

## Biochemical Evaluation of *Withania somnifera* Root Powder on Adjuvant-Induced Arthritis in Rats

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### Abstract

The present investigation was carried out to evaluate the biochemical effect of *Withania somnifera* Linn. Solanaceae, commonly known as ashwagandha on adjuvant induced arthritic rats. Results were compared to Indomethacin, a non steroidal anti-inflammatory drug. Arthritis was induced by an intra dermal injection of Complete Freund's Adjuvant (0.1 mL) into the right hind paw of Wistar albino rats. *Withania somnifera* root powder (1000 mg/kg/day) and Indomethacin (3 mg/kg/day) were orally administered for 8 days (from 11<sup>th</sup> to 18<sup>th</sup> day) after adjuvant injection. After the experimental period, all the animals were sacrificed and serum, liver and spleen samples were collected for further biochemical analysis. A significant increase in the activities of gluconeogenic enzymes, tissue marker enzymes, blood glucose level, WBC, platelet count, erythrocyte sedimentation rate, and acute phase proteins (hyaluronic acid, fibrinogen and ceruloplasmin) was observed in adjuvant-induced arthritic rats, whereas the activities of glycolytic enzymes, body weight, levels of hemoglobin, RBC count, and packed cell volume were found to be decreased. These biochemical alterations observed in arthritic animals were ameliorated significantly after the administration of *Withania somnifera* root powder (1000 mg/kg/b.wt) and Indomethacin (3 mg/kg/b.wt). Our results suggest that *Withania somnifera* root powder is capable of rectifying the above biochemical changes in adjuvant arthritis and it may prove to be useful in treating rheumatoid arthritis.

### Abstrak

**Evaluasi Biokimiawi Bubuk Akar *Withania somnifera* pada Tikus yang Diinduksi Adjuvant-Arthritis.** Penelitian saat ini dilakukan untuk mengevaluasi efek biokimiawi dari *Withania somnifera* Linn. Solanaceae, yang juga dikenal sebagai ashwagandha, pada tikus yang diinduksi *adjuvant-arthritic*. Hasil penelitian kemudian dikomparasi terhadap Indomethacin, yang merupakan obat anti peradangan non-steroid. Arthritis diinduksi dengan menggunakan injeksi Complete Freund's Adjuvant (0,1 mL) secara intra-dermal ke telapak kaki belakang tikus Wistar albino. Akar *Withania somnifera* bubuk (1000 mg/kg/hari) dan Indomethacin (3 mg/kg/hari) diberikan secara oral selama 8 hari (dari hari ke 11-18) pasca dilakukannya injeksi adjuvant. Setelah masa eksperimen, seluruh hewan percobaan dikorbankan, kemudian sampel limpa, hati, dan serum dikumpulkan untuk analisis biokimiawi lebih jauh. Pada tikus-tikus yang diinduksi *adjuvant-arthritic*, terdapat peningkatan signifikan dalam aktifitas enzim glukoneogenesis, enzim petanda jaringan, level glukosa darah, jumlah sel darah putih (WBC), jumlah keping darah, tingkat sedimentasi eritrosit, dan protein fase akut (asam *hyaluronic*, fibrinogen dan ceruloplasmin). Sementara itu, terjadi penurunan aktifitas enzim glikolisis, berat tubuh, level hemoglobin, jumlah sel darah merah (RBC), dan volume sel yang dimampatkan (PCV). Kondisi perubahan biokimiawi yang terjadi pada hewan penderita arthritis ini membaik secara signifikan setelah pemberian bubuk akar *Withania somnifera* (1000 mg/kg/b.wt) dan Indomethacin (3 mg/kg/b.wt). Hasil penelitian mengindikasikan bahwa bubuk akar *Withania somnifera* dapat menyembuhkan perubahan biokimiawi pada *adjuvant-arthritis* yang disebutkan di atas. Hasil ini dapat bermanfaat dalam perawatan kondisi *rheumatoid-arthritis*.

