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Therapeutic Potential of *Withania somnifera* Extract on Experimental Model of Arthritis in Rats: Histological Study

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ABSTRACT

In Ayurvedic medicine, the *Withania somnifera*, known as Ashwagandha, is a common medicinal herb. The purpose of this research was to investigate the efficacy of Ashwagandha extract as an anti-arthritic agent in animal model. Treatment of rats with adjuvant-induced arthritis (AIA) with Ashwagandha extract, as well as standard drug (Diclofenac Potassium) (10 mg/kg), was orally started on day 2 after the administration of complete Freund's adjuvant and continued until the 21st day of the experiment. Animals were divided into ten groups (n=5). Group 1 represented the control group, group 2 presented the rheumatoid arthritis (RA) group which received complete Freund's adjuvant. Groups 3, 4, and 5 were treated with different doses of Ashwagandha (50, 100, or 200 mg/kg, respectively), group 6 treated with a standard drug. While groups 7, 8, and 9 were RA-rats and treated with Ashwagandha (50, 100, or 200 mg/kg, respectively). The tenth group represented RA rats treated with standard drug. The arthritic index and histological analysis of the rat ankle joint were used to evaluate the therapeutic impact in arthritic rats. Treatment arthritic rats with plant extract considerably restored the arthritic index. The level of edema and arthritis improved in the Ashwagandha-treated group (100-200 mg/kg), as compared with arthritic group that received no treatment. In addition, histopathological analysis demonstrated that the plant extract significantly ($p< 0.05$) reduced joint inflammation, pannus development, and vascularity in treated groups in a dose-dependent manner in comparison to the arthritic control. In conclusion, *Withania somnifera* has anti-arthritic action, which is made possible by an improvement in the histological changes.

1. Introduction

Rheumatoid arthritis (RA) is considered as the most common musculoskeletal system disorder and is considered the main cause of reduced movement among the elderly. RA affects the structure of the joints with gradual changes in cartilage and subchondral bone, as well as the appearance of synovitis [1]. Ignoring the symptoms might lead to chronic joint damage and deformity due to a variety of inflammatory mediators. The prevalence of RA is stable throughout the world, affecting between 0.5 and 1.0 percent of people[2]. It is most common in adults aged 25 to 55 [3]. Women are three times more likely to be impacted than men [4]. Synovial hyperplasia, angiogenesis, and mononuclear infiltration are all part of the characteristics of RA [5]. There are three phases of progression: the first is synovial swelling, which results in symptoms such as joint pain, warmth, and stiffness.

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The second stage is fast cell division and proliferation, which results in thickening of the synovial lining. A final consequence of inflammation is that the affected joint may change its form and alignment, resulting in severe discomfort and loss of mobility [6]. The complete Freund's adjuvant (FCA) generated arthritic model of chronic inflammation, which is driven by immunological mechanisms, is regarded to be the best accessible experimental model of RA [7].

For the identification of medications that are long acting anti-inflammatory agents with minimal side effects, researchers are turning to traditional medicine [8]. Plant-derived medications are still a valuable resource in the fight against severe illnesses, especially in impoverished nations [9]. About 60-90 percent of RA patients have used complementary and alternative treatment, with traditional Chinese medicine being the most common [10].

Several researchers have investigated traditional herbal medicinal resources as adjuvant therapeutic agents in the treatment of RA [11]. *Withania somnifera* are a crucial medicinal plant widely utilized in different traditional systems of medicine for the treatment of various conditions

[12]. For example, *Withania somnifera* can be used as an antioxidant [13],as an anticancer [14], and diabetes-fighting [15] agent as well. [16] showed that *Withania somnifera* inhibited collagenase type 2 gelatinase activity in vitro in a substantial and repeatable manner.

Accordingly, the purpose of this study was to investigate the histopathological changes that occur in the ankle joint after rats have been induced to develop arthritis, as well as the potential therapeutic efficacy of *Withania somnifera* root extract in alleviating these alterations.

2. Materials and Methods

2.1 Study Material

A water-based extract of *Withania somnifera* root was obtained from Now Foods at 395 S Glen Ellyn Rd., Bloomingdale, USA. Using a magnetic stirrer, 2000 mg of extract was stirred in 10 ml of deionized water.

