11. IN VIVO ANTI-ARTHRITIC ACTIVITY

11.1 MATERIALS AND METHODS

Institutional Animal Ethics Committee Approval:

The experimental protocol was approved by the Institutional Animal Ethics Committee and the IAEC approval No. IAEC / XXXV / SRU / 308 / 2013.

11.1.1 Acute Oral Toxicity Study

Acute oral toxicity study was performed according to the OECD test guideline 423- Acute toxic class method. Young healthy adult Sprague Dawley female rats, weighing between 140-180 gm b.wt. were divided into two groups with 3 rats in each group. The animals were housed in a well-ventilated polypropylene cage (3 rats/cage) and 12 h light/12 h dark artificial photoperiod was maintained. The rats were maintained at 22°C (± 3°C) temperature and 50–80 % relative humidity. The rats had free access to pelleted feed (M/s. Provimi Animal Nutrition Pvt Ltd, India) and reverse osmosis purified water (Rios, USA) ad libitum.

The Experimental rats were grouped into two, with 3 rats in each group.

Group I : EESC (2000 mg/kg b.wt.)

Group II : EESS (2000 mg/kg b.wt.)

The extracts were administered to the experimental animals once orally via gastric intubation. Lethality and abnormal clinical signs were observed on the day of dosing and thereafter for 14 days. Body weight was recorded before dosing and
thereafter once in a week till completion of the experiment. Gross pathological changes were also observed at the end of the experiment.

11.1.2 Evaluation of In vivo Anti-Arthritic Activity

11.1.2.1 Complete Freund’s Adjuvant induced arthritis (Pearson & Wood, 1959)

Pearson (1956) found that rats immunized with CFA (Complete Freund’s Adjuvant) developed arthritis. This model is called as Adjuvant-Induced Arthritis (AIA), which has been widely used as an arthritic animal model (Pearson 1956).

Adjuvant induced arthritis in rats is a chronic inflammatory disease, characterized by infiltration of synovial membrane with an involvement of joint destruction resembles rheumatoid arthritis in humans. After adjuvant injection, increase in edema of hind paw along with increased activity of lysosomal enzymes were reported (Anderson 1969), which are involved in the degradation of structural macromolecules in connective tissue and cartilage proteoglycans. Also it may contribute to the tissue injury in rheumatic diseases. Acute inflammatory response induced by CFA is associated with leukocyte infiltration, mast cell activation and release of cytokines and free radicals.

Freund’s adjuvant is irreplaceable component of induction protocols of many experimental animal models of autoimmune disease. Evidence collected from various types of studies provides more insight in the specific alterations of the immune response caused by CFA. Early events include rapid uptake of adjuvant components by dendritic cells, enhanced phagocytosis, secretion of cytokines by
mononuclear phagocyte, and transient activation and proliferation of CD4 lymphocytes.

11.1.2.2 Methodology

Female Sprague Dawley rats (180-230 gm) were divided into 6 groups of 6 animals each. On day zero, all the animals were injected with 0.05 ml of Complete Freund’s Adjuvant (Chondrex, USA) in the sub-plantar region of the right hind paw. The Complete Freund’s Adjuvant contains 10 mg/ml of heat-killed Mycobacterium tuberculosis.

Group I : Arthritic control

Group II : Methotrexate treated (0.1 mg/kg b.wt. i.p. alternate days)

Group III : EESC treated (200 mg/kg. b.wt.)

Group IV : EESC treated (400 mg/kg. b.wt.)

Group V : EESS treated (200 mg/kg. b.wt.)

Group VI : EESS treated (400 mg/kg. b.wt.)

The dosing was started on 14th day and continued up to 28th day, body weight and paw volume were measured at the interval of 7 days. The paw volume was measured by using Digital plethysmometer. At the end of the 28th day, the blood samples were collected for the study of biochemical parameters. The animals were sacrificed and the ankle joints with hind paw and spleen were taken for histopathological studies.
11.1.2.3 Histopathological studies

The rats from each group were euthanized using anesthetic ether at the end of the experimental period. The hind paws from rats were amputated proximal to the ankle joints. They were fixed in 10% neutral buffered formalin for 24 h followed by decalcification in 10% formic acid for approximately 4 days. After completion of decalcification, the digits were trimmed off and the ankle joints were transected in a mid-sagittal plane. The ankle joint and paw were processed for paraffin embedment, sectioned and then stained with hematoxylin and eosin (Bancroft & Gamble, 2008) for microscopic evaluation.

