Proceedings S.Z.M.C. Vol: 40(2): pp. 239-244, 2026. *PSZMC-1039-40-2-2026*

Uric Acid Lowering Effect of Thymoquinone in Potassium Oxonate-Induced Hyperuricemic Rats

Syed Muhammad Saad Gardezi[1], Moneeb Ashraf[2], Sadaf Humayun Khan[3], Hassan Farooq[3], Amer Hassan Siddiqui[4], Sana Tufail[1]

Affiliation:
[1]Quaid e Azam Medical College, Bahawalpur.
[2]King Edward Medical University, Lahore.
[3]Allama Iqbal Medical College, Lahore.
[4]Ameer Ud Din Medical College, Lahore.
Correspondence:
Dr. Sadaf Humayun Khan, Assistant Professor, Department of Pharmacology, Allama Iqbal Medical College, Lahore.
E-mail: sadafhumayunkhan@gmail.com
Received: Nov 4, 2025, **Accepted:** June 22, 2026, **Published:** June 30, 2026.
DOI: https://doi.org/10.47489/szmc.v40i2.854
Data Availability: The corresponding author can provide access upon request.
Funding: No funding received
Competing Interests: No competing interest to declare.

Author Contributions:
SMSG, MA, SHK, AHS, ZK, ST

- Each author made substantial contributions to the conception and design of the study, or acquisition, analysis, and interpretation of data.
- All authors were involved in drafting The manuscript or critically revising it for important intellect content.
- All authors approved the final version of the manuscript to be published and agree to be accountable for all aspects of the work

ABSTRACT

Background: High serum uric acid levels lead to hyperuricemia, which, if left untreated, can result in gout, metabolic syndrome and can also be an aggravating factor in renal and cardiovascular conditions. Nowadays xanthine oxidase inhibitors and uricosuric drugs are used to treat hyperuricemia. Thymoquinone, derived from Nigella Sativa Linn, can be explored for its potential uric acid lowering effects.
Objective: To evaluate the effect of Thymoquinone on serum and urinary uric acid in potassium oxonate induced hyperuricemic rats.
Method: This experimental study was conducted at the Post Graduate Medical Institute, Lahore from January 2021 - January 2022. Twenty-four rats were randomly divided into four groups (A-D). Group A and B were taken as normal and disease control respectively. Groups B- D were injected intraperitoneal potassium oxonate on days 1, 3 and 7 to induce hyperuricemia. Group C was given oral Allopurinol (5mg/kg) whereas Group D was treated with Thymoquinone at 20 mg/kg orally for seven consecutive days. Serum and urine samples were taken on days 0, 1, 3 and 7, while serum and urinary creatinine levels on day 0 and 7 were obtained to estimate fractional excretion of uric acid. Data was analyzed and processed by SPSS version 24. (p value ≤ 0.05 was considered significant)
Results: A significant reduction in serum uric acid levels (*p*-value <0.001) was seen from day 1 to 7 in Thymoquinone treated group when compared to the disease control, whereas this difference was insignificant when compared to Allopurinol treated group. Thymoquinone treated group also showed significantly increased urinary uric acid levels on day 7 (*p*-value <0.001).
Conclusion: Thymoquinone effectively lowers serum uric acid levels and shows an increase in urinary uric acid levels. It also enhances urine volume and fractional excretion of uric acid (FEUA %). These effects suggest its potential in preventing or treating hyperuricemia.
Keywords: Hyperuricemia, Thymoquinone, Potassium Oxonate, Allopurinol

INTRODUCTION: Despite advances in medical sciences, Hyperuricemia remains a major health concern. It is a key risk factor in conditions like gout, hypertension, cardiovascular diseases, chronic kidney disease as well as metabolic syndrome and is defined as elevated levels of uric acid in the blood (above 6.6 mg/dl in men and 7.7 mg/dl in women). Globally, the prevalence of hyperuricemia varies, with rates of 21% in the USA, 8.4% in Saudi Arabia, and 6.2% in China [1]. Whereas in Pakistan, the prevalence is reported to be 27.9% in males and 49.3% in females [2]. Studies conducted in Karachi and Sukkur also reported increasing prevalence with age [3-5].

