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ORIGINAL ARTICLE

Evaluation of anti-inflammatory effect of *Withania somnifera* root on collagen-induced arthritis in rats

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Abstract

Context: *Withania somnifera* (Linn.) Dunal (Solanaceae) has long been used as an herb in Ayurvedic and indigenous medicine and has received intense attention in recent years for its chemopreventive properties.

Objective: The present study focuses on the effect of *W. somnifera* root powder on the behavioral and radiological changes in collagen-induced arthritic rats.

Materials and methods: The rats were randomly divided into five groups: normal control, arthritic control, arthritic rats treated with *W. somnifera* root powder (at dose levels 600 and 800 mg kg^{-1}) and arthritic rats treated with methotrexate (at dose level 0.3 mg kg^{-1}). The treatment with *W. somnifera* (daily) and methotrexate (weekly) was initiated from the 20th day post collagen immunization and continued up until the 45th day. Arthritis was assessed macroscopically by measuring paw thickness, ankle size and body weight. Arthritic pain was assessed by toe-spread and total print length of the affected paw. Functional recovery due to the oral treatment of *W. somnifera* and methotrexate was assessed by sciatic functional index and rota rod activity.

Results: Administration of *W. somnifera* root powder (600 mg kg^{-1}) to the arthritic rats significantly decreased the severity of arthritis by effectively suppressing the symptoms of arthritis and improving the functional recovery of motor activity and radiological score.

Discussion and conclusion: *W. somnifera* root has a protective effect against collagen-induced arthritis (CIA) in rats. The results suggest that *W. somnifera* root powder acts as an anti-inflammatory and antioxidant agent in decreasing the arthritic effects in collagen-induced arthritic rats.

Keywords

Arthritic rat, Ashwagandha, motor coordination, paw thickness, radiological score, rheumatoid arthritis

History

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Introduction

Rheumatoid arthritis (RA) is a chronic, progressive, autoimmune inflammatory disorder marked by synovial hyperplasia with a local invasion of bone and cartilage leading to joint destruction. It is a symmetric polyarticular arthritis that primarily affects the diarthrodial joints of the hands and feet. RA affects ∼1% of the population worldwide (Recklies et al., 2000). The disease can occur at any age, but it is most common among those aged 40–70 years (Alamanos et al., 2006), with women being afflicted more than men at a ratio approximately 3:1 (Kinne et al., 2007).

The collagen-induced arthritis (CIA) model of RA has been extensively studied to identify and understand the potential pathogenic mechanisms of autoimmunity, the role played by the individual cell types in disease onset and progression, and to design and test new therapeutics. The model shares several pathological features with the disease, including synovial hyperplasia, mononuclear cell infiltration and cartilage degradation. The CIA model is suitable for testing anti-inflammatory drugs for potential use in RA (Inglis et al., 2007).

Withania somnifera (Solanaceae) (Dafni & Yaniv, 1994) is commonly known as Ashwagandha or Indian ginseng. It is a reputed herb used in Ayurvedic and indigenous medicine for over 3000 years (Winters, 2006). The pharmacological activities of *W. somnifera* like anti-inflammatory (Alhindawi et al., 1992), antitumor (Mishra et al., 2000), antibacterial (Owais et al., 2005), antioxidant (Bhattacharya et al., 2001), anticonvulsive (Kulkarni et al., 1998) and immunosuppressive properties (Furmanowa et al., 2001) have been studied.

Many pharmacological studies have been conducted to investigate the properties of *W. somnifera* in an attempt to authenticate its use as a multi-purpose medicinal agent. Phytochemically, this plant is unique because it possesses the largest and most diverse set of withanolides (Alam et al., 2011). The chemistry of *Withania* species has been extensively studied and several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannins, and saponins, etc., have been identified, extracted, and isolated (Mir et al., 2012). At present, more than 12 alkaloids, 40 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated

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and reported from aerial parts, roots and berries of *Withania* species, with withanolides being the main bioactive constituent (Mirajili et al., 2009). Roots of this plant are considered most active for therapeutic purposes by virtue of significant accumulation of active constituents, withanolides (Pawar et al., 2011).

The present study was aimed to see the effect of *W. somnifera* root powder on the behavioral and radiological changes in collagen induced arthritic rats.

Materials and methods

Experimental model

Albino female rats (Wistar strain), 6–10 week of age were purchased from a central animal house facility, Banaras Hindu University, Varanasi and were acclimatized in the animal house conditions with a 12:12 h light: dark schedule. The choice of the sex of the animals, i.e., females, was based on the findings that autoimmune arthritis is mediated by sex hormones (Holmdahl, 1995), and that female rats are more susceptible to arthritis as compared to the males (Van den Berg, 2004). Free access to food and water was given *ad libitum*. Six rats were used per study group. Rats were subdivided into the following groups: normal control rats; arthritic control rats; *W. somnifera* ($600\,mg\,kg^{-1}$) treated arthritic rats; *W. somnifera* ($800\,mg\,kg^{-1}$) treated arthritic rats and methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats. All the experimental protocols were pre-approved by the animal ethical committee, Jiwaji University, Gwalior and Banaras Hindu University, Varanasi, India.

Induction of CIA

CIA in rats was developed according to Remmers et al. (2002). Collagen from bovine tracheal cartilage type II (CII) (obtained from Sigma Chemical Company St. Louis, MO) was dissolved in cold 0.1 N acetic acid ($2\,mg\,ml^{-1}$) and was emulsified with an equal volume of freshly opened, cold Freund's adjuvant incomplete (IFA) (Sigma, St. Louis, MO). The emulsion was made by using a three-way stopcock with syringe. Rats were injected intradermally at several sites on the back with a dose of $2\,mg\,kg^{-1}$ of body weight. On the seventh day after the primary immunization, the rats were re-immunized with 0.1 ml (100 μg) of similarly prepared collagen/IFA emulsion injected intradermally at the base of the tail.

Collection of *W. somnifera* and dose preparation

W. somnifera was collected from the botanical garden, Banaras Hindu University, Varanasi, in October, 2011 and was scientifically approved in the Department of Botany, Banaras Hindu University, Varanasi (identified by Prof. M.P. Singh, a well known plant taxonomist). The roots were collected, washed with sterile distilled water and dried at 80 °C in an oven. The dried roots of *W. somnifera* were ground with a pestle and mortar into powdered form and used as oral feed for experimental rats post mixing with distilled water to get the desired concentration of 600 and $800\,mg\,kg^{-1}$, for the purpose of therapeutic treatment for experimental rats.

Dose schedule

The water suspensions (1 ml) of *W. somnifera* (600 and $800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) were administered orally to the arthritic rats with the help of a syringe cannula. *W. somnifera* (600 and $800\,mg\,kg^{-1}$) was administered at 10 am, daily. However, methotrexate ($0.3\,mg\,kg^{-1}$) was given once a week, at the same time. Sterile water was, however, given to the control as well as untreated arthritic control rats. The treatment of *W. somnifera* and methotrexate was started from day 20th post collagen immunization and continued up to 45th day.

Assessment of arthritis and arthritic score

Rats were screened for the development and progression of arthritis daily from day 0 to 45th day with 5 day interval. The severity of arthritis was graded according to Brand et al. (2007):

Grade 0 = No sign of arthritis
Grade 1 = Redness and swelling in paw
Grade 2 = Deformity in paw
Grade 3 = Ankylosis in paw
Grade 4 = Maximal swelling and deformity with ankylosis

The arthritic score of a diseased rat was sum of the maximum grades of arthritis in the involved paws. The data were expressed as mean ± SEM of six animals per group from day 0 to 45th day recorded at an interval of 5th day.

