

Effects of *Solanum nigrum* on Liver Enzymes and Hematological Profile in Complete Freund's Adjuvant-Induced Arthritic Rats

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ABSTRACT

Background: Disease-modifying anti-rheumatic drugs (DMARDs) used for rheumatoid arthritis (RA) have a steady onset and multiple adverse effects. The objective was to evaluate the effects of the ethanolic extract of *Solanum nigrum* (SN) on methotrexate-associated hepatotoxicity and pancytopenia in Complete Freund's adjuvant (CFA)-induced arthritic rats.

Methods: 30 male Wistar albino rats were used in a 4-week pre-clinical experimental study conducted at Ziauddin University Karachi from November 2021 to December 2021. Animals were divided into 5 groups; Group-I Negative control (0.9% normal saline), Group-II positive control (0.9% normal saline), Group-III Standard (Methotrexate 1.5mg/kg), Group-IV (SN 100mg/kg), Group-V (SN 200mg/kg). To develop arthritis, 0.1mL of CFA was administered intraarticularly in the right knee joints of all groups except Group-I at day 0. For euthanasia, 100mg/kg pentobarbital was injected intraperitoneally in all animals on the 29th day. 10ml of blood was collected in vacutainer tubes for CBC and LFT. SPSS was used to analyze the results and ANOVA was applied for intergroup and intragroup comparisons. A P-value less than 0.05 was considered significant at a 95% confidence interval.

Results: Diseased controls increased WBCs, but Groups III, IV, and V considerably decreased WBCs. Group III also caused suppression in RBCs and hemoglobin levels. SN showed a significant decrease in WBCs, but it did not suppress RBCs, hemoglobin, and platelet levels. MTX significantly increased total bilirubin, direct bilirubin, SGPT, and alkaline phosphatase. Contrary to this, SN did not significantly increase liver enzymes when compared with the negative controls.

Conclusion: Unlike methotrexate, the ethanolic extract of SN improved the hematological and liver profile.

Keywords: *Solanum nigrum*, Methotrexate, Hematology, Liver Disease.

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INTRODUCTION

Rheumatoid arthritis (RA) is a progressive disease with chronic illness, with a prevalence of 0.5% to 1%¹. For the management of pain and joint inflammation, RA patients usually use DMARDs along with immunosuppressive steroids and non-steroidal anti-inflammatory drugs (NSAIDs)². Steroids and NSAIDs do not interfere with the progression of disease and are used only for symptomatic pain relief. While conventional (methotrexate, sulfadiazine, and hydrochloroquine) and biological (TNF-alpha inhibitors, IL-6 inhibitors) DMARDs slow down the disease progression and prevent further joint damage³. American rheumatologists recognized Methotrexate (MTX) in the 1990s as the initial drug of choice and recommended medication for RA treatment⁴. It serves as a chemotherapeutic agent at higher concentrations by inhibiting the enzyme dihydrofolate reductase (DHFR), which halts the synthesis of folic acid. At lower doses, it suppresses the activation of T-cells and downregulates B-cells by inhibiting adenosine metabolism by blocking the AICAR transformylase enzyme⁵. MTX is associated with multiple potential adverse effects including bone marrow suppression, hepatotoxicity, renal toxicity, and pulmonary fibrosis⁶. Before starting MTX medication, a clinical evaluation of the patient is recommended, which includes a liver function test (LFT), complete blood count (CBC), renal function test, and often a chest X-ray⁷.

Natural plants, which offer a wide range of bioactive chemicals with medicinal qualities, have been essential in the development of medications. Research on herbal extracts as synthetic drug substitutes is growing in order to address drug resistance and adverse drug reactions that are frequently linked with conventional drugs. These extracts, which are abundant in bioactive components, have demonstrated encouraging treatment outcomes for a range of medical disorders. *Solanum nigrum*, also referred to as black nightshade or makoh, is a plant that belongs to the Solanaceae family and has been used traditionally for a number of illnesses, such as inflammation, edema, and joint discomfort⁸. The major biologically active components of *Solanum nigrum*, especially the saponins and steroidal alkaloids, play a crucial role in its therapeutic applications. The distilled extract of *Solanum nigrum*, arq-e-makoh, has reported antibacterial, antioxidant, gastroprotective, hypocholesterolemic, hypoglycemic, diuretic, and hepatoprotective properties. The distilled extract of *Solanum nigrum*, arq-e-makoh, has reported antibacterial, antioxidant, gastroprotective, hypocholesterolemic, hypoglycemic, diuretic, and hepatoprotective properties⁹.

