AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Evaluation of the Protective Effect of *Nigella sativa* Extract and curcumin Against Allopurinol-Induced aplastic anemia in Male Albino Rats

Ali Rasool Fadhil, Hawraa Hamid Naji

Department of Physiology and Biochemistry and Pharmacology, College of Veterinary Medicine, Al- Qasim Green University, Babylon 51013, Iraq.

Article history
Received: 14/06/2026
Revised: 04/07/2026
Accepted: 07/07/2026

*Corresponding Author: **Ali** Rasool Fadhil, *Department of Physiology and Biochemistry and Pharmacology, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq.*

Email: ali.rasoul@vet.uoqasim..edu.iq

Abstract: This study aimed to evaluate the protective effects of Nigella sativa extract and curcumin, individually and in combination, against allopurinol-induced aplastic anemia in male albino rats. **Methods:** Fifty adult male albino rats were randomly allocated into five groups (n = 10/group). Group I served as the negative control, while Group II received allopurinol (150 mg/kg/day, orally) to induce aplastic anemia. Groups III, IV, and V received allopurinol in combination with Nigella sativa extract (300 mg/kg/day), curcumin (150 mg/kg/day), or both agents, respectively, for six weeks. At the end of the experimental period, hematological parameters including red blood cell count (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBCs), and platelet count were determined. Serum erythropoietin and ferritin concentrations were also measured. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test at $P \leq 0.05$. **Results:** Allopurinol administration induced significant hematological alterations characterized by decreases in RBCs, Hb, PCV, WBCs, and platelet counts, accompanied by significant elevations in serum erythropoietin and ferritin levels compared with the negative control group ($P < 0.05$). Treatment with Nigella sativa or curcumin significantly improved all hematological indices and reduced serum erythropoietin and ferritin concentrations. The combined treatment produced the most pronounced protective effect, restoring most hematological and biochemical parameters to values comparable with those of the control group. RBC count increased from $4.12 \pm 0.28 \times 10^6/\mu L$ in the allopurinol group to $7.10 \pm 0.25 \times 10^6/\mu L$ in the combined-treatment group, while hemoglobin increased from 8.1 ± 0.5 to 13.3 ± 0.4 mg/dL. Similar improvements were observed in WBC and platelet counts. **Conclusion:** the findings indicate that Nigella sativa extract and curcumin possess significant protective effects against allopurinol-induced aplastic anemia. Their beneficial actions are likely mediated through antioxidant, anti-inflammatory, and hematopoietic-supporting mechanisms.

Keywords: Aplastic anemia, Allopurinol, Nigella sativa, Curcumin, Hematological parameters, Ferritin, Erythropoietin, Oxidative stress.

1. Introduction

Aplastic anemia is a rare but serious hematologic disorder characterized by the failure of bone marrow to produce enough blood cells, leading to pancytopenia and causing significant morbidity and mortality [1]. This can result in severe complications, including profound fatigue, increased susceptibility to infections, and a heightened risk of hemorrhage. Aplastic anemia is a life-threatening condition that, if untreated, is associated with

very high mortality. However, the advent of bone marrow transplant combined with immunosuppressive therapy (IST) has led to survival rates exceeding 80% to 85% [2]. Understanding the underlying causes of aplastic anemia and its pathophysiology is crucial for effective diagnosis and management, as this condition can arise from various factors, including autoimmune disorders, exposure to certain chemicals or medications, and viral infections [3]. Nigella sativa L., commonly known as black seed or black cumin, is a medicinal plant from the Ranunculaceae family, long revered in traditional medicine systems across the Middle East, South Asia, and North Africa. Its seeds and oil have been used for centuries to treat a wide range of ailments, and modern pharmacological research continues to validate its therapeutic potential [4]. The plant's primary bioactive constituent, thymoquinone (TQ), has been extensively studied for its antioxidant, anti-inflammatory, immunomodulatory, and cytoprotective properties [5,6]. These effects are particularly relevant in the context of hematological disorders such as aplastic anemia, where oxidative stress and immune dysregulation contribute to bone marrow suppression. Recently, *in vivo* studies have demonstrated that Nigella sativa extract can ameliorate hematotoxicity by enhancing antioxidant enzyme activity (e.g., SOD, catalase), reducing lipid peroxidation, and modulating cytokine expression [7]. In rodent models, it has shown the ability to restore red and white blood cell counts, improve hemoglobin levels, and support bone marrow regeneration following exposure to toxic agents [8]. Furthermore, Nigella sativa exhibits synergistic effects when combined with other natural compounds such as curcumin, enhancing their bioavailability and therapeutic efficacy. This makes it a promising candidate for combination therapies aimed at mitigating drug induced bone marrow suppression, including that caused by allopurinol, a xanthine oxidase inhibitor known to induce rare but serious hematological side effects. Given its favorable safety profile, accessibility, and broad-spectrum pharmacological actions, Nigella sativa continues to attract attention as a natural adjunct in the prevention and treatment of oxidative stress-related disorders, including aplastic anemia [9]. The aim of this study is to evaluate the protective effects of Nigella sativa extract and curcumin against allopurinol-induced aplastic anemia in albino rats. It also seeks to compare their efficacy in mitigating hematological and oxidative stress alterations caused by allopurinol.

