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Antiperoxidative Effect of *Withania somnifera* Root Powder on Liver Lipid Peroxidation and Antioxidant Status in Adjuvant-induced Arthritic Rats

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Abstract: The present study was carried out to evaluate the antiperoxidative effect of *Withania somnifera* Linn. Dunal (family-Solanaceae) on liver lipid peroxidation and antioxidant status in adjuvant induced arthritic rats. Results were compared with those for Indomethacin, a non steroidal anti-inflammatory drug. Arthritis was induced by intra dermal injection of complete freund's adjuvant (0.1 mL) in to the right hind paw of Wistar albino rats. *Withania somnifera* root powder (1000 mg kg^{-1} b.wt.) and indomethacin (3 mg kg^{-1} b.wt.) were orally administered for 8 days beginning 11 days after adjuvant injection. The antiperoxidative effect of *Withania somnifera* root powder was investigated by measuring changes in lipid peroxidation and antioxidant status of liver in arthritic animals. Results of the present investigation showed significant decrease in the level of lipid peroxides, constituents with the increased enzymic antioxidants and depleted non-enzymic anti-oxidant status in arthritic animals. The oral administration of *Withania somnifera* root powder (1000 mg kg^{-1} b.wt.) modulated the above altered lipid peroxidation and antioxidant status to near normal levels in arthritic animals.

Key words: *Withania somnifera* root powder, adjuvant-induced arthritis, lipid peroxidation, antioxidant status, indomethacin

INTRODUCTION

Withania somnifera L. Dunal (family-Solanaceae) commonly known as ashwagandha is an evergreen tomentose shrub, grown wild and also cultivated for medicinal use in many parts of India. Of all parts of this plant, *Withania somnifera* root has been considered to be the most active for therapeutic purposes. The roots of *Withania somnifera* contain several alkaloids, withanolides, a few flavanoids and reducing sugars (Rasool and Varalakshmi, 2006a, b). These reports suggest that *Withania somnifera* root powder is a rich source of bioactive compounds. *Withania somnifera* has been used as a drug since ancient times for all age groups of both sexes and even during pregnancy, without toxic effects. Different investigators have reported that *Withania somnifera* possesses antisertogenic, anticancer, anabolic and beneficial effects in the treatment of arthritis, geriatric problems and stress (Rasool and Varalakshmi, 2007). It also possesses adaptogenic, cardiotropic, cardio protective and

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anticoagulant properties (Hamsa *et al*., 2007). Our preliminary studies confirm its anti-inflammatory, immunomodulatory and lysosomal membrane stabilizing action and antioxidant effect on adjuvant and gouty arthritis in rats (Rasool *et al*., 2000; Rasool and Varalakshmi, 2006a, b). In addition, our earlier studies proved its antiperoxidative action on spleen lipid peroxidation and antioxidant status in adjuvant-induced arthritic rats (Rasool and Varalakshmi, 2007). By considering this data, in the current study, the antiperoxidative effect of *Withania somnifera* root powder in liver was investigated using adjuvant-induced arthritis, a well known experimental model for rheumatoid arthritis in rats. The antiperoxidative effect of *Withania somnifera* root powder was assessed by measuring changes in lipid peroxidation and antioxidant status in liver of arthritic animals. For comparison purposes, non-steroidal anti-inflammatory drug indomethacin was used.

MATERIALS AND METHODS

Animals

The study was performed with Wistar strain albino rats, 120-150 g, of either sex. The rats were obtained from Tamil Nadu Veterinary College, Chennai, India. Rats were acclimatized for a week in a light and temperature-controlled room with a 12 h dark-light cycle. The rats were fed with commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was freely available. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Ministry of Culture, Chennai. Experimental protocol was approved by our departmental ethical committee.