Patients with RA who are treated with DMARDs often face delayed onset of action, gradual loss of effica-

cy, and a range of adverse effects due to the nature of these medications. Previous literature and our recent study have reported significant anti-edematous and anti-inflammatory effects of *Solanum nigrum* that were comparable to MTX¹⁰. So, there is a critical need for specific herbal alternatives that may be used in combination with conventional drugs like MTX for the attenuation of their adverse effects including hepatotoxicity and pancytopenia. Thus, the purpose of this study was to investigate the effects of the natural herb *Solanum nigrum* on methotrexate-associated hepatotoxicity and pancytopenia.

METHODS

It was a pre-clinical experimental study conducted at Ziauddin University, Karachi. The study was conducted from November 2021 to May 2022. *Solanum nigrum* was purchased from the local nursery and authentication was done from the Karachi University herbarium (Voucher # 97676). Plant extraction was done at Karachi University, Department of Pharmacognosy. The leaves were washed, allowed to air dry at room temperature and then coarsely ground into particles. For ten days, this powder was immersed in pure ethyl alcohol where it was stirred two to four times a day. The solution was filtered through Whatman filter paper 1. The obtained extract was then further concentrated by rotary evaporation and then kept in an airtight flask in a refrigerator¹¹. CFA (F5881-10ML) was purchased from Sigma Aldrich, Germany. Phenobarbital, dimethyl sulfoxide (DMSO), and alcohol were ordered from Laboratory Scientific Supplies Pvt. Limited, Karachi. Methotrexate vials were purchased from a local pharmacy.

In this study, 30 male Wistar albino rats were used with an approximate weight of 200±20g. All the animals used in this study were retained under standard conditions in their conventional cages in a 12/12-hour light-dark cycle with ad libitum access to food and water at room temperature¹². The investigational procedures done in this study were approved by the Animals Ethics Committee (AEC) of Ziauddin University (Protocol number: 2021-004/MM) and were performed according to the "Canadian Council on Animal Care- Revised on April 2020"¹³. The rats were randomly divided into 5 groups (n = 6). Group-I: Negative (non-diseased) control (0.9% normal saline); Group-II: Positive (diseased) control (0.9% normal saline); Group-III: Methotrexate (MTX) 1.5mg/kg; Group-IV: *Solanum nigrum* 100mg/kg; Group-V: *Solanum nigrum* 200mg/kg.

Arthritis was induced in all groups, except group I (negative controls), by an intra-articular injection of 0.1ml CFA into the right knee joint space at day 0. The concentration of CFA was 1mg/ml¹⁴. This was then followed by intraperitoneal injections of

standard drug (MTX 1.5mg/kg) and research herb (SN) extract, of 100mg/kg and 200mg/kg concentrations, on days 0,7,14 and 21 according to the groups mentioned below. Group I and Group II were treated with 0.9% normal saline on days 0, 7, 14, and 21. Group III was treated with an intraperitoneal injection of 1.5mg/kg methotrexate as a standard dose on days 0, 7, 14, and 21. Group IV and Group V were treated with intraperitoneal injections of ethanolic extract of SN at 100mg/kg and 200mg/kg, respectively at the same intervals once weekly for four weeks. These dosages were selected based on several previous investigations that evaluated the anti-arthritic effect in rats^{15,16,17}.

After overnight food deprivation, on the 29th day of the study, rats were deeply anesthetized and sacrificed by 100mg/kg pentobarbital intraperitoneal injection as per American Veterinary Medical Association

AVMA and Institutional Animal Ethics Committee (IAEC) guidelines¹⁸. 8ml to 10ml of blood samples were collected by cardiac puncture from each rat and then transferred into vacutainer tubes. 5ml of blood was separated in a purple top tube for complete blood count (CBC) while the remaining 5ml was centrifuged at 3000 rpm for 10 minutes in a yellow top vacutainer tube. After centrifugation, sera were separated for liver function tests (LFTs).

