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Original Article

A COMPARATIVE STUDY OF ANTIARTHRTIC EFFICAY OF Cardiospermum halicacabum (L) AND Cissus vitiginea (L) FREUND’S COMPLETE ADJUVANT INDUCED ARTHRITIS

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Abstract

The anti-arthritic potential of Cardiospermum halicacabum, Cissus vitiginea and combined extracts of Cardiospermum halicacabum and Cissus vitiginea were evaluated by taking complete Freund’s adjuvant (CFA) induced model. Arthritis was induced by injecting 0.1ml of complete Freund’s adjuvant below the plantar aponeurosis of the right hind paw. Treatment with the plant extracts started on the day of induction of CFA and continue up to 21 days. In this study the combined extracts significantly (p<0.01) decrease the paw edema on 21st day. They also significantly rectified the deranged hematological parameters which is observed from the studies. The combined extracts found to show more effect than Cardiospermum halicacabum and Cissus vitiginea extracts in the terms of % of inhibition. The study gives a scientific rational to the use of this plant in arthritis and related conditions.

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Keywords:- Cardiospermum halicacabum, Cissus vitiginea, Complete Freund’s adjuvant, Hematological parameter, Biochemical parameters.

1. INTRODUCTION

Arthritis, the joint inflammation, refers to a group of diseases that cause pain, swelling, stiffness and loss of motion in the joints. Rheumatoid Arthritis (RA) is one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact (Buch and Emery, 2002). Rheumatoid Arthritis (RA) is considered the most common chronic inflammatory autoimmune disease, occurring in 1 to 2% of the worldwide population (Firestein et al., 2005). The epidemiological ratio of arthritis in female and male is 3:1 and the prevalence is 1% of the world population. The peak age of onset is between 30 and 55. Annually, the incidence of RA is 30 per 100,000 people are reported every year in both developed and developing countries. Due to the fact that women are affected more than men, the prevalence of RA in women over the age of 65 is around 5% (Spector, 2010). Lower incidences of rheumatoid arthritis are reported every year in East Asia. The prevalence of arthritis is approximately in the West (Lipsky, 2005). The prevalence of RA in India subcontinent is 1.5–2 percent of population. The true picture of annual incidence rate of rheumatoid arthritis in India has not been well documented. In many joint diseases, pro-inflammatory factors such as cytokines and prostaglandins, together with Reactive Oxygen Species (ROS) and Nitrogen Species (NS) are released at sites of inflammation (Abbas and Mohsenzadegan, 2008).

Currently available Anti-inflammator drugs have been associated with a number of side effects. Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants have been associated with a number of side effects. Use of plants for treating various ailments of both man and animal is as old practice as man himself. India is richly endowed with a wide variety of plants having medicinal value. These plants have been used as folk remedies or indirectly as pharmaceutical preparation of modern medicine. Owing to the global trend towards improved ‘quality of life’, there is considerable evidence of an increase in demand for medicinal plant. Several plant products are known to exhibit creditable medicinal properties for the treatment of arthritis instead of allopathic medicine. In order to evaluate the anti-arthritic activity of Cardiospermum halicacabum, Cissus vitiginea and combined extracts of Cardiospermum halicacabum and
Cissus vitiginea on CFA induced arthritis rats.

2. MATERIALS AND METHODS

2.1 Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2°C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided ad libitum. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

2.2 Chemicals

Complete Freund’s adjuvant was obtained from Sigma Aldrich (Saint Louis, Missouri, USA) and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

2.3 Plant material

The fully mature Cardiospermum halicacabum, (Plate-1), Cissus vitiginea (Plate-2) leaves were collected in April 2014 from akkaravattam and near new bus stand Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified with the help of the Flora of Tamilnadu Carnatic by Matthew (1983). Specimen was further origin with reference to Herbarium sheets (Plate-3) available in the Rapinat Herbarium, St. Joseph’s College, Tiruchirappalli, Tamilnadu, India.

2.4 Preparation of powder

The Cardiospermum halicacabum and Cissus vitiginea leaves were collected and dried under shade. Separately these dried leaves were mechanically powdered and stored in an airtight container. These powdered materials were used for further analysis.