2.2 Experimental animals

The animals were purchased from the Suez Canal University's Faculty of Veterinary Medicine's Lab Animal House in Ismailia, where they had been held for study, allowed to aclimatize for two weeks before taking part in the experiment. In this experiment, male Albino rats weighing 120–130g and aged 2–3 months were used. To avoid uncomfortable contact with a hard surface, they were maintained in polystyrene cages with sawdust on the bottom. The rats were kept in a 12 hour light-dark cycle with unrestricted food and drink. The room had a temperature of 25°C.

2.3 Determination of LD50 of extract in male rats:

The method of Lorke (1983) was utilized for the completion of an acute toxicity (LD50) study. During the first part of the experiment, nine rats were randomly split into three groups of three rats each. Each group received either10, 100, or 1000 mg of extract per kilogram of body weight orally (through a gavage tube), respectively. For a full twenty-four hours, the rats were monitored for any symptoms of side effects or mortality. In the second part of the investigation, the procedure was carried out once more with three rats, each of which was assigned to one of three groups at random and given a different amount of extract per kilogram of body weight: 1600, 2900, and 5000 mg. In addition, the rats were monitored for indicators of toxicity and mortality for a period of twenty-four hours.

2.4 Freund's adjuvant induced arthritis and treatment protocol

Adjuvant arthritis was established on day 0 by injecting 200 µL of CFA (complete Freund's adjuvant) purchased from Sigma USA into the plantar surface of the rat's right hind paw with a 25-gauge hypodermic needle [17]. The inflammation, which appeared as redness, edema, and hypersensitivity to unpleasant stimuli, was restricted to the injected paw. Inflammation began quickly after the injection and peaked between 12 and 24 hours later. The CFA-injected rat had normal grooming and activity levels, and the influence of hyperalgesia on their regular behavior appeared to be modest.

The animals were divided into ten groups. The first five healthy groups of rats were divided into the first group that served as a control group, the second to the fourth groups received different doses of the extract (50, 100, 200 mg/kg) respectively and the fifth group received the standard drug (Diclofenac potassium at dose 10 mg/kg). The other five groups were injected with CFA into the sub plantar region of the left hind paw of each animal under light anesthesia. The time of adjuvant injection was referred to as day 0. Two days after induction of arthritis, daily oral treatments were started for 21 days. Group 6 received solvent (distilled water) and considered as positive control, group (7, 8 and 9) receive different doses of the extract (50, 100, 200 mg/kg) respectively and the tenth group treated with the standard drug (Diclofenac potassium at dose 10 mg/kg).

2.5 Arthritis evaluation

The changes in paw volume were assessed on various days up to 21 days after the Freund's adjuvant injection, and the results were analyzed. It was possible to quantify the change in the inflammatory response by using a caliper on days 3, 6, 9, 12, 15, 18, and 21 following the administration of the adjuvant. With the use of a caliper, the degree of RA, as indicated by joint swelling, was determined by measuring two perpendicular diameters of the joint at two different angles (Lange Caliper; Cambridge Scientific Industries, Cambridge). In order to compute joint circumference, the geometric formula: circumference =2 (sqrt (a2 + b2/2), where "a" is the latero-lateral diameter and "b" is the anteroposterior diameter was used [18]

2.6 Histological examination

The hind paws of rats were cut off above the knee joint and preserved in 10% formaldehyde solution at the end of the experiment. One week in 10% formic acid decalcified the paws, followed by paraffin embedding and mid-sagittal sectioning. The tarsal joint articulation was stained with hematoxylin and eosin according to Wick [19]. Histopathological grading system was used to evaluate pannus development, lymphocytic infiltration, and vascularity in synovial tissues [20]. It was determined that the invasion of mononuclear cells had the following score: Infiltration levels range from 0 (no infiltration) to 3 (strong infiltration), ranging from light to severe. Cartilage and subchondral bone loss by pannus development were graded as follows: 0 (no change), 1 (mild change), 2 (moderate change), and 3 (severe change) (pannus invasion into the subchondral bone. Scores for vascularity were calculated using the following score: the number of blood vessels ranges from 0 (almost none) through 1, 2, and 3, depending on how many they are.

2.7 Statistical analysis

The data is summarized as mean standard deviation to compare the groups statistically, one-way analysis of variance was used (ANOVA). The Duncan multiple range test was used to examine the differences in mean values. At a p-value of 0.05, the differences were deemed significant.