Macroscopic assessment of arthritis

Severity of arthritis was assessed macroscopically by the quantification of the changes in paw thickness, body weight, and ankle size. Measurement of paw thickness and ankle size was made with a dial gauge caliper from 0 to 45th day at an interval of every 5th day. The data were expressed as mean ± SEM of six animals per group.

Measurement of arthritic pain

Arthritic pain of arthritic control, *W. somnifera* (600 and $800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats were evaluated using the foot-print assessment of pain that reflects the spontaneous pain behavior Kumar, 2011). The rat's hind paws were dipped in ink and the animals were allowed to walk on white paper for a distance of 60 cm from day 20th to 45th day. The data were recorded at an interval of every 5th day and expressed as mean ± SEM of six animals per group.

The footprints were scored as follows:

0 = Normal footprint
1 = Partial footprint (no heel)
2 = Fingers only
3 = Absence of one footprint
4 = Total absence of footprint

Measurement of total print length, 1–5 toe spread and 2–4 toe spread

According to Bain et al. (1989) and Walker et al. (1994), the functional recovery of normal control, arthritic control, *W. somnifera* (600 and $800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats was measured by allowing

the animal to explore in a 10 cm wide and 60 cm long wooden corridor on a sheet of ink absorbing paper, with their hind paws dipped in blue ink on day 45th post collagen immunization. Walking on the sheet resulted in at least three to four prints of each foot. Individual walking print length, 1–5 toe spread and 2–4 toe spread values were measured with a dial gauge caliper. The data were expressed as mean ± SEM of six animals per group.

Sciatic functional index

For evaluation of functional recovery, sciatic functional index (SFI), developed by De Medinaceli et al. (1982), and experimental toe spread were used. Footprints were collected as per Patro et al. (2008) by allowing the animal to explore in a 10 cm wide and 60 cm long wooden corridor on a sheet of ink absorbing paper, with their hind paws dipped in blue ink on day 0th to 45th day at an interval of every 5th day post collagen immunization. Walking on the sheet resulted in at least three to four prints of each foot. Paper containing prints were allowed to dry and vernier caliper was used to take the measurements. At least three footprints per animal per time point were measured. These footprints were analyzed twice. The parameters measured for normal control, arthritic control, and treated groups were footprint length (PL), total toe spread (TS or distance between first and fifth toe), and intermediate toe spread (IT or the distance between second and fourth toe). These observations were used in the following formula proposed by Bain et al. (1989):

$$\mathrm{SFI} = 38.3 \times \frac{\mathrm{EPL} - \mathrm{NPL}}{\mathrm{NPL}} + 109.5 \times \frac{\mathrm{ETS} - \mathrm{NTS}}{\mathrm{NTS}} + 13.3 \times \frac{\mathrm{EIT} - \mathrm{NIT}}{\mathrm{NIT}} - 8.8$$

where:
SFI = Sciatic functional index
EPL = Experimental print length
NPL = Normal print length
ETS = Experimental toe spread
NTS = Normal toe spread
EIT = Experimental intermediate toe spread
NIT = Normal intermediate toe spread

Rota rod test for motor coordination

Motor coordination (in terms of time) of normal control, arthritic control, *W. somnifera* (600 and 800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treated arthritic rats were evaluated with the rota rod test. This forced motor activity has subsequently been used to determine the functional recovery in rats. This is a very sensitive test of muscular coordination. Animals with joint pain fall off quickly from rotating wheel. Rotamex-5 (Columbus Instruments, Columbus, OH) was used for the purpose. This instrument is completely software based with a computer interface.

Experimental conditions for Rotamex

The animals were acclimatized for 3 days with the maximum speed of 10 rpm for 100 sec prior to the experiment. The animals who failed the acclimatization test were omitted and those succeeded were allowed to run on the rotating wheel, scheduled for 420 sec, in increments of 2 rpm per 2 sec to the maximum speed of 40 rpm. Moreover, testing at various time intervals, time response curves could be obtained. Final data were collected and analyzed with the software Rotamex version 1.2.3.

The Rotamex unit was controlled with the computer interface having software named Rotamex version 1.2.3. The infrared beams connected with the instrument detect the position of the rat (on the rotating wheel). As soon as the rat falls from the rotating rod, the exact reading in the form of time or path length can be recorded up to the accuracy of two decimal points. This instrument allows automatic recording of the time that each rat was able to stay on the rod at different rotational speeds. The data so generated were automatically transferred to the interfaced computer in the form of excel files.

Radiography

Radiographic analysis of normal and arthritic hind paws was performed using a X-ray machine (Philips X12 Germany) with a 40 kW exposition for 0.01 s. The hind paws of rats were used for radiological scoring as described by Nishikawa et al. (2003). All radiographs were taken with X-ray film (Kodak Diagnostic Film).

The following radiograph criteria were considered:
0 = No bone damage
1 = Tissue swelling and oedema
2 = Joint erosion
3 = Bone erosion and osteophyte formation

Statistical analysis

The values were presented as means ± SEM. Results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test (all pair wise multiple comparison procedure) (Sigma Stat 3.5, Systat Software Inc., Chicago, IL). A value of $^{*}p < 0.05$ was considered significant to arthritic control versus normal and treated groups.

Results

Arthritis assessment

The sign of arthritis started from the 15th day post collagen immunization with slight swelling and redness, reached a significant level at the 20th day and attained its maximum level at the 45th day. The macroscopic sign of severe arthritis at the 45th day included swelling, redness, deformity and ankylosis in hind paws and ankle joints. Such symptoms were, however, found in forelimbs as well. The hind paw of normal rats showed significant difference from the hind paw of arthritic control rats. Whereas arthritic rats treated with *W. somnifera* (800 mg kg^{-1}) showed redness and swelling only, the arthritic rats treated with *W. somnifera* (600 mg kg^{-1}) showed almost no sign of arthritis and appeared essentially similar to normal rats. In contrast, methotrexate (0.3 mg kg^{-1})