**Keywords:** *adjuvant-induced arthritis, indomethacin, non-steroidal anti-inflammatory drug, Withania somnifera root powder*

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### Introduction

*Withania somnifera* Linn. Dunal, a solanaceae, commonly known as Ashwagandha, is an Indian ayurvedic medicinal

plant used in several indigenous drug preparations prescribed for common diseases of respiratory and reproductive tracts.<sup>1</sup> It is an evergreen tomentose shrub, grown wild and also cultivated to improve overall

physical and mental health in many parts of India. Currently *Withania somnifera* root extract is used as a dietary supplement in the United States, and has received worldwide attention for its pharmacological activities. *Withania somnifera* has been shown to have promising antibacterial, anti tumour, and immune modulating properties.<sup>2</sup> *Withania somnifera* has been used as a remedy since ancient times for all age groups of both sexes and even during pregnancy without toxic effects.<sup>3</sup> *Withania somnifera* root has been considered to have therapeutic purposes.<sup>4</sup>

The root of *Withania somnifera* contain several alkaloids, with anolides, a few flavanoids and reducing sugars and was shown to have free radical scavenging activity. It has been reported that *Withania somnifera* contain active compounds like withaferin A, sitoindosides VII–X, 5-dehydroxywithanolide-R, with asomniferin-A, 1-oxo-5 $\beta$ ,6 $\beta$ -epoxy-witha-2-ene-27-ethoxy-olide, 2,3-dihydro withaferin A, 24,25-dihydro-27-desoxywithaferin A, 27-O- $\beta$ -D-glucopyranosylphysagulin D, physagulin D, withanoxide I–VII, 27-O- $\beta$ -D-glucopyranosylviscosalactone B, 4,16-dihydroxy-5 $\beta$ , 6 $\beta$ -epoxyphysagulin D, viscosalactone B, and diacetylwithaferin A and they are suggested to have anti-cancer, anti-oxidative and anti-mutagenic properties.<sup>5,6</sup> These above reports indicate that *Withania somnifera* is a rich source of bioactive compounds. Our preliminary studies indicated that *Withania somnifera* root powder has promising anti-arthritis activity by way of decreasing paw diameter and lysosomal enzyme activities.<sup>7</sup>

Rheumatoid arthritis is a chronic auto immune disorder characterized by non-specific, usually symmetric inflammation of the peripheral joints.<sup>8</sup> The long-term prognosis of this disease is characterized by significant morbidity, loss of functional capacity, and increased mortality. This disease affects about 1% of the general population worldwide.<sup>9</sup> Non-steroidal anti-inflammatory drugs such as Indomethacin and naproxen are frequently used as first-line therapies for rheumatoid arthritis; however, these agents may have serious side effects such as gastrointestinal toxicity, renal toxicity, or gastrointestinal bleeding.<sup>10</sup> Therefore we focused our research to find out a drug with long acting anti-inflammatory activity and minimum side effects from plant resources. Hence this study was carried out to reveal the effect of *Withania somnifera* root powder on carbohydrate metabolism, hematological constituents and tissue enzymes in adjuvant induced arthritis, an experimental model for rheumatoid arthritis in rats. The standard non-steroidal anti-inflammatory drug, Indomethacin, was used as reference drug for purposes of comparison.

## Methods

**Animals.** The study was performed with Wistar strain albino rats, 120-150grams, of either sex. The rats were

brought from Tamilnadu Veterinary College, Chennai India. Rats were acclimated for a week in a light and temperature-controlled room with 12 hour dark-light cycle. The rats were fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was freely available.

**Pharmacological agents.** The commercially available powdered root of *Withania somnifera* was obtained from Indian Medical Practitioners Co-operative Stores and Society (IMCOPS), Adyar, Chennai, India and its aqueous suspension in 2% gum acacia was used at a dose of 1000 mg/kg/day. Indomethacin (Tamil Nadu Dadha Pharmaceuticals, Chennai, India) was dissolved in 2% gum acacia solution and then was administered at a dosage of 3 mg/kg/day.<sup>7</sup> All other reagents like glucose, glucose-6-phosphate, fructose 1,6-bisphosphate, dinitrophenyl hydrazine, ATP, disodium phenyl phosphate and sodium pyruvate were used and purchased locally.