2.5 Preparation of extracts

The powder material of Cardiospermum halicacabum (10g) and Cissus vitiginea (10g) leaves were macerated with 70% ethanol and 30% water at room temperature for 3 days. The combined extract as equal quantity of Cardiospermum halicacabum (5g) and Cissus vitiginea (5g) leaves were macerated with 70% ethanol and 30% water at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

2.6 Freund’s Complete Adjuvant induced Arthritic Model

2.6.1 Experimental design

Adult Wistar male rat with an initial body weight of 180 to 220g were taken, and divided into five groups each containing six animals (plate-4). On day zero, all rats were injected into the sub plantar region of the left hind paw with 0.1ml of Freund’s complete adjuvant. This consists of Mycobacterium butyricum suspended in heavy paraffin oil by thorough grinding with motor and pestle to give a concentration of 6mg/ml (Shivanand Pandey et al., 2010). Dosing with the Cardiospermum halicacabum, Cissus vitiginea and combined extracts were started on the first day and continued for 21 days according to the following schedule: Group I : Normal rats. Group II: Adjuvant induced arthritic rats. Group III : Arthritis induced rats administered with extract of Cardiospermum halicacabum leaves (500mg/kg body weight/rat/day for 21 days p.o.). Group IV : Arthritis induced rats administered with extract of Cissus vitiginea leaves (500mg/kg body weight/rat/day for 21 days p.o.). Group V : Arthritis induced rats administered with combination extract of Cissus vitiginea and Cardiospermum halicacabum (500mg/kg body weight/rat/day for 21days p. o.). The degree of inflammation was measured by a mercury displacement method. The edema formation and the percentage of inhibition were calculated as follows.

\[
\text{Vc} - \text{Vt} \quad \text{-------} \times 100 \\
\text{Vc}
\]

Where Vc is the edema volume of the control group and Vt is the edema volume of the treated group.

2.6.2 Collection of blood and preparation of serum sample

At the end of the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 37°C and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for biochemical analysis.

2.7 BIOCHEMICAL ESTIMATIONS

Maldondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1979). Reduced glutathione was estimated by method of Moron et al (1979). The serum SGOT and SGPT were estimated by the method of Reitman and Frankel (1957). Haemoglobin was estimated by Cyanmethaemoglobin method (Dacie and Lewis, 1968) (Beacon Diagnostic Kit). ESR sedimentation rate WBC, RBC counted by the method of Ochei and Kolhatkar, (2000). The qualitative C - reactive protein and Rheumatoid Factors were followed by the method of Singer et al., (1957) and Scherffarth et al., (1970).

2.8 Statistical Analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and p< 0.05 were considered to be significant.

3. RESULTS

The present study was carried out to evaluate the
Table I: Effect of Cardiospermum halicacabum, Cissus vitiginea and combined extracts on % of inhibition of paw volume in Freund’s adjuvant induced arthritis in experimental rats

<table>
<thead>
<tr>
<th>Days</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 4</td>
<td>-</td>
<td>-</td>
<td>48.23±4.97</td>
<td>44.44±3.08</td>
<td>51.85±3.57</td>
</tr>
<tr>
<td>Day 8</td>
<td>-</td>
<td>-</td>
<td>57.21±3.99</td>
<td>52.45±3.64</td>
<td>69.25±4.83</td>
</tr>
<tr>
<td>Day 15</td>
<td>-</td>
<td>-</td>
<td>66.10±4.62</td>
<td>63.78±4.41</td>
<td>80.56±5.60</td>
</tr>
<tr>
<td>Day 21</td>
<td>-</td>
<td>-</td>
<td>88.54±6.16</td>
<td>86.32±6.04</td>
<td>95.26±6.66</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.