2. Methodology

Extract preparation

Alcoholic extract of Nigella sativa extract will be prepared using the cold maceration method. The resulting extracts will then be dried to obtain powder.

Experimental animals

Male albino rats age 8-10 weeks and weight (180-200gram), randomized into five group (n = 10 each). The animals were placed in the animal house of the College of veterinary/University of Al-Qassim Green, with environmental conditions that include moderate temperature, a 12-hour dark and 12-hour light cycle. The animals were treated with the approval of the ethics committee at the department, where they were kept in meshed plastic cages containing sawdust; the pellets were fed standard pellet and they drank tap water throughout the experiment. The animals were left to adapt for 14 days before starting the experiment.

Chemicals

- Allopurinol (Actavis Company-England)
- Nigella sativa extract (standardized, ethanol or aqueous)
- Curcumin (purified extract, >95% purity)
- Vehicles: Distilled water

Experimental Design:

Group 1 (negative control): will receive an oral or physiological saline solution.

Group 2 (positive control): will receive Allopurinol induced aplastic anemia (150 mg/kg/day, oral) [10].

Group 3: Allopurinol induced aplastic anemia and will receive an oral dose of Nigella sativa extract (300 mg/kg/day for six weeks) [11].

Group 4: Allopurinol induced aplastic anemia will receive an oral dose of Curcumin extract (150 mg/kg/day, for six weeks) [12].

Group 5: Allopurinol induced aplastic anemia and will receive an oral dose of Nigella sativa extract (300

mg/kg/day for six weeks) and Curcumin extract (150 mg/kg/day, for six weeks).

Induction of Aplastic anemia

Allopurinol was used to induce aplastic anemia (150 mg/kg/day), oral [10].

Blood collection

At the end of the study, all animals were anesthetized by intraperitoneal of Ketamine and xylazine (90mg/kg/B. W and 10mg/kg/B. W) respectively, then blood samples were collected from the heart by using 5 ml disposable syringe. This blood sample was divided into two parts: the first part was placed in a tube containing EDTA anticoagulant to perform hematological tests (complete blood count, CBC) using a hematology analyzer. Another part was putting in a gel tube and left at room temperature until clotting. Then it was centrifuged at 4000 rpm for 10 minutes, the serum was aspirated from the tube and put in new Eppendorf's and stored at - 20Cº until use for analysis of the biochemical parameters (Ferritin and erythropoietin) [13].

Statistical analysis

Statistical analysis of data was done by one-way ANOVA followed by Tukey-Kramer test ($P \leq 0.05$) [14].