Spleen was collected from rats of all groups at the end of the experimental period and fixed in 10% neutral buffered formalin for 48 h. The representative samples of spleen from all groups were trimmed, dehydrated in series of graded alcohol and processed for paraffin embedment. 3-4 µ thickness of tissue section were prepared and stained with haematoxylin and eosin (Bancroft & Gamble 2008) for histopathological evaluation.

11.1.2.4 Immunohistochemical studies

The antibodies of CD4 (#SC7219), IL-2 (#SC7896), TGF-β (#SC-146) and TNF-α (#SC52746) were purchased from Santa Cruz, U.S.A and used for the immunohistochemical studies. The spleen of various groups were trimmed, dehydrated in series of graded alcohol, embedded in paraffin wax and 3-4 µ thickness of tissue section were prepared. The sections were deparaffinized in xylene and hydrated through descending grades of alcohol. Heat-induced antigen
retrieval was carried out by microwaving the sections for 20 min using citrate buffer (pH 6.0) for TNF-α and Tris-EDTA buffer (pH 8.0) for CD4, IL-2 and TGF-β. Each step was preceded by three washings in PBST (Phosphate buffered saline with 0.05 % tween 20).

Endogenous peroxidase quenching was done by incubation with 3 % H$_2$O$_2$ for 30 min followed by blocking with 5 % goat serum in 1 % BSA for 30 min. Primary antibody incubation was done at 4°C overnight and biotinylated secondary antibody incubation was carried out for 30 min. at room temperature. Avidin-Biotin peroxidase incubation was carried out for 30 min at room temperature. The sections were stained with DAB (3,3’-diaminobenzidine) chromogen for 15-20 min and counterstained with Harris hematoxylin for 30 sec. The sections were dehydrated in alcohol, xylene and then mounted in DPX (Distrene –Plasticiser -Xylene).

11.1.2.5 Statistical Analysis

The collected data were analysed with SPSS 16.0 version. Mean and SEM were used to describe about the data descriptive statistics. For the multivariate analysis, Kruskal-Wallis test was used. Mann-Whitney U test was used to find the significant difference between and independent groups. In all the above statistical tools, the probability value P < 0.05 was considered as significant level and P < 0.01 was considered as highly significant.
11.2 RESULTS AND DISCUSSION

11.2.1 Acute Oral Toxicity Study

The ethanolic extracts of *Stereospermum colais* (EESC) and *Stereospermum suaveolens* (EESS) were administered at a dose of 2000 mg/kg b.wt. orally to the fasted female *Sprague Dawley* rats. There were no treatment related deaths, abnormal clinical signs or remarkable body weight changes observed in all the experimental animals. No gross pathological observation was recorded in all the experimental animals. So LD$_{50}$ of the extracts were found to be greater than 2000 mg/kg b.wt. Hence, the extracts “EESC and EESS” falls in the “category-5 or unclassified” in accordance to the Globally Harmonised System of classification of chemicals.

11.2.2 In vivo Anti-Arthritic Activity

The anti-arthritic activity of the ethanolic extracts of the roots of *Stereospermum colais* (EESC) and *Stereospermum suaveolens* (EESS) was evaluated by Complete Freund's Adjuvant induced arthritic model (Pearson & Wood, 1959). This arthritic model is widely used and has a close similarity to human rheumatoid arthritis.