Table II: Effect of Cardiospermum halicacabum, Cissus vitiginea and combined extracts on MDA and GSH in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/L)</td>
<td>9.84±0.39</td>
<td>10.31±0.75*</td>
<td>8.89±0.27*</td>
<td>9.31±0.54*</td>
<td>8.68±0.48*</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>8.13±0.61</td>
<td>5.11±0.94#</td>
<td>8.33±0.50*</td>
<td>7.81±0.37*</td>
<td>9.80±1.21*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I; # Significantly different from Group II

Table III: Effect of Cardiospermum halicacabum, Cissus vitiginea and combined extracts on SGOT, SGPT activities and creatinine in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (IU/dl)</td>
<td>38.02±6.49</td>
<td>48.69±3.02*</td>
<td>38.36±2.99</td>
<td>42.82±5.27</td>
<td>37.57±3.02</td>
</tr>
<tr>
<td>SGPT (IU/dl)</td>
<td>35.29±4.05</td>
<td>53.54±7.78*</td>
<td>29.45±7.09</td>
<td>45.20±6.38</td>
<td>25.95±6.94</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.87±0.06</td>
<td>1.39±0.14*</td>
<td>1.03±0.09</td>
<td>1.09±0.48</td>
<td>0.88±0.51</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I; # Significantly different from Group II

Table IV: Effect of Cardiospermum halicacabum, Cissus vitiginea and combined extracts on CRP and RA factor activities in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>C- Reactive Protein (CRP)</td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Rheumatoid Arthritis Factor (RA)</td>
<td>--</td>
<td>++</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

(-) Negative  (+++) Positive

Table V: Effect of Cardiospermum halicacabum, Cissus vitiginea and combined extracts on Hb, RBC, WBC and ESR content in experimental rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>17.16±1.53</td>
<td>10.94±1.12*</td>
<td>18.45±1.53*</td>
<td>16.89±2.25*</td>
<td>15.49±1.75*</td>
</tr>
<tr>
<td>RBC (Million/cu.mm)</td>
<td>4.63±0.50</td>
<td>3.38±0.528*</td>
<td>4.68±0.57*</td>
<td>4.20±0.58*</td>
<td>4.50±0.66*</td>
</tr>
<tr>
<td>WBC (Cells/cu.mm)</td>
<td>8491.66±372.89</td>
<td>13483.33±655.90*</td>
<td>8483.33±364.16*</td>
<td>7413.33±187.7*</td>
<td>8108.33±212.059*</td>
</tr>
<tr>
<td>ESR (mm)</td>
<td>4.21±0.23</td>
<td>6.32±0.45*</td>
<td>4.25±0.22*</td>
<td>4.89±0.32*</td>
<td>4.59±0.25*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD for six rats in each group.
* Significantly different from Group I; # Significantly different from Group II
Rheumatoid Arthritis. The observations made on different groups of experimental and control animals were compared as follows. Table-I represent the % of inhibition of paw volume in Freund’s adjuvant induced arthritis in experimental rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly inhibited the paw volume day by day. The combination extracts (GroupV) of Cardiospermum halicacabum and Cissus vitiginea leaves significantly inhibited the paw volume day by day. The highest inhibition was observed in Combined extract as 95.26% followed by Cardiospermum halicacabum (88.54%) and Cissus vitiginea (86.32%) leaves extract.

Table-II represents the levels of MDA and GSH in serum of normal and experimental rats. Group II Arthritis rats showed a significant increased in the level of MDA when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly decreased in the level of MDA when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased the MDA content. The decreasing order is Combined extract < Cardiospermum halicacabum < Cissus vitiginea were observed.

Group II Arthritis rats showed a significant decreased in the level of GSH when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly increased in the level of GSH when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly increased the GSH content. The increasing order is Combined extract < Cardiospermum halicacabum < Cissus vitiginea were observed.

Table-III represents the activity of SGOT, SGPT and creatinine in serum of normal and experimental rats. Group II Arthritis rats showed a significant increased in the activity of SGOT when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly decreased in the activity of SGOT when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased the SGOT activity. The decreasing order is Combined extract < Cardiospermum halicacabum < Cissus vitiginea were observed.

Group II Arthritis rats showed a significant increased in the activity of SGPT when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly decreased in the activity of SGPT when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased the SGPT activity. The decreasing order is Combined extract < Cardiospermum halicacabum < Cissus vitiginea were observed.

Group II Arthritis rats showed a significant increased in the level of Creatinine when compared to Group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased the Creatinine content. The decreasing order is Combined extract < Cardiospermum halicacabum < Cissus vitiginea were observed.