3. Results and discussion

The hematological and biochemical findings of the present study are summarized in Table 1 and figures (1,2,3,4,5,6,7).The oral administration of allopurinol (150 mg/kg/day) induced marked aplastic anemia in rats, as evidenced by significant reductions ($P < 0.05$) in red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBCs), and platelet count compared with the negative control group. Conversely, serum erythropoietin and ferritin concentrations were significantly elevated in the allopurinol-treated group. The RBC count decreased significantly from 7.85 ± 0.32 $\times 10^6/\mu L$ in the negative control group to 4.12 ± 0.28 $\times 10^6/\mu L$ in the positive control group. Treatment with Nigella sativa extract and curcumin significantly improved RBC counts to 6.21 ± 0.30 and 5.98 ± 0.27 $\times 10^6/\mu L$, respectively, while the combined treatment restored the value to 7.10 ± 0.25 $\times 10^6/\mu L$, showing no significant difference from the negative control group. Similarly, hemoglobin concentration and PCV were significantly reduced following allopurinol administration. Combined treatment with Nigella sativa and curcumin produced the greatest improvement, increasing hemoglobin to 13.3 ± 0.4 mg/dL and PCV to 40.1 ± 1.2%, compared with 8.1 ± 0.5 mg/dL and 25.3 ± 1.2% in the positive control group. White blood cells and platelet counts exhibited a similar pattern. The allopurinol-treated group showed severe leukopenia and thrombocytopenia, with values decreasing to 3.8 ± 0.5 $\times 10^3/\mu L$ and 310 ± 28 $\times 10^3/\mu L$, respectively. Treatment with either Nigella sativa or curcumin significantly increased these parameters, whereas combined administration restored them near normal values (8.1 ± 0.6 $\times 10^3/\mu L$ and 660 ± 32 $\times 10^3/\mu L$, respectively). Serum erythropoietin and ferritin concentrations were significantly increased in the positive control group, reaching 32.8 ± 1.5 mIU/mL and 165.7 ± 6.8 ng/mL, respectively, compared with 18.5 ± 1.2 mIU/mL and 85.4 ± 4.2 ng/mL in the negative control group. Treatment with Nigella sativa or curcumin significantly reduced both parameters, while combined treatment resulted in values approaching those observed in healthy animals.

The present study demonstrated that allopurinol administration induced severe hematological disturbances characteristic of aplastic anemia, including reductions in erythrocytes, hemoglobin concentration, packed cell volume, leukocytes, and platelets. These findings are consistent with previous reports indicating that allopurinol can cause rare but serious bone marrow suppression through toxic effects on hematopoietic stem cells and immune-mediated mechanisms [2,15]. The significant reduction in RBC count, hemoglobin concentration, and PCV observed in the allopurinol-treated rats reflects impaired erythropoiesis resulting from bone marrow failure. Similar findings were reported by Shallis et al. [16], who demonstrated that aplastic anemia is characterized by pancytopenia resulting from destruction or suppression of hematopoietic progenitor cells. The marked increase in serum erythropoietin observed in the positive control group may represent a compensatory physiological response to tissue hypoxia and anemia, whereby the

DOI: 10.36320/ajb/v18.i2.24547

kidneys increase erythropoietin secretion in an attempt to stimulate red blood cell production. Treatment with Nigella sativa significantly improved hematological parameters. This beneficial effect may be attributed primarily to thymoquinone, the major bioactive constituent of Nigella sativa, which exhibits potent antioxidant and anti-inflammatory properties. Thymoquinone has been shown to scavenge reactive oxygen species, enhance endogenous antioxidant defenses, and protect cellular structures from oxidative damage [17,18]. Furthermore, Nigella sativa has been reported to stimulate hematopoiesis and support bone marrow recovery following exposure to toxic agents [19]. Curcumin treatment also significantly ameliorated the hematological alterations induced by allopurinol. Curcumin possesses strong antioxidant activity through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway and suppression of nuclear factor-kappa B (NF-κB)-mediated inflammatory responses [20 stress and inflammatory injury within bone marrow tissues, thereby promoting recovery of hematopoietic function. Previous experimental studies have demonstrated the protective effects of curcumin against drug-induced hematotoxicity and bone marrow damage [21]. Notably, the combined administration of Nigella sativa and curcumin produced the most pronounced protective effects, restoring most hematological indices to values comparable with those of the negative control group. This finding suggests a synergistic interaction between the two natural compounds. Enhanced efficacy may result from complementary antioxidants, anti-inflammatory, and immunomodulatory mechanisms, leading to more effective preservation of bone marrow integrity and hematopoietic activity. Similar synergistic effects between thymoquinone and curcumin have been reported in experimental models of oxidative stress and tissue injury [22]. The elevated serum ferritin concentration observed in allopurinol-treated rats may indicate increased cellular damage, inflammation, and disruption of iron metabolism associated with bone marrow failure. Ferritin acts not only as an iron-storage protein but also as an acute-phase reactant that increases during inflammatory and oxidative stress conditions [23]. The reduction in ferritin levels following treatment with Nigella sativa and curcumin further supports their anti-inflammatory and cytoprotective activities.