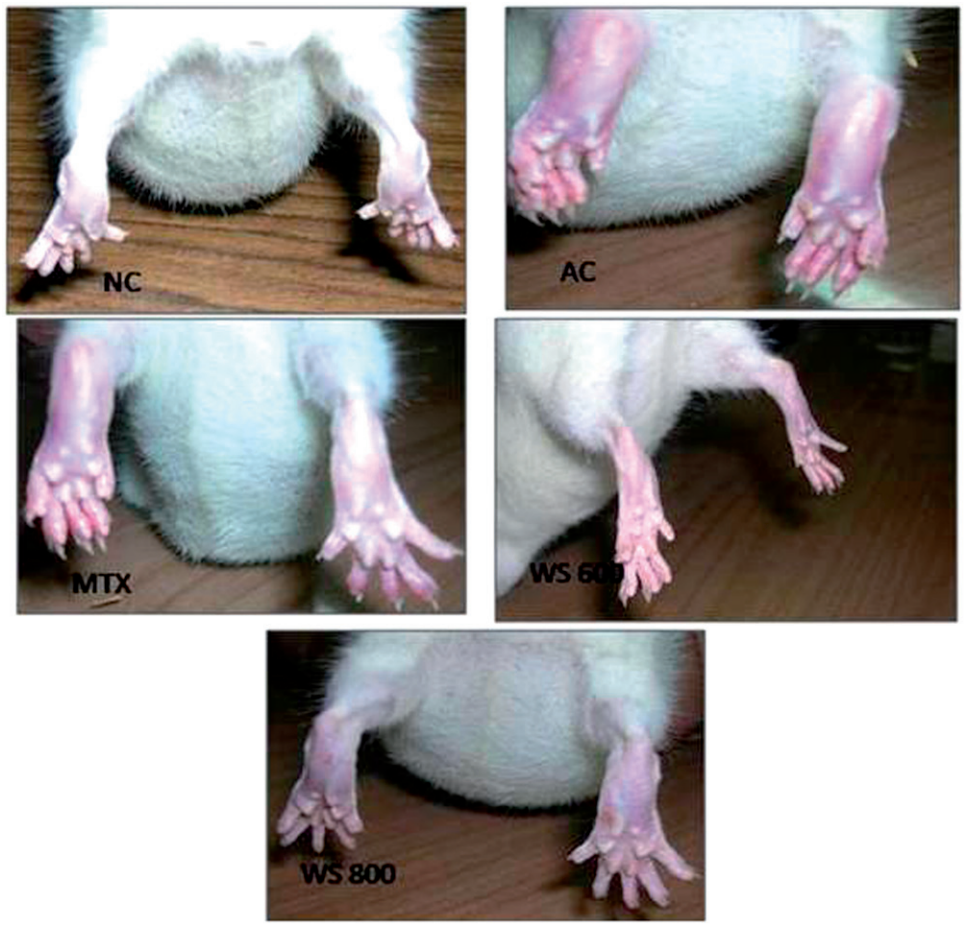

Figure 1. Hind paws and ankle joint of representative rat groups at the 45th day. NC: normal control rat, AC: arthritic control rat showing redness, swelling, deformity and ankylosis with severe arthritis (symptoms maximum in the group), MTX: methotrexate treated rat with moderate arthritic symptoms, swelling only with moderate arthritis (symptoms maximum in the group), (WS 600) *W. somnifera* treated (600 mg kg^{-1}) rat showing almost no sign of arthritis and appeared essentially normal (symptoms minimum in the group) and (WS 800) *W. somnifera* treated (800 mg kg^{-1}) rat showing redness. Pictures are representative of six distinct rats per group.

treated arthritic rats showed redness and swelling with moderate arthritis at the 45th day when the animals were sacrificed (Figure 1).

Paw thickness

A significant increment in hind paw thickness from the 15th (5.53 ± 0.47 mm), 20th (6.22 ± 0.29 mm), 30th, 40th, and 45th day (5.9 ± 0.21 mm) was observed in arthritic control rats during development of arthritis. Arthritic control rats, however, showed a significant difference in hind paw thickness from normal control rats from 3.47 ± 0.13 to 4.05 ± 0.06 mm on the 15th to 45th day, respectively. Whereas, *W. somnifera* (600 mg kg^{-1}) treatments to the arthritic rats resulted in a significant decline in their paw thickness from the 30th (4.55 ± 0.41 mm) to 45th day (4.2 ± 0.22 mm), the arthritic rats treated with *W. somnifera* (800 mg kg^{-1}), however, showed significant decline in their paw thickness from the 35th (4.65 ± 0.29 mm) to 45th day (4.48 ± 0.25 mm) when compared with their arthritic control counterparts during development of arthritis. Methotrexate (0.3 mg kg^{-1}) treated arthritic rats also showed significant decline in their paw thickness from the 40th (5.02 ± 0.21 mm) to 45th day (4.95 ± 0.23 mm) when compared with their arthritic control counterparts during development of arthritis (Figure 2).

Ankle size

A significant increment in the ankle size of arthritic control rats was recorded from the 20th (0.6 ± 0.01 cm) to 45th day (0.68 ± 0.01 cm) when compared with the ankle size of their normal control counterpart [20th (0.36 ± 0.004 cm) to 45th day (0.5 ± 0.008 cm)]. Whereas, *W. somnifera* (600 mg kg^{-1}) treatments to the arthritic rats resulted in a significant decline in their ankle size (0.51 ± 0.006 cm) at the 45th day, the arthritic rats treated with *W. somnifera* (800 mg kg^{-1}), however, showed non-significant decline in their ankle size from the 25th (0.64 ± 0.04 cm) to 45th day (0.57 ± 0.03 cm). Methotrexate (0.3 mg kg^{-1}) treated arthritic rats also showed a decline in their ankle size from the 25th (0.74 ± 0.04 cm) to 45th day (0.63 ± 0.02 cm) when compared with the ankle size of their arthritic control counterparts. The decrease in ankle size was not, however, found to be statistically significant at $p \leq 0.05$ (Figure 3).

Change in body weight

An absolute increment in the body weight of all the groups of rats was found to be similar in the first 12 days and no significant differences were observed between them. However, after 12 days, a loss in the body weight was observed in the arthritic control, *W. somnifera* and methotrexate treated

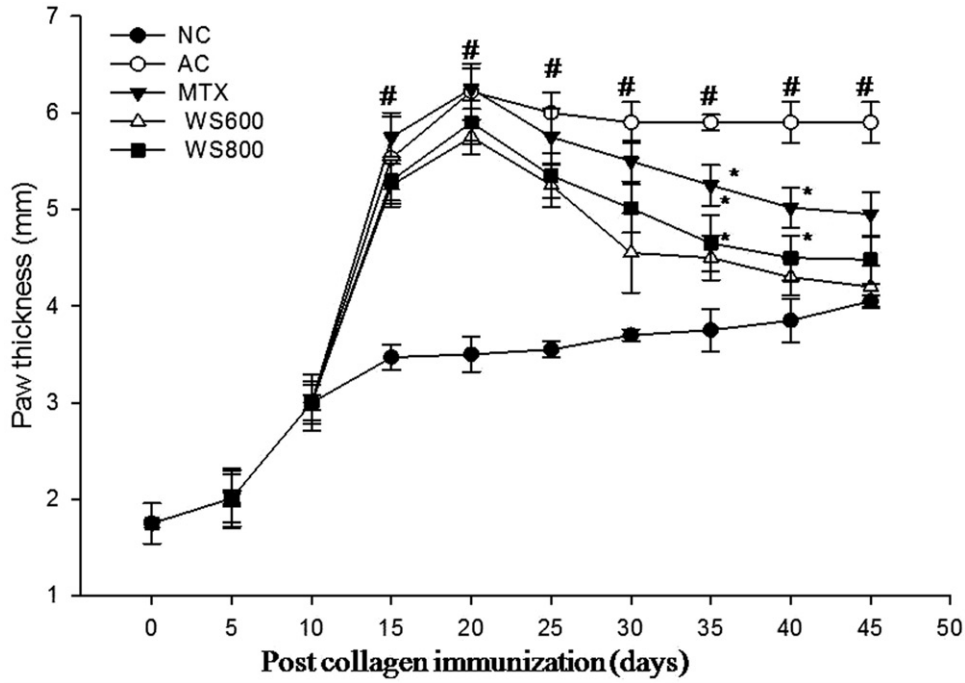

Figure 2. Paw thickness of rats from day 0 to the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600: *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