Uric acid levels in the body are closely regulated and any disruption in this regulation can lead to increased serum levels. Hyperuricemia is either caused by an under excretion or overproduction of uric acid, often influenced by dietary factors [6]. It leads to the formation of monosodium urate crystals in joints and soft tissues,

ultimately causing gout [7]. Whereas Gout remains the most common inflammatory arthritis with constantly increasing global prevalence [8].

Hyperuricemia can be treated by reducing the synthesis of uric acid (xanthine oxidase inhibitors), increasing the excretion of uric acid (URAT1 inhibitors) and regulating the metabolic hydrolysis of uric acid (uricase inhibitors). Xanthine oxidase inhibitors, being the most commonly used drugs are classified as purine analogs (e.g. allopurinol) and non-purine analog (e.g. febuxostat and topiroxostat) [9,10]. However, the need for the development of more efficient treatment options for better management of this condition continues. For centuries different herbs have been employed in traditional medicine, which should be explored more thoroughly for their potential benefits in treating this condition.

Thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone) is extracted from Nigella Sativa Linn which is also known as black cumin, black seed or kalonji. Although its usefulness as an anti-cancer [11] and antimicrobial drug [12] has been demonstrated in some studies as well as its use in conditions like hyperlipidemia [13] and diabetes mellitus [14] is well documented but it has never been used to treat conditions like hyperuricemia. In this study, thymoquinone was evaluated for its potential to reduce uric acid levels. The outcomes were statistically compared with those of diseased rats and allopurinol-treated rats.

METHOD: This randomized controlled experimental animal study with parallel group design was conducted at the Post Graduate Medical Institute, Lahore, after the approval from Advanced studies & research board, University of Health Sciences, Lahore, Pakistan (UHS/Education/126-20/826 dated 18-03-2020) from January 2021 to January 2022. It involved twenty- four healthy adult male Sprague Dawley rats, randomly allocated into four groups (Group A to D), each consisting of six rats. Inclusion criteria included adult male sprague dawley rats weighting between 140-180 grams whereas rats with any signs of disease were excluded from the study. The animals were taken care of and experimental protocols were followed according to the criteria outlined in the 'Guide for the care and use of laboratory animals'.

Except normal control, Potassium oxonate, 250 mg/kg via intraperitoneal injection was given to all the groups on days 1, 3 and 7 for induction of hyperuricemia [15,16]. Group A and B were taken as Normal and Disease control whereas Group C was administered Allopurinol 5mg/kg [17] orally and Group D was provided with Thymoquinone 20mg/kg [18] orally, daily for seven consecutive days. Serum and urine samples were collected on days 0, 1, 3 and 7, while serum and urinary creatinine levels on days 0 and 7 were obtained to estimate fractional excretion of uric acid.