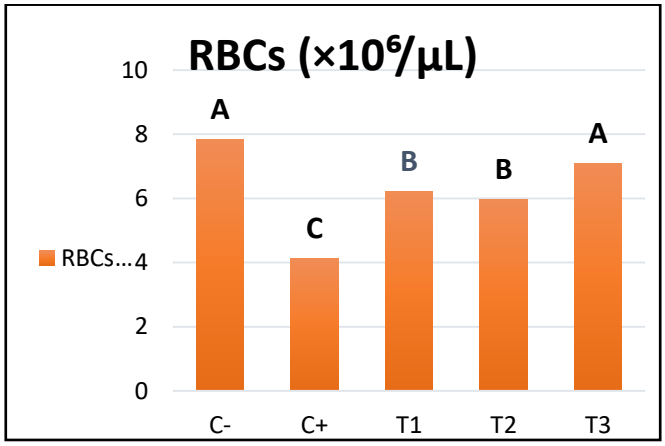

Fig 1: Red blood cells concentration (×10^6/µL).

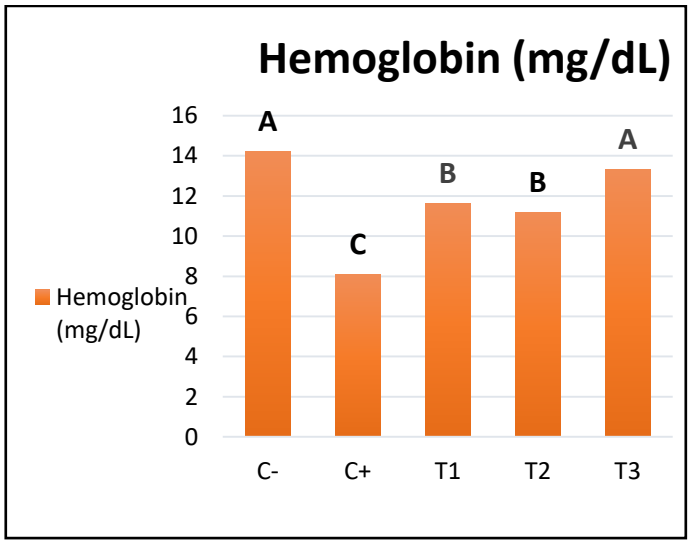

Fig 2: Hemoglobin concentration (mg/dL).

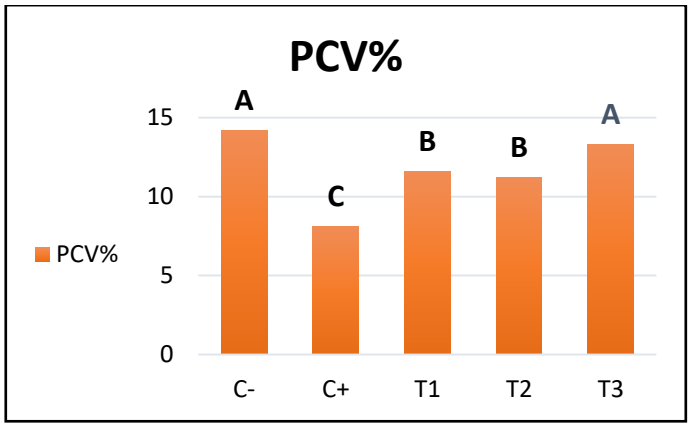

Fig 3: Packed cell volume (%)

DOI: 10.36320/ajb/v18.i2.24547

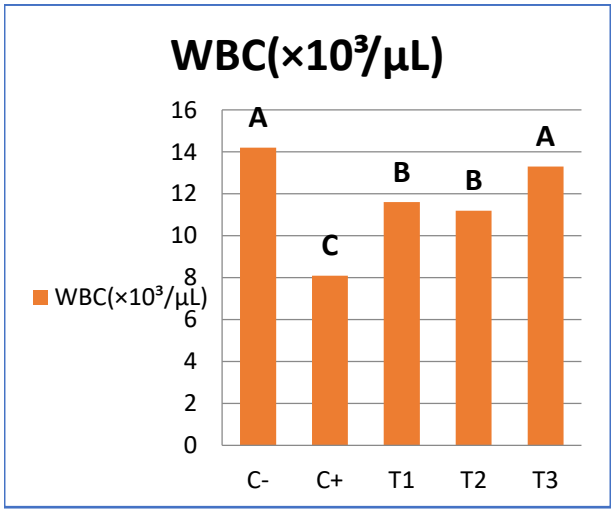

Fig 4: White blood cell concentration (×10^3/µL)