The results were analyzed by SPSS software version 22. For numeric variables mean and standard deviation were calculated. According to Shapiro Wilk's analysis, the data was found to be parametric. Hence, ANOVA followed by post hoc Tukey's test was applied for inter and intra-group comparison. P-value <0.05 was considered significant at a 95% confidence interval.

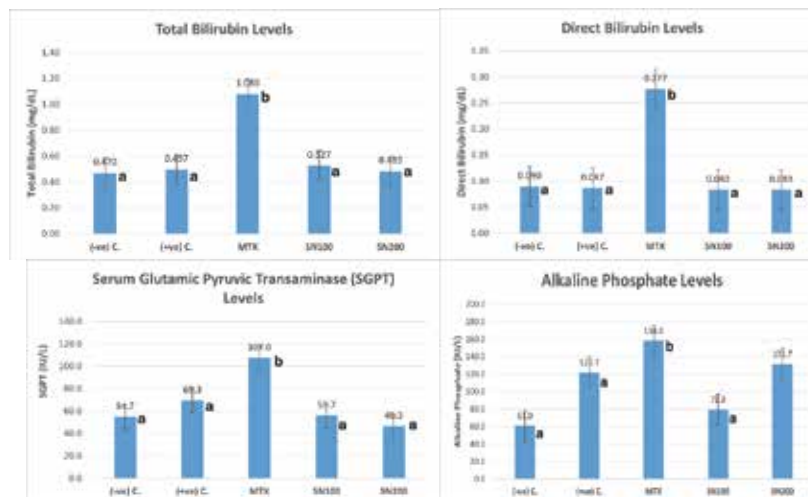
RESULTS

Table 1: Comparison of RBCs, HGB, WBCs, and platelet levels in various experimental groups at day 28.

	-ve Control (Healthy)	+ve Control (Diseased)	Methotrexate 1.5mg/kg	Solanum nigrum 100mg/kg	Solanum nigrum 200mg/kg
RBCs	6.24 ^a	4.91	3.66	7.21 ^a	6.59 ^a
HGB	15.30 ^a	15 ^a	11.17 ^b	14.70 ^a	14.57 ^a
WBCs	6.37 ^b	20.10 ^a	9.07 ^b	6.07 ^b	8.67 ^b
Platelets	962 ^a	925 ^a	629 ^b	891 ^a	945 ^a

a shows significant (p<0.05) difference between MTX group and other groups; **b** shows significant (p<0.05) difference between positive control and other groups

MTX group showed significant suppression of RBCs & hemoglobin when compared with herbal groups. Like MTX, both herbal groups showed notable suppression of WBCs when compared with the diseased controls. Platelets were also significantly decreased in the MTX group when compared with all other groups (**Table 1**).



a shows significant (p<0.05) difference between MTX group and other groups; **b** shows significant (p<0.05) difference between positive control and other groups

Figure 2: Comparison of Total Bilirubin, Direct Bilirubin, SGPT, and Alkaline Phosphate Levels in Various Experimental Groups at Day 28.