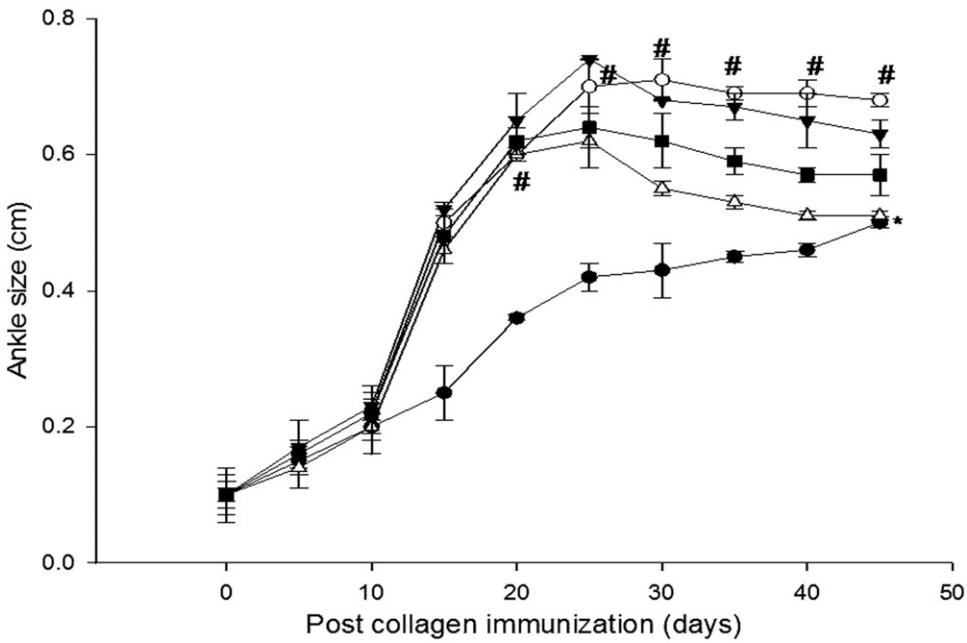

Figure 3. Ankle size of rats from day 0 to the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

arthritic rats from 15th to 20th day when compared with the body weight of their normal control counterparts. The body weight of arthritic control rats declined significantly from 15th (143.33 ± 1.63 g) to 45th day (147 ± 1.66 g) when compared with the body weight of their normal control counterpart. *W. somnifera* (600 mg kg^{-1}) treated arthritic rats showed a significant increment in their body weight from the 30th (158.67 ± 4.30 g) to 45th day (171.33 ± 0.60 g) when compared with their arthritic control counterparts. Arthritic rats treated with *W. somnifera* (800 mg kg^{-1}) showed a significant increment in their body weight from the 30th (157.67 ± 4.51 g) to 45th day (169.0 ± 0.89 g), methotrexate (0.3 mg kg^{-1}) treated arthritic rats, however, showed a significant increment in their body weight on the 25th (153.33 ± 2.60 g), 30th (156.33 ± 3.11 g), 40th (160.0 ± 2.07 g) and 45th day (162.0 ± 1.03 g) when compared with the body weight of their arthritic control counterparts (Figure 4).

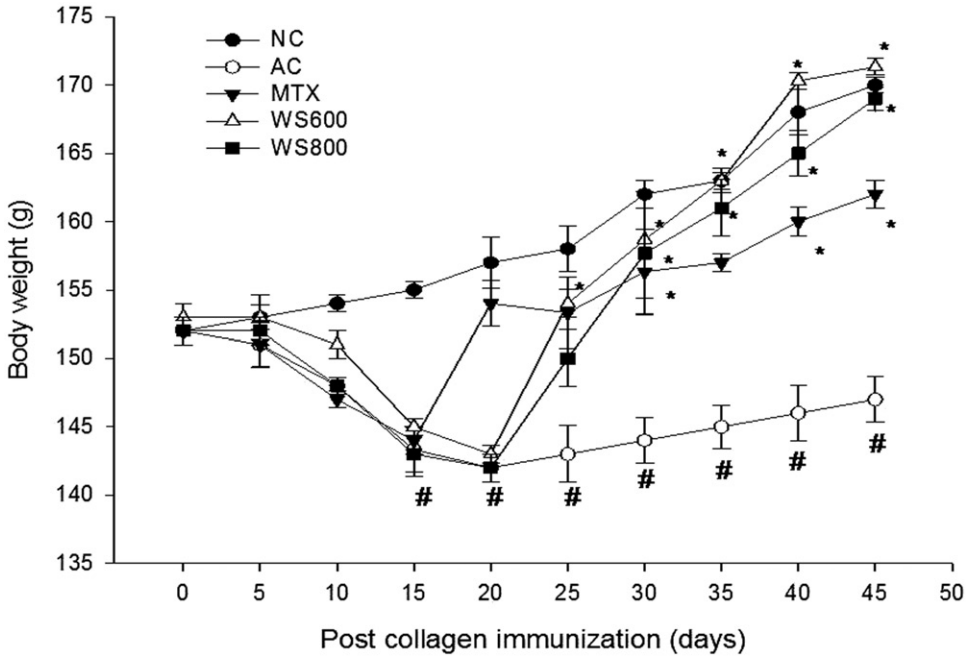

Figure 4. Body weight of rats from day 0 to the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate ($0.3\,mg\,kg^{-1}$), WS 600; *W. somnifera* ($600\,mg\,kg^{-1}$), WS 800: *W. somnifera* ($800\,mg\,kg^{-1}$) treated rats with ± SEM, $N = 6$, $*p < 0.05$ versus AC, $\#p < 0.05$ versus NC.

Arthritic score

A significant increment in the arthritic score was observed in arthritic control rats from the 15th (1.75 ± 0.31), 20th (3.5 ± 0.22), 30th (3.96 ± 0.05), 40th (3.92 ± 0.08) and 45th day (3.92 ± 0.08) during development of arthritis. However, treatment of arthritic rats with *W. somnifera* ($600\,mg\,kg^{-1}$) resulted in a significant decline in their arthritic score from the 25th (3.5 ± 0.22), 30th (2.33 ± 0.32), 35th (1.67 ± 0.20), 40th (1.0 ± 0.28) and 45th day (0.50 ± 0.22) during development of arthritis when compared to the arthritic score of their arthritic control counterparts. In contrast, *W. somnifera* ($800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats showed lower arthritic score as compared to the arthritic score of their arthritic control counterparts but they did not show a significant decline at the $p \leq 0.05$ level during development of arthritis (Figure 5).

Arthritic pain

A significant increment in arthritic pain was observed in arthritic control rats from the 20th (2.75 ± 0.29) to 45th day (3.83 ± 0.01) during development of arthritis when compared with the arthritic pain of their normal control counterparts. However, arthritic rats treated with *W. somnifera* ($600\,mg\,kg^{-1}$) resulted in a significant decline in their arthritic pain from the 40th (1.25 ± 0.16) to 45th day (0.50 ± 0.22) during development of arthritis. In contrast, *W. somnifera* ($800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) treatment to the arthritic rats resulted in a decline in their arthritic pain but did not show a significant difference in their arthritic pain when compared with their arthritic control counterparts at the $p \leq 0.05$ level during development of arthritis (Figure 6).

1–5 toe spread

A significant spread between 1 and 5 toe spread of hind paws was observed in the arthritic rats treated with 600 (1.51 ± 0.04) and $800\,mg\,kg^{-1}$ (1.41 ± 0.04) *W. somnifera,* at 45th day. No significant difference in 1–5 toe spread was, however, observed in methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats when compared with their arthritic control counterparts (Figure 7).