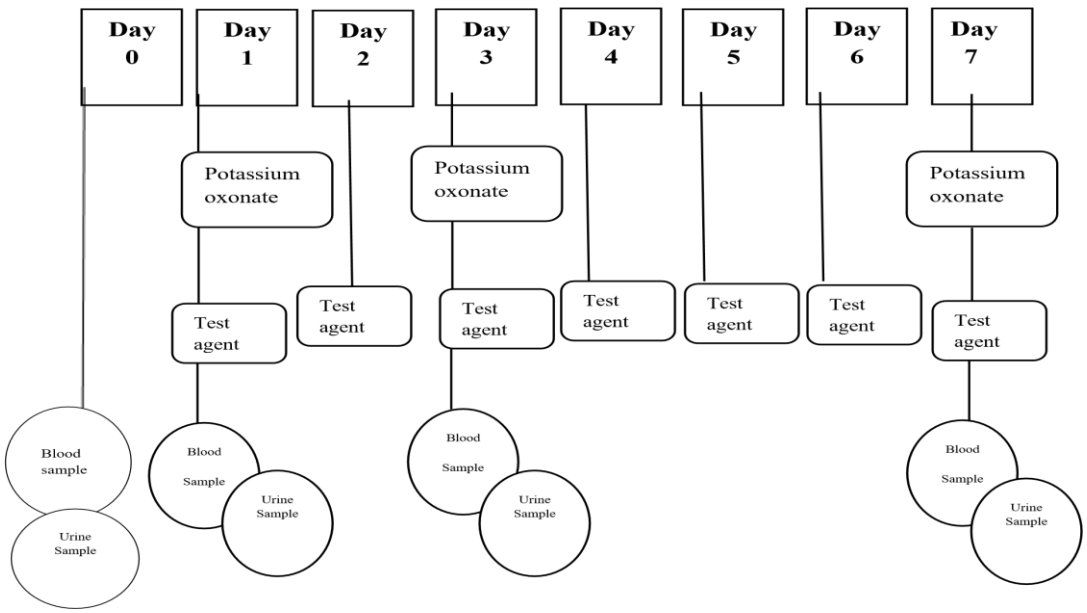

Figure 1: Experimental Design

Estimation of uric acid level was done by Uric acid meter (UA Sure). It was used to measure serum levels on day 1 and 3. While at day 0 and 7 the levels were measured by enzymatic colorimetric method using standard

diagnostic kit [19] similar measures were taken for estimation of urinary uric acid levels. Estimation of creatinine levels both serum and urinary, was done on days 0 and 7 by Jaffe method using standard diagnostic kit [20].

Fractional excretion of urate was measured by serum (S) and urinary (U) concentration of uric acid and creatinine (Cr) by using the formula

$$FEUa = \frac{\text{Uric acid (U)}_x \text{Cr (S)}}{\text{Uric Acid (S)}_x \text{Cr(U)}}^{x} 100$$

Outcome assessment and statistical analysis were performed by investigators blinded to the treatment groups. SPSS version 24 was used for data analyses. Quantitative variables (serum and urinary uric acid, serum and urinary creatinine, fractional excretion of urate) were presented in Mean ± SD. ANOVA was applied to check any significant difference between the groups. To test the significance of results in each group between start and end of study paired t test was applied. Repeated measure ANOVA was applied for multiple sampling. Post hoc Tukey's test was used to observe which group means differs.

RESULTS: All randomized animals were included in the final analysis. Results revealed that there was no significant difference in mean ± SD body weight among rat groups (p-value 0.176). Serum uric acid significantly increased on days 1, 3 and 7 in disease control (p-value of 0.001). Allopurinol and Thymoquinone treated groups showed significant decrease in serum uric acid levels on days 1, 3 and 7 when compared to the disease control group (p-value 0.000). Thymoquinone treated group showed a significant decrease in serum uric acid levels from day 1 (6.0 ±2.0) to day 7 (3.6 ± 0.8) with a p- value of 0.001. No significant difference was observed when Allopurinol treated group was compared to Thymoquinone (p -value 0.844).