11.2.2.1 Effect on Body weight

Decrease in body weight is also one of the indication of arthritis development (Jalalpure et al. 2011). So, the body weight was recorded on 0$^{th}$, 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day and the mean value of body weight of all the groups are presented in Table 11.1. Mild reduction in body weight was observed in the arthritic
control group and methotrexate treated group, which was restored by EESC and EESS treated groups (Figure 11.1)

11.2.2.2 Effect on Paw volume

CFA induced arthritis is the most extensively used chronic test model in which the clinical and pathological changes are similar to those seen in human RA (Ward & Cloud 1966). Chronic inflammation in the CFA model is manifested as a progressive increase in the volume of the injected paw. As measurement of paw volume is an important parameter in the evaluation of arthritis (Jalalpure et al. 2011), the paw volume was recorded on 0th, 7th, 14th, 21st and 28th day and the mean values of all the groups are presented in Table 11.2. The arthritic control group showed significant increase in paw volume which indicates the sign of arthritis development [Figure 11.4 (a)]. The methotrexate treated group showed a significant reduction in paw volume (64.95 %, P < 0.01) compared with the arthritic control group. Among the extract treated groups, the EESC treated group showed more reduction (58.97 %, P < 0.01) than the EESS treated group (20.51 %, P < 0.01). When comparison was made between the low and high dose groups of EESC and EESS, in both the cases, the reduction in paw volume was found to be dose dependent (Figure 11.2).
### Table 11.1 Effect of EESC and EESS on body weight of arthritic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0(^{th}) Day</td>
</tr>
<tr>
<td>Arthritic Control</td>
<td>201.67 ± 6.89</td>
</tr>
<tr>
<td>Methotrexate treated</td>
<td>203.33 ± 5.40</td>
</tr>
<tr>
<td>EESC treated (200 mg/kg)</td>
<td>205.67 ± 8.65</td>
</tr>
<tr>
<td>EESC treated (400 mg/kg)</td>
<td>205.67 ± 8.01</td>
</tr>
<tr>
<td>EESS treated (200 mg/kg)</td>
<td>205.33 ± 5.60</td>
</tr>
<tr>
<td>EESS treated (400 mg/kg)</td>
<td>205.67 ± 7.54</td>
</tr>
</tbody>
</table>

n = 6, Values are Mean ± SEM, Kruskal-Wallis followed by Mann-Whitney U test, P > 0.05 vs. Arthritic control

(EESC – Ethanolic Extract of *Stereospermum colais*, EESS – Ethanolic Extract of *Stereospermum suaveolens*)
Table 11.2 Effect of EESC and EESS on paw volume of arthritic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>PAW VOLUME (ml)</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0th Day</td>
<td>7th Day</td>
</tr>
<tr>
<td>Arthritic Control</td>
<td>1.22 ± 0.07</td>
<td>2.62 ± 0.16</td>
</tr>
<tr>
<td>Methotrexatetreated</td>
<td>1.21 ± 0.03</td>
<td>1.99 ± 0.01**</td>
</tr>
<tr>
<td>EESC treated (200 mg/kg)</td>
<td>1.21 ± 0.06</td>
<td>2.40 ± 0.06a</td>
</tr>
<tr>
<td>EESC treated (400 mg/kg)</td>
<td>1.20 ± 0.04</td>
<td>2.27 ± 0.07**a</td>
</tr>
<tr>
<td>EESS treated (200 mg/kg)</td>
<td>1.20 ± 0.04</td>
<td>2.56 ± 0.23a</td>
</tr>
<tr>
<td>EESS treated (400 mg/kg)</td>
<td>1.21 ± 0.05</td>
<td>2.48 ± 0.18a</td>
</tr>
</tbody>
</table>

n = 6, Values are Mean ± SEM, Kruskal-Wallis followed by Mann-Whitney U test,
*P < 0.05, ** P < 0.01 vs. Arthritic control and aP < 0.01 vs. Methotrexate treated

(EESC – Ethanol Extract of Stereospermum colais, EESS – Ethanol Extract of Stereospermum suaveolens)
Figure 11.1  Effect of EESC and EESS on body weight of arthritic rats

\( n = 6 \), Values are Mean ± SEM, Kruskal-Wallis followed by Mann-Whitney U test

P > 0.05 vs. Arthritic control

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)

Figure 11.2  Effect of EESC and EESS on paw volume of arthritic rats

\( n = 6 \), Values are Mean ± SEM, Kruskal-Wallis followed by Mann-Whitney U test

*P < 0.05, **P < 0.01 vs. Arthritic control and *P < 0.01 vs. Methotrexate treated.

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)
11.2.2.3 Biochemical studies

The levels of biochemical markers such as LDH, ALP, AST, ALT, total protein, urea, creatinine, CRP-hs (C-Reactive Protein- high sensitive) were determined and the results were tabulated (Table 11.3 and Figure 11.3).