Table IV represents the effect of Cardiospermum halicacabum and Cissus vitiginea on CRP and RA factor activities in experimental rats. Group II Arthritis rats showed a positive results were observed when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea shows the negative results when compared to group II. The combined extracts of Cardiospermum halicacabum and Cissus vitiginea shows the negative results.

Table-V represents the levels of Hb, RBC and WBC Experimental rats. Group II Arthritis rats showed a significant decreased in the level of Hb when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly increased in the level of Hb when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly increased the Hb content. The increasing order is Cardiospermum halicacabum < Combined extract < Cissus vitiginea were observed.

Group II Arthritis rats showed a significant decreased in the count of RBC when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea significantly increased in the count of RBC when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly increased the RBC count. The increasing order is Cardiospermum halicacabum < Combined extract < Cissus vitiginea were observed.

Group II Arthritis rats showed a significant increased in the count of WBC when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased the WBC count. The increasing order is Cardiospermum halicacabum < Combined extract < Cissus vitiginea were observed.

Group II Arthritis rats showed a significant increased in the ESR when compared to Group I rats. Group III and IV Arthritis rats treated with Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased in the ESR when compared to group II. The combination extract of Cardiospermum halicacabum and Cissus vitiginea leaves significantly decreased the ESR. The increasing order is Cardiospermum halicacabum < Combined extract < Cissus vitiginea were observed.

4. Discussion

Rheumatoid arthritis is one of the most common inflammatory diseases worldwide. Rheumatoid Factors (RFs) and C - reactive protein (CRP) are group of antibodies directed to determinants in the Fc portion of
immunoglobulin G molecule. Although, RFs are found in a number of rheumatoid disorders. It is central role in clinic lies in its utility as an aid in the diagnosis of RA (Frederick wolf et al., 1991). In the present study, we also observed the positive results of RF and CRP in arthritis rats. Supplementation of Cardiospermum halicacabum, Cissus vitiginea and combined extracts to arthritis rats negative results RFs and CRP.

Free radicals are enormously produced at the site of inflammation and tissue injuries (Gotia et al., 2001). Malondialdehyde (MDA) is the major aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid. MDA, a secondary product of lipid peroxidation is used as an indicator of tissue damage by series of chain reactions. Lipid peroxides that are generated at the site of inflammation of tissue injury diffuses into blood and can be estimated in serum or plasma, which inturn reflect the severity of the tissue damage (Gutteridge, 1995). In the present study, elevated levels of MDA and decline multidirectional antioxidant status were noticed in arthritis as compared to control rats. Inflammation and tissue injury related oxidative stress has been implicated in the pathogenesis of rheumatoid arthritis (Ostrakhovich and Afanas’ev, 2001). Susceptibility of erythrocytes to peroxide stress is increased in several diseased conditions (Wagner et al., 1998). Thus, the elevated serum lipid peroxidation observed in the present study in arthritis induced rats can be related to excessive lipid peroxidation observed with consequent leakage into serum or as a result of excessive generation and diffusion of lipid peroxides from the inflamed or injured joints of arthritis. Supplementation of Cardiospermum halicacabum, Cissus vitiginea and combined extracts to arthritis rats decreased MDA content in serum. This indicate that the Cardiospermum halicacabum, Cissus vitiginea and combined extracts decrease the oxidative damage in arthritis rats.

The present study was carried out to evaluate the efficiency of Cardiospermum halicacabum, Cissus vitiginea and combined extracts against a inflammatory disease, that is, arthritis. In the present study, rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to accumulation of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid diseases. The determination of paw swelling is apparently simple, sensitive and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs (Singh et al., 1996).

The effect of Cardiospermum halicacabum, Cissus vitiginea and combined extracts on adjuvant arthritis is shown in Table 1. In the control group, maximum swelling was seen on the 8th day and thereafter there was gradual decline in the swelling up to 15th day. The swelling was found to increase again and become maximum on the 21st day. In addition to this, a swelling was also noticed in the uninfected paw as well as in the fore paws on 8th day. The nature of the secondary lesions was also modified from severe degree to moderate by Cardiospermum halicacabum, Cissus vitiginea and combined extracts. The sudden alteration of physical parameters observed in our results at 15th day, namely the sharp increase in paw circumference and volume, Lack of mobility suggest that day 15 may be the beginning of a second stage where the secondary reactions began. The decrease of paw volume and circumference, after day 15 until day 21. suggests that the second stage is established within that period of time. The test drugs significantly decrease the paw volume in both the phases. Cardiospermum halicacabum, Cissus vitiginea and combined extracts significantly decrease the paw edema significantly from the 1st day. At a dose of 500mg/kg it suppresses both the phases of inflammation. At 500mg/kg combined extract is found to significantly reduce the paw volume than Cardiospermum halicacabum and Cissus vitiginea the inflammation from the first day of study.

