., vol. 4, no. 7, pp. 654–658, 2015.

[25]K. Sharma, A. Tayade, J. Singh, and S. Walia, "Bioavailability of nutrients and safety measurements," in Functional Foods and Nutraceuticals, 2020, pp. 543–593.

[26]B. S. Obeidat and A. M. Alqudah, "Black cumin meal (Nigella sativa) as an alternative feed resource during the suckling period of Awassi ewes," Anim. Feed Sci. Technol., vol. 306, p. 115820, 2023.

[27]A. Sheinenzon et al., "Serum albumin levels and inflammation," Int. J. Biol. Macromol., vol. 184, pp. 857–862, 2021.

[28]P. Balyan et al., "Monosaccharide-mediated glycoxidation of bovine serum albumin and its prevention by Nigella sativa," ACS Omega, vol. 9, no. 40, pp. 41722–41731, 2024.

[29]S. Alfonso et al., "Comparative analysis of blood protein fractions in two Mediterranean farmed fish," BMC Vet. Res., vol. 20, no. 1, p. 3, 2024.

[30]H. S. Mahmoud et al., "The effect of dietary supplementation with Nigella sativa mediates immunological function in male Wistar rats," Sci. Rep., vol. 11, no. 1, p. 7542, 2021.

[31]G. R. Gandhi et al., "Anti-inflammatory natural products modulate interleukins in IBD," J. Pharm. Anal., 2023.

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

ISSN: 2312-8135 | Print ISSN: 1992-0652

info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

الخلاصة:

الخلفية :حبة البركة أو الكمون الأسود (Nigella sativa L.) نبات عشبي ذو خصائص طبية وعطرية، ينتمي إلى عائلة Ranunculaceae، ويُستخدم في الطب التقليدي لفوائده الصحية المتعددة، مثل دعم الجهاز المناعي، وتعزيز وظائف الدماغ، والوقاية من الأمراض المزمنة. تهدف هذه الدراسة إلى تقييم تأثير مستخلص الكمون الأسود على مستويات الكوليسترول والبروتين الكلي (الألبومين والجلوبيولين) في ذكور الأرانب السليمة.

المواد والطرق:تم استخدام 24 أرنب نيوزيلندي سليم، تراوحت أعمارهم بين 5 و6 أشهر، وأوزانهم بين 1200–1500 غم. قُسّمت الأرانب إلى ست مجموعات، مجموعة سيطرة وخمس مجموعات جرعت بمستخلص مائي من الحبة السوداء بجرعات تصاعدية (80، 130، 180، 230، 280 ملغم/كغم من وزن الجسم). تم قياس مستويات الكوليسترول، البروتين الكلي، الألبومين، والجلوبيولين في الدم بعد الجرعات

النتائج:أظهرت النتائج انخفاضًا معنويًا في الكوليسترول في الجرعتين 80 و130 ملغم/كغم، وارتفاعًا معنويًا في الجرعات الأعلى (180–280 ملغم/كغم). كما لوحظ ارتفاع معنوي في البروتين الكلي والجلوبيولين في الجرعات العالية، خصوصًا المجموعة E، بينما لم يطرأ تغيير معنوي على الألبومين.

الاستنتاج:الجرعات المنخفضة من الحبة السوداء تخفض الكوليسترول، بينما الجرعات العالية ترفعه، مع زيادة في البروتين الكلي والجلوبيولين، دون تغيير في الألبومين.

الكلمات المفتاحية : الحبة السوداء، الكلوليسترول، االبروتين الكلي ، الالبومين، ، الجلوبيولين.