ORIGINAL ARTICLE

Antiarthritic effects of Solanum *nigrum* in CFA-induced rat model – a histological analysis

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Biomedica - Official Journal of University of Health Sciences, Lahore, Pakistan
Volume 41(1):3-9
https://doi.org/10.24911/BioMedica/5-1192

ABSTRACT

Background and Objective: Conventional disease-modifying anti-rheumatic drugs used for the treatment of rheumatoid arthritis are known for their steady onset of action and multiple adverse effects. The objective of this study was to evaluate the anti-arthritic activity of the ethanolic extract of *Solanum nigrum* (SN) in the Complete Freund's adjuvant-induced arthritic rat model.

Methods: A total of 30 male Wistar albino rats were used in a 4-week pre-clinical experimental study. The animals were divided into 5 groups; Group-I negative (healthy) control (0.9% normal saline), Group-II positive (diseased) control (0.9% normal saline), Group-III standard group (Methotrexate 1.5 mg/kg), Group-IV (SN 100 mg/kg), and Group-V (SN 200 mg/kg). To develop arthritis, 0.1ml of Complete Freund's Adjuvant was administered intraarticularly in the right knee joints of all groups except Group-I at day 0. Knee joint circumference was assessed by using a Vernier caliper once weekly for 4 weeks. For euthanasia, 100 mg/kg pentobarbital was injected intraperitoneally in all animals on the 29th day for the assessment of histopathological changes in the knee joints. SPSS version 22 was used to analyze the results, and ANOVA was applied for intergroup and intragroup comparisons.

Results: Edema in the positive controls (group-II) increased continuously throughout the study duration. While group-III methotrexate (MTX) showed an initial increase in edema, but slight reduction was observed in the last two weeks. Group-V (SN200) with a higher dose of herbal extract showed maximum reduction in edema. Histological assessment showed the maximum arthritic score (3) in group-II. While 66% of animals in group-III MTX and group-IV (SN100) showed mild scoring exhibiting scattered inflammatory cells, Group-V showed maximum improvement in the histopathological changes, and 66% of animals showed normal scoring, while 33% animals showed few inflammatory cells ($p < 0.05$).

Conclusion: Ethanolic extract of SN demonstrated decreased knee joint edema and improved histopathological outcomes in animal model, suggesting therapeutic potential for arthritis management in humans, in future.

Keywords: Arthritis, Rheumatoid arthritis Histology, Edema, Solanum nigrum.

Received: 31 August 2024 **Revised 1:** 02 November 2024 **Revised 2:** 13 December 2024 **Accepted:** 28 December 2024
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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder and a major contributor to the global burden of disability, morbidity, and mortality. It is characterized by symmetric polyarthritis, particularly of the small joints, along with synovial hyperplasia and bone damage.[1] Globally, around 0.2%-1% of the population is affected by RA. Most of the affected RA patients are middle-aged women, although it can occur at any age.[2] The data on the prevalence of RA in Pakistan are limited; however, few studies have reported a 0.14%-0.22% prevalence of RA in the Pakistani population[3], while another study reported a range of 0.14%-0.6%, with RA being more common in females.[4]

Non-steroidal anti-inflammatory drugs have long been used to manage RA, but corticosteroids were first introduced in the 1950s for symptomatic relief. Around the same time, conventional disease-modifying anti-rheumatic drugs (DMARDs) like hydroxychloroquine in the 1950s, azathioprine in the 1960s, and sulfasalazine and methotrexate (MTX) in the mid-1980s were also introduced for RA management.[5] MTX was recognized by American rheumatologists in the 1990s as the initial drug of choice and recommended medication for managing RA.[6] Although

MTX is preferred over other DMARDs due to its higher tolerance and cost-effectiveness, several adverse effects are associated with its use. Regarding its clinical benefits against cancer and immunosuppression, it has a unique mechanism. At higher concentrations, it acts as a chemotherapeutic agent by inhibiting dihydrofolate reductase, thereby halting folic acid synthesis. While at lower doses, as required in autoimmune conditions, it inhibits adenosine metabolism by inhibiting the AICAR transformylase, leading to extracellular adenosine accumulation, which suppress B-cells function and T-cells activation.[7] Due to its frequent adverse effects like bone marrow suppression, hepatotoxicity, renal toxicity, and pulmonary fibrosis, a regular clinical evaluation of the patient is advised, which includes a complete blood count, liver function test, renal function test, and occasionally a chest radiography before initiating MTX therapy.[8]

