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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF THE ALCOHOLIC EXTRACT OF *SIDA RHOMBIFOLIA* ROOTS IN ARTHRITIC RATS

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ABSTRACT

The alcoholic extract of *Sida rhombifolia* (SRE) roots, which exhibited significant anti-inflamatory activity, was evaluated for the possible mode of action by studying its antioxidant potential in adjuvant induced rats. The biological defense system constituting the superoxide dismutase, glutathione peroxidase, ascorbic acid showed a significant increase while the lipid peroxide content was found to decrease to large extent on SRE treatment thereby indicating the extracts free radical scavenging property. Histopathological studies too supported anti-rheumatic potential of the roots of *Sida rhombifolia*.

Keywords: *Sida rhombifolia* roots, Free Radical Scavenging Activity; Arthritic Rats.

INTRODUCTION

In traditional system of medicine the practitioners use various indigenous plants for the treatment of different types of arthritic conditions. One such plant drug used by Siddha practitioners is *Sida rhombifolia* commonly called Bala or Atibala is claimed by folklore for various ailments like rheumatism, seminal weakness, and diarrhea (K.D. Rainsford, 1980). Earlier studies on the ethanolic extact of the roots (SRE) revealed a significant anthelminthic, antifungal and antibacterial activity**.** Since no scientific validation on the anti rheumatic activity is carried on the roots, the roots are selected for the study.

MATERIAL AND METHODS

Preparation of Extract

Sida rhombifolia roots **[**authenticated by Dr. P. Jayaraman., Director, Plant Anatomy Research Centre, Tambaram, Chennai] were procured from Sri Ramachandra College of Pharmacy medicinal garden and a voucher specimen (Pharma No 06/07) has been retained in the herbarium of the college of pharmacy, Sri Ramachandra Medical College and Research Institute, Chennai.

Shade dried and coarsely powdered roots of *Sida rhombifolia* (3 kg) were extracted with 90% ethanol in an aspirator bottle at room temperature for 72 h. Nearly 80% of the solvent was removed by distillation over boiling water bath at atmospheric pressure and the remaining under reduced pressure. This extract (SRE yield 1.95%) was suspended in 5% gum acacia and used for animal experiments. SRE when subjected to phytochemical screening revealed the presence of alkaloids, sterols, triterpenes, phenolics and tannins (Harbone, 1973).

Animal Studies

Wistar strain male albino rats, weighing 170-225 grams were used for this experiment, maintained in animal experimental laboratory of Sri Ramachandra University at room temperature (25^0 C), relative humidity of 75 % and 12 hrs, dark light cycle, were used for experiments. The animals had access to standard laboratory feed (M/s. Hindustan Levers Ltd.) and water *ad libitum*.

Free Radical Scavenging Activity

The rats were divided into four groups each consisting of six animals. The first group served as control which received only the vehicle (0.2 % Carboxy Methyl Cellulose). Arthritis was induced to other three groups with 0.1 ml of Complete Freund's Adjuvant (CFA) by intra-dermal injection in the right hind paw. The second group animals received o.2 % Carboxy Methyl Cellulose only while the third and fourth groups were administered the standard drug (Diclofenac sodium, 0.5 mg/kg) and SRE (100 mg/kg) as emulsion in carboxy methyl cellulose daily for a period of 6 weeks by oral route (Williams et al., 1994). The body weight was measured periodically.

The hind paw swelling was recorded every week using plethysmograph. The animals were sacrified by cervical dislocation on the 42^{nd} day and the blood was collected by cardiac puncture prior to sacrifice. The liver and spleen were rapidly removed and washed with ice-cold saline. The tissues were cut into small pieces and homogenized using Tris buffer (0.01 M, pH7.4) at 4^0c to give 10% homogenate. The haemolystae was extracted as per the method (Quist 1980). The collected blood with anti-coagulant was centrifuged to remove the plasma. The packed cells were washed first with isotonic saline to remove the buffy coat and then thrice with isotonic Tris-HCI buffer (0.3M, pH7.4). the haemolysate was prepared by suspending washed red blood cell with hypotonic buffer (Tris-HCI buffer, 0.01M, pH7.2).

Histopathological Examination

Histopathological studies were done in hind limb joints of the animals. The tissues were fixed in formalin, decalcified and embedded in paraffin blocks. Sections prepared with the microtome were stained with hematoxylin and eosin and examined under microscope and photomicrographs taken (Gordon and Bradbury, 1990).

