Antiarthritic and anti-inflammatory activity of *Solanum nigrum* leaves extract on CFA-induced arthritic rat model

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ABSTRACT

OBJECTIVE:

To evaluate antiarthritic and anti-inflammatory activity of *Solanum nigrum* leaves extract on CFA-induced arthritic rat model.

METHODOLOGY:

It was a 4-week pre-clinical experimental study, including 30 male Wistar albino rats divided in 5 groups as; Group-I Negative control, Group-II Positive control; Group-III Methotrexate (MTX); Group-IV *Solanum nigrum* (SN) 100mg/kg; Group-V *Solanum nigrum* (SN) 200mg/kg. Arthritis was induced by administering 0.1mL of CFA in right hind paw from Group-II to Group-V at day 0. Intraperitoneal injections of 0.9% normal saline (Group-I and II); MTX (Group-III), SN100 (Group-IV) and SN200 (Group-V) were administered at days 0, 7, 14 and 21. Paw size was measured by using Vernier caliper on day 0, 7, 14, 21 and 28. On the 29th day, animals were anesthetized by an intraperitoneal injection of pentobarbital 100mg/kg and 10ml of blood was collected by cardiac puncture. Serum was separated by centrifugation to analyze IL-1, IL-2, IL-6, TNF-α and PG-E2 by ELISA. Results were analyzed by using SPSS version 22. ANOVA was applied for intergroup and intragroup comparison. P-value <0.05 will be considered significant at 95% confidence interval.

RESULTS:

Paw circumference was significantly and equally decreased in MTX, SN100 and SN200 groups when compared with positive control group showing equal efficacy of SN as an anti-inflammatory agent. ELISA results revealed significant increase in all pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF-α and PG-E2) is positive control group. MTX and herbal treatment groups (IV and V) significantly suppressed these cytokines but SN200 exhibited maximum reduction.

CONCLUSION:

Solanum nigrum can be used as an effective adjunctive with standard DMARDs like MTX to increase the efficacy in the treatment of rheumatoid arthritis.

INTRODUCTION:

Amongst the various contributors to the major disability burden worldwide, arthritis acquires the prime position (1). Arthritis, the inflammation of joints is the most common cause of multiple joint pain in elderly people. Multiple types of arthritis have been studied but the most debilitating type is rheumatoid arthritis (2). Rheumatoid arthritis is a long-standing autoimmune disease distinguished by the destruction of cartilage, bone, and synovial membrane leading to joint damage and possibly permanent disability (3). The global prevalence of RA is 1% and the male-to-female ratio is 1:3 (4). The increasing prevalence of RA is a serious issue of concern, especially in developing countries like Pakistan. Various etiological pathways of RA are described in the literature but the exact pathogenesis of RA is still unclear and needs to be investigated. According to the literature, the etiology of RA is based on genetic variability along with a few triggering factors such as infection or environmental influences (5). Besides the articular damage, many patients may develop extra-articular manifestations which include respiratory system complications (pulmonary nodulosis), skeletal complications (osteoarthritis), and most importantly cardiovascular-related morbidities and mortalities (6, 7). Therapeutics prescribed and used for RA do not cure the disease entirely but either may modify the progression of the disease (Disease-modifying anti-rheumatic drugs - DMARDs) or provide symptomatic relief (Non-steroidal anti-inflammatory drugs - NSAIDs) (8). Long-term medicinal use of DMARDs and NSAIDs has very common and frequently reported adverse effects including gastrointestinal toxicity, hepatic and pulmonary fibrosis, and cardiovascular disorders (9). Therefore, it is a dire need of time to discover better therapeutic agents with the least adverse effects (10).

In many regions of the world, medicinal plants are being in use for various ailments since ancient times. Extracts of different herbs and plants, which have biologically active constituents, are now replacing synthetic drugs to avoid many adverse effects and drug resistance (11). One such herb, *Solanum nigrum* (SN), belongs to a family of flowering plants called *Solanaceae*. Commonly it is known as 'black nightshade' or '*makoh*' and has been in use for joint pain since long (12). Various researchers have discovered multiple pharmacological effects of SN which include

its hepatoprotective, anti-inflammatory, antinociceptive, antioxidant, antitumor, antiulcerogenic & antipyretic effects (13). In recent times, this plant has become the center of attention owing to its marked antitumor potential (14). Previously, it has been used for the treatment of tracheitis, asthma, edema, and hepatic damage (15). Biologically active constituents namely steroidal alkaloids, polyphenols, tannins, steroidal glycosides, and flavonoids are vastly present in SN and have also reported anti-inflammatory and antioxidant activity (16). Furthermore, this herb is cost-effective and easily available therefore can be integrated with conventional medicine for RA in future.