3. Results

No observed side effects were recorded even with the highest dose of Ashwagandha extract (5000mg/kg).The anti-inflammatory activity of the Ashwagandha extract, shown in figure 1, revealed that the tested extract exhibited a statistically significant (P<0.05) inhibition of paw volume (ankle circumference) in a dose-dependent manner. The experimental period showed significant inhibition of paw edema with three tested doses (50, 100 and 200 mg/kg). However, maximum inhibition of paw edema was found in the group receiving 200 mg/kg of Ashwagandha extract.

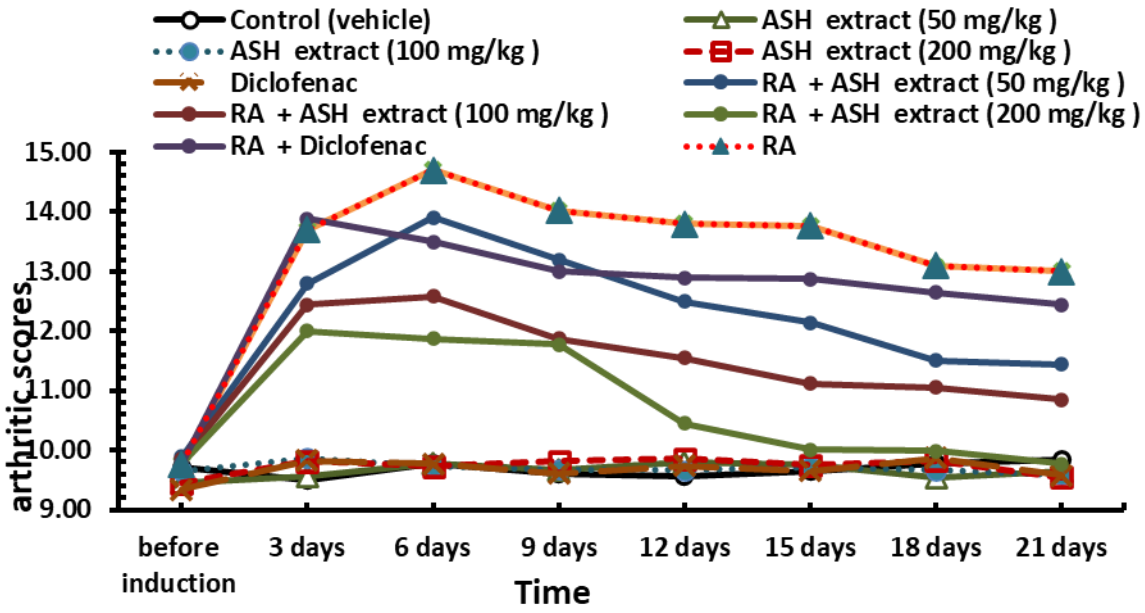

Figure 1: Effect of ethanolic Ashwagandha extract on ankle circumferences of CFA-induced ankle inflammation in rats.

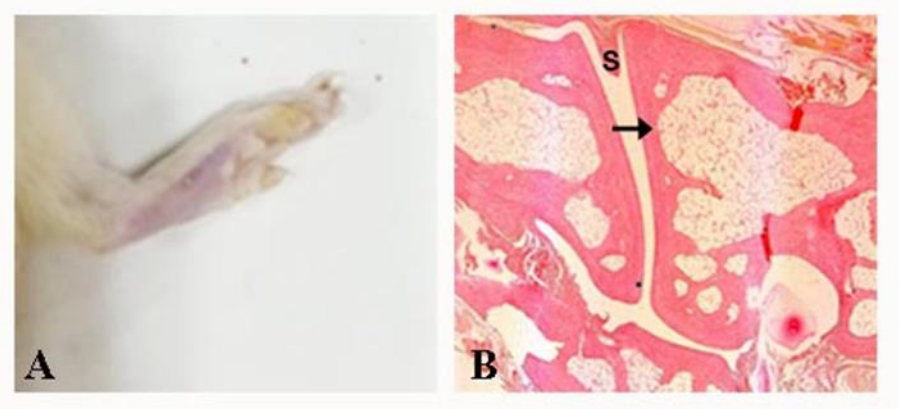

Figure 2: Photographs of gross morphological and histological examination of normal control. (A) Gross morphology of Control normal rat showed no signs of joint inflammation (B) H&E-stained-sagittal section of control rat ankle joints showing a normal joint with no infiltrates in the synovium (S)and intact cartilage surface (arrow) (200X).