Biochemical Estimations

The levels of lipid peroxide (Okhawa et al., 1979), total reduced glutathione ascorbic acid and enzymes such as superoxide dismutase (Marklund and Marklund, 1974), glutathione peroxidase (Rotruck et al., 1973) were estimated. Protein was estimated by the method of Lowry et al., 1951.

Statistics

The results of the physical parameters such as body weight and paw volume were analysed using student t test. The results of the biochemical parameters were analysed by the application of one-way analysis of variance (ANOVA). Values of $P < 0.05$ were considered statictically significant.

RESULTS AND DISCUSSION

Toxicity

SRE did not produce any perceptible changes in the autonomic and behavioural responses in rats other than insensitiveness to pain stimulus. There was no mortality upto the dose of 3.2 g/kg body weight even after 7 days.

Body Weight

The disease induced animals in group-II showed only a slight increase in the body weight compared to control and other groups (Fig.1).

Paw Volume

The paw volume was measured using plethysmograph. There was an appreciable increase in paw volume in group-II. A significant reduction in paw volume was observed in both SRE and standard drug treated rats compared to the disease induced group. There was a sharp reduction in the paw volume observed in the third week of drug treatment in SRE and diclofenac treated groups. The results are shown in (Fig. 2).

Histopathology

Histopathological studies revealed marked infiltration of leukocytes and eosinophilic inflammatory exudates in the synovial membrane on 15^{th} day (Fig. 3.1) and mild focal infiltration of cells in the synovial region and transformation of flattened epithelium to cuboidal cells on 42^{nd} day (Fig. 3.2) of arthritic induced group. Congestion of vessels, complete regeneration of synocytes and disappearance of inflammatory exudates, few cuboidal cells lining the synovial membrane

were observed in standard drug treated group on 42nd day (Fig. 3.3). In SRE treated rats, the joints showed mild focal infiltration of cells in synovial region transformation of flattened epithelium to cuboidal cells at focal area and complete bridging of synovial cells and disappearance of inflammatory exudates with mild fibroplasias (Fig. 3.4). Complete repair of synovial membrane further supports anti-arthritic potential (Fig. 3.5).

Lipid Peroxidation (LPO)
The lipid peroxide activity significantly increased in the tissues and plasma of the arthritis-induced group animals when compared to control. SRE-treated animals showed remarkably reduced lipid peroxide activity, which was comparable to that of standard drug treated group.

Superoxide Dismutase Activity (SOD)
A reduced activity of SOD was observed in the disease induced group, whereas a substantial increase in the same was found in SRE and diclofenac treated groups.

Glutathione Peroxidase Activity (GPX)
A significant increase in GPX was observed in both SRE and diclofenac treated groups compared to the disease induced group.

Total Reduced Glutathione (TRG)
A significant increase in SRE content was observed in the tissues of SRE treated group when compared to disease induced control animals. The increase in TRG in liver of SRE treated group was remarakable when compared to control and diclofenac treated groups.

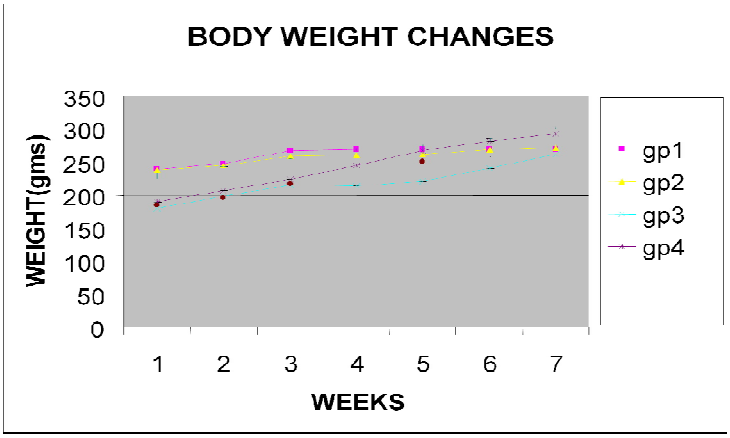
Ascorbic Acid Content (ASA)
A reduced level of ascorbic acid was found mainly in the spleen and plasma of the disease induced animals when compared to normal control group. However, in SRE and standard drug treated animals it showed an appreciable increase. The above results are shown in Table 1.