In line with this background, this study is designed to evaluate *Solanum nigrum* as an antiarthritic and anti-inflammatory agent for the treatment of RA in albino rats.

METHODOLOGY:

It was a pre-clinical experimental study conducted at Ziauddin University, Karachi. The study was conducted from November 2021 to May 2022.

Plant material and extraction:

Solanum nigrum (SN) was purchased from the local nursery and authentication was done from the Karachi University herbarium. The leaves were washed and air-dried at room temperature away from the sunlight and ground into coarse particles. This powder was soaked in absolute ethyl alcohol for 10 days and stirring was done daily 2 to 4 times. To obtain the material the solution was filtered through Whatman filter paper 1. Rotary evaporation was done to concentrate the extract and then stored in a refrigerator in an air-tight flask.

Materials:

Complete Freund's adjuvant (CFA) (F5881-10ML) was ordered from Sigma Aldrich, Germany. Phenobarbital, dimethyl sulfoxide (DMSO), and alcohol were ordered from Laboratory Scientific Supplies Pvt. Limited, Karachi. Methotrexate was purchased from a local pharmacy.

Animal Protocols:

30 Male Wistar albino rats, weighing 200±20g, were used in the study. Animals were kept in the animal house of the College of Pharmacy, Ziauddin University, Karachi. The animals were retained under standard conditions in their conventional cages in a 12/12-hour light-dark cycle with ad libitum access to water and food at room temperature. All the investigational procedures done in the study 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".

Induction of arthritis:

Arthritis was induced by injecting 0.1mL of CFA subcutaneously at the palmar surface of the right hind paw of rats on day 0 except in the negative control group followed by intraperitoneal injections of standard drug and research herb extract on days 0,7,14 and 21 according to the following groups.

Grouping of animals:

The rats were randomly assigned to 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: SN 100mg/kg; Group-V: SN 200mg/kg.

Procedure:

Following induction of arthritis, 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. While 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. These doses were selected based on different preliminary studies that were done to evaluate anti-arthritic activity in rats (17, 18).

Physical parameter estimation:

Paw edema was measured by a manual Vernier caliper at day 0 before induction of arthritis and on days 7, 14, 21, and 28.