2–4 toe spread

A significant reduction in 2–4 toe spread of hind paws was observed in arthritic control rats when compared with their normal control counterparts. A significant spread between 2 and 4 toe was observed in the arthritic rats treated with 600 (0.69 ± 0.02) and $800\,mg\,kg^{-1}$ (0.66 ± 0.07) *W. somnifera,* at 45th day. No significant difference in 2-4 toe spread was, however, observed in methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats (0.53 ± 0.04) when compared with their arthritic control counterparts (Figure 8).

Total print length

Functional assessments of rats were performed using walking track analysis. Individual walking total print length values were measured in normal control, arthritic control and *W. somnifera* (600 and $800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) treated arthritic rats at 45th day post collagen immunization. The oral dose of *W. somnifera* (600 and $800\,mg\,kg^{-1}$) and methotrexate ($0.3\,mg\,kg^{-1}$) to the arthritic rats affected all the footprint measurements. A significant reduction in total print length was observed in arthritic control rats (1.78 ± 0.08) at the 45th day post collagen immunization when compared with the total print length of their normal control (2.72 ± 0.05) counterparts. A significant difference in total print length was recorded in 600 (2.82 ± 0.22) and

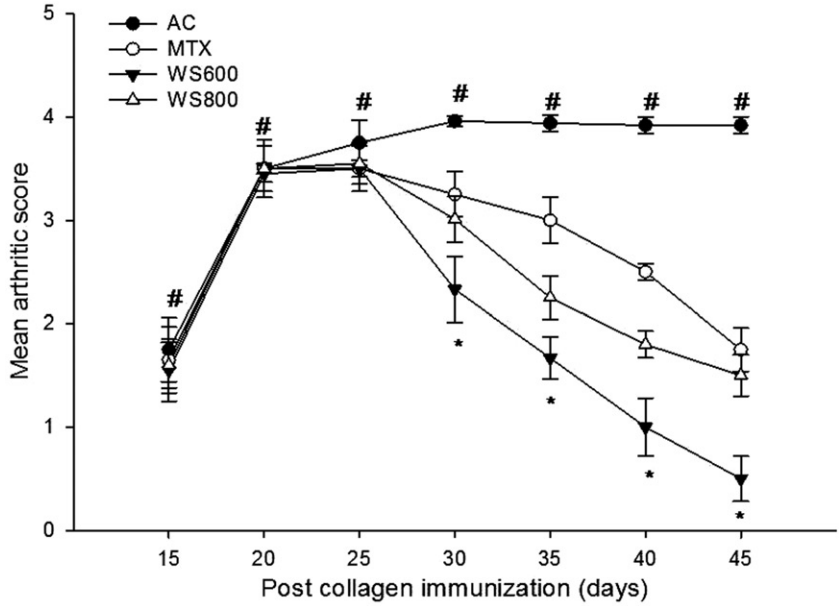

Figure 5. Mean arthritic score of rats from day 0 to the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

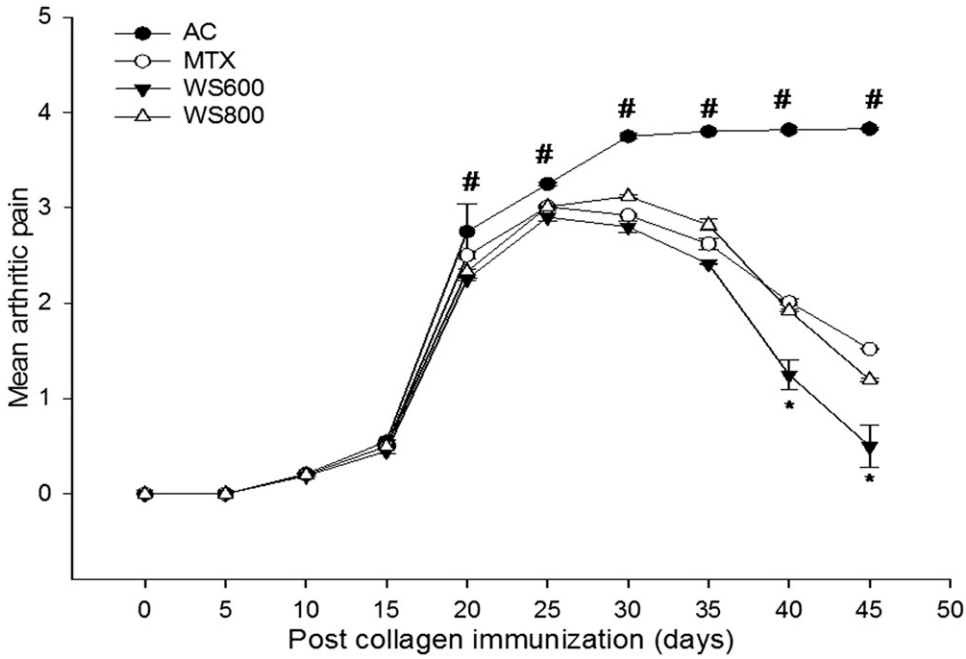

Figure 6. Mean arthritic pain of rats from day 0 to the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

800 mg kg^{-1} (2.55 ± 0.06) *W. somnifera* treated arthritic rats at the 45th day. No significant difference in total print length was, however, observed in methotrexate (0.3 mg kg^{-1}) treated arthritic rats (2.41 ± 0.13) when compared with their arthritic control counterparts (Figure 9).

Sciatic functional index

At the 20th day post collagen immunization, the arthritic control, *W. somnifera* (600 and 800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treated arthritic rats were unable to put their steps on paper sheet due to considerable swelling, arthritic pain and stress. The sciatic functional index assessments of rats were performed using walking track analysis. *W. somnifera* (600 and 800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treatments to the arthritic rats affected all sciatic functional index measurements from the 25th to 45th day post collagen immunization. A significant reduction in sciatic functional index of arthritic control rats was recorded at the 25th (−62.38 ± 1.31), 30th (−53.79 ± 6.99) and 45th day (−57.18 ± 1.92) when compared with the sciatic functional index of their normal control counterparts at 25th (−11.04 ± 4.64) to 45th day (−15.43 ± 7.21). Arthritic rats treated with *W. somnifera* (600 mg kg^{-1}) showed a significant increment in their sciatic functional index when compared with the sciatic functional index of their arthritic control counterparts from the 25th (11.51 ± 3.34) to 45th day (2.73 ± 5.74). *W. somnifera* (800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treated arthritic rats, however, showed a

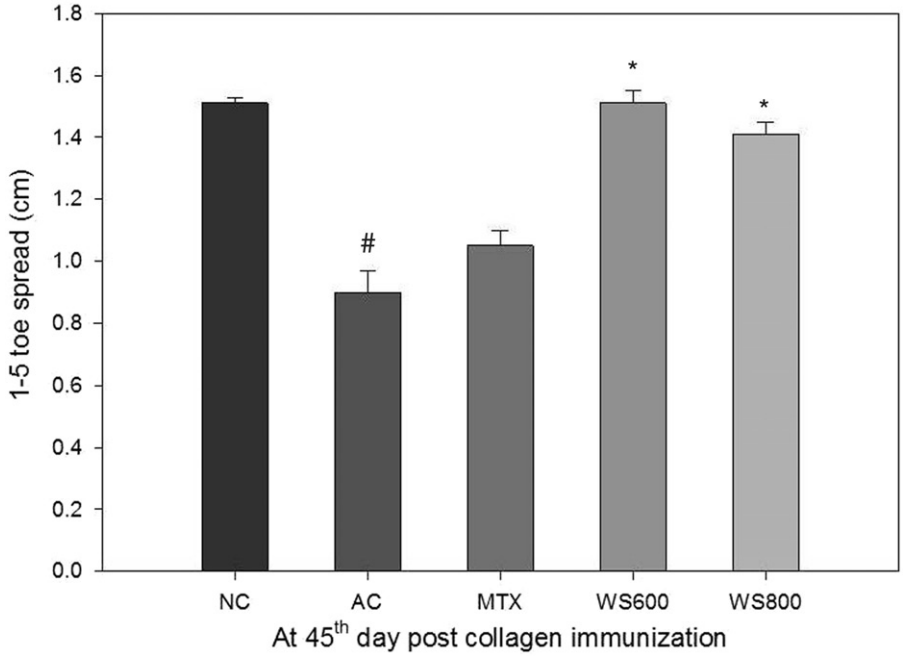

Figure 7. 1–5 toe spread of rats at the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