The articular surface of the control rats was smooth and there was a consistent tidal mark separating the articular from the subchondral bone that was underneath it. Each and every one of the chondrocytes was encased in a consistent matrix (Fig. 2). Gross morphological examination of rat hind paw revealed diffused soft tissue swelling and redness with joint thickening in the arthritic rats (AIA rats) (Figure 3A). Rats treated with low dose (Figure 4A) and moderate dose of Ashwagandha (Figure 5A) revealed mild swelling, whereas there were no signs of joint thickening, redness, or swelling in rat treated with high dose (200 mg/kg) of Ashwagandha extract (Figure 6A). however gross morphology of joint in AIA rats treated with standard drug revealed signs of joint thickening, redness, or swelling (Figure 7A). The control group injected with sterile saline solution and all plant extract-treated groups at different doses did not show any change in the histological changes of rat ankle joint cartilage, and no inflammation or tissue destruction was observed (Figure 2B). In contrast, ankle joints of AIA rat showed

severe joint destruction with extensive inflammation, and erosion of cartilage and bone (Figure 3B and 3C). Ashwagandha extract at a dose of 100 and 200 mg/kg clearly inhibited the joint destruction, although a slight hypertrophy of synovium and mild inflammatory cell infiltration remained (Figure 4B and 5 B respectively). Ashwagandha extract at a dose of 50 mg/kg mildly prevent those arthritic changes (Fig. 6B). Induction of arthritis and treatment with the standard drug (Diclofenac Potassium) (Figure 7B) had significant effect reduction of inflammation as compared with positive control (AIA-group).

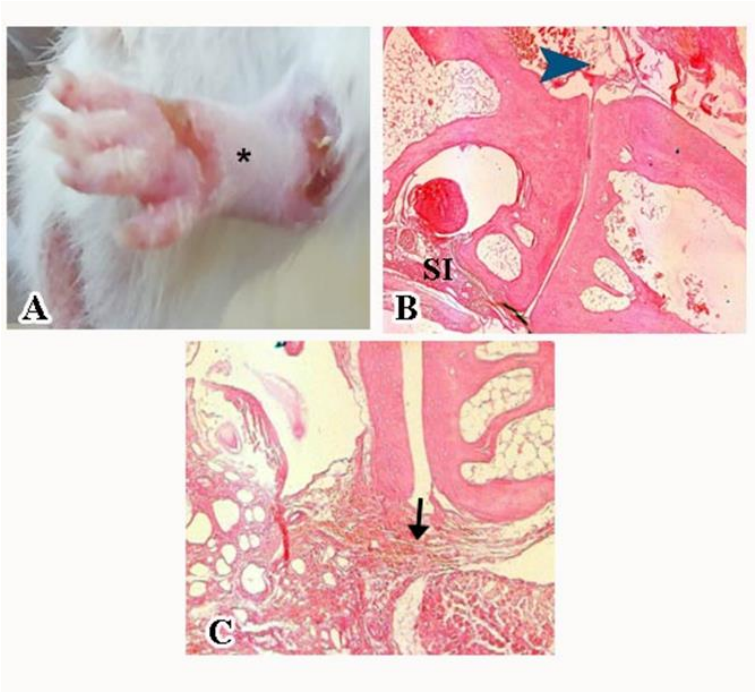

Figure 3: Gross morphology and paws histology of AIA rat. (A) Gross morphology of rat paws showing oedema (*) in the hind paws of experimental animals on day 21 after CFA injection (B) H&E-stained sagittal section of rat ankel joint suffering rheumatoid arthritis, demonstrated marked synovial hypertrophy and hyperplasia (head arrow) and a marked infiltration of the synovial tissues (SI) (200X). (C) Magnified synovium showed pannus formation (arrow) (400X).