**Experimental design.** The rats were divided into six groups, each group comprising of six animals. Group I served as the control group. In Group II arthritic control, arthritis was induced by intradermal injection of Complete Freund's Adjuvant (0.1 mL) into the right hind paw.<sup>7</sup> The adjuvant (Tuberculosis Research Center, Chennai, India) contained heat-killed *Mycobacterium tuberculosis* (10 mg) in paraffin oil (1 mL). Group III and IV drug controls were treated with *Withania somnifera* (1000 mg/kg b.wt) and Indomethacin (3 mg/kg b.wt), respectively, for 8 days. Groups V and VI comprised of arthritic rats that were treated with *Withania somnifera* and Indomethacin, respectively, from days 11 to 18 after the administration of Complete Freund's Adjuvant.

Body weight changes of the experimental groups at different periods up to 19 days, following the injection of Complete Freund's Adjuvant were recorded. On the 19<sup>th</sup> day, at the end of the experimental period, the animals were sacrificed by cervical decapitation, and the blood was collected. The liver and spleen were immediately dissected out and homogenized in ice-cold 0.01 M, Tris HCL buffer, pH 7.4 to give a 10% homogenate. The serum, liver and spleen tissue homogenate were used for assaying the following hematological and biochemical parameters.

The activities of glucose metabolizing enzymes, hexokinase,<sup>11</sup> aldolase,<sup>12</sup> phosphoglucoisomerase,<sup>13</sup> fructose-1-6-diphosphatase,<sup>14</sup> and glucose-6-phosphatase<sup>12</sup> were determined in the liver and spleen. Blood glucose was estimated by the method of Sasaki, et al. (1972).<sup>15</sup> and the protein content was measured by the method of Lowry, et al. in 1951.<sup>16</sup> Tissue enzymes namely aspartate transaminase,<sup>17</sup> alanine transaminase,<sup>17</sup> alkaline phosphatase,<sup>18</sup> and lactate dehydrogenase<sup>12</sup> were

estimated in plasma, liver, and spleen respectively to investigate the anti-arthritis affect of *Withania somnifera*. Hematological constituents namely hemoglobin,<sup>19</sup> RBC, WBC,<sup>20</sup> platelet count,<sup>21</sup> erythrocyte sedimentation rate,<sup>22</sup> packed cell volume,<sup>23</sup> serum hyaluronic acid,<sup>24</sup> fibrinogen,<sup>25</sup> and ceruloplasmin<sup>26</sup> were estimated.

**Statistical analysis.** The results were expressed as mean±SD and statistical analysis was performed using ANOVA, to determine the significant differences between the groups, followed by Student's Newman-Keul's test, p<0.05 implied significance.

**Results and Discussion**

Figure 1 shows the changes in body weight of the control group and experimental group rats. The growth of arthritic rats (Group II) was found to be retarded, compared to normal rats. The body weight of *Withania somnifera* treated arthritic rats (Group V) was found to increase when compared to that of control rats.

Table 1 presents the glycolytic and gluconeogenic enzyme activities in the liver and spleen of experimental animals. The activities of glycolytic enzymes were significantly decreased, whereas gluconeogenic enzymes and blood glucose level were found to be increased in Group II arthritic rats, when compared to Group I control rats. The administration of *Withania somnifera* root powder and Indomethacin to arthritic rats reversed the above changes to normal level considerably, the effect being equal to that of Indomethacin.