The incidence and impairments associated with RA, in addition to the limitations of DMARDs—including delayed onset of action, gradual loss of efficacy, and side effects remain major clinical concerns. Although the recently developed biologic DMARDs have slightly succeeded in achieving lower disease activity, the high cost of these medications remains a significant problem, particularly in low-income countries. Therefore, there is an urgent need for affordable therapeutic agents with a lower adverse profile that can be used either as a standalone therapy or in combination with existing therapies.[9]

Herbal traditional phytomedicine is a centuries-old practice that has been used by various ethnicities across the globe in different cultures and civilizations. Extracts from natural plants and herbs have long served as the primary source for the synthesis of various medications. Literature reports that about 85%–90% of the world's population relies on traditional herbal remedies for managing various health conditions.[10] To overcome drug resistance and the side effects of synthetic medications, herbal extracts containing multiple bioactive constituents are being extensively studied and have shown commendable therapeutic potential.

Solanum nigrum (SN), commonly known as black nightshade or *makoh*, belongs to the Solanaceae family and has traditionally been used for joint pain, edema, and inflammation. Some of the most significant biologically active constituents of SN with potential therapeutic properties are steroidal saponins, steroidal alkaloids, and polyphenols.[11] Arq-e-makoh, a distillate prepared from SN, has demonstrated antimicrobial, antioxidant, gastroprotective, hypocholesterolemic, hypoglycemic, diuretic, and hepatoprotective effects. It has a remarkable free radical scavenging activity and has shown notable effectiveness in conditions such as hepatosplenomegaly and fatty liver disease.[12]

Therefore, this study was designed to explore the potential therapeutic role of SN in the management of RA, given its reported organo-protective and multi-targeted pharmacological properties.

Methods

It was a pre-clinical experimental study conducted at the Department of Pharmacology, Ziauddin University, Karachi, from November 2021-May 2022. SN leaves were acquired from a local nursery, and authentication was done from the herbarium of Karachi University (Voucher # 97676). The leaves were washed, air-dried at room temperature, and coarsely ground. This powder was immersed in pure ethyl alcohol for 10 days, with stirring thrice daily. The mixture was filtered through Whatman filter paper 1, and the filtrate was concentrated by rotary evaporation. The resulting extract was kept in an airtight container in a refrigerator.[13] The percentage yield of the extract was 19.1%.[14]

Complete Freund's Adjuvant (CFA) (F5881-10 ml) was procured from Sigma Aldrich, Germany. Pentobarbital, dimethyl sulfoxide (DMSO), and alcohol were procured from Laboratory Scientific Supplies Pvt. Limited, Karachi. MTX was acquired from a local registered pharmacy.

Animal protocols

A total of 30 male Wistar rats, weighing 200 ± 20 g, were included in the study. Rats were kept in the animal house of the College of Pharmacy, Ziauddin University, Karachi, under standard laboratory conditions in their cages in a 12/12-hour light-dark cycle with *ad libitum* access to water and food at 22°C-26°C.[15] All experimental procedures were approved by the Animals ethics committee of Ziauddin University (Protocol number: 2021-004/MM) and were performed according to the "Canadian Council on Animal Care - Revised on April 2020".[16] Body weight of all rats was measured on days 0, 7, 14, 21, and 28 of the study using a weighing balance.