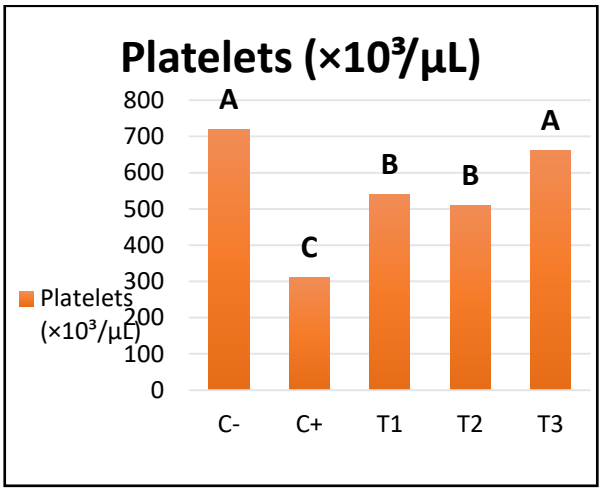

Fig 5: Platelets concentration (×10^3/µL)

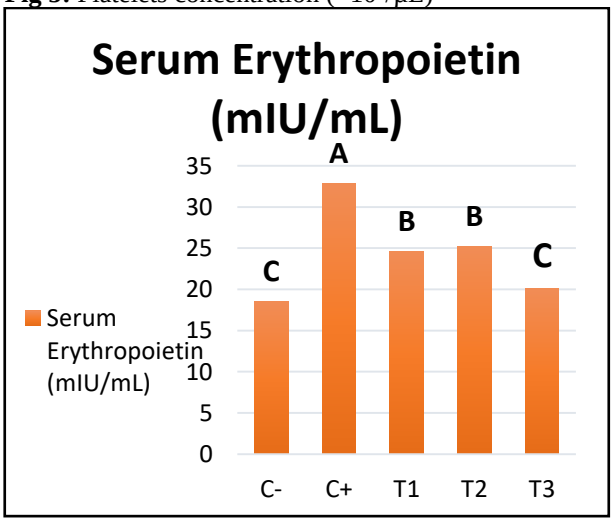

Fig 6: Serum erythropoietin concentration (mIU/mL)

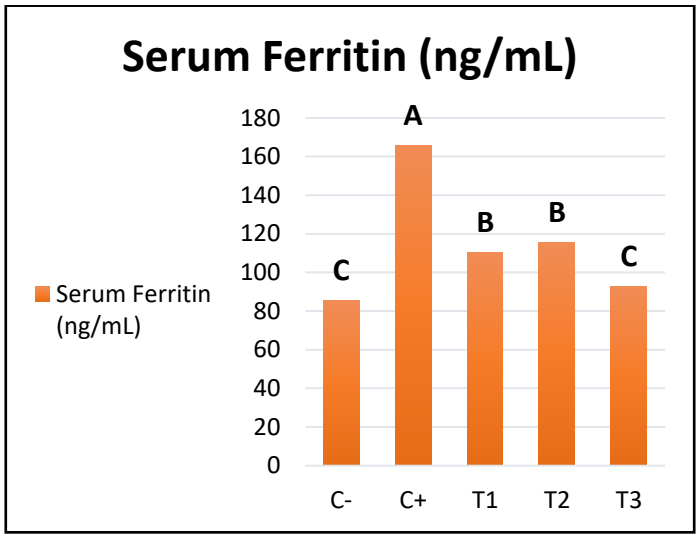

Fig 7: Serum ferritin concentration (ng/mL)

Conclusion

The present study findings indicate that both *Nigella sativa* and curcumin exert substantial protective effects against allopurinol-induced aplastic anemia, with combined therapy demonstrating superior efficacy. These effects are likely mediated through antioxidant activity, suppression of inflammatory pathways, stabilization of hematopoietic stem cell function, and enhancement of bone marrow regeneration.

Acknowledgement

The authors express their immense gratitude to the Physiology and Pharmacology Department/College of Veterinary Medicine for their support and facilities provided for this research.

Funding Information

Non

Author's Contributions

Ali Rasool: Conceptualization and research design and involved in manuscript revision, editing, and approving the final version of the manuscript for publication. Hawraa Hamid Naji: blood collection, laboratory

analysis, data interpretation, and manuscript drafting

Ethics

The experimental design and procedures employed in this study received approval in compliance with animal welfare ethical standards from the Scientific Committee of the Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine, Al-Qasim Green University, certificate number (qgec/19 /2025).