Cytoplasmic cellular enzymes, like Lactate Dehydrogenase (LDH) are serving as indicators of the cellular integrity induced by pathological conditions. Other cellular enzymes, such as Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) are indicators of phagocytic activity, can also be used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions. These changes are in agreement with the decreased lysosomal stability in adjuvant induced arthritis (Olsen et al. 1990 & Geetha 1993).

The serum transaminases AST and ALT were reported to play a major role in the release of chemical mediators such as bradykinins in inflammatory condition (Glenn et al. 1965). A study proving the increased activity of serum Alkaline Phosphatase in RA was reported by Niino et al (1998). An investigation done among the RA patients envisage that the ALP activity was detectable at a high prevalence of about 30 % of population (Aida et al. 1992). Further elevated level of serum biochemical markers such as AST, ALT and ALP were reported in adjuvant induced arthritic rats (Hungl et al. 2006).

The results of this study revealed that elevated level ALP, AST and ALT were observed in the arthritic control group and the treatment groups significantly
reduced ALP, AST and ALT levels. Among the treatment groups, EESS (400 mg/kg) treated group showed significant reduction in the ALP level (P < 0.01) and EESC (400 mg/kg) treated group showed significant reduction in the AST and ALT levels (P < 0.01) when compared with the arthritic control group.

Lipid peroxidation produces marked alteration in molecular organization of membrane lipid resulting in increased membrane permeability and leakage of cytoplasmic markers such as LDH into circulation (Mason et al. 1997). In the present study, the activity of lysosomal enzymes was markedly increased in the adjuvant induced arthritic rats and significantly reduced by treatment groups. When compared with other groups, methotrexate and EESC (400 mg/kg) treated group produced significant decrease (P < 0.01) in the level of LDH. An important mechanism of anti-inflammatory activity is the membrane stability modulating effect (Subrata et al. 1994). The treatment groups may exert its effects by modifying the lysosomal membrane activity or by inhibiting the release of lysosomal enzymes (Carevic & Djokic, 1988).

Urea and serum creatinine are the biomarkers of kidney injury (Edelstein et al. 2008). It was hypothesized that substantial fraction of blood urea in arthritic rats comes from arginine synthesized in the kidneys (Filho et al. 2003). Elevated level of blood urea and serum creatinine was found in arthritic control group. This indicates the kidney dysfunction in arthritic rats. These alterations were significantly reduced by treatment with methotrexate, EESC and EESS. Among the treatment groups EESS (400 mg/kg) showed a significant reduction (P < 0.01) in creatinine level and EESC (400 mg/kg) showed a marked reduction in the urea level (P < 0.01).
C - Reactive Protein (CRP) is a member of the class of acute phase reactants and rises dramatically during inflammatory processes (McConkey et al. 1973). Marked increase in CRP levels was observed in arthritic control group. The level of C-reactive protein was found to be significantly reduced in the standard and extract treated groups. Treatment with EESC and EESS, at the dose of 400 mg/kg, significantly decreased (P < 0.01) the CRP level.
### Table 11.3 Effect of EESC and EESS on biochemical parameters