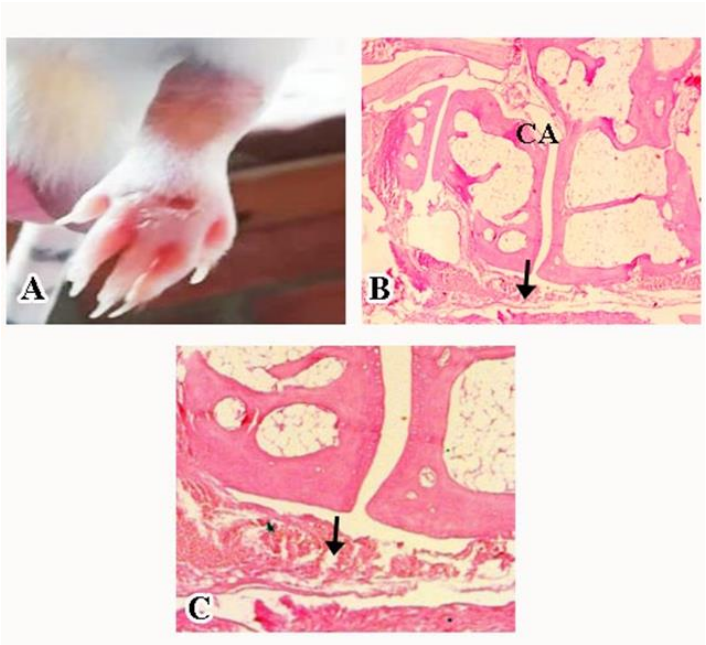

Figure 4: Gross morphology and histology of AIA rat treated with low dose (50 mg/kg) of Ashwagandha extract. (A) Gross morphology of rat paws showing moderate oedema (B) H&E-stained sagittal section of rat ankle joints displayed cartilage degeneration (CA) (200X), (C) Magnified synovium showed decrease of synovial lining hyperplasia (arrow) compared with AIA rats. (400X).

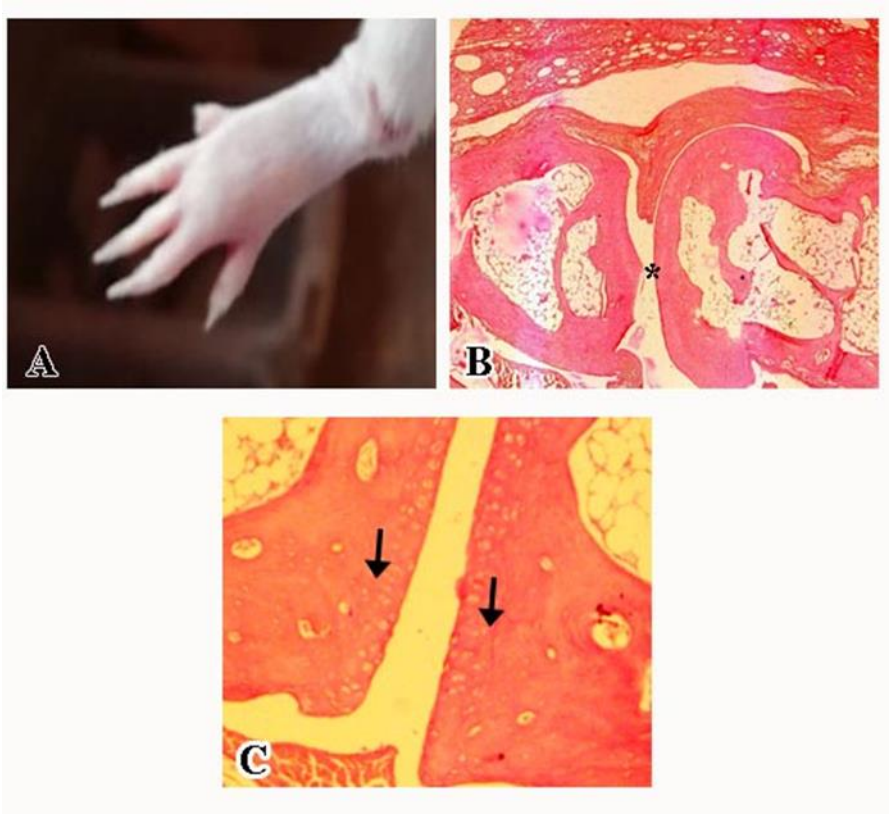

Figure 5: Gross morphology and histology of AIA rat treated with moderate dose (100 mg/kg) of Ashwagandha extract. (A) Gross morphology of rat paws revealed mild oedema (B) H&E staining of sagittal sections of rat ankle joints showed normal space around the joint (*) (200X). (C) showed intact surface of cartilage (arrow) (400X).