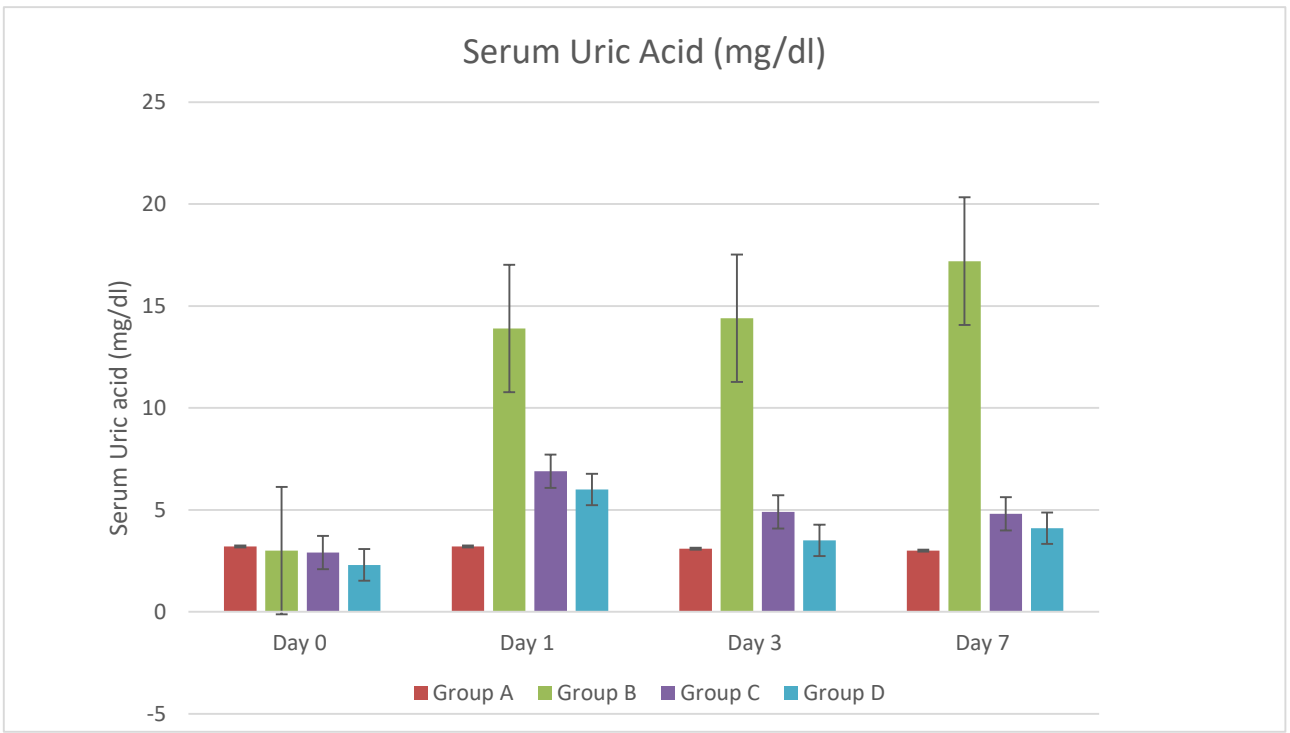

Figure 2: Comparison of Serum uric acid (mg/dL) among rat groups on day 0, 1, 3 and 7 (n=6) Where: Group A (Positive control); B (Disease control); C (Allopurinol treated); D (Thymoquinone treated)

Regarding urinary uric acid an initial rise on day 1 (p-value 0.027) followed by a significant reduction on day 7 (p-value 0.003) was seen in Allopurinol treated group. Thymoquinone treated group showed significantly reduced urinary uric acid levels on day 1 and 3 as compared to the Allopurinol treated group (*p*-value 0.027) followed by significantly increased levels on day 7(*p*-value <0.001).