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ALP(U/L)</th>
<th>ALT(U/L)</th>
<th>AST(U/L)</th>
<th>CR(mg/dL)</th>
<th>LDH(U/L)</th>
<th>TP(g/dL)</th>
<th>Ur(mg/dL)</th>
<th>CRP-hs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritic Control</td>
<td>415.09 ± 19.67</td>
<td>91.99 ± 3.16</td>
<td>123.91 ± 6.87</td>
<td>1.00 ± 0.02</td>
<td>203.80 ± 11.63</td>
<td>9.12 ± 0.08</td>
<td>43.98 ± 0.91</td>
<td>5.07 ± 0.12</td>
</tr>
<tr>
<td>Methotrexate treated</td>
<td>277.03 ± 13.31**</td>
<td>71.57 ± 0.94**</td>
<td>102.14 ± 1.30*</td>
<td>0.68 ± 0.07**</td>
<td>120.55 ± 4.98**</td>
<td>6.15 ± 0.04**</td>
<td>40.38 ± 0.53**</td>
<td>3.26 ± 0.07**</td>
</tr>
<tr>
<td>EESC treated (200mg/kg)</td>
<td>269.13 ± 12.76**</td>
<td>76.07 ± 0.86**#</td>
<td>123.64 ± 1.74**</td>
<td>0.67 ± 0.16**</td>
<td>154.73 ± 4.27**#</td>
<td>6.56 ± 0.20**</td>
<td>36.32 ± 0.82**#</td>
<td>3.07 ± 0.06**</td>
</tr>
<tr>
<td>EESC treated (400mg/kg)</td>
<td>269.81 ± 12.89**</td>
<td>70.26 ± 0.66**</td>
<td>107.23 ± 0.92**</td>
<td>0.67 ± 0.07**</td>
<td>141.51 ± 5.57**</td>
<td>6.12 ± 0.04**</td>
<td>34.27 ± 0.79**#</td>
<td>2.57 ± 0.12**#</td>
</tr>
<tr>
<td>EESS treated (200mg/kg)</td>
<td>269.80 ± 8.83**#</td>
<td>75.28 ± 0.69**#</td>
<td>110.67 ± 1.92</td>
<td>0.68 ± 0.06**</td>
<td>145.43 ± 0.56**#</td>
<td>5.97 ± 0.03**#</td>
<td>41.07 ± 0.62**</td>
<td>3.13 ± 0.06**</td>
</tr>
<tr>
<td>EESS treated (400mg/kg)</td>
<td>241.11 ± 7.73**#</td>
<td>70.97 ± 0.85**#</td>
<td>120.68 ± 2.89**</td>
<td>0.58 ± 0.12**</td>
<td>146.79 ± 5.82**#</td>
<td>7.08 ± 0.03**#</td>
<td>39.60 ± 0.87**</td>
<td>2.75 ± 0.08**#</td>
</tr>
</tbody>
</table>

n = 6, Values are Mean ± SEM, Kruskal-Wallis followed by Mann-Whitney U test

*P < 0.05, **P < 0.01 vs. Arthritic control and #P < 0.05, aP < 0.01 vs. Methotrexate treated

[EESC – Ethanol Extract of Stereospermum colais, EESS – Ethanol Extract of Stereospermum suaveolens ALP – Alkaline Phosphatase, ALT- Alanine Transaminase, AST – Aspartate Transaminase, CR – Creatinine, LDH – Lactate Dehydrogenase, TP – Total Protein, Ur – Urea, CRP-hs- C – Reactive Protein (high sensitive)]
n = 6, Values are Mean ± SEM, Kruskal-Wallis followed by Mann-Whitney U test
*P < 0.05, **P < 0.01 vs. Arthritic control and #P < 0.05, ##P < 0.01 vs. Methotrexate treated


Figure 11.3 Effect of EESC and EESS on biochemical parameters
Figure 11.4 Photographs of arthritis induced rat hind paw

(a) Arthritic control
(b) Methotrexate treated
(c) EESC (200mg/kg) treated
(d) EESC (400mg/kg) treated
(e) EESS (200mg/kg) treated
(f) EESS (400mg/kg) treated

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)
11.2.2.4 Histopathology of Joint and Hind paw

The haematoxylin and eosin stained ankle joints and paw tissues of rats were evaluated for general histopathological changes. The arthritic control group revealed severe degree of panniculitis, skeletal muscular necrosis, synovitis, infiltration by inflammatory cells (polymorphonuclear cells, multinucleated giant cells). Moderate degree of articular cartilage erosion and synovial invasion was also observed. [Figure 11.5 (a) & Figure 11.6 (a)].

Methotrexate treated group revealed mild degree of synovitis and inflammatory cells infiltration, panniculitis, articular cartilage erosions and moderate degree of muscular necrosis [Figure 11.5 (b) & Figure 11.6 (b)]. Significant reduction in the severity of synovitis, panniculitis, subcutaneous inflammation, muscular necrosis and inflammatory cells infiltration was observed in the EESC (400 mg/kg) treated group [Figure 11.5 (d) & Figure 11.6 (d)] and mild reduction was observed in EESC (200 mg/kg) treated group [Figure 11.5 (c) & Figure 11.6 (c)]. EESS (400 mg/kg) treated group showed moderate reduction in the severity of synovitis, panniculitis, subcutaneous inflammation, inflammatory cell infiltration [Figure 11.5 (f) & Figure 11.6 (f)] and mild reduction has been observed in EESS (200 mg/kg) [Figure 11.5 (e) & Figure 11.6 (e)]. The pathological findings suggested that EESC and EESS decreased the severity of arthritis and other associated lesions in the ankle joints and the paw tissues of arthritic rats.
11.2.2.5 Histopathology of spleen