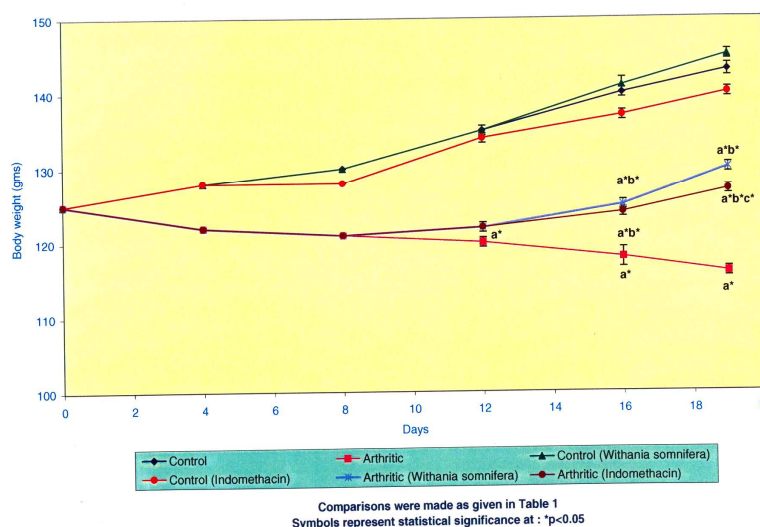
Table 2 depicts the levels of hematological constituents, serum hyaluronate, and acute phase proteins of the control group and experimental group rats. The arthritic rats (Group II) showed a significant decrease in the level of hemoglobin, RBC count, packed cell volume,

and an increase in WBC, platelet count, erythrocyte sedimentation rate (ESR), hyaluronic acid, fibrinogen, and ceruloplasmin when compared to normal animals. These alterations were significantly reversed to near normal levels in *Withania somnifera* root powder treated arthritic rats (Group V), the effect being better than that of Indomethacin.

Table 3 shows the effect of *Withania somnifera* root powder on the changes of alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase in the control and experimental groups. A marked increase in alanine transaminase, aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase was observed in Group II arthritic rats. *Withania somnifera* root powder treated arthritic rats (Group V) showed a significant decrease in the level of alanine transaminase, aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase than Indomethacin, when compared to arthritic control rats (Group II).

Rheumatoid arthritis is a chronic relapsing immune inflammatory multisystem disease with predominant synovial proliferation and destruction of the articular cartilage.<sup>27</sup> Etiology of rheumatoid arthritis still remains obscure despite extensive research. The pathogenesis of rheumatoid arthritis is multifactorial and recent research has implicated oxygen free radicals as mediators of tissue damage.<sup>28</sup> Changes in the body weight are a useful index to assess the course of the disease and the response to therapy of anti-inflammatory drugs in quest.<sup>29</sup>

The loss of body weight observed in arthritic rats may be due to the reduced absorption of glucose and leucine in the intestine of the rat.<sup>30</sup> The increase in body weight during *Withania somnifera* root powder administration reveals the restoration of absorption capacity of the intestine in the arthritic rats.



**Figure 1. Effect of *Withania somnifera* and Indomethacin on Body Weight Changes**

**Table 1. Effect of *Withania somnifera* Root Powder and Indomethacin on Glycolytic and Gluconeogenic Enzymes Activity in Control and Experimental Arthritic Rats**