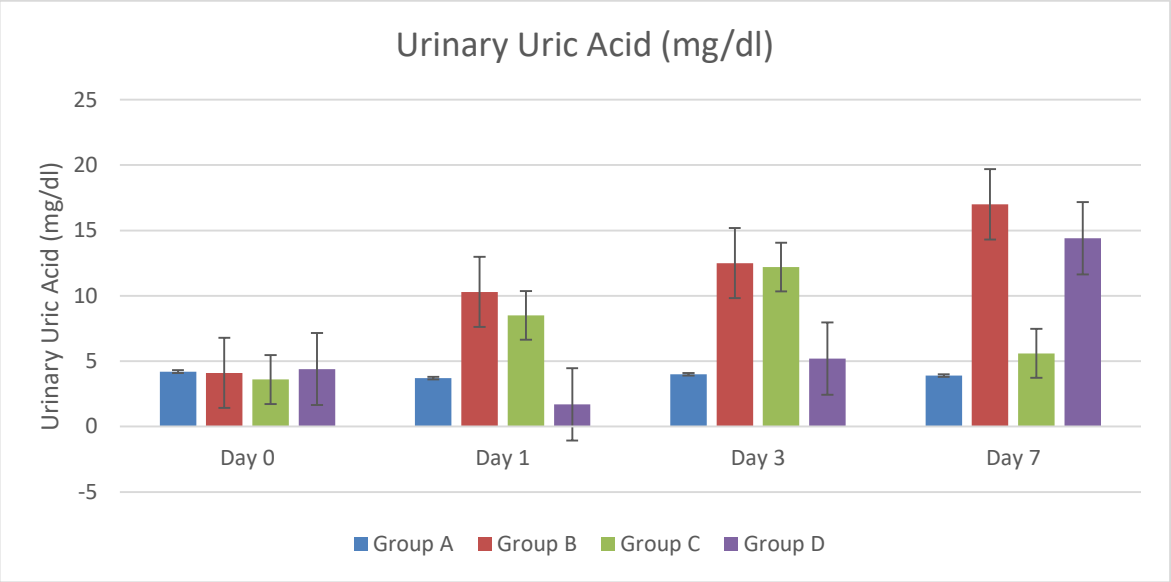

Figure 3: Comparison of urinary uric acid (mg/dL) among rat groups on day 0, 1, 3 and 7 (n=6) Where: A (Normal control); B (Disease control); C (Allopurinol treated); D (Thymoquinone treated)

No significant change was noted from day 0 to 7 in Normal control (group A), Allopurinol treated (group C) and Thymoquinone treated (group D) groups in fractional excretion of urate.

DISCUSSION: Although allopurinol is a key therapeutic option for hyperuricemia, it has also been linked to serious hypersensitivity reactions and adverse cardiovascular and renal outcomes [21]. Thymoquinone is considered a relatively safe drug. Safety profile of Thymoquinone is assessed in many studies. Its LD 50 is 2-3 g/kg in acute phase. Even at 100 mg/kg, it does not alter liver enzymes or produce any other toxicity [22]. Our findings revealed that, in our rat model, thymoquinone administration at a dose of 20 mg/kg resulted in a significant decrease in uric acid levels.

The significant reduction in serum uric acid levels observed in the thymoquinone-treated group is consistent with previous in vitro studies in which thymoquinone significantly inhibited xanthine oxidase activity [23] as well as with an experimental study conducted by Hafeez et al. on hyperuricemic rats, which also documented the uric acid-lowering effects of black seed powder in hyperuricemic rats [24].

Since this reduction was significant when compared to disease control (*p*-value <0.001) whereas no significant difference was observed when compared to Allopurinol treated group (*p* value > 0.05), it can be concluded that thymoquinone possesses a serum uric acid-lowering effect comparable to that of allopurinol.

Thymoquinone treated group exhibited significant increase in urinary uric acid levels only on day 7 ($p < 0.001$) whereas, on days 1 and 3, its levels were lower than those of allopurinol. In contrast, allopurinol showed an early rise in urinary uric acid, likely due to increased production, followed by a reduction on day 7. These findings suggest that thymoquinone not only inhibits xanthine oxidase but may also enhance uric acid excretion, possibly through effects on renal transporters, a mechanism that requires further investigation.

Thymoquinone also demonstrated diuretic activity by significantly increasing urine volume throughout the study period. In addition, the fractional excretion of uric acid (FEUA%) was significantly higher in the thymoquinone-treated group than in the allopurinol-treated group. These findings are supported by the study of Dera, Ayed A., et al., which reported that thymoquinone exerts protective effects against hyperuricemia-induced renal oxidative stress and mitochondrial abnormalities [25]. These findings suggest that this drug may be useful not only in

reducing serum uric acid levels but also in providing protective effects against hypertension and renal oxidative stress.

This study is limited by being an animal study with a small sample size. Furthermore, different doses of thymoquinone should be investigated, and molecular studies should be conducted to better elucidate its effects on the renal transport system and its potential role in the management of hyperuricemia. In addition, well-designed human clinical studies should be conducted in the future to confirm its efficacy and safety and to facilitate the translation of these findings into clinical practice.

CONCLUSION: Thymoquinone is a component of commonly available herb with well-established safety profiles, its usefulness as an anti hyperuricemic agent should be further studied for future medicinal purposes. This study concludes that Thymoquinone at 20 mg/kg, decreases serum and urinary uric acid.