Drugs

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 1000 mg kg^{-1} b.wt. (Rasool and Varalakshmi, 2007). This dosage was fixed based on their dose dependent anti-inflammatory effect on inhibiting paw edema without any toxic effect (unpubilished data). Indomethacin (Tamilnadu Dadha Pharmaceuticals, Chennai, India) was dissolved in 2% gum acacia solution and 3 mg kg^{-1} b.wt. was administrated orally. All other reagents used were standard laboratory reagents of analytical grade and purchased locally

Experimental Protocol

Rats were divided into six groups each comprising of six animals. Group 1 served as controls. In Group 2, arthritis was induced by intradermal injection of Complete Freund's Adjuvant (CFA) (0.1 mL) into the right hind paw (Rasool and Varalakshmi, 2007). The adjuvant (Tuberculosis Research Center, Chennai, India) contained heat-killed *Mycobacterium tuberculosis* (10 mg) in paraffin oil (1 mL). Group 3 and 4 were treated with *Withania somnifera* and Indomethacin, respectively, for 8 days. Groups 5 and 6 comprised of arthritic rats were treated with *Withania somnifera* and Indomethacin, respectively, from day 11 to 18 after the administration of Complete Freund's Adjuvant (CFA).

On the 19th day, at the end of the experimental period, the animals were sacrificed by cervical decapitation. The liver was immediately dissected out and homogenized in ice-cold 0.01 M, Tris HCl buffer, pH 7.4 to give a 10% homogenate. Liver tissue homogenate was used for assaying the following biochemical investigations.

Biochemical Estimations

Liver lipid peroxidation was carried out by the procedure of Hogberg *et al.* (1974) using thiobarbutric acid as the colouring agent. Malondialdehyde (MDA) produced during peroxidation of lipids served as an index of lipid peroxidation. MDA reacts with TBA to generate a colour product, which absorbs at 532 nm.

Liver lipid peroxidation with inducer system namely, 10 mM $FeSO_4$/0.2 ascorbate/10 mM H_2O_2 was measured by the method of Devasagayam and Tarachand (1987). The Malondialdehyde contents of the samples were expressed as nmoles of MDA formed/mg/protein.

Superoxide dismutase (SOD) activity in liver was determined by the method of Marklund and Marklund (1974). The degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH by SOD was used as a measure of the enzyme activity. Catalase and Glutathione peroxidase (Gpx) activities in liver were estimated by the method of Sinha (1972) and Rotruk *et al.* (1973).The activity of catalase was expressed as µg of H_2O_2 consumed/min/mg protein. Glutathione peroxidase was expressed as µg of glutathione utilized/min/mg/protein. Non enzymic antioxidants-reduced glutathione (Moron *et al*., 1979), ascorbic acid (Omaye *et al*., 1979), vitamin E (Desai, 1984), total sulphydryl and non protein sulphydryl (NPSH) (Sedlack and Lindsay, 1968) were estimated in liver.

Statistical Analysis

Results were expressed as mean±SD and statistical analysis was performed using ANOVA, to determine significant differences between groups, followed by student's Newman-Keul's test. $p<0.05$ implied significance.

RESULTS AND DISCUSSION

In group 2 arthritic rats, lipid peroxide level in liver was decreased significantly when compared to control group, whereas administration of *Withania somnifera* root powder to group 5 arthritic rats altered the above changes by regulating the lipid peroxide level to nearly that of normal levels (Table 1).

In group 2 arthritic rats, the enzymic antioxidants were significantly increased in arthritic rats when compared to control rats, whereas the non-enzymic antioxidants-reduced glutathione, total sulphydryl, nonprotein sulphydryls, vitamin C and E were significantly decreased in arthritic condition. Administration of *Withania somnifera* root powder modulated the enzymic and non-enzymic antioxidant levels to near normal control levels in Group 5 arthritic rats considerably, which indicates its antiperoxidative action (Table 2).

