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Hemato-Biochemical Changes Due to Ethanolic Extract of *Cardiospermum halicacabum* in Arthritic Rats

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Abstract The study was envisaged to explore the possible antiarthritic effects of *Cardiospermum halicacabum* against a proprietary topical antiarthritic preparation in adjuvant induced arthritis in rats. *Cardiospermum halicacabum* and diclofenac ointments were applied from 1st to 21st day to the respective groups twice daily. On 21st day animals were sacrified and blood was collected for the evaluation of hematological parameters and antioxidant assay. The arthritic rats showed hematological perturbations suh as an increase in the percentage of neutrophils and WBC, decrease in the percentage of lymphocytes and Hb level. These changes were significantly reversed by application of 10 and 20% ointment of *Cardiospermum halicacabum* of which higher dose was identical to diclofenac. Antioxidant assay showed significant decrease in the level of superoxide dismutase, catalase, reduced glutathione and increase in malonaldehyde in the arthritic control group which was reversed by treatment groups. It is concluded that the treatment with of *Cardiospermum halicacabum* ointment produced significant anti-arthritic activity in dose dependent manner at par with standard drug diclofenac.

Keywords *Cardiospermum halicacabum,* Diclofenac, Ointment, Antioxidant assay, Anti-arthritic.

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Introduction

Artritis can be serious threat to the well being of an animal. It is painful and can lead to permanent deformity and crippling. Morphological, biochemical and molecular studies undertaken in recent years revealed that oxidative stress played a primary role in the development of many degenerative changes in the cells and tissues. Since all the cell and tissues are having antioxidant enzymes like superoxide dismutase, catalase, serum malondialdehyde, reduced

glutathione and glutathione peroxidise, they dispose free radicals and protect the cells and tissues from oxidative attack. Normally balance is maintained between the oxidative attack of free radicals and antioxidant defense system prevailing in the cells and tissues. However, if the balance is tilted more towards the generation of free radicals, then there is a damage to cell structures, DNA, lipids and proteins. The hormonal and metabolic side effects of the steroidal drugs led to the development of nonsteroidal anti-inflammatory agents. Use of NSAIDs also leads to the development of severe gastrointestinal and renal problems. Despite considerable progress in the treatment of arthritis by NSAIDs and other drugs, search for newer drugs continues because, the existing synthetic drugs have several limitations. The modern medicine has also started admitting that ayurveda and herbal medicine, has a lot of positive influence on the treatment of arthritis. Wide range of phytoconstituents responsible for anti-arthritic activity includes alkaloids, glycosides, tannins, phenols, anthocyanins, sterols and triterpenoids. *Cardiospermum halicacabum* has been investigated in animal models for anti-inflammatory, analgesic, antipyretic and anti-ulcer activities [1]. Topical application of *Cardiospermum halicacabum* at different concentrations to the rats affected by arthritis is likely to reverse the major pathological effects of arthritis.

Materials and Methods

In the present study, thirty six Wistar rats of either sex, weighting approximately 140-160 g were divided into six groups (Group I—VI). Group I,II and III served as control, arthritic control and herb control respectively. While group IV and V were treated with 10and 20% herbal extract ointment and group VI was treated with diclofenac ointment. Animals were kept in the laboratory animals house of the Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal and maintained under normal condition with free access to feed and water.The experiment was approved by institutional animal ethical committee (IAEC).

All the animals were fasted over night before sacrifice. On 21st day, they were sacrificed under deep ether anaesthesia. Blood was collected in anticoagulant coated vials for estimation of hematological parameters and blood smear were made for assessing RBC and WBC status. Blood was also collected in test tubes, centrifuged and serum was separated for antioxidant assay. Following hematological parameters were done as per the method described by Benjamin [2]. Hemoglobin concentration (Hb,g/dl), total erythrocyte count (TEC, millions/µl), total leukocyte count (TLc, thousands/µl), erythrocyte sedimentation rate (ESR, mm) and differential leukocyte count (DLC %). For differential leukocyte count, the smears from fresh whole blood were prepared and stained by modified copper peroxidase method and examined under oil immersion. Antioxidant assay were done manually for superoxide dismutase (SOD), catalase (CAT) , reduced glutathione (GSH) and serum malondialdehyde (MDA), by using spectrophotometer. The data were analysed by one way ANOVA for significance as per the method of Snedecor and Cochran [3].

Results and Discussion

Treatment with *C. halicacabum* and diclofenac significantly improved the level of RBC and Hb when compared to arthritic control group. The results were in accordance with Ekambaram et al. [4] and Yerragunta et al. [5] wherein they reported similar effect for *S. potatorum* Linn. and *Butea monosperma* respectively. ESR and WBC counts which drastically increased in artritic control group have been remarkably counteracted by the standard drug diclofenac, 10 and 20% ointment of *C. halicacabum*. This concurs with the results of Elumalai and Yoganandham [6]. In arthritic condition there is mild to marked rise in WBC count due the release of IL-1B which increases the production of both granulocytes and macrophage colony stimulating factors [7]. FCA induced arthritic group showed hematological perturbations, such as increase in the percentage of neutrophils and decrease in the percentage of lymphocytes which got significantly altered by 10 and 20% *C.halicacabum* and diclofenac ointment which are in agreement with the result of Ravichandran and Panneerselvam [8].