Grouping of animals

The rats were randomly assigned to 5 groups (n = 6) using an online random number generator to ensure unbiased allocations: I—Negative control (0.9% normal saline), II—positive control (0.9% normal saline), III—methotrexate 1.5 mg/kg, IV—*SN* 100 mg/kg, and V—*SN* 200 mg/kg.[17] The sample size of 6 animals per group was determined based on similar studies.[18]

Induction of arthritis

Arthritis was induced in all groups except the negative controls group by an intra-articular injection of 0.1 ml of concentration 1 mg/ml CFA into the right knee joint on day 0.[19] This was followed by intraperitoneal injections of MTX

1.5 mg/kg[20], SN 100 mg/kg, and SN 200 mg/kg on days 0,7,14, and 21 according to the assigned groups.[21]

Experimental procedure

Following induction of arthritis, the negative and positive control groups were treated with 0.9% normal saline intraperitoneally on days 0, 7, 14, and 21. This was done to ensure that all groups underwent the same injection procedure, thereby allowing differences in the results to be attributed to the treatments rather than to the vehicle or injection process. MTX group was treated with an intraperitoneal injection of 1.5 mg/kg MTX as a standard dose on days 0, 7, 14, and 21. SN100 and SN200 groups were treated with intraperitoneal injections of the ethanolic extract of SN at 100 mg/kg and 200 mg/kg, respectively, at the same intervals. These dosages were selected following several preliminary investigations that assessed the anti-arthritic effect in rats.[21,22] 10% DMSO was employed as a vehicle for the administration of herbal extract and MTX due to its effective solvent properties for hydrophobic compounds while maintaining low toxicity levels. Studies support the safe use of 10% DMSO in rodent models, where it does not produce any significant adverse effects or interfere with physiological parameters.[23]

On day 29, after overnight fasting, rats were anesthetized and euthanized by 100 mg/kg pentobarbital intraperitoneal injection as per the guidelines of American Veterinary Medical Association and Institutional Animal Ethics Committee.[24]

Histological assessment of joints

After euthanasia, the right knee joints of all rats were excised and fixed in 10% formalin for 48-72 hours. The joints were then placed in a decalcifying solution (10% formic acid) for 2-3 days. Tissues were processed and embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin.[25] Histopathological changes in joints were evaluated under a light microscope to assess inflammatory changes. To avoid bias, the observers were blinded to group assignments. Furthermore, the histological assessments were independently performed by two observers, and inter-observer reliability was evaluated to ensure consistency and reproducibility of the results. The arthritic severity was scored based on neutrophil infiltration and bone/cartilage destruction at 0–3 scale, where 0 = normal (negligible cellular infiltration), 1 = mild (scattered inflammatory cells), 2 = moderate (dense cellular infiltration), and 3 = severe (pannus formation and bone erosion along with infiltration of plasma cells).[26]

Statistical analysis

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

Results

Histology

The positive control group showed marked arthritis, evidenced by synovial proliferation, pannus formation, and plasma cell infiltration in the knee joint cavity, as shown in Figure 1. There was a statistically significant (p-value < 0.05) decrease in mean histological score observed in the MTX 1.5 mg/kg group (0.67 ± 0.33) when compared to the positive controls (2.83 ± 0.71), showing scattered inflammatory cells. The SN 100 mg/kg group showed mild arthritis with scattered inflammatory cells and had a mean histological score of 1.33 ± 0.42, which was found to be significantly (p-value < 0.05) decreased when compared to the positive controls (2.83 ± 0.71). However, the SN 200 mg/kg group showed negligible cellular infiltration, corresponding to a mean histological score of 0.33 ± 0.22, which was significantly decreased when compared to positive controls. Figure 2 shows the mean histological scores of all experimental groups.

Body weight

The body weight of rats was measured at day 0 before inducing arthritis and subsequently once weekly on days 7, 14, 21, and 28. There were no statistically significant changes observed in the body weight of rats throughout the study period, although an insignificant increase in the body weight of the positive control group was observed (Figure 3).