The haematoxylin and eosin stained spleens were evaluated for general histopathological changes. The spleen of arthritic control as well as treatment groups did not reveal pathological lesions and showed normal architecture (Figure 11.7).
Bone erosion and invasion of synovial cells with presence of bone cells and inflammatory cells (40x)

Muscular necrosis, infiltration of inflammatory cells (40x)

Mononuclear cells and giant cell infiltration in synovium (40x)

Minimal mononuclear cells infiltration (40x)

Mononuclear infiltration (40x)

Minimal mononuclear infiltration (40x)

Figure 11.5 Histopathology of rat hind paw
Figure 11.6 Histopathology of the ankle joint of rat

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)
Figure 11.7 Histopathology of spleen

(a) Arthritic control (10x)  
(b) Methotrexate treated (10x)  
(c) EESC (200mg/kg) treated (10x)  
(d) EESC (400mg/kg) treated (10x)  
(e) EESS (400 mg/kg) treated (10x)

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)
11.2.2.6 Immunohistochemical studies

Rheumatoid Arthritis is a systemic inflammatory disease characterized by chronic synovial inflammation resulting in cartilage and bone damage, leading to joint destruction. There are several cell types and their mediators are involved in the tissue-destructive inflammation such as T cells, B cells, monocyte/macrophages and pro-inflammatory cytokines such as TNF α, IL-1β and IL-2 etc., (Jocea et al. 2004).

Adjuvant induced arthritis is a model of chronic inflammation induced in rats by a mycobacterial suspension, which is T-cell mediated. Now a days there are studies performed, to determine the involvement of several cell subpopulation and the relationship between immune-competent cells in lymphoid organs and lesions in synovial joints (Carol et al. 2000). The spleen is an important lymphoid organ involved in immune responses against all types of antigen that appear in the circulation and it provides a readily available source of cells known to be involved in adjuvant arthritis (Jerne et al. 1963). An attempt has been made to analyse the involvement of spleen in the disease pathogenesis along with the immune response by the immuno-histochemical analysis of spleen tissue.

Immunoglobulin production is regulated by T lymphocytes. The T cells are classified into CD4 and CD8 positive T cells having helper and suppressive functions respectively, where CD stands for "Cluster of Differentiation", a historical term that was coined to define cell-surface molecules that are recognized by a given set of monoclonal antibodies (Sprent 1993).
CD4 cell numbers significantly increase in the splenic compartment of arthritic control group [Figure 11.8 (a)]. Sagawara et al. (1995) also described an increase in spleen CD4 lymphocyte percentage in arthritic condition. The increase in spleen T cells might be the result of *in situ* lymphocyte proliferation or T cell migration from lymph nodes. The presence of CD4 cells are more in the arthritic control group indicates the pathogenesis of disease. The other groups showed less number of CD4 cells when compared with arthritic control group.

Since Methotrexate is an immunosuppressant, it suppresses the regulatory T cells function, which was observed through the reduction in the CD4 cells distribution [Figure 11.8 (b)]. The EESC and EESS treated groups (400 mg/kg) also had the suppressive action on the CD4 cells which is comparable with the effect of methotrexate [Figure 11.8 (c-f)]. The results revealed that the extracts exert the anti-arthritic activity through the T-cell mediated inflammatory pathway. Among the two plants *Stereospermum colais* was found to be more effective in suppressing the CD4 cell activation and proliferation than *Stereospermum suaveolens*.

Cytokines have been reported to be expressed at significant levels in chronic state of inflammatory diseases (Ulfgren et al. 1995). It is well established that TNF-α, plays an important role in the pathology of RA. Since it induces collagenase production which may lead to cartilage destruction and bone resorption in RA. (Alvaro et al. 1990 & Migita et al. 1996). Several lines of recent evidence have also suggested that pro-inflammatory cytokines such TNF-α play a pivotal role in the pathogenesis of RA. TNF-α contributes to synoviocyte proliferation and increases
the production of tissue enzymes such as matrix metalloproteinase resulting in cartilage degradation (Bingham 2002).