DISCUSSION

CFA is effective in mimicking the immunological and pathological features of these diseases, making it a valuable tool for researchers¹⁹. The positive control group's WBCs showed a noteworthy surge, but the other three treatment groups MTX, SN100, and SN200 showed a considerable decrease in WBCs. MTX used as a standard drug, caused a significant decrease in WBC levels but also caused suppression in RBC and HGB levels. In a similar manner, a different trial that used MTX as treatment revealed a noteworthy decrease in WBCs, RBCs, and HGB levels, demonstrating the drug's suppressive effects on blood cells²⁰. In relation to SN, a study conducted a hematological study in rats with arthritic conditions generated by CFA. Three distinct dosages of SN, 200 mg/kg, 400 mg/kg, and 800 mg/kg, demonstrated a dose-dependent decrease in WBC count, indicating the herb's ability to mitigate inflammation²¹. This report also showed a decline in platelets upon increasing the dose of the herb while, contrary to this, in our study, there was no significant difference in platelet levels in both treated and untreated groups. Similar to our findings, another study found no discernible difference in the effects of different dosages of SN on platelets and RBCs. The study's findings also demonstrated that SN treatment had no obvious effect on HGB, MCV, or MCH, demonstrating the its non-bone marrow suppressive qualities²². According to our findings, SN attenuated the bone marrow suppressive impact of MTX by not significantly affecting red blood cells, hematocrit, or hemoglobin levels in the groups treated with it. Therefore, our results support this argument that, unlike MTX, SN does not have bone-marrow suppressive properties rather it can be used adjunctively to prevent bone marrow regression has been reported with MTX, MTX exhibited a significant increase in total bilirubin. MTX also caused a significant increase in the SGPT levels as MTX is well known to cause serum SGPT elevation²³. Our results also exhibited a significant increase in alkaline phosphate in the MTX group when compared with the negative control group. Few other recent studies also reported that the MTX group notably increased the bilirubin, SGPT and alkaline phosphate levels when compared with controls^{24, 25}. Individuals who use MTX for an extended period of time may develop liver damage as a result of intracellular MTX-polyglutamate (MTX-PG) buildup. It is a metabolite of MTX that triggers inflammation, oxidative stress, fibrosis, and apoptosis in hepatocytes therefore LFTs are usually recommended during the course of its treatment²⁶. The results reported that both SN groups showed no significant effects on total bilirubin levels rather it may decrease the hepatotoxic potential of MTX if used in combination. Similar outcomes have been reported in another investigation, demonstrat-

ing a noteworthy reduction in bilirubin levels upon SN treatment. Alkaline phosphate and SGPT levels were also found to decline in a dose-dependent manner in the same study¹². Literature has also reported a decrease in total bilirubin, SGPT, and alkaline phosphate in SN-treated animals²⁷. Apart from its suppressive effects on pro-inflammatory mediators, SN also has reported hepatoprotective, gastroprotective, nephroprotective, anti-inflammatory, antioxidant, and analgesic properties^{28,29,30}.

CONCLUSION

In conclusion, unlike methotrexate which showed myelosuppressive effects, *Solanum nigrum* did not show any suppressive effects on the hematological profile. *Solanum nigrum* demonstrated its hematopoietic potential. Furthermore, *Solanum nigrum* outperformed MTX in reducing the hepatic parenchymal damage brought on by MTX and did not significantly increase the liver enzymes. Thus, *Solanum nigrum* can be further evaluated in clinical trials as an adjunct to MTX for the comprehensive therapy of RA following additional in vivo research with dose optimization.

LIST OF ABBREVIATIONS

CFA - Complete Freund's Adjuvant
SN - *Solanum Nigrum*
CBC - Complete Blood Count
HGB - Hemoglobin
LFT - Liver Function Test
SGPT - Serum Glutamic Pyruvic Transaminase
MTX - Methotrexate

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The study was approved by the Animal Ethics Committee (AEC) of Ziauddin University having protocol number 2021-004/MM.

AUTHORS' CONTRIBUTIONS

Study conception and design by **HR**, **KI**, and **OI**. Manuscript writing, data acquisition, interpretation and analysis were done by **AA** and **HR**. **HA** and **SM** were major contributors to writing and manuscript preparation.