REFERENCES

1. Song P, Wang H, Xia W, Chang X, Wang M, An L. Prevalence and correlates of hyperuricemia in the middle-aged and older adults in China. Sci Rep. 2018;8(1):4314. https://www.nature.com/articles/s41598-018-22570-9
2. Qudwai W, Jawaid M. Frequency of uric acid levels symptomatic and asymptomatic hyperuricemia among the Pakistani population. Mid East J Fam Med. 2017;15:52-7. http://www.mejfm.com/September2017/Hyperuricemia.pdf
3. Raja S, Kumar A, Aahooja RD, Thakuria U, Ochani S, Shaukat F. Frequency of hyperuricemia and its risk factors in the adult population. Cureus. 2019;11(3). https://doi.org/10.7759/cureus.4198
4. Shaikh AA, Altaf A. Prevalence of hyperuricemia in Sukkur; Pakistan: A cross sectional survey. Prof Med J. 2019;26(09):1567-9. https://doi.org/10.29309/TPMJ/2019.26.09.4027
5. Muzzammil M, Qadir A, Mughal A, Effendi J, Minhas MS, Jahanzeb S. The prevalence of hyperuricemia and its associated risk factors in patients presenting with joint pain in Karachi. Int J Res Orthop. 2020;6(6):1151. https://dx.doi.org/10.18203/issn.2455-4510.IntJResOrthop20204579
6. Shaikhomar O, Header E. Dietary Etiological Factors Contributing to the Prevalence of Hyperuricemia in Makkah Region. Prensa Med Argent S. 2020;2:2-7. https://www.researchgate.net/profile/Osama-Shaikhomar/publication/348975708_Dietary_Etiological_Factors_Contributing_to_the_Prevalence_of_Hyperuricemia_in_Makkah_Region/links/6019a669a6fdcc37a8fc06b1/Dietary-Etiological-Factors-Contributing-to-the-Prevalence-of-Hyperuricemia-in-Makkah-Region.pdf.
7. Bardin T, Richette P. Definition of hyperuricemia and gouty conditions. Curr Opin Rheumatol. 2014;26(2):186-91. https://doi.org/10.1097/BOR.0000000000000028
8. Singh JA, Gaffo A, editors. Gout epidemiology and comorbidities. Semin Arthritis Rheum. 2020;50(3S):S11-S16. https://doi.org/10.1016/j.semarthrit.2020.04.008
9. Hou Z, Ma A, Mao J, Song D, Zhao X. Overview of the pharmacokinetics and pharmacodynamics of URAT1 inhibitors for the treatment of hyperuricemia and gout. Expert Opin Drug Metab Toxicol. 2023;19(12):895-909. https://doi.org/10.1080/17425255.2023.2287477
10. Cicero AF, Fogacci F, Kuwabara M, Borghi C. Therapeutic strategies for the treatment of chronic hyperuricemia: an evidence-based update. Medicina. 2021;57(1):58. https://doi.org/10.3390/medicina57010058
11. Alhmied F, Alammar A, Alsultan B, Alshehri M, Pottoo FH. Molecular mechanisms of thymoquinone as anticancer agent. Comb Chem High Throughput Screen.2021;24(10):1644-53. https://doi.org/10.2174/1386207323999201027225305
12. Chatterjee G, Saha AK, Khurshid S, Saha A. A Comprehensive Review of the Antioxidant, Antimicrobial, and Therapeutic Efficacies of Black Cumin (Nigella sativa L.) Seed Oil and Its Thymoquinone. J Med Food. 2025;28(4):325-39. https://doi.org/10.1089/jmf.2024.k.0149
13. Wang F, Yao W, Yu D, Hao Y, Wu Y, Zhang X. Protective role of thymoquinone in hyperlipidemia-induced liver injury in LDL-R−/− mice. BMC Gastroenterol. 2023;23(1):276. https://doi.org/10.1186/s12876-023-02895-0
14. Mahomoodally MF, Aumeeruddy MZ, Legoabe LJ, Montesano D, Zengin G. Nigella sativa L. and its active compound thymoquinone in the clinical management of diabetes: A systematic review. Int J Mol Sci. 2022;23(20):12111. https://doi.org/10.3390/ijms232012111
15. Chen Y, Li C, Duan S, Yuan X, Liang J, Hou S. Curcumin attenuates potassium oxonate-induced hyperuricemia and kidney inflammation in mice. Biomed Pharmacother. 2019;118:109195. https://doi.org/10.1016/j.biopha.2019.109195
16. Haidari F, Rashidi MR, Keshavarz SA, Mahboob SA, Eshraghian MR, Shahi MM. Effects of onion on serum uric acid levels and hepatic xanthine dehydrogenase/xanthine oxidase activities in hyperuricemic rats. Pak J Biol Sci.2008;11(14):1779-84. https://doi.org/10.3923/pjbs.2008.1779.1784
17. Zajączkowski S, Ziółkowski W, Badtke P, Zajączkowski MA, Flis DJ, Figarski A, et al. Promising effects of xanthine oxidase inhibition by allopurinol on autonomic heart regulation estimated by heart rate variability (HRV) analysis in rats exposed to hypoxia and hyperoxia. PLoS One. 2018;13(2):e0192781. https://doi.org/10.1371/journal.pone.0192781