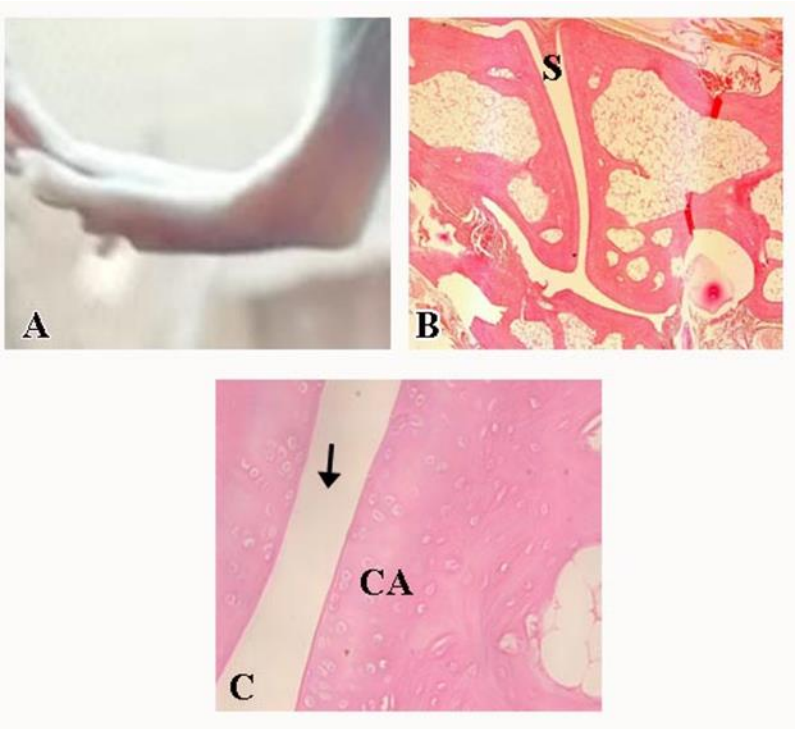

Figure 6: Gross morphology and histology of AIA rat treated with high dose (200 mg/kg) of Ashwagandha extract. (A) Gross morphology of rat paws showed reduced severity of paw inflammation (B) H&E-stained sagittal section of rat ankle joints normal synovial space around the joint (S) (200X). (C) magnification of joint displayed normal population of chondrocytes (CA) besides normal joint space (arrow) (400X).

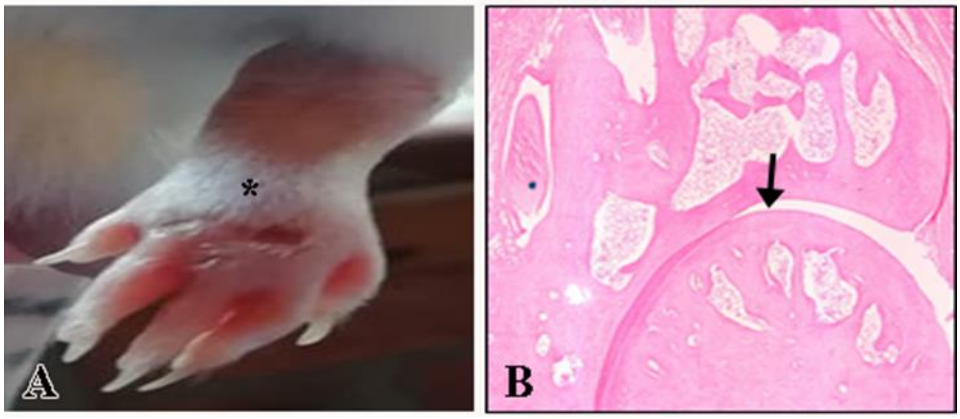

Figure 7: Gross morphological and histological examination of AIA rats treated with standard drug. (A) Gross observation of rat paws displayed edema and inflammation (B) H&E staining of sagittal sections of control rat ankle joints demonstrating disrupted articular surface, and reduction of joint space (arrow) (200X).