REFERENCES

1. Tański W, Dudek K, Tomaszewicz A, Świętoniowska-Lonc N. Sexual dysfunction and quality of life in

- patients with rheumatoid arthritis. *International journal of environmental research and public health*. 2022 Mar 6;19(5):3088. doi: 10.3390/ijerph19053088
2. Paglia MDG, Silva MT, Lopes LC, Barberato-Filho S, Mazzei LG, Abe FC, et al. Use of corticoids and non-steroidal anti-inflammatories in the treatment of rheumatoid arthritis: Systematic review and network meta-analysis. *PLoS One*. 2021 April 7;16(4):e0248866. doi: 10.1371/journal.pone.0248866
 3. Li JM, Yao YD, Luo JF, Liu JX, Lu LL, Liu ZQ, Dong Y, Xie Y, Zhou H. Pharmacological mechanisms of sinomenine in anti-inflammatory immunity and osteoprotection in rheumatoid arthritis: a systematic review. *Phytomedicine*. 2023 Dec 1;121:155114. doi: 10.1016/j.phymed.2023.155114
 4. Garcia-Gonzalez CM, Baker J. Treatment of early rheumatoid arthritis: methotrexate and beyond. *Current Opinion in Pharmacology*. 2022 Jun 1;64:102227. doi: 10.1016/j.coph.2022.102227
 5. Alqarni AM, Zeidler MP. How does methotrexate work? *Biochemical Society Transactions*. 2020 Apr 29;48(2):559-67. doi: 10.1042/BST20190803
 6. Hamed KM, Dighirri IM, Baomar AF, Alharthy BT, Alenazi FE, Alali GH, Alenazy RH, Alhumaidi NT, Alhulayfi DH, Alotaibi YB, Alhumaidan SS. Overview of methotrexate toxicity: a comprehensive literature review. *Cureus*. 2022 Sep 23;14(9). doi: 10.7759/cureus.29518
 7. Khan S, Batool W, Naveed S, Ahmad SM. A fatal fate: a medical error leading to acute methotrexate toxicity. *Cureus*. 2022 Oct;14(10). doi: 10.7759/cureus.30659
 8. Gul B, Anwar R, Saleem M, Ahmad M, Ullah MI, Kamran S. Attenuation of CFA-induced arthritis through regulation of inflammatory cytokines and antioxidant mechanisms by *Solanum nigrum* L. leaves extracts. *Inflammopharmacology*. 2023 Dec;31(6):3281-301. doi: 10.1007/s10787-023-01357-z
 9. Harish K, Sivasankaran SM, Manoharan S. Indian Medicinal Plants with Multiple Pharmacological Efficacies: A Comprehensive Review. *Journal of Drug Delivery & Therapeutics*. 2024 Feb 1;14(2). doi: 10.22270/jddt.v14i1.6347
 10. Rasheed H, Ismail K, Ismail O, Ali A, Imad R, Anwar J. Assessment of anti-inflammatory and anti-arthritic potential of ethanolic extract of *Solanum nigrum* L. leaves in CFA-induced arthritic rat model. *ANNALS OF ABBASI SHAHEED HOSPITAL AND KARACHI MEDICAL & DENTAL COLLEGE*. 2024 Aug 31;29(3):298-305. doi: 10.58397/ashkmdc.v29i3.790
 11. Syahputra RA, Sutiani A, Silitonga PM, Rani Z, Kudadiri A. Extraction and phytochemical screening of ethanol extract and simplicia of moringa leaf (*Moringa oleifera* Lam.) from sidikalang, north Sumatera. *International Journal of Science, Technology & Management*. 2021 Nov 20;2(6):2072-6. doi: 10.46729/ijstm.v2i6.381
 12. Cui P, Qu F, Sreeharsha N, Sharma S, Mishra A, Gubbiyappa SK. Antiarthritic effect of chitosan nanoparticle loaded with embelin against adjuvant-induced arthritis in Wistar rats. *IUBMB life*. 2020 May;72(5):1054-64. doi: 10.1002/iub.2248
 13. Care CCoa. Canadian Council on animal Care guidelines 2020 [Available from: <https://ccac.ca/en/guidelines-and-policies/the-guidelines/>].
 14. Li S, Li R, Xu Y-X, Baak J, Gao J-H, Shu J-Q, et al. Traditional Chinese medicine aconiti radix cocta improves rheumatoid arthritis via suppressing COX-1 and COX-2. *Evidence-Based Complementary and Alternative Medicine*. 2021 Sep 8;2021. doi: 10.1155/2021/5523870
 15. Sheng Z, Zeng J, Huang W, Li L, Li B, Lv C, et al. Comparison of therapeutic efficacy and mechanism of paclitaxel alone or in combination with methotrexate in a collagen-induced arthritis rat model. *Zeitschrift für Rheumatologie*. 2020 Dec 15. doi: 10.1007/s00393-020-00940-x
 16. Indela KR, Yerragopu AK, Chamarthi SK. A REVIEW ON PLANTS HAVING ANTI INFLAMMATORY ACTIVITY. 2020 Sep 20. doi: 10.20959/wjpr202012-18738
 17. Saibu G, Adu OB, Faduyile F, Iyapo O, Adekunle K, Abimbola S, et al. Investigation of the antioxidant potential and toxicity of the whole leaf of *Solanum nigrum* in albino rats. *Journal of Research and Review in Science*. 2020 Dec;7(1):9-16. doi: 10.36108/jrrslasu/0202.70.0120
 18. Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. Retrieved on March. 2020 Mar;2013 (30):2020-1.
 19. Patel R, Kadri S, Gohil P, Deshpande S, Shah G. Amelioration of complete Freund's adjuvant-induced arthritis by *Calotropis procera* latex in rats. *Future Journal of Pharmaceutical Sciences*. 2021 Dec;7:1-11. doi: 10.1186/s43094-021-00361-w
 20. Tummala Srinivas DB, Kumar BK, Lakshman M, Reddy AG. Oxidative stress induced hematological alteration of methotrexate attenuates *Bacopa monnieri* in rats. *pathogenesis*. 2021 Jul 9;4(5):6.
 21. Alamgeer SA, Uttra AM, Hasan UH. Alkaloids, flavonoids, polyphenols might be responsible for potent antiarthritic effect of *Solanum nigrum*. *Journal of Traditional Chinese Medicine*. 2019 Oct 15;39(05):632.
 22. Aduwamai UH, Abimbola MM, Ahmed ZH. Effect of *Solanum nigrum* methanol leaf extract on phenylhydrazine induced anemia in rats. *Jordan J Biol Sci*. 2018 Mar 21;11(1):65-71.
 23. Dogra A, Gupta D, Bag S, Ahmed I, Bhatt S, Nehra E, et al. Glabridin ameliorates methotrexate-induced liver injury via attenuation of oxidative stress, inflammation, and apoptosis. *Life Sciences*. 2021 Aug 1;278:119583.
 24. Elsayy H, Algefare AI, Alfwuaires M, Khalil M, Elmenshawy OM, Sedky A, et al. Naringin alleviates methotrexate-induced liver injury in male albino rats and enhances its antitumor efficacy in HepG2 cells. *Bioscience reports*. 2020 Jun 10;40(6):BSR20193686. doi: 10.1042/BSR20193686