Table 1: Effect of *Sida rhombifolia* root extract on enzyme and non-enzyme levels of blood and tissues of organs

Treatment	Normal control	Disease control	Standard drug treated (Diclofenac 0.3 mg/kg)	SRE treated 100 mg/kg
LPO (µg / mg protein or / ml haemolysate) Liver Plasma	 0.12±0.03 0.15±0.02	 0.17±0.02 0.34±0.03	 0.09±0.03 0.20±0.02	 0.11±0.03 0.24±0.02
SOD (u / mg protein or / ml haemolysate) Liver Plasma	 0.0443±0.00227** 24.27±2.868**	 0.019±0.0023* 4.967±1.289	 0.04017±0.0043* 29.11±8.833*	 0.0390±0.0036* 19.58±3.190*
GPX(nm of GSH oxidized / min / mg protein or / ml Plasma) Liver Plasma	 7.512±1.183* 17.22±2.014**	 1.280±0.4375 5.630±1.751	 1.680±1.397** 15.41±1.767*	 6.0448±1.095* 7.153±2.287
TRG (µg of GSH / mg protein) Liver	 144.8±21.96*	 11.87±2.302	 202.0±25.67*	 275.2±92.24**
ASA(µg / mg tissue protien Or µg / mg plasma) Liver Plasma	 4.913±0.5692** 1032±1.661**	 1.417±0.2683 1.735±0.2915	 3.535±0.2466** 7.708±1.326*	 3.922±0.4060** 7.708±1.326*

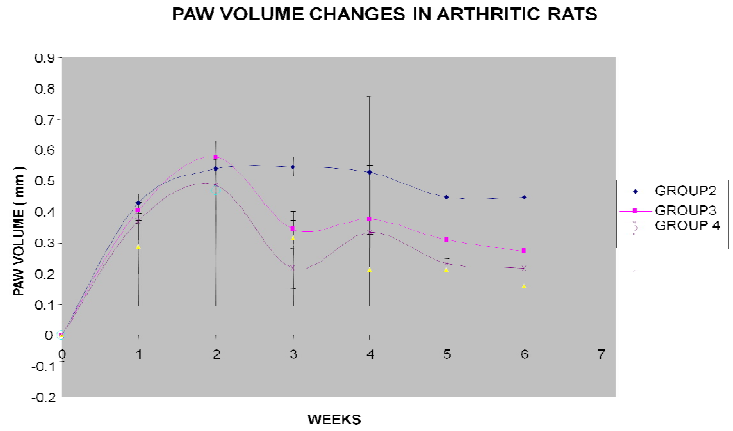
The values represents mean±S.D. for six rats. LPO: lipid peroxides; SOD: superoxide dismutase; GPX: glutathione peroxidase; TRG: total reduced glutathione; ASA: ascorbic acid.

Fig. 1: Body Weight Changes



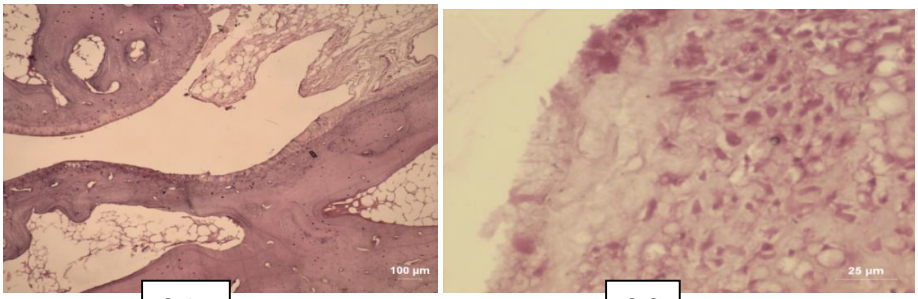
Each point represents the mean ± SEM of 6 observations