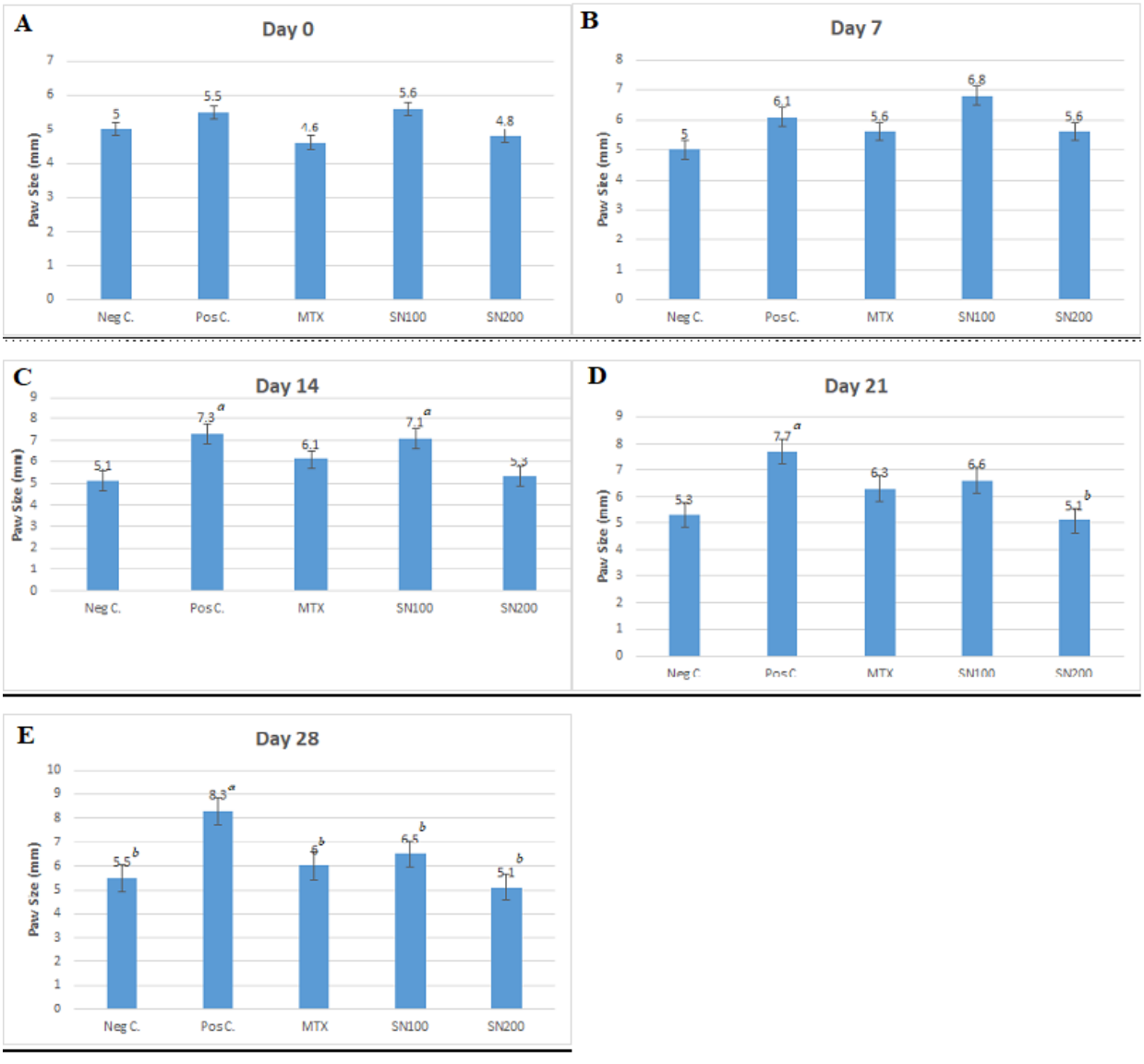
Measurement of pro-inflammatory mediators in serum via ELISA:

On the 29th day, after overnight food deprivation, all the animals were anesthetized by intraperitoneal injection of pentobarbital 100mg/kg (American Veterinary Medical Association AVMA) as per Institutional Animal Ethics Committee (IAEC) guidelines 1998. 8-10ml of blood was collected by cardiac puncture and transferred into vacutainer tubes that were then immediately centrifuged at 3000rpm for 10 minutes. After centrifugation, the serum was separated to quantify levels

of pro-inflammatory markers such as interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and prostaglandin-E2 (PG-E2) through ELISA.

RESULTS:

Effects on paw edema:



a shows significant difference exists between negative control and other groups

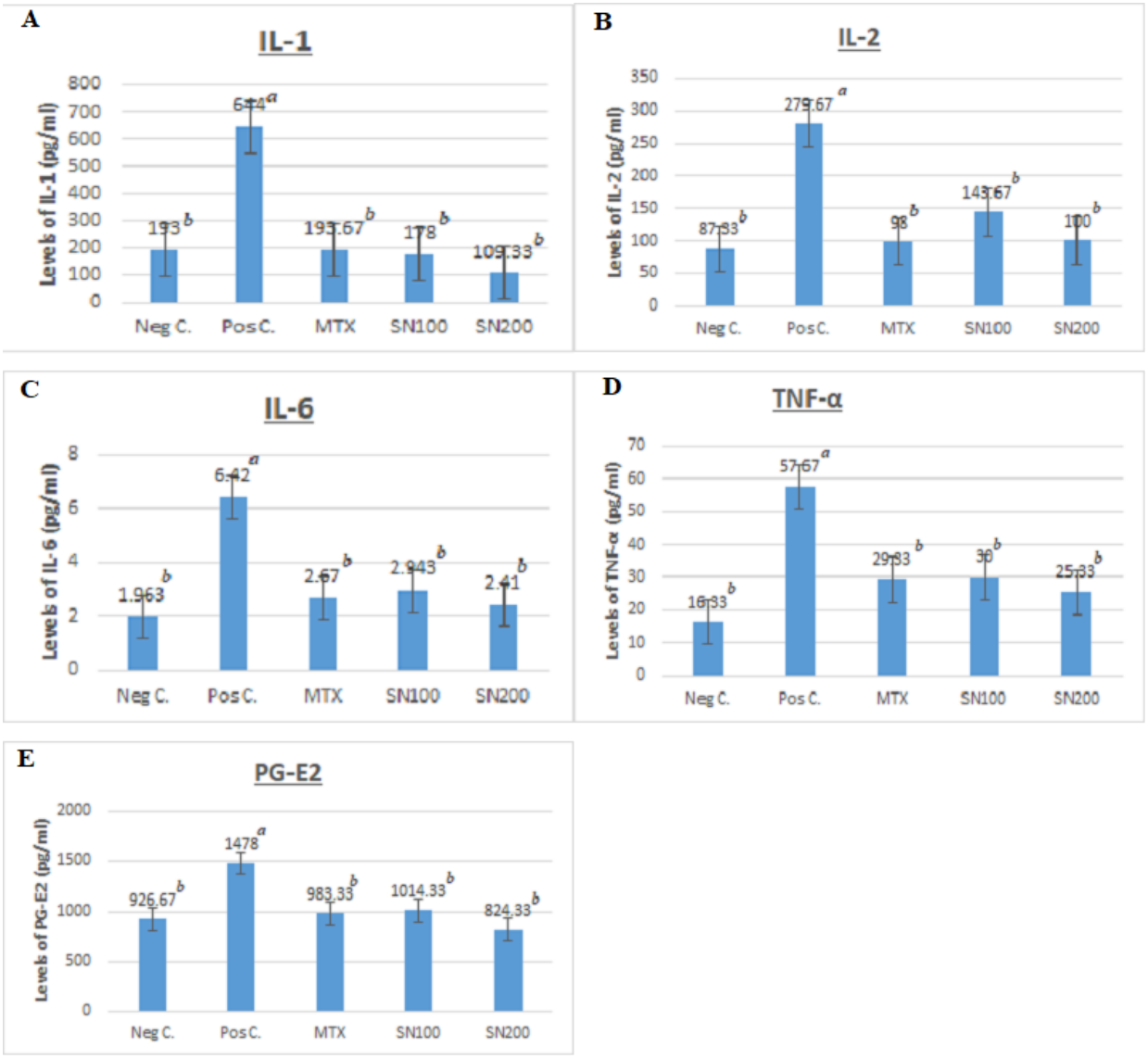
b shows a significant difference exists between positive control and other groups

Figure 1: Post-treatment effects of SN on paw edema size in CFA-induced arthritic rat model

Paw edema of all the Wistar albino rats was measured on days 0, 7, 14, 21, and 28. On day 0, there was no significant difference in paw edema of both treated and untreated groups (Fig. 1A). While on day 7, a significant increase in paw edema in all the arthritic induced-groups (Positive control, MTX, SN100, and SN200) was observed (Fig. 1B). Measurements taken at the last day of study, day 28, revealed continuous increment in paw size of the positive control group (Fig. 1E). On the same day, there was no notable increase in paw size of the negative control group. When the MTX group was compared with the diseased (positive) control group, it expressed a significant decrease in edema size nearly equal to as of the negative (non-diseased) control group (Fig. 1E).

Similarly**,** On the 28th day, the herbal group SN100 also expressed a significant decrease in edema size as compared to the positive control, almost equally effective as the MTX group. The second herbal group SN200 showed a remarkable decrease in edema size when compared with the positive control while a non-significant difference exists between SN200 and the MTX group (Fig. 1E).