Discussion

In the past few decades, various adjuvant-induced arthritic models have been explored to investigate the pathogenesis of arthritis and new therapeutic approaches. CFA is considered the "gold standard adjuvant" for inducing cell-mediated immunity in autoimmune disease research, particularly in RA and experimental autoimmune encephalomyelitis.[27]

In this study, 0.1 ml of CFA was administered in the knee joints of rats. In the negative control group, the histological assessment of knee joint sections reported minimal infiltration of inflammatory cells. While the positive control group, treated with CFA, showed severe infiltration of inflammatory cells, pannus formation, and arthritic dismantling caused by the adjuvant. A statistically significant difference (p-value < 0.05) was observed between the positive control and negative control groups. The MTX 1.5 mg/kg group showed scattered inflammatory cells and was scored 1 (mild inflammation), confirming the anti-arthritic

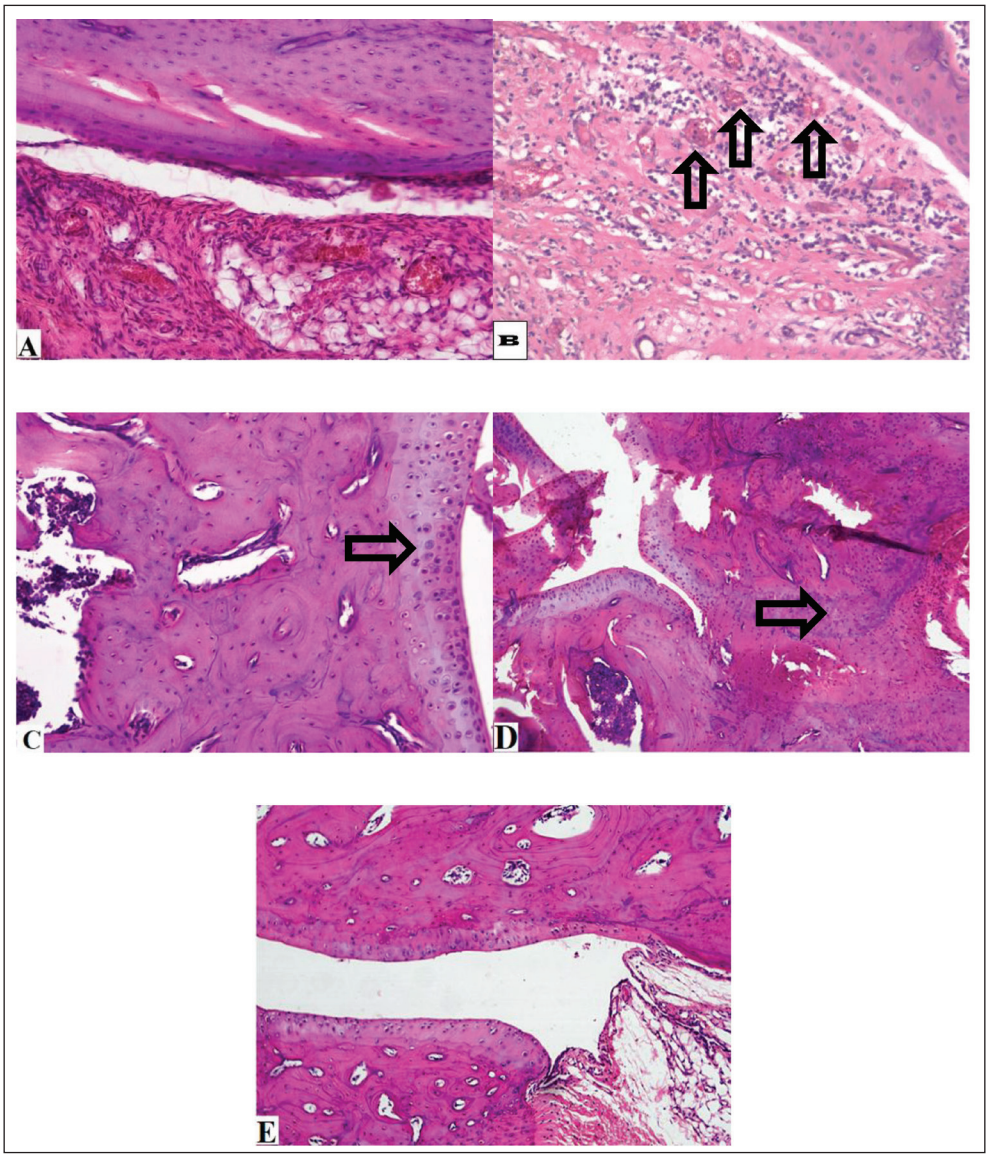

Figure 1. *Histological analysis of the knee joint at 20X on the 29th day. A = Negative control group, B = Positive control group, C = MTX 1.5 mg/kg group, D = SN 100 mg/kg group, and E = SN 200 mg/kg group. Vertical arrow (↑) showing increased cellular infiltration, infiltration of plasma cells, and pannus formation; Horizontal arrows (→) showing scattered inflammatory cells.*