Enzymes	Group I	Group II	Group III	Group IV	Group V	Group VI
<b>Liver</b>						
Hexokinase	20.44±1.76	15.63±1.35a*	20.51±1.77	20.64±1.78	19.20±1.66 b*	18.51±1.60 b*
Aldolase	18.20±1.57	13.54±1.17a*	18.36±1.59	18.21±1.57	17.26±1.49 b*	16.54±1.43 b*
Phosphoglucosomerase	19.30±1.67	14.63±1.26a*	19.41±1.68	19.28±1.67	18.54±1.60 b*	16.26±1.49 c*
Glucose -6- phosphatase	17.54±1.52	21.12±1.82a*	17.26±1.49	17.17±1.48	16.39±1.41 b*	17.72±1.53 b*
Fructose 1,6- diphosphatase	18.63±1.61	21.72±1.87a*	18.71±1.61	18.81±1.63	19.16±1.65 b*	19.21±1.66 b*
<b>Spleen</b>						
Hexokinase	12.54±1.08	9.20±0.79 a*	12.63±1.09	12.71±1.09	11.79±1.01 b*	10.83±2.01 a*b*
Aldolase	13.54±1.17	10.26±0.88a*	13.24±1.14	13.13±1.13	12.82±1.11 b*	12.56±1.08 b*
Phosphoglucosomerase	16.24±1.40	12.54±1.08a*	16.33±1.41	16.28±1.40	15.78±1.36 b*	15.26±1.31 b*
Glucose -6- phosphatase	16.08±1.39	21.26±1.83a*	16.27±1.41	16.11±1.40	17.43±1.50 b*	18.36±7.59 b*
Fructose 1,6-diphosphatase	21.54±1.86	26.82±2.32a*	21.26±1.83	21.18±1.83	22.61±1.96 b*	23.12±2.00 b*
Blood glucose	101.27±9.44	129.48±5.88a*	100.29 ±9.55	100.75±9.75	110.40 ±5.30 b*	108.34±3.42 b*

Values are mean±SD for six animals in each group

One unit of enzyme activity is expressed as: Hexokinase–nanomoles of glucose–6-phosphate; Aldolase–nanomoles of glyceraldehyde, Phosphoglucosomerase–nanomoles of fructose –formed/minute mg protein at 37°C. Glucose-6-phosphatase and fructose-1,6-diphosphatase –nanomoles of inorganic phosphorus released/minute/mg protein at 37°C. Comparisons are made between: <sup>a</sup>Group I and Group II, III, IV, V, VI; <sup>b</sup>Group II and Group V, VI; <sup>c</sup>Group V and Group VI. The symbol represent statistical significance at-p<0.05

**Table 2. Effect of *Withania somnifera* Root Powder and Indomethacin on Haematological Parameters, Serum Hyaluronate and Acute Phase Proteins in Control and Experimental Arthritic Rats**

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Hb (g/dL)	14.40±0.79	10.36±1.21 a*	14.60±1.13	14.30±1.38	13.51±1.16 b*	12.33±1.56 a*
RBC	3.71±0.19	3.32 ±0.22 a*	3.77±0.26	3.74±0.26	3.63±0.26	3.54 ±0.25
WBC	7.43 ±0.55	17.03 ±1.21 a*	7.30±0.58	7.52±0.54	9.56±0.79 a* b *	11.32± 0.78 a*b*c*
Platelet count	2.55 ±0.14	4.44 ±0.36 a*	2.57±0.17	2.50±0.15	2.62±0.18 b*	2.86±0.20 a*b*
Packed cell volume %	33.73±2.54	26.61 ±2.14 a*	34.0 ±2.34	34.26±3.00	30.31±2.17 b*	29.38±2.84 a*
ESR (mm)						
30 min	3.02±0.22	10.35 ±0.95 a*	2.59 ±0.17	2.77±0.16	6.23±0.40 a* b*	8.92±0.74 a*b*c*
60 min	5.03±0.34	14.45 ±0.96 a*	4.77±0.29	5.49±0.45	8.4 0±0.61 a*b*	11.60 ±0.85 a*b*c*
Hyaluronate (µg/mL)	90.09±5.68	183.00±15.8 a*	85.34±9.13	95.26±9.37	102.20±7.20 b*	126.50±11.6 a*b*c*
Fibrinogen (mg/dL)	47.95±4.46	201.84±11.7 a*	53.43±6.38	52.21±5.68	88.93±9.31 a*b*	127.92±11.82 a*b*c*
Ceruloplasmin (mg/dL)	16.68±2.11	31.27±4.1a*	17.02±2.49	16.96±1.93	19.79±3.27 b*	23.31±2.56 a*b*c*

Values are mean±SD for six animals in each group

Comparisons are made between: <sup>a</sup>Group I and Group II, III, IV, V, VI; <sup>b</sup>Group II and Group V, VI; <sup>c</sup>Group V and Group VI. The symbol represent statistical significance at-\*p<0.05