Effects on serum mediators:



a shows significant difference exists between negative control and other groups

b shows a significant difference exists between positive control and other groups

Figure 2: Post-treatment effects of SN on IL-1. IL-2, IL-6, TNF-α, and PG-E2 production in CFA-induced arthritic rat model on day 28

Serum ELISA of all 5 groups was done after the completion of the study. It showed a remarkable increase in pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF-α) and prostaglandin E2 (PG-E2) levels in the positive-control group. MTX and SN100 groups showed low levels of

IL-1 when compared with the positive controls and it was equal to the negative-control group. While SN200 expressed a much more decrease in IL-1 levels even lower than the negative controls (Fig. 2A). MTX and SN200 showed equal levels of reduction in IL-2 levels when compared with the positive controls. The SN100 group also had reduced IL-2 levels but was not as effective as MTX and SN200 groups (Fig. 2B).

MTX and SN200 groups resulted in a nearly equal decrease in IL-6 levels when compared with the positive controls. SN100 also showed a significant reduction but slightly less than the other standard and herbal treatment groups (Fig. 2C). Regarding TNF-α, the SN100 group showed equal effectiveness as the standard group. While SN200 showed more effectiveness in reducing TNF-α levels (Fig. 2D). Results of PG-E2 analysis showed that all the treatment groups decreased the PG-E2 levels when compared with the positive controls. SN100 showed a minimum decrease in-between all other treatment groups. A maximum decrease in PG-E2 levels was expressed by SN200 which was even lower than the negative control PG-E2 levels (Fig. 2E).

Results were analyzed by using SPSS software version 22. ANOVA was applied for intergroup and intragroup comparison. P-value <0.05 will be considered significant at 95% confidence interval.

DISCUSSION:

Complete Freund's adjuvant has been reported as a gold standard adjuvant for inducing cell-mediated immunity in research models of autoimmune diseases especially experimental autoimmune encephalomyelitis and rheumatoid arthritis (19). It is composed of inactivated and desiccated *mycobacterium butyricum/tuberculosis* suspended in sterile non-metabolizable paraffin oil (20). The CFA-triggered RA exhibit chronic synovial and cartilaginous damage (21). The most widely recognized mechanism of CFA is depot formation at the injection site causing antigen trapping. This prolonged release of antigen from the depot site causes a constant stimulation of the immune system leading to induction of cytokines, recruitment of immune cells, enhancement of antigen uptake and presentation, and production of antibodies leading to a chronic inflammatory reaction (22).

In our study, CFA was induced in all treatment groups except the non-diseased control Group-I. A maximum increase in paw edema size was observed in the diseased-control group which was not treated with any drug or herb. Various other studies have also reported a significant

increase in paw size due to inflammatory and edematous effects produced by CFA (3, 23). MTX, a standard DMARD used in RA, caused a notable reduction in paw edema size in Group-III showing its anti-inflammatory and anti-arthritic activity. Various other studies have reported the suppressive effect of MTX on paw edema size (24, 25). The herb used in our study, *Solanum nigrum*, was used at two different doses that were 100mg/kg and 200mg/kg. Both doses had shown a noteworthy decline in inflammatory edema size but the higher dose (200mg/kg) resulted in more significant suppression of paw edema and performed even better than the standard drug MTX. Another study reported a significant decrease in paw edema size when treated with SN herbal extract (26). Steroidal alkaloid Solanine A, flavonoids, and polyphenols isolated from SN have also shown notable suppression of inflammatory edema (26, 27).

Results obtained from ELISA have revealed that CFA-induced arthritis caused a remarkable increase in the levels of all pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF-α) and PG-E2, as evident in Group-II of our study. Multiple other studies have reported that CFA induction causes an increase in pro-inflammatory cytokines (28, 29). As documented in the literature, our study has also shown a halting effect of MTX on rising pro-inflammatory cytokines and PG-E2 (25, 30, 31). When compared with the diseased-control, both 100mg/kg and 200mg/kg doses of SN reported downregulation of the pro-inflammatory cytokines and PGE2 but the SN200 group has shown more efficacy. Previous literature has also reported the immunomodulatory effect of SN and down-regulation of pro-inflammatory cytokines (32, 33). Phytosterols, steroidal saponins, and alkaloids isolated from SN have reported their anti-inflammatory activity by their suppressive action on pro-inflammatory cytokines (34-36).

Conclusion:

Thus, we can summarize that *Solanum nigrum* can be used as an effective adjunctive with standard DMARDs like MTX to increase the efficacy in the treatment of rheumatoid arthritis. After further validation by clinical trials, this herb can be come up as integrated medicine in the future.

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