properties of MTX. Comparing MTX-treated rats using joint histology, previous investigations have also shown minimal infiltration of inflammatory cells in MTX-treated animals.[28] Another study using collagen-induced arthritis demonstrated that MTX improved histopathological changes and exerted suppressive effects on arthritis-associated joint damage.[29] In our research, dose-dependent antiarthritic activity was observed in the SN-treated animals. The lower dose group, SN 100 mg/kg group, showed scattered inflammatory cells, while the higher dose group, SN 200 mg/kg group, showed negligible changes and minimal infiltration of the inflammatory cells in the joint cavity. Similar findings were observed in another study that showed an 800 mg/kg dose of SN caused a minimum influx of inflammatory cells, and significant inhibition of cartilage destruction with no pannus formation.[30] Additionally, SN has been reported to inhibit osteoclastic differentiation and improve histopathological changes in ovariectomy-induced osteoporosis model.[31] Taken together, these findings, along with previous literature, highlight the SN's potential to improve the histopathological changes associated with arthritis.

The weight of the animals was also measured on days 0, 7, 14, 21, and 28. There was no statistically significant increase in the body weights across the groups. A non-significant increase in body weight was recorded in the positive control group. A similar trend was reported in another study comparing

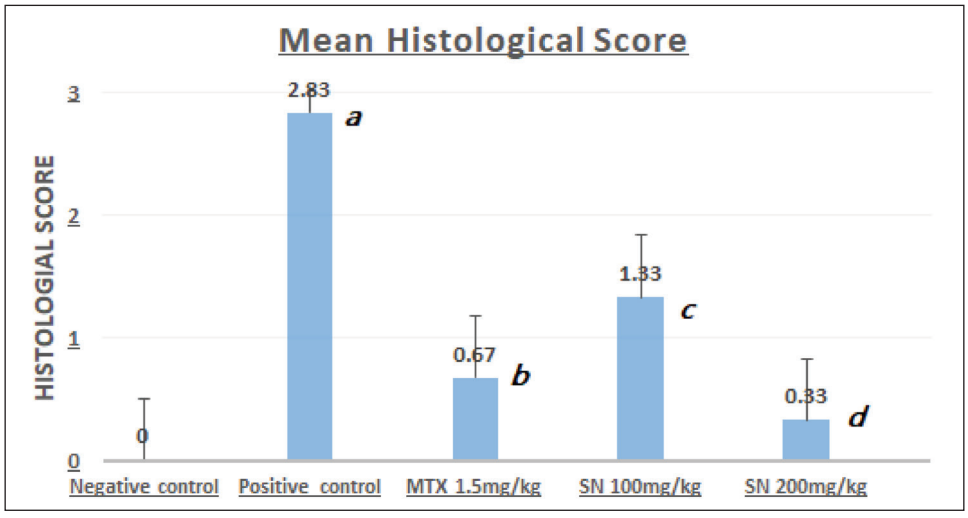

Figure 2. *Mean histological scoring of all experimental groups, where, arthritic score 0 = normal (Negligible cellular infiltration), 1 = mild (Scattered inflammatory cells) 2 = moderate (Dense cellular infiltration or cartilage destruction) 3 = severe (Pannus formation and bone erosion along with infiltration of plasma cells) a = significant difference (p-value < 0.05) between positive control and other groups; b = significant difference (p-value < 0.05) between MTX group and positive control; c = significant difference (p-value < 0.05) between SN 100 mg/kg and positive controls; d = significant difference (p-value < 0.05) between SN 200 mg/kg and positive controls.*