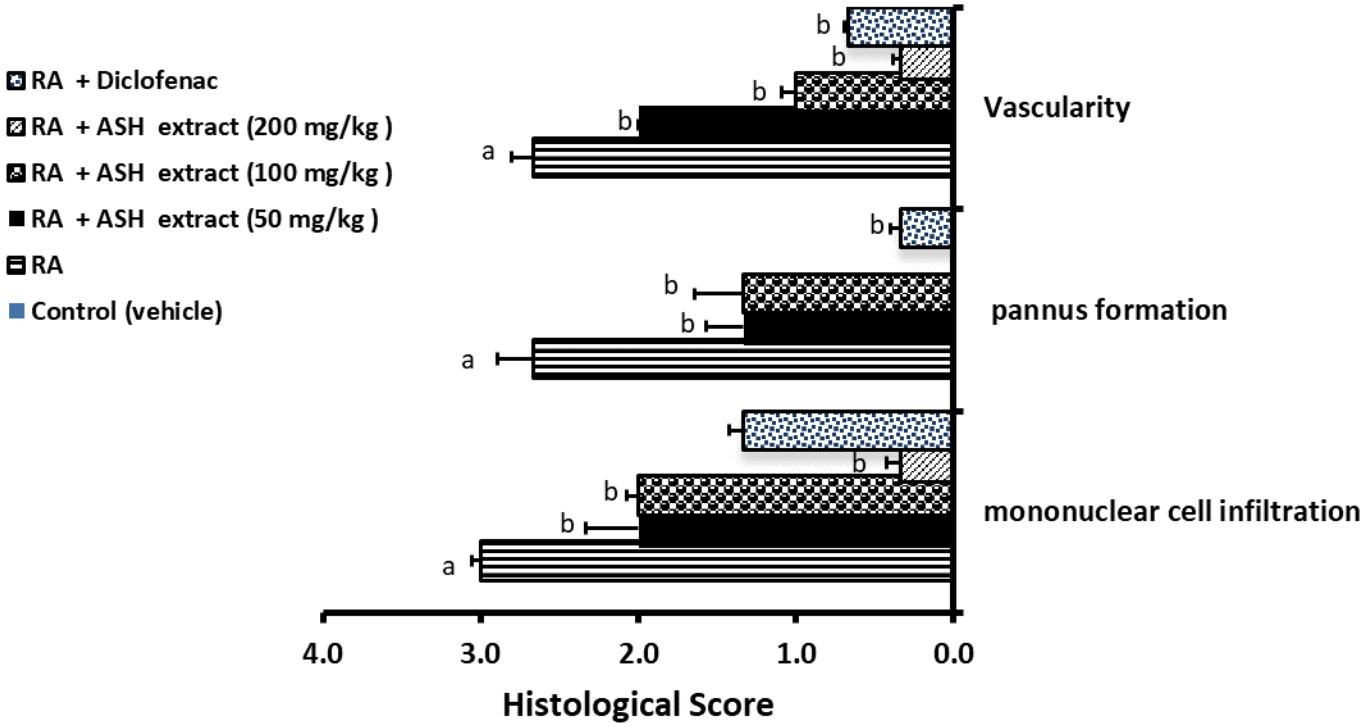

Figure 8: Ankle histological score in Ashwagandha extract-treated rats with normal and adjuvant arthritis. Data presented mean ± S.E. (n= 5/ group). a Significant different from normal control, bSignificant different compared with adjuvant arthritis (AIA) group.

In addition, the histological score that was determined by leukocyte infiltration, pannus invasion, and the degree of vascularity was significantly lower in the extract-treated group in comparison to the AIA group in a dose-dependent way (Fig. 8).

4. Discussion

An inflammatory disorder of synovial tissue known as rheumatoid arthritis (RA) results in joint damage [21] . It can lead to joint deformity and bone and cartilage deterioration if this tissue stays inflamed for an extended period of time [22]. Joint deterioration can still occur even with the present therapy for RA, which solely addresses the symptoms. Nonsteroidal anti-inflammatory medicines (NSAIDs) [23], corticosteroids, disease modifying antirheumatic drugs (DMARDs) [24], and biological response modifiers (BRMs) [25] all have a long list of side effects. Gastrointestinal side effects of NSAIDs can vary from mild discomfort to life-threatening ulceration. Up to 15%–60% of individuals may have common side effects such nausea, gastritis and stomach ulcers [26]. DMARDs

have gastrointestinal, liver, renal, and stomatitis side effects, as well as bone marrow suppression [27].