Arthritic control showed significant decrease in

Table 1. Effect of *Cardiospermum halicacabum* ointment on hematological parameters (Mean ± SE) in AIA rats. n=6, Menas bearing different superscripts within row differ significantly ($p<0.05$).

Parameters	Groups I	II	III	IV	V	VI
Hb (g/m/dl)	13.998^{d}±0.177	9.668^{a} ± 0.112	13.901^{d} ± 0.191	12.516^{b} ± 0.149	13.446^{c} ± 0.171	14.053^{d} ± 0.028
TLC (cells/cm)	7627.50^{ab} ± 156.60	11125.30^{c} ± 216.04	7643.00^{ab} ± 110.36	7954.80^{b} ± 226.29	7392.00^{a} ± 207.31	7249.33^{a} ±100.25
TEC (million/cm)	5.668^{c} ± 0.112	4.575^{a} ± 0.142	5.645^{c} ± 0.099	5.080^{b} ± 0.027	5.628^{c} ± 0.040	5.801^{c} ± 0.013
Lymphocyte (%)	68.000^{c} ±0.069	43.730^{a} ± 0.199	67.910^{c} ±0.115	67.020^{b} ± 0.090	68.050^{c} ± 0.051	68.060^{c} ± 0.058
Neutrophils (%)	25.050^{a} ± 0.162	49.350^{d} ± 0.219	25.040^{a} ± 0.259	27.960^{c} ± 0.307	26.050^{b} ± 0.311	25.220^{a} ± 0.049
Eosinophils (%)	1.230^{a} ± 0.395	2.210^{b} ± 0.140	2.216^{b} ± 0.105	2.113^{b} ± 0.081	2.070^{b} ± 0.107	1.661^{ab} ± 0.340
Basophils (%)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Monocytes (%)	0.311^{ab} ± 0.131	0.093^{a} ± 0.045	0.123^{ab} ± 0.060	0.333^{b} ± 0.039	0.083^{a} ± 0.037	0.200^{ab} ± 0.070
ESR 30 min (mm)	7.801^{a} ± 0.224	22.063^{d} ± 0.548	8.030^{a} ± 0.256	13.150^{c} ± 0.180	9.180^{b} ± 0.139	8.590^{ab} ± 0.234
SOD (U/mg protein)	0.506^{c} ± 0.005	0.330^{a} ± 0.028	0.490^{c} ± 0.009	0.390^{b} ± 0.029	0.500^{c} ± 0.004	0.513^{c} ± 0.011
CAT (µ moles/ min/mg protein)	0.251^{d} ± 0.017	0.043^{a} ± 0.001	0.245^{cd} ± 0.031	0.107^{b} ± 0.008	0.196^{c} ± 0.009	0.226^{cd} ± 0.010
GSH (nmol/ml)	4.676^{d}± 0.127	3.160^{a} ± 0.077	4.480cd ± 0.029	3.563^{b} ± 0.101	4.270^{c} ± 0.020	4.773^{d} ± 0.156
MDA (nmol/ml)	3.520^{a} ± 0.090	12.530^{c} ± 0.192	3.420^{a} ± 0.061	4.910^{b} ± 0.131	3.610^{a} ± 0.094	3.460^{a} ± 0.034

antioxidant enzymes such as GSH, SOD, CAT and significant increase in MDA level. Similar results were reported by venukumar and Latha [9] in brain and kidney of AIA rats. The failure of antioxidant defence mechanism to keep pace with oxidant generation may either be due to decreased antioxidant defence or increased generation of oxidants in arthritis.

The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress. The cellular antioxidant action is reinforced by the presence of dietary antioxidants [10]. Topical application of 10 and 20% ointment of *Cardiospermum halicacabum* caused significant reduction in serum MDA level (Table 1) and similar results wre reported by Mawsouf [11]. Significant increase in the mean values of serum GSH was noticed in all the treatment groups as compared to arthritic control group. This results concurs with that of El-Dakhakhny et al. [12]. All the treatment groups (10,20 and diclofenac ointment) showed marked increased in serm CAT and SOD level. This result concurs with the result of Pathak et al. [13].

The antioxidant effect of *C. halicacabum* is a balance of two activities in free radical scavenging effect and reducing power of ferrous ion [14]. *C. halicacabum* was found to contain rutin, a flavanol glycoside comprising of flavonol quercetin and disaccharides rutinose which increase glutathione level and thereby reduce oxidative damage in inflammatory condition [15].

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