Table 1: Effect of *Withania somnifera* and Indomethacin on basal and induced lipid peroxidation in liver of control and experimental animals

	Groups					
Parameters	1	2	3	4	5	6
Liver						
Basal	1.92±0.17	1.23±0.05a*	1.850±0.20	1.95±0.17	1.80±0.10b*	2.51±0.22a*b*c*
$FeSO_4$ induced	6.00±0.52	4.97±0.43a*	6.025±0.53	6.12±0.53	6.00±0.34b*	8.37±0.76a*b*c*
Ascorbate induced	4.88±0.42	3.83±0.34a*	4.830±0.40	4.94±0.43	4.79±0.41b*	5.35±0.68a*b*c*
H_2O_2 induced	2.53±0.21	1.61±0.14a*	2.560±0.22	2.60±0.22	2.44±0.21b*	2.59±0.31a*b*c*

Treatment of groups are as follows: Group 1: Control; Group 2: Arthritic rats; Group 3: Control rats treated with *Withania somnifera* (1000 mg kg^{-1} b.wt.) for 8 days from 11th to 18th day; Group 4: Control rats treated with Indomethacin (3 mg kg^{-1} b.wt.) for 8 days from 11th to 18th day; Group 5: Arthritic rats treated with *Withania somnifera* (1000 mg kg^{-1} b.wt.) from 11th to 18th day post adjuvant and Group 6: Arthritic rats treated with Indomethacin (3 mg kg^{-1} b.wt.) from 11th to 18th day post adjuvant. Comparisons are made as follows: a: Group 1 vs groups 2, 3, 4, 5 and 6; b: Group 2 vs groups 5 and 6 and c: Group 5 vs group 6. Each value represent mean±SD (n = 6). Values are expressed as: nanomoles of malondialdehyde formed/mg protein; plasma- mg dL^{-1}. The symbols represent statistical significance at: *$p<0.05$

Table 2: Effect of *Withania somnifera* and Indomethacin on the enzymic and nonenzymic antioxidant levels in liver of control and experimental animals

	Groups					
Parameters	1	2	3	4	5	6
Superoxide Dismutase (SOD)	3.11±0.27	3.95±0.34a*	3.13±0.27	3.13±0.27	3.33±0.28b*	2.54±0.13a*b*c*
Glutathione peroxidase (Gpx)	7.28±0.65	9.78±0.39a*	7.25±0.64	7.31±0.65	7.95±0.68b*	5.02±0.43a*b*c*
Catalase	27.13±12.72	45.63±12.04a*	25.76±11.03	26.60±10.98	33.80±8.81b*	34.16±9.28b*
Glutathione	6.78±0.59	6.39±0.59	6.75±0.59	6.81±0.60	6.68±0.60	5.06±0.43a*b*c*
Totalsulphydryl (TSH)	18.19±1.68	14.47±1.40a*	18.16±1.66	17.62±1.62	18.24±1.65b*	17.67±1.61b*
Non-protein sulphydryl (NPSH)	5.06±0.43	3.97±0.34a*	5.07±0.44	5.10±0.44	4.77±0.42b*	4.05±0.35a*
Vitamin C	2.64±0.23	1.86±0.16a*	2.51±0.21	2.73±0.23	2.47±0.21b*	2.21±0.19a*b*
Vitamin E	0.81±0.07	0.58±0.05a*	0.80±0.07	0.81±0.07	0.78±0.06b*	0.68±0.06a*b*c*

Treatment of groups are as follows: Group 1: Control; Group 2: Arthritic rats; Group 3: Control rats treated with *Withania somnifera* (1000 mg kg^{-1} b.wt.) for 8 days from 11th to 18th day; Group 4: Control rats treated with Indomethacin (3 mg kg^{-1} b.wt.) for 8 days from 11th to 18th day; Group 5: Arthritic rats treated with *Withania somnifera* (1000 mg kg^{-1} b.wt.) from 11th to 18th day and Group 6: Arthritic rats treated with Indomethacin (3 mg kg^{-1} b.wt.) from 11th to 18th day. Comparisons are made as follows: a: Group 1 vs groups 2, 3, 4, 5 and 6; b: Group 2 vs group 5 and 6 and c: Group 5 vs group 6. Values are expressed as mean±SD (n = 6). Enzyme units are expressed as; SOD-units/mg protein (unit-Amount of enzyme required to inhibit the auto-oxidation reaction by 50%); Gpx-µg of GSH utilized/min/mg protein; Catalase-µmol of H_2O_2 consumed/min/mg protein. Glutathione, Total sulphydryl (TSH), Non-protein sulphydryl (NPSH), Vitamin C-µg/mg protein. Vitamin E-mg/g tissue. The symbols represent statistical significance at: *$p<0.05$