Fig. 2: Paw Volume Changes



Each point represents the mean ± SEM of 6 observations

Fig. 3: Histopathological Changes



3.1 3.2

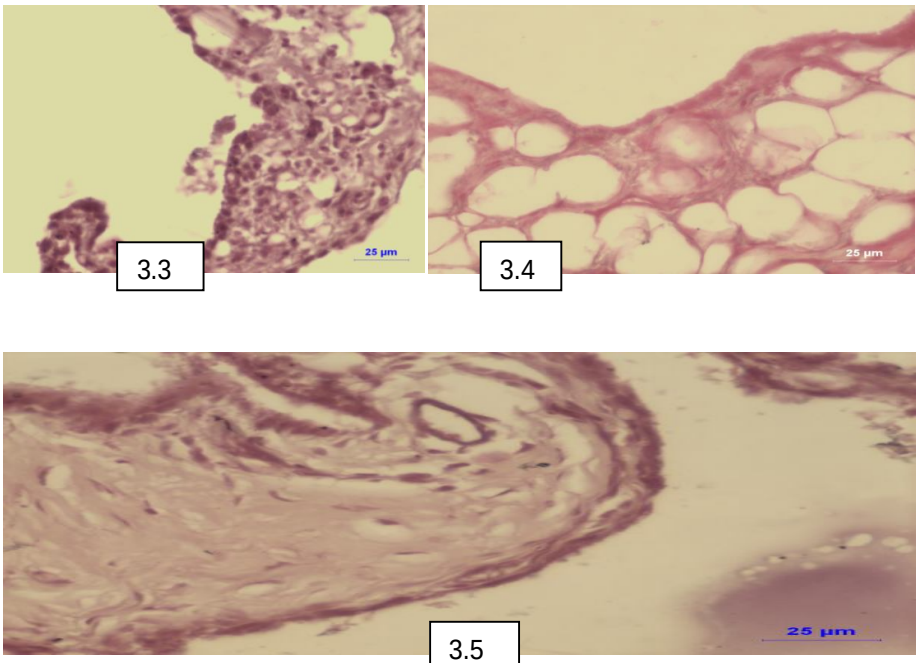


Fig. 3.1: Arthritic rat at 15th day (group II)
Fig. 3.2: Arthritic rat at 15th day (group II)
Fig. 3.3: Arthritic rat treated with standard drug (Diclofenac sodium, 0.5 mg/kg) at 42nd day (group III)
Fig. 3.4: Arthritic rat treated with SRE (100mg/kg) at 42nd day (group IV)
Fig. 3.5: Arthritic rat treated with SRE (100mg/kg) at 42nd day (group IV)

CONCLUSIONS

In arthritic condition, the granulocytes and macrophages accumulate in the affected area and produce large amounts of superoxide and hydrogen peroxide radicals and the estimation of these active species in the disease induced and the drug treated animals help in assessing the free radicals scavenging property and indirectly the anti-arthritic potential of the plant drug.

In the present study, the alcoholic extract of the roots of the plant *Sida rhombifola* (SRE) was considered for the study based on the plants anti-arthritic potential. Administration of various doses of SRE to the animals did not show any toxicity thereby confirming the safety of the extract. The body weight increase thouh less, observed in arthritic-induced animals may be due to the systemic or local action of cytokines resulting from chronic inflammation. However, the difference in body weight changes were not that significant (Fig. 1).

Paw swelling is another major factor in evalauating the degree of inflammation and therapeutic efficacy of the drugs. The arthritic rats showed soft tissue swelling around the ankle joints during the acute phase of arthritis and it was considered to be edema of the particular tissues. The swelling has been found to be increasing in the initial phase of inflammation and then becomes constant in two weeks. In SRE and diclofenac-treated groups, reduction in paw swelling from third week onwards may be due to immunological protection rendered by them.

The extent of lipid peroxidation is measured through malondialdehyde activity (MDA), a pro-oxidant factor, which determines the oxidative damage. In the present study, MDA content of spleen, liver and the plasma of the arthritis-induced animals (group-II) were found to be significantly increased compared with the normal group. This indicates that the tissues are subjected to increased oxidative stress while a statistically significant ($P<0.05$) reduction of lipid peroxide activity was observed in standard drug SRE treated groups.

Glutathione is intra-cellular thiol rich tripeptide which plays a major role in the protection of cells and tissue structures. Low concentration of glutathione has been implicated in rheumatoid arthritis. In the present study, glutathione content was found to be lower in the disease induced group while the same has been elevated in SRE and standard groups. However, in the liver of SRE-treated group, an elevated level of glutathione was observed thereby showing the protective role of SRE in rheumatoid arthritis. The total reduced glutathione content was found to be statistically significant ($P<0.05$) in SRE-treated group.

Glutathione peroxide catalyses the reduction of hydrogen peroxide in the presence of glutathione to form water and oxidized glutathione. A reduced glutathione peroxidase activity was observed in disease induced group compared to normal, SRE and standard drug treated group.

Superoxide dismutase, an enzymic antioxidant, catalytically scavenges the superoxide radical damage. A reduced activity of SOD was observed in the disease induced group compared to SRE and standard drug treated groups. In liver and spleen, the enzyme activity was found to be more than standard drug-traeted group.

Ascorbic acid, a cellular aqueous phase antioxidant, has been shown to exert protective against oxidative stress. The increase in ascorbic acid content in plasma and tissues of SRE (group IV) and standard drug treated groups might be due to the immunological role of the secondary metabolites present in the roots of *Sida rhombifolia* in arthritic condition. The compete repair of synovial membrane by SRE in Histopathological parameter further proved the anti-arthritic potential of SRE.

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