The expression of inflammatory cytokines such as TNF α, IL-2 and TGF β were also studied. Localization of large amounts of inflammatory cytokines TNF – α and IL- 2 were seen in the splenic tissue of the arthritic control animals [Figure 11.9 (a) & 11.10 (a)]. This was evident from the intense brown staining. A reduction in the expression of these cytokines was observed in the methotrexate [Figure 11.9 (b) & 11.10 (b)] and extracts treated animals as compared to the arthritic control, whereas minimal expression was observed in the EESC and EESS treated groups at the dose of 200 mg/kg [Figure 11.9 (c, e) & 11.10 (c, e)] and EESC and EESS treated groups at the dose of 400 mg/kg [Figure 11.9 (d, f) & 11.10 (d, f)] reduced the expression to a greater extent than the standard methotrexate treated group.

TNF - α is a pleiotropic cytokine, which plays a critical role in both acute and chronic inflammations. Several inflamagens have the ability of inducing the synthesis of TNF-α, which facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells. Some studies suggested that blocking of TNF-α in specific can reduce the severity of inflammation. Among the two plants, Stereospermum colais significantly reduced the TNF-α expression than Stereospermum suaveolens.

TGF-β has both pro- and anti-inflammatory effects. It functions as a biological switch, antagonizing or modifying the action of other cytokines and growth factors. TGF-β suppresses the proliferation and differentiation of T cells and B cells and limits IL-2, IFN-g, and TNF-α production. (Kingsley et al. 1994).
Expression of TGF-β was found to be more in arthritic control group [Figure 11.11 (a)]. EESC & EESS treated groups at the dose of 200 mg/kg significantly reduced the TGF-β expression [Figure 11.11 (c,e)], whereas moderate reduction was observed in standard as well as the extracts treated group at the dose of 400 mg /kg [Figure 11.11 (d,f)]. This may be due to the immuno-modulatory effect of the extracts
Figure 11.8 Effect of EESC and EESS on the expression of CD4 cells in splenic tissue
Figure 11.9 Effect of EESC and EESS on the expression of IL-2 in splenic tissue

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)
Figure 11.10  Effect of EESC and EESS on the expression of TNF-α in splenic tissue

(a) Arthritic control (40x)  (b) Methotrexate treated (40x)  (c) EESC (200 mg/kg) treated
(d) EESC (400 mg/kg) treated  (e) EESS (200 mg/kg) treated (40x)  (f) EESS (400 mg/kg) treated

(EESC – Ethanoic Extract of Stereospermum colais, EESS – Ethanoic Extract of Stereospermum suaveolens)
Figure 11.11 Effect of EESC and EESS on the expression of TGF-β in splenic tissue

(EESC – Ethanolic Extract of Stereospermum colais, EESS – Ethanolic Extract of Stereospermum suaveolens)
11.3 CONCLUSION

The anti-arthritic activity of the EESC and EESS were evaluated by Complete Freund's Adjuvant induced arthritic model. The present preclinical study has revealed evidences for the traditional use of roots of *Stereospermum colais* and *Stereospermum suaveolens* in the treatment of arthritis.

The reduction in the paw volume exhibited the anti-inflammatory potential of the ethanolic extract of both the plants. The extracts at the dose of 400 mg/kg has markedly inhibited the change in joint architecture as compared to arthritic control. Also the study revealed that the extracts may possibly act by affecting the T cell mediated inflammatory process which was evident by decreased expression of CD 4 cells, IL-2 and TNF-α. The presence of phytochemicals such as terpenoids, flavonoids, quinones, phenols and tannins may probably attribute to the anti-arthritic property of the plant.

The study concludes that ethanolic extract of both the plants (EESC and EESS) exhibited significant anti-arthritic activity at the dose 400 mg/kg than the lower dose 200 mg/kg and *Stereospermum colais* was found to be more potent than *Stereospermum suaveolens*.

11.4 REFERENCES


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