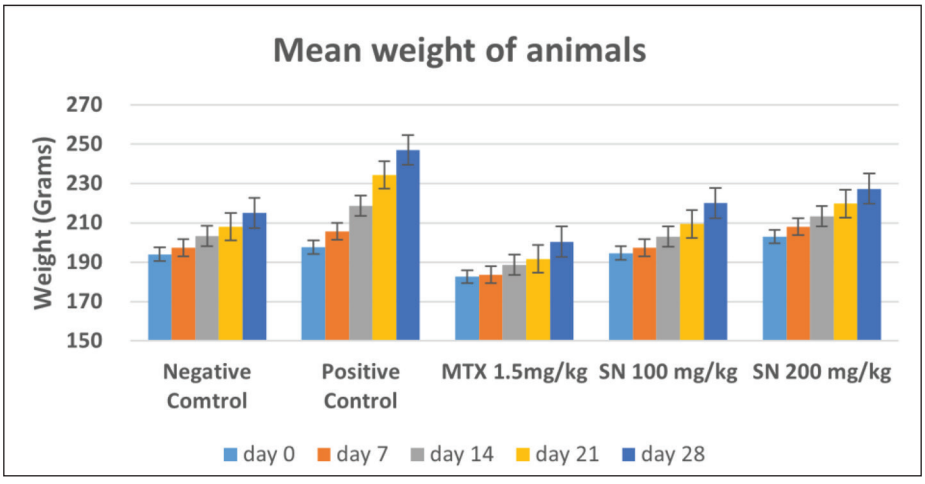

Figure 3. *Comparison of weight in various experimental groups at different intervals.*

the weights of negative and positive control groups.[32] But contrary to this, multiple studies reported a reduction in body weights of the arthritic control group animals.[33] The possible reason for the increase in body weight of positive controls is due to reduced physical activity, leading to less energy expenditure, combined with potentially increased food intake.

Limitations of the study

This study has several limitations that should be considered while interpreting the findings. First, the experiment was conducted on a small sample size of rats under controlled laboratory conditions, which may limit the generalizability of results to larger populations or clinical settings. Second, only histological parameters were evaluated; additional biochemical and molecular markers of inflammation and oxidative stress could have provided a more comprehensive understanding of the antiarthritic mechanisms of *S. nigrum*. Third, the study duration was relatively short, and the long-term efficacy and safety of *S. nigrum* could not be assessed. Fourth, only two doses of the extract were tested, leaving the possibility that other dose ranges might yield different therapeutic responses. Finally, the study did not explore the potential toxicity or adverse effects of *S. nigrum*, which is essential before considering its translation into clinical use.

Conclusion

In conclusion, treatment with *S. nigrum* has demonstrated significant improvements in histological outcomes compared to the positive controls. These effects may be attributed to its potential anti-inflammatory and antioxidant properties. However, further *in vivo* studies are needed to validate these findings, evaluate long-term safety, and determine the optimal therapeutic dose.

Acknowledgement

The authors would like to acknowledge Dr. Faraz Baig, Professor of Pathology, Ziauddin University, Karachi for his histologic assessment and academic support during the research work. Further, we are thankful to all staff of Pharmacology Department of Ziauddin University, Karachi for their logistic support during the conduction of this study.

List of Abbreviations

ANOVA	Analysis of variance
CFA	Complete Freund's Adjuvant
DMARDs	Disease modifying anti-rheumatic drugs
DMSO	Dimethyl sulfoxide
MTX	Methotrexate
RA	Rheumatoid arthritis
SN	*Solanum nigrum*

Conflict of interest

None to declare.

Grant support and financial disclosure

None to disclose.

Ethical approval

The ethical approval of the study was granted by the Animal Ethics Committee (AEC) of Ziauddin University, Karachi, Pakistan vide Letter no: 2021-004/MM dated 03 June, 2021.

Authors' contributions

HUR, KI, MOI: Conceived the study, designed the methodology and drafting of manuscript, reviewed and revised the manuscript, and critical intellectual input.

AA, HA, SZA: Collection and assembly of data, drafting of the manuscript, data analysis, and interpretation of results.

ALL AUTHORS: Approval and full responsibility for the final version of the manuscript to be published.

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