During arthritic condition, glycolysis and citric acid cycle enzyme activities were found to be altered.<sup>31,32</sup>

Our present study agreed those previous reports because the glycolytic enzyme activities were significantly decreased, whereas the gluconeogenic enzymes were found to be increased in the liver and spleen of arthritic rats. Reduced glucose transport or accumulated gluconeogenic substrates at tissue sites may inhibit glycolytic enzymes in the arthritic rats (Group II). Enlargement of the adrenal gland during adjuvant induced arthritis coincides with the detection of increased amount of plasma corticosterone.<sup>33</sup> The increased plasma corticosterone level in arthritic condition stimulates gluconeogenic activity in the tissues, with a resultant increase in the activities of these gluconeogenic enzymes.

Inflammatory changes in the joints may alter the normal equilibrium between the entry and utilization of glucose.<sup>34</sup> The decreased glycolytic and increased

gluconeogenic enzymes or the impaired transport of glucose into the joints might cause an increase in the blood glucose level during arthritis. The administration of *Withania somnifera* root powder and Indomethacin to arthritic animals brought the above changes to near normal levels. The effect of *Withania somnifera* in arthritic animals is almost equal to that of Indomethacin.

The decrease in hemoglobin content seen in arthritic rats may contribute to the anemia which may be due to reduced erythrocyte deformability and an increase in spleen weight.<sup>35,36</sup> The reduced deformability leads to a shortened life span of erythrocytes,<sup>37</sup> which results in the depression of RBC levels. In arthritic rats, WBC, and platelet count were increased which are responsible for the destruction of invading pathogenic microorganisms.<sup>38</sup> Clinical observations and measurement of erythrocyte sedimentation rate serves as a useful marker for rheumatoid arthritis.<sup>39</sup> The level of serum hyaluronate

**Table 3. Effect of *Withania somnifera* Root Powder and Indomethacin on the Activities of Tissue and Serum Enzymes in Control and Experimental Arthritic Rats**

Parameters Units/mg protein	Group I Control	Group II Arthritis	Group III <i>Withania somnifera</i> root powder	Group IV Indomethacin	Group V Arthritis + <i>Withania somnifera</i> root powder	Group VI Arthritis+ Indomethacin
<b>Plasma</b>						
Alanine transaminase	0.58±0.07	1.12±0.11a	0.61±0.07	0.63±0.07	0.70±0.07a*b*	0.84±0.09 a*b*c*
Aspartate transaminase	0.52±0.06	0.98±0.12a*	0.48±0.06	0.51±0.07	0.61±0.05a*b*	0.66±0.07a*b*c*
Alkaline phosphatase	2.70±0.20	5.96±0.4 8a*	2.55±0.27	2.78±0.28	2.75±0.34 a*	2.72±0.25 b*
Lactate dehydrogenase	5.44±0.47	10.87±1.3 a*	5.68±0.48	5.47±0.53	6.88±0.68 a*b*	8.25±0.76a*b*c*
<b>Liver</b>						
Alanine transaminase	0.13±0.02	0.36±0.05 a*	0.15±0.03	0.13±0.01	0.19±0.04a*b*	0.22±0.03 a*b*
Aspartate transaminase	0.15±0.04	0.33±0.05 a*	0.15±0.02	0.17±0.03	0.19±0.03a*b*	0.23±0.03 a*b*
Alkaline phosphatase	1.29±0.13	3.51±0.23 a*	1.24±0.17	1.28±0.14	1.46±0.16 b*	1.79±0.17 a*b*c*
Lactate dehydrogenase	10.81±1.51	19.2±1.9 a*	10.64±1.52	9.93±1.37	10.83±1.60 b*	14.48±1.72a*b*c*
<b>Spleen</b>						
Alanine transaminase	0.16±0.01	0.41±0.04 a*	0.18±0.01	0.16±0.03	0.28±0.05 a*b*	0.31±0.04 a*b*
Aspartate transaminase	0.23±0.02	0.67±0.06 a*	0.21±0.02	0.25±0.02	0.41±0.07 a*b*	0.51±0.04 a*b*c*
Alkaline phosphatase	0.55±0.04	0.90±0.10 a*	0.53±0.05	0.52±0.08	0.62±0.05 b*	0.71±0.06 a*b*c*
Lactate dehydrogenase	4.85±0.48	9.56±0.85 a*	4.54±1.08	4.96±0.40	6.06±0.29 a*b*	7.85±0.65 a*b*c*