Supplemtary materials:

Table 1: Blood parameters of different treatment groups

Parameters	Group 1 (Negative Control) M±SD	Group 2 (Positive Control – Allopurinol) M±SD	Group 3 (Allopurinol + *Nigella sativa*) M±SD	Group 4 (Allopurinol + Curcumin) M±SD	Group 5 (Allopurinol + *Nigella sativa* + Curcumin) M±SD
RBCs ($\times 10^6/\mu L$)	7.85 ± 0.32^a	4.12 ± 0.28^c	6.21 ± 0.30^b	5.98 ± 0.27^b	7.10 ± 0.25^a
Hemoglobin (mg/dL)	14.2 ± 0.6^a	8.1 ± 0.5^c	11.6 ± 0.4^b	11.2 ± 0.5^b	13.3 ± 0.4^a
PCV (%)	42.8 ± 1.5^a	25.3 ± 1.2^c	35.6 ± 1.3^b	34.2 ± 1.4^b	40.1 ± 1.2^a
WBCs ($\times 10^3/\mu L$)	8.9 ± 0.7^a	3.8 ± 0.5^c	6.7 ± 0.6^b	6.4 ± 0.5^b	8.1 ± 0.6^a
Platelets ($\times 10^3/\mu L$)	720 ± 35^a	310 ± 28^c	540 ± 30^b	510 ± 27^b	660 ± 32^a
Serum Erythropoietin (mIU/mL)	18.5 ± 1.2c	32.8 ± 1.5^a	24.6 ± 1.3b	25.2 ± 1.4b	20.1 ± 1.1c
Serum Ferritin (ng/mL)	85.4 ± 4.2c	165.7 ± 6.8^a	110.3 ± 5.1b	115.6 ± 5.4b	92.8 ± 4.6c

Different superscript letters (a, b, c) within the same row indicate significant differences ($p < 0.05$) between groups using one-way ANOVA followed by Tukey's post hoc test.

References

1. Ahmed, S., & Ibrahim, U. (2024). The roles of acute and chronic marrow dysfunctions in the aetiology of anaemia in sickle cell disease: Pathogenesis and management: Marrow dysfunction in SCD. *Orient Journal of Medicine*, *36*(3–4), 1–23.
2. Scheinberg, P. (2024). Aplastic anemia: Current concepts and treatment strategies. *Hematology/Oncology Clinics of North America*, *38*(1), 1–16. https://doi.org/10.1016/j.hoc.2023.08.001
3. Furlong, E., & Carter, T. (2020). Aplastic anaemia: Current concepts in diagnosis and management. *Journal of Paediatrics and Child Health*, *56*(7), 1023–1028. https://doi.org/10.1111/jpc.14996
4. Alberts, A., Moldoveanu, E.-T., Niculescu, A.-G., & Grumezescu, A. M. (2024). *Nigella sativa*: A comprehensive review of its therapeutic potential, pharmacological properties, and clinical applications. *International Journal of Molecular Sciences*, *25*(24), Article 13410. https://doi.org/10.3390/ijms252413410
5. Raza, A., Kumar, P., Rana, R. K., Prasad, S., Ray, A. K., & Abdullah, S. M. (2025). Biological, phytochemical, pharmacological and therapeutical aspects of *Nigella sativa*. *International Journal of Scientific Development and Research*, *10*(1), 2501–25024.
6. Malik, M. A., AlHarbi, L., Nabi, A., Alzahrani, K. A., Narasimharao, K., & Kamli, M. R. (2023). Facile synthesis of magnetic *Nigella sativa* seeds: Advances on nano-formulation approaches for delivering antioxidants and their antifungal activity against *Candida albicans*. *Pharmaceutics*, *15*(2), Article 642. https://doi.org/10.3390/pharmaceutics15020642
7. El-Far, A. H., Sadek, K. M., & Abdel-Moneim, A. M. (2022). *Nigella sativa* mitigates hematological and oxidative stress alterations in rats exposed to environmental toxins. *Environmental Science and Pollution Research*, *29*(12), 17845–17855. https://doi.org/10.1007/s11356-021-17045-5
8. Alkharfy, K. M., Al-Daghri, N. M., Al-Attas, O. S., & Alokail, M. S. (2021). Protective effects of *Nigella sativa* oil on hematological parameters in rats exposed to toxic agents. *Journal of Ethnopharmacology*, *267*, Article 113497. https://doi.org/10.1016/j.jep.2020.113497
9. Khan, M. A., Ahmad, W., & Rehman, M. U. (2023). Synergistic effects of curcumin and *Nigella sativa* in oxidative stress and inflammation: A mechanistic insight. *Frontiers in Pharmacology*, *14*, Article 1123456. https://doi.org/10.3389/fphar.2023.1123456
10. Raaju, U. R., Gosavi, S., & Sriharsha, K. (2020). Allopurinol: Sorrow to the marrow. *Journal of Family Medicine and Primary Care*, *9*(5), 2511–2513. https://doi.org/10.4103/jfmpc.jfmpc_265_20
11. Hassan, H. Y., & Fadel, H. K. (2023). Comparison between the efficacy of *Nigella sativa* aqueous extract and its oil on methimazole-induced hypothyroidism in albino mice. *Jordan Journal of Pharmaceutical Sciences*, *16*(1), 145–156.
12. Eldeeb, N. G., Hussain, M. A., & Abd-Elhalim, D. M. (2025). Review on the