Rheumatoid arthritis is a chronic relapsing immuno-inflammatory multisystem disease with predominant synovial proliferation and destruction of the articular cartilage. Etiopathogenesis of rheumatoid arthritis still remains obscure despite extensive research. Although the pathophysiology basis of rheumatoid arthritis is not yet fully understood, reactive oxygen species have been implicated in its pathogenesis. Reactive oxygen species are highly reactive transient chemical species (nitric oxide, super oxide anion, hydrogen peroxide and hydroxyl radical) with the potential to initiate cellular damage (to proteins, lipids, etc.) in joint tissues especially in rheumatoid arthritis (Rasool and Varalakshmi, 2007; Choi, 2007). In the present study, liver lipid peroxide levels were found to be decreased in arthritic conditions. Suppression of lipid peroxidation in liver of arthritic rats may be due to damage of the ascorbic acid-Fe^{2+} dependent mechanisms and decline in the level of hepatic cytochrome P450. This cytochrome is involved in NADPH dependent lipid peroxidation. Similar reports are reported by Geetha *et al*. (1998).

SOD may play an important role in protecting cells against reactive oxygen species. Increased Super Oxide Dismutase (SOD) activity observed in group 2 arthritic rats appear to be reflux mechanism to guard against extracellular oxygen free radicals. Increased production of NADPH from hexose monophosphate shunt during arthritis may cause an increase in SOD activity (Marklund *et al*., 1987). GPx protects the cell against cell damage resulting from the increased level of peroxides. Liver has been reported to be a major site of lipid peroxide metabolism. Lipid peroxides are metabolized in the liver by GPx (Kasama *et al*., 1988). This may be the reason for the absence of noticeable change in liver lipid peroxides in arthritis. The main role of catalase is to detoxify H_2O_2. Its activity is higher in liver and erythrocytes. In rheumatoid arthritis, its concentration is very low, to expect considerable protection against H_2O_2 (Blake *et al*., 1981; Geetha *et al*., 1998). Glutathione is endogenously synthesized in the liver and is the first line of defense against peroxidation. In arthritic rats, liver glutathione was not affected. This might be due to the fact that liver glutathione is utilized and resynthesized with great rapidity. The observed non-enzymic antioxidants depression in adjuvant-induced arthritis is associated with the protracted inflammatory phase of the disease. Protein-

sulphahydryl groups play an important role in the destruction of peroxides formed during phagocytosis by granulocytes. Protein-sulphahydryl level reduction may be due to the activation of inflammatory mediators (Lorber *et al*., 1975). Vitamins C and E were significantly reduced in arthritic rats. This reduction was due to increased oxidative stress, which leads to damage of the membranes of various cell organelles. After *Withania somnifera* treatment, the alterations produced in arthritic rats with respect to antioxidant concentrations were modulated considerably near to normal levels. The compounds such as Sitoindosides VII-X, Withaferin A (Glycowithanolides), polyphenols and flavonoids in *Withania somnifera* root powder was proved to have antioxidant activity (Jovanoic and Simic, 2000). Thus the antioxidant efficacy observed in *Withania somnifera* root powder treated arthritic rats was due to the presence of potential sources of above antioxidants in *Withania somnifera* root powder. Many literatures reveal supporting evidences of antiperoxidative activity of *Withania somnifera* root powder in different experimental conditions (Chaurasia *et al*., 2000; Dhuley, 1998).

In conclusion, the present biochemical findings support the antiperoxidative property of *Withania somnifera* and correlates with its therapeutic potential to produce symptomatic relief in rheumatoid arthritis. Thus the antiperoxidative effect observed in *Withania somnifera* treated arthritic animals could be mediated through phytosterols, polyphenols, flavanoids and vitamin C in *Withania somnifera* root powder. A further research is on underway with its active principles to identify the exact mechanism of action..

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