Complete Freund Adjuvant has been used extensively in the development of animal models of RA [28]. CFA-induced arthritis is a model of chronic polyarthritis with features that resemble RA. The medicinal value of medicinal plants is of great importance to the health of individual and communities [29]. *Withania somnifera* (Ashawagandha) is very revered herb of the Indian Ayurvedic system of medicine [30]. It is used for various kinds of disease processes. The available scientific data support the conclusion that Ashwagandha is a real potent regenerative tonic, due to its multiple pharmacological actions like anti-stress, neuroprotective, antitumor, anti-arthritic, analgesic, and anti-inflammatory etc. It is useful for different types of diseases like Parkinson, dementia, memory loss, stress induced diseases, malignoma and others [31].

The present study aims to investigate the potential anti-inflammatory effect of Ashawagandha extract on RA rat model. In the present study tested plant exhibited no significant cytotoxic activity on albino rats which had normal behavior and did not show any change in the general appearance during the observation period. Histopathological examination of RA rat knee joint showed the presence of an inflammatory synovitis with synovial hyperplasia (pannus), aggregation of fibrin that float freely in the joint and adheres to the articular surface, inflammatory cell infiltration also observed that occurs when inflammatory cells infiltrate around the blood vessels, destruction of bone and joint cartilage (cartilage erosion) also observed while treatment with different doses of Ashawagandha extract displayed rehabilitated histopathological changes in a dose dependent manner in AIA-induced animals .

The present study displayed that high dose of ashwagandha extract (200 mg/kg) have been shown to minimize the production of pannus in rheumatoid arthritis joints. The findings of the study showed that rats treated with Ashwagandha extract at low and moderate dosages (50 and 100 mg/kg, respectively) had some joint healing, although it was less than the results of the study using the maximum dose. With untreated rheumatoid arthritis group, the joint surfaces are not perfectly smooth and level, and the joint surface appears flat and irregular, as seen in the illustration. Furthermore, after the ashwagandha treatment, CFA-induced proliferation of synovial cells and a large amount of inflammatory cell infiltration were alleviated to varying degrees. The recoveries in the ashwagandha groups were showed doses dependent manner, thus, these findings indicated that the ashwagandha had potent anti-inflammatory activity.

The creation of pannus, which increases the generation of free radicals, results in joint injury [32]. In rheumatoid arthritis, synovial tissue ordinarily establishes a link with joint cartilage at the periphery [33]. In and around the cartilage, synovial tissue has grown in an inflammatory state. Pannus is the name given to the extra tissue that develops. In most cases, the pannus is vascularized and has an iron deposit. Mononuclear cells predominate in pannus, with a fibroblastic appearance common [34]. The majority of macrophages are formed from monocytes attracted to the joint by chemotactic proteins [35]. TNF-α was among the cytokines generated by macrophages. An increase in the synthesis of several sorts of products was seen in the synovialis when TNF- was activated in the cells. (MCP-1). In addition to IL-1 and TNF- α, bone resorption was enhanced [36].

Ashwagandha extract acts as a protective agent for the treatment of complete Freund's adjuvant induced rheumatoid arthritis by preventing neutrophil accumulation and infiltration. It has been shown that Ashwagandha includes alkaloids, flavanols, alkanolides and glycosides compounds [37]. Sterols and phenolic acids have also been found in previous phytochemical research on Ashwagandha [38]. steroidal lactones (withanolides, withaferins) and saponins are the root's main bioactive components [39]. Anti-inflammatory properties can be found in withanolides [40].

Conclusion

In conclusion, the present research showed that extract of ashwagandha had an anti-inflammatory impact, which it displayed by reducing the production of pannus and the number of inflammatory cells that infiltrated wounded joints. In the arthritic control group, histopathological alterations such as hyperplasia of capillary, infiltration of inflammatory cells, changes in the structure of sub-synovial collagen fibre, and thickening of extracellular matrix were observed. These alterations were seen as a result of the progression of the disease. The treatment of the arthritic rats with an extract of ashwagandha resulted in a significant attenuation of the pathological alterations caused by RA. According to the findings of the histological score, the extract greatly reduced the lymphatic infiltration that was brought on by arthritis. Pannus and the vascularity of synovium were dramatically relieved in extract-treated groups in comparison to the arthritic control group in a dose-dependent manner.

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