18. Hosseinzadeh H, Parvardeh S, Masoudi A, Moghimi M, Mahboobifard F. Attenuation of morphine tolerance and dependence by thymoquinone in mice. Avicenna J Phytomed. 2016;6(1):55. https://pmc.ncbi.nlm.nih.gov/articles/PMC4884218/
19. Che Sulaiman I, Chieng B, Osman M, Ong K, Rashid J, Wan Yunus W, et al. A review on colorimetric methods for determination of organophosphate pesticides using gold and silver nanoparticles. Microchim Acta. 2020;187(2):131. https://doi.org/10.1007/s00604-019-3893-8
20. Toora B, Rajagopal G. Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. Indian J Exp Biol. 2002;40(3):352-4. https://europepmc.org/article/med/12635710
21. Yang C-Y, Chen C-H, Deng S-T, Huang C-S, Lin Y-J, Chen Y-J, et al. Allopurinol use and risk of fatal hypersensitivity reactions: a nationwide population-based study in Taiwan. JAMA Intern Med.2015;175(9):1550-7. https://doi.org/10.1001/jamainternmed.2015.3536
22. Goyal SN, Prajapati CP, Gore PR, Patil CR, Mahajan UB, Sharma C, et al. Therapeutic potential and pharmaceutical development of thymoquinone: a multitargeted molecule of natural origin. Front Pharmacol.2017;8:656. https://doi.org/10.3389/fphar.2017.00656
23. Ahjel SW, Humadi SS, Awad SM, El-Shehry MF, Mansour YE, Elkader El-Rashedy AA. Novel pyrimidine derivatives and black cumin as xanthine oxidase inhibitors: Synthesis, docking study and formulation. Pak J Pharm Sci. 2024;37(5). http://doi.org/10.36721/PJPS.2024.37.5.REG.1151-1161.1
24. Hafeez A, Rehan AM, Hakim Z, Munir A, Khan RN, Khokhar A, editors. Nigella sativa Seeds Protective Ability in Pyrazinamide Induced Hyperuricemia in Mice. Proc. 2022. https://doi.org/10.47489/PSZMC-825361-44-48
25. Dera AA, Rajagopalan P, Alfhili MA, Ahmed I, Chandramoorthy HC. Thymoquinone attenuates oxidative stress of kidney mitochondria and exerts nephroprotective effects in oxonic acid-induced hyperuricemia rats. Biofactors. 2020;46(2):292-300. https://doi.org/10.1002/biof.1590

The Authors:

- Dr. Syed Muhammad Saad Gardezi, Senior Demonstrator, Department of Pharmacology, Quaid E Azam Medical College, Bahawalpur, Pakistan.
- Dr. Moneeb Ashraf, Professor & Head of Department, Department of Pharmacology, King Edward Medical College, Lahore Pakistan.
- Dr. Sadaf Humayun Khan, Assistant Professor, Department of Pharmacology, Allama Iqbal Medical College, Lahore Pakistan.
- Dr. Hassan Farooq, Associate Professor, Department of Pharmacology, Allama Iqbal Medical College, Lahore, Pakistan.
- Dr. Amer Hassan Siddiqui, Assistant Professor, Department of Pharmacology, Ameer ud din Medical College, Lahore, Pakistan
- Dr. Sana Tufail, Professor & Head of Department, Department of Pharmacology, Quaid-e-Azam Medical College, Bahawalpur