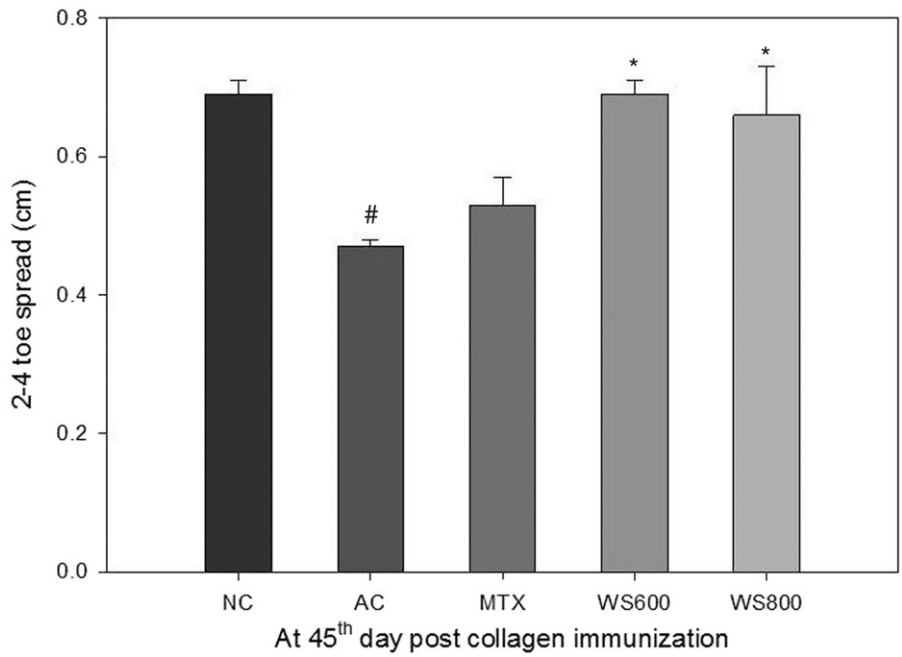

Figure 8. 2–4 toe spread of rats at the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

significant difference in their sciatic functional index at the 25th day only (Figure 10).

Rota rod activity

Functional assessments of experimental rats were performed by rota rod analysis. The functional recovery was measured in arthritic control, *W. somnifera* (600 and 800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treated arthritic rats during development of arthritis. *W. somnifera* (600 and 800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treatments to the arthritic rats affected their rota rod activity. A significant reduction in rota rod activity was observed in arthritic control rats from the 20th (6.90 ± 1.19) to 45th day (18.41 ± 2.10) when compared with their normal control counterparts. A significant difference in rota rod activity was recorded in both 600 (34.31 ± 4.45 to 46.16 ± 1.96) and 800 mg kg^{-1} (30.82 ± 5.37 to 40.72 ± 2.29) *W. somnifera* treated arthritic rats from the 25th to 45th day. Methotrexate (0.3 mg kg^{-1}) treated arthritic rats, however, showed a significant difference in their rota rod activity from the 30th (26.78 ± 2.07) to 45th day (30.58 ± 1.80) when compared with the rota rod activity of their arthritic control counterparts (Figure 11).

Radiological analysis

Radiographic severity of joint destruction was examined at the end of the experiment (45th day). Bone erosion and joint space narrowing were detected in the ankle joint of arthritic rats. Arthritic changes were significantly reduced in *W. somnifera* (600 mg kg^{-1}) treated arthritic rats when compared with their arthritic control counterparts. Whereas,

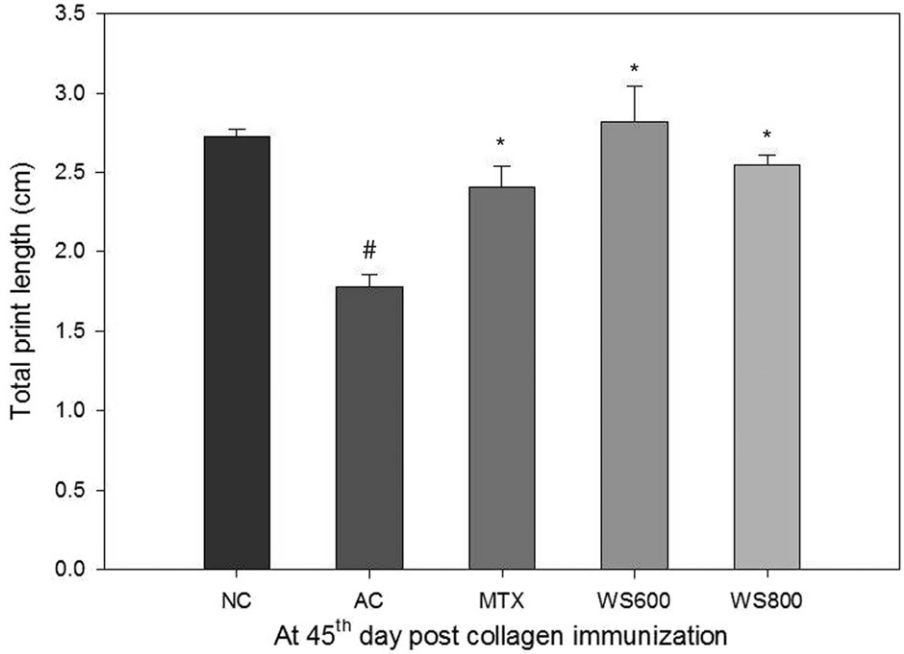

Figure 9. Total print length of rats at the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

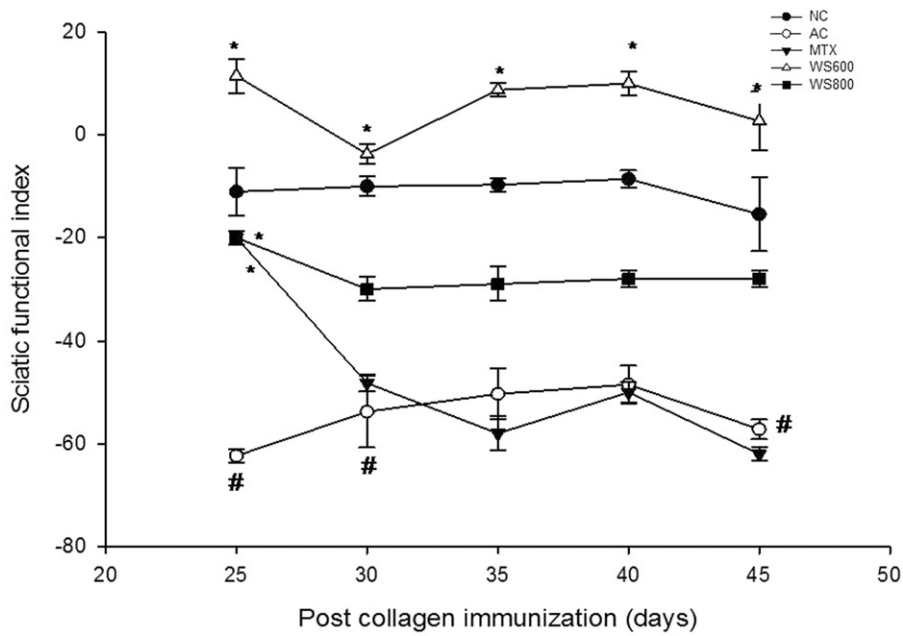

Figure 10. Sciatic functional index of rats from the 25th to 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

W. somnifera (800 mg kg^{-1}) treatment to the arthritic rats resulted in a decrease in the soft tissue swelling score, methotrexate treatment to the arthritic rats, however, showed decreased inflammation but the inflammation level remained slightly higher than those with *W. somnifera* (600 mg kg^{-1}) treated rats (Figure 12).