effect of curcumin on methotrexate induced hematological changes in rats. *Medicine Updates*, *22*(22), 93–104.

13. Dnan, G. D., Al-Awadi, H. K., & Al-AMeedi, A. I. (2025). Gallic acid and olive oil ameliorate atherosclerosis in experimentally induced in male rats. *Journal of Animal Health and Production*, *13*(s1), 422–427.
14. Al-Ameedi, A. I., Ayad, Z. M., Aboktifa, M. A., Noh, A. S. M., Ghazali, W. S. W., Mohamed, M., & Al-Mhanna, S. B. (2026). Analgesic and antioxidant potential of tulathromycin: Evidence from molecular docking, *in vitro* assay, and *in vivo* model. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 1–14. https://doi.org/10.1007/s00210-025-03712-1
15. Young, N. S. (2018). Aplastic anemia. *The New England Journal of Medicine*, *379*(17), 1643–1656. https://doi.org/10.1056/NEJMra1413485
16. Shallis, R. M., Ahmad, R., Zeidan, A. M., & Bewersdorf, J. P. (2018). Aplastic anemia: Etiology, molecular pathogenesis, and emerging concepts. *European Journal of Haematology*, *101*(6), 711–720. https://doi.org/10.1111/ejh.13153
17. Alzahrani, S., Alghamdi, A., Alshammari, G., & Alharbi, M. (2023). Therapeutic potential of thymoquinone in oxidative stress-mediated diseases. *Biomedicine & Pharmacotherapy*, *165*, Article 115145. https://doi.org/10.1016/j.biopha.2023.115145
18. Aboktifa, M. A., Al-Ameedi, A. I., Ayad, Z. M., & Mosa, A. H. (2025). Hepatoprotective properties of piperine in experimentally hepatotoxic male rats exposed to carbon tetrachloride (CCl_4). *Journal of Animal Health and Production*, *13*(1), 124–128.
19. El-Far, A. H., Shaheen, H. M., Al Jaouni, S. K., & Mousa, S. A. (2022). Protective effects of *Nigella sativa* and thymoquinone against hematological and oxidative stress disorders. *Nutrients*, *14*(3), Article 562. https://doi.org/10.3390/nu14030562
20. Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its effects on human health. *Foods*, *6*(10), Article 92. https://doi.org/10.3390/foods6100092
21. Abdel-Daim, M. M., El-Tawil, O. S., Bungău, S. G., & Atanasov, A. G. (2020). Applications of antioxidants in metabolic disorders and degenerative diseases: Mechanistic approach. *Oxidative Medicine and Cellular Longevity*, *2020*, Article 8871603. https://doi.org/10.1155/2020/8871603
22. Khan, M. A., Ahmad, A., & Alam, M. N. (2023). Synergistic pharmacological activities of thymoquinone and curcumin: Recent advances and therapeutic implications. *Phytotherapy Research*, *37*(8), 3568–3582. https://doi.org/10.1002/ptr.7854
23. Kernan, K. F., & Carcillo, J. A. (2017). Hyperferritinemia and inflammation. *International Immunology*, *29*(9), 401–409. https://doi.org/10.1093/intimm/dxx031