Values are mean ± SD for six animals in each group

Comparisons were made as in Table 1. Enzymes unit: Aminotransferases -μ moles x 10<sup>-2</sup> of pyruvate; ALP-μ moles x of phenol; LDH-μ moles x 10<sup>-1</sup> of pyruvate liberated /min/mg protein. The symbols represent statistical significance at: \*p<0.05

is another reliable index of arthritic severity.<sup>40,41</sup> Normally serum hyaluronate is low but in active and destructive rheumatoid arthritis, the levels increase significantly. In the present study, serum hyaluronate increased in adjuvant arthritis may be due to its release from tissues and inflamed joints in the arthritic animals. The increase in plasma fibrinogen might be due to the result of liver stimulation by the products of tissue disintegration.<sup>42</sup> Plasma fibrinogen is deposited excess as fibrin in synovial fluid in rheumatoid arthritis. It plays a vital role in the inflammatory joint diseases and its level serves as a useful marker to assess the progression of arthritis.<sup>43</sup>

Ceruloplasmin acts as an extracellular scavenger of superoxide radicals<sup>44</sup> and is likely to participate in the inhibition of lipid peroxidation formation, during inflammation, through oxidation of ferrous molecules required for free radicals generation.<sup>45</sup> The increased secretion of hormones and mediators like interleukin-1, epinephrine, and glucocorticoids released during arthritis might be responsible for the elevation of serum ceruloplasmin in Group II arthritic rats. Ceruloplasmin is synthesized in the liver in response to tissue injury and is released into the blood circulation.<sup>46</sup> The elevated serum ceruloplasmin level in arthritic animals appears to correlate with the increased antioxidant activity.<sup>47,48</sup> Administration of *Withania Somnifera* root powder significantly decreased the level of serum hyaluronate and acute phase proteins and normalized the hematological changes in arthritic rats, the effect being better than that of Indomethacin.

Tissue damage was assessed by measuring the activities of enzyme in the serum and in the respective organs. The increase in aminotransferases is due to the release from the cells of the liver, since liver impairment is also a feature of adjuvant arthritis.<sup>49</sup> Alkaline phosphatase has been reported to be present mainly in the blood vessels, pia arachnoid and choroid plexus. Alkaline phosphatase activity has been reported to increase during the morphological and functional development of the tissues. Lactate dehydrogenase was significantly increased in arthritic rats. Reduction in the lactate dehydrogenase level after *Withania somnifera* root powder treatment may be due to the inhibition of the release of osteosarcoma collagenase and neutrophil, which indicate the membrane stabilizing effect of *Withania somnifera*. Alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase were significantly reduced in arthritic rats after the administration of *Withania somnifera* root powder than Indomethacin. This reducing effect of *Withania somnifera* may be related to their anti-inflammatory activity. The beneficial effect of *Withania somnifera* observed in this study might be due to the presence of various active principles in its roots. It is known that *Withania somnifera* root powder is popularly used as a home remedy for several diseases such as arthritis, geriatric problems, fever, tuberculosis, asthma, and it is an official drug mentioned in the Indian Pharmacopoeia. Based on the significance observed between *Withania somnifera* (Group-V) and Indomethacin treated (Group-VI) arthritic animals in our study, it may be suggested that *Withania somnifera*

exerts a more promising anti-inflammatory effect than Indomethacin. *Withania somnifera* can be used as a potential anti-arthritic drug as an alternative to Indomethacin in the treatment of rheumatoid arthritis.

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