Radiological score

Radiographic arthritic changes in hind paws of experimental rats were measured on the basis of tissue swelling, edema, joint erosion, bone erosion and osteophyte formation at 45th day post collagen immunization. A statistically significant difference in radiological score was recorded in arthritic rats (2.0 ± 0.33) when compared with their normal control counterparts. *W. somnifera* (600 mg kg^{-1}) treatment to arthritic rats resulted in a significant reduction in the arthritic symptoms (0.17 ± 0.20) when compared with their arthritic control counterparts. *W. somnifera* (800 mg kg^{-1}) and methotrexate (0.3 mg kg^{-1}) treatments to the arthritic rats whereas decreased the radiological score (0.50 ± 0.22) and (0.83 ± 0.28), respectively, no significant difference was, however, recorded at the $p \leq 0.05$ level when compared with arthritic control counterparts (Figure 13).

Discussion

Disease modifying anti-rheumatic drugs (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs) either simultaneously or in combination have been the principal therapies for RA in the last decade (Kremer, 2001; Moreland et al., 2001). However, the value of these drugs is limited by their toxicity and the fact that these drugs provide symptomatic relief but have no significant effect on the underlying disease process

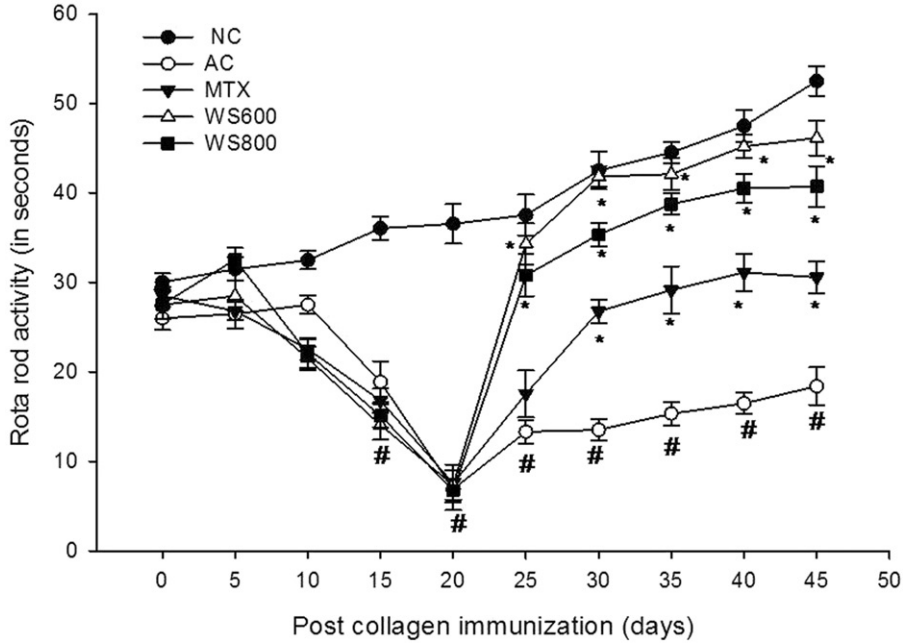

Figure 11. Rota rod activity of rats from day 0 to the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

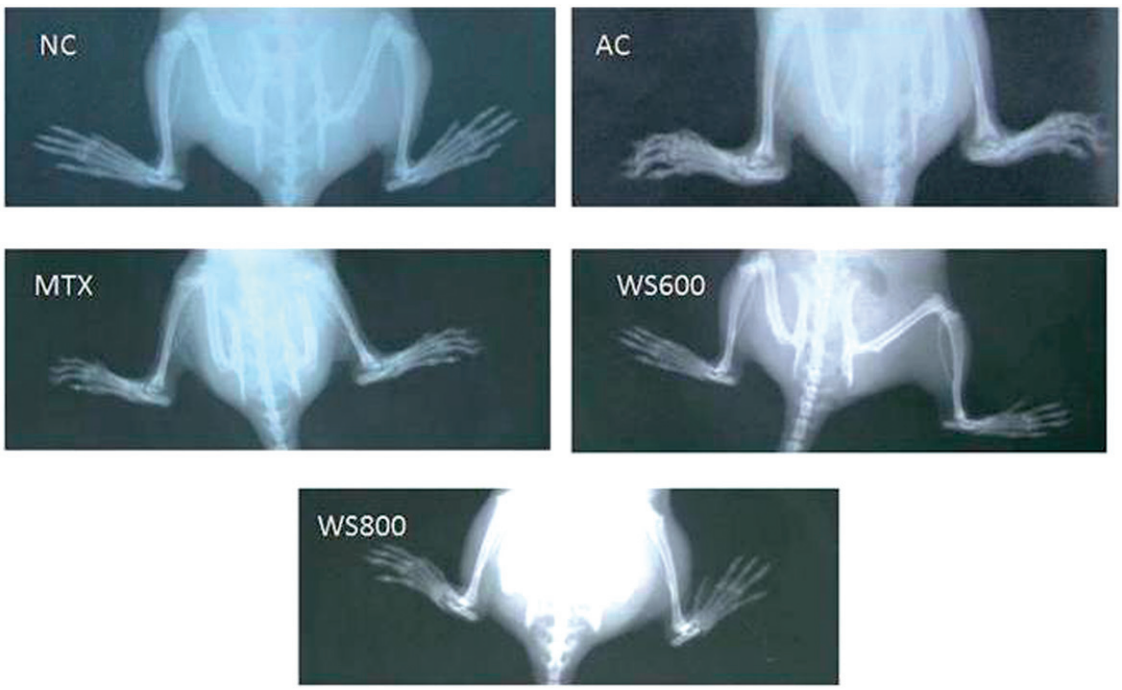

Figure 12. Radiological analysis (X-ray) of joints of rats.

(Ahmed et al., 2005). The last few years have witnessed the advent of several new therapeutic agents for RA which are safer than the usual DMARDs; however, these drugs have their own drawbacks. Plants are considered to be a rich source to tackle complex inflammatory conditions with minimum side effects (Pawar et al., 2011), hence the effective herbs with anti-oxidant activity have been recently reviewed (Rahimi et al., 2009). *W. somnifera* is gaining more attention as a source of potential pharmaceuticals due to its pharmacologic properties (Mishra et al., 2000).