Assessment of the levels of SGOT, SGPT and creatinine provides an excellent and simple tool to measure the antiarthritic activity of the target drug (Kataoka et al., 2002). The activities of aminotransferases and creatinine were significantly increased in arthritic rats, since these are good indices of liver and kidney impairment, which are also considered the features of adjuvant arthritis (Rainsford et al., 1982). Serum SGOT and SGPT have been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process (Myles Glenn et al., 1965). Also, Niino-Nanke et al (1998) confirmed a positive correlation between the increased activity of serum SGOT and SGPT and the disease activity in RA. Elevated levels of serum SGOT, SGPT and ALP in arthritis induced rats can be due to increase in the liver fraction or due to an increase of both isoenzymes. This in turn implicates a localized liver intoxication and bone loss in the form of bone erosion and periarticular osteopenia, as the enzyme is released into circulation in the course of bone formation and resorption (Rehman and Lane, 2001). The decreased enzyme levels on treatment of Cardiospermum halicacabum, Cissus vitiginea and combined extracts emphasizes the decreased bone loss and organ protective role of it against adjuvant induced arthritic rats, since treatment of NSAIDs which are hepatotoxic result in elevated levels of SGOT and SGPT in RA (Fries et al., 1990). From this it may be suggested that the anti-inflammatory effect is maybe due the rectification of biochemical parameters. The significant protection of these parameter is might be due to the combined effect of the phytochemical present in them.

In the present study, the arthritic rats exhibited a reduced RBC count, reduced Hb level. All these indicate the anemic condition, which is a common diagnostic feature in patients with chronic arthritis (Allar et al., 1977). Our Cardiospermum halicacabum, Cissus vitiginea and combined extracts significantly (p<0.05) rectify these condition. The RBC count increased with the treatment of standard dexamethasone 500mg/kg of Cardiospermum halicacabum, Cissus vitiginea and combined extracts respectively. Which indicate the usefulness of these extracts in alleviating the anemic conditions induced by Freund’s adjuvant. Erythrocyte Sedimentation Rate (ESR) is an estimate of the suspension stability of RBC’s in

plasma. It is related to the number and size of the red blood cells and to the relative concentration of plasma proteins, especially fibrinogen, α and β globulins. Increase in the rate is an indication of active but obscure disease processes. The rise in ESR responds to stress or inflammation like injection, injury, surgery and tissue necrosis. The treatment with the Cardiospernum halicacabum, Cissus vitiginea and combined extracts improved the ESR to a near normal level indicating the significant recovery from the arthritis progress thus justifying its significant role in arthritic conditions27. White blood cells (WBC) are a major component of the body’s immune system. Indications for a WBC count include infectious and inflammatory diseases (Mowat and Semin, 1971). In arthritis condition there is a mild to moderate rise in WBC count due to release of IL-IB. IL-IB increases the production of both granulocyte and macrophages colony stimulating factor28. WBC count was increased in arthritic group. The migration of leukocytes is significantly suppressed in extract treated groups as seen from the significant decrease in the WBC count. All the rectification of hematological parameters supports the antiarthritic effect of Cardiospernum halicacabum, Cissus vitiginea and combined extracts.

In the light of the above results it might be concluded that among the different treatments as Cardiospernum halicacabum, Cissus vitiginea and combined extracts to arthritis rats, combined extracts exhibited a potent anti-arthritic effect by the paw volume in the test animals and modifying the deranged hematological and biochemical parameters. The effect of combined extracts of Cardiospernum halicacabum and Cissus vitiginea can be co-related with the presence of phytoconstituents such as flavonoids, tannins, saponin it. This work gives a scientific rational to the use of this plant in arthritis and related conditions.

5. Acknowledgments

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6. References

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