25. Alfwuaires MA. Galangin mitigates oxidative stress, inflammation, and apoptosis in a rat model of methotrexate hepatotoxicity. *Environmental Science and Pollution Research*. 2022 Mar;29(14):20279-88. doi: 10.1007/s11356-021-16804-z
26. Ezhilarasan D. Hepatotoxic potentials of methotrexate: Understanding the possible toxicological molecular mechanisms. *Toxicology*. 2021 Jun 30;458:152840. doi: 10.1016/j.tox.2021.152840
27. Chester K, Zahiruddin S, Ahmad A, Khan W, Paliwal S, Ahmad S. Bioautography-based identification of antioxidant metabolites of *Solanum nigrum* L. and exploration its hepatoprotective potential against D-galactosamine-induced hepatic fibrosis in rats. *Pharmacognosy Magazine*. 2019 Apr;15(Suppl 1):S104-S110. doi: 10.4103/pm.pm_359_18
28. Alam A, Sahar A, Sameen A, Faisal MN. The antioxidant and hepatoprotective potential of *Solanum nigrum* against oxidative stress. 2022 May 10. doi: 10.21203/rs.3.rs-1590719/v1
29. Fallahzadeh A, Mohammadi S. Assessment of the antinociceptive, anti-inflammatory, and acute toxicity effects of *solanum dulcamara* essential oil in male mice. *Journal of Babol University of Medical Sciences*. 2020 Mar 10;22(1):162-8. doi: 10.22088/jbums.22.1.162
30. Paumgartten F, de Souza G, da Silva A, De-Oliveira A. Analgesic properties of plants from the genus *Solanum* L.(Solanaceae). *Treatments, Mechanisms, and Adverse Reactions of Anesthetics and Analgesics*: Elsevier; 2022 Jan 1. p. 457-71. doi: 10.1016/B978-0-12-820237-1.00039-9.
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