The results of the present study demonstrated that oral administration of the root powder of *W. somnifera* effectively suppressed the symptoms of CIA in rats, as evidenced by a decrease in the macroscopic symptoms of severe arthritis-including swelling, redness, deformity, and ankylosis in hind paw and ankle joints, and radiological score. Protective efficacy of *W. somnifera* was also confirmed by assessing sciatic functional index for functional recovery of motor activity of *W. somnifera* treated arthritic rats. The present study reports the potential anti-inflammatory activity of the root powder of *W. somnifera* against CIA in rats.

In RA, systemic complaints include fatigue, fever and weight loss. The body weight of arthritic animals significantly decreases progressively during the development of arthritis (Egan et al., 2004; Granado et al., 2005; Rasool et al., 2006). In the present study, a significant loss in the body weight was also

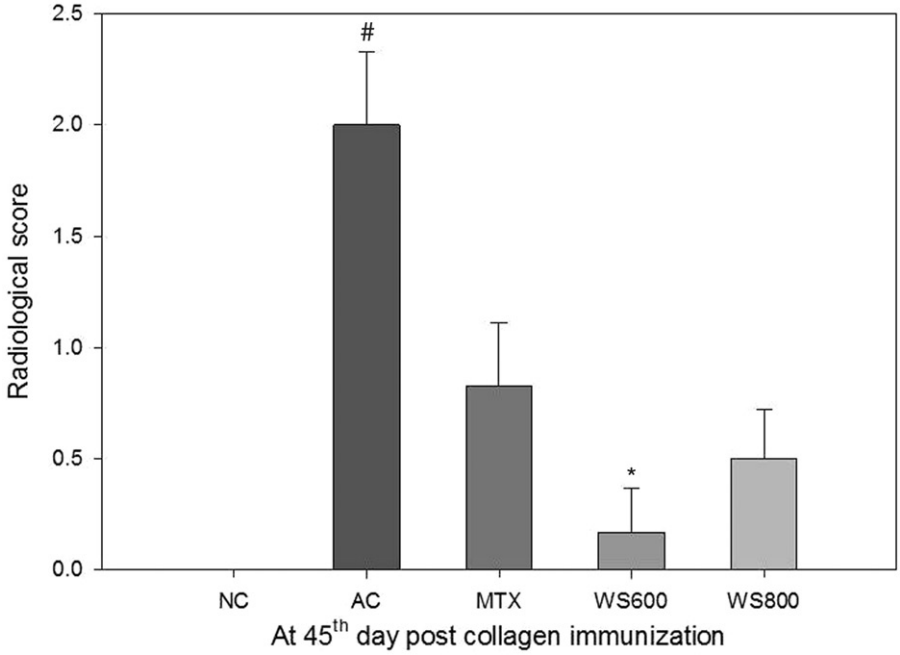

Figure 13. Mean radiological score of rats at the 45th day post collagen immunization. NC: normal control, AC: arthritic control, MTX: methotrexate (0.3 mg kg^{-1}), WS 600; *W. somnifera* (600 mg kg^{-1}), WS 800: *W. somnifera* (800 mg kg^{-1}) treated rats with ± SEM, $N = 6$, *$p < 0.05$ versus AC, #$p < 0.05$ versus NC.

observed in the arthritic rats when compared with their normal control counterparts. However, *W. somnifera* treated arthritic rats at a dose of 600 mg kg^{-1} showed a significant increase in their body weight in comparison to their arthritic control counterparts. Similar increment in body weight in CIA rat following their treatment with *Spirulina platensis* and *Spirulina fusiformis* has also been reported (Kumar et al., 2009; Rasool et al., 2006).

Inflammation in the joints is the major cause of joint pain most commonly associated with RA (Fiorentino et al., 2008) that leads to loss of joint mobility and impaired quality life. The increased inflammatory response contributes to the severity of the arthritic condition and, hence the pain. The footprint assessment showed a significant difference in the pain experienced by the arthritic control and the *W. somnifera* treated rats.

Disability associated with RA is largely due to the development of hyperalgesia exacerbated by several inflammatory mediators that lead to decreased motor activity (Hakkinen et al., 2004). Functional recovery in the motor activity was assessed by toe-spread experiments. Significant spread between the 1 and 5 and 2 and 4 toe and total print length in hind paws were observed in *W. somnifera* (600 mg kg^{-1}) treated arthritic rats at the 45th day. The decrease in the toe spread and total print length is due to the neurophysiological process that leads to the generation of the painful sensation. During inflammation, joint nerves become sensitized to mechanical stimuli through the action of neuropeptides, eicosanoids, proteinase-activated receptors and ion channel ligands (McDougall, 2006).

Joint pain caused by inflammation of joints, leads to the peripheral sensitization of primary sensory afferents and activation of microglia leading to impaired motor function (Fiorentino et al., 2008; Patro et al., 2011). *W. somnifera* (600 mg kg^{-1}) treatment to the arthritic rats resulted in an improvement in swelling, arthritic pain and motor coordination in contrast to the arthritic control rats that were unable to put their step on the sheet due to considerable swelling, arthritic pain, and stress.

W. somnifera (600 mg kg^{-1}) treated arthritic rats showed significant reduction in their radiological score implying its capability of controlling joint inflammation and bone erosion. It has been suggested that inhibition of TNF can potentially prevent radiological progression and thereby prevent disability (Breedveld et al., 2006; Emery et al., 2008; Goekoop-Ruiterman et al., 2005).

The effectiveness of *W. somnifera* in a variety of rheumatologic conditions may be, in part, due to its anti-inflammatory (Anbalagan & Sadique, 1981, 1984; Begum & Sadique, 1988) and antioxidant properties (Bhattacharya et al., 1997; Dhuley, 1998; Mishra et al., 2000; Prithviraj et al., 2013; Singh et al., 2010) which are likely to contribute to the chemoprevention action (Prakash et al., 2002).

Rasool et al. (2000) and Rasool and Varalakshmi (2006), by using the adjuvant induced arthritic rat model, have also demonstrated the immunomodulatory role and anti-inflammatory activity of *W. somnifera* roots. The antioxidant and anti-inflammatory property of *W. somnifera* was attributed to sitoindosides VII-X and withaferin A (glycowithanolides) (Bhattacharya et al., 1997; Dhuley, 1998; Uddin et al., 2012). The presence of other potential sources such as polyphenols, flavonoids and vitamin C (Devipriya & Shyamaladevi, 1999; Udayakumar et al., 2010; Visavadiya & Narasimhacharya, 2007) can also attribute to the antioxidant efficacy of *W. somnifera*.

Overall, the data showed that *W. somnifera* (600 mg kg^{-1}) treatment improves paw thickness, ankle size, body weight, arthritic score, arthritic pain, 1–5 and 2–4 toe spread, total print length, motor coordination, and sciatic functional index in the arthritic rats. The significant decline in arthritic symptoms like arthritic score, ankle size and paw thickness in *W. somnifera* treated arthritis rats may be due to its anti-

inflammatory and anti-oxidant activity that may be responsible for the improvement in behavioral changes and functional recovery in the arthritic rats.

Conclusion

The present study clearly demonstrates that *W. somnifera* root powder (600 mg kg^{-1}) effectively suppressed the behavioral changes in collagen induced arthritic rats. This suppressing ability of *W. somnifera* could be due to its anti-inflammatory ability. Thus, *W. somnifera* treatment could be a potential therapeutic strategy for the treatment of RA**.**

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of this article. This study was financially supported by University Grants Commission, New Delhi in the form of Major Research Project [(F. 39-363/2010 (SR)].

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