Anti-arthritis activity of different extracts of *S. zeylanica* and *S. ovalifolia* in Wistar Albino rats

7.1. Introduction

Arthritis is characterized by inflammation of the synovial membrane, pain and restricted joint movement and is the most common joint problem caused by hypersensitive mechanisms (Pearson, 1959). Rheumatoid arthritis (RA) is a chronic progressive, systemic inflammatory disorder affecting the synovial joints which eventually leads to joint destruction. Arthritis is more prevalent in Western countries and the prevalence of RA in Indian subcontinent is 1.5-2% of the population. Studies disclosed that the prevalence of RA is 1% of the world population and the epidemiological ratio of arthritis in female and male is 3:1 (Babushetty and Chandrashekhar, 2012). To study the pathogenesis of arthritis as well as to assess the anti-arthritis potential of drugs and molecules, researchers choose animal models of rheumatoid arthritis. The criteria behind the selection of an animal model involve the capacity to predict efficacy of agents in humans, reproducibility of data, ease of performing the model, reasonable duration of test period and similar pathology and/or pathogenesis to that of human disease. Animal model also gives an idea about the efficacy of the molecule or drug under investigation along with the severity in the toxicity of the same (Bendele, 2001).

Adjuvant-induced arthritis has been widely used as an experimental model of poly arthritis. It has been widely used for preclinical testing of numerous anti-arthritic agents which are either under preclinical or clinical investigation or are currently used as therapeutics in this disease (Pearson, 1956; Carlson *et al*., 1985; Bendele, 2001). The
characteristics of this model are reliable onset and progression of disease, poly articular inflammation, marked bone resorption periosteal bone proliferation and is easily measureable. Mild cartilage destruction occurs in comparison to the inflammation and bone destruction (Bendele, 2001).

Adjuvant induced arthritis in rats is a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joints which resembles RA in humans. Induction of adjuvant disease can be done with either Freund’s complete adjuvant (FCA) supplemented with Mycobacterium or by injection of the synthetic adjuvant N, N-dioctyl dodecyl- N’, N-bis (2-hydroxy-ethyl) propanediamine (LA). Adjuvant can be injected at the base of the tail or in one of the foot pads. If injected into the footpad, the acute inflammatory reaction in local area can be studied and the immunological reaction develops approximately 9 days later in the contralateral paw and various organs. From the 9th day onwards, hind paw swelling will be monitored.

7.2. Materials and Methods

7.2.1. Preparation of Extracts and Doses

The test plant extracts were prepared as mentioned earlier by soaking the plant materials in respective solvents. The filtered extracts were concentrated by evaporation of solvents in the rotary flash evaporator. All doses were prepared shortly prior to administration. About 200mg/kg and 400 mg/kg of extracts were prepared by dissolving the extracts in 2.5% Tween 80. The volume administered did not exceed 2ml/100gm of animal. Dose selection of the test extracts were based on the toxicity studies conducted in the earlier part of the research work. It was found that all the test extracts did not cause mortality until a dose of 5000mg/kg bwt.
7.2.2. Animals

Eight weeks old male Wistar Albino rats were randomly selected as control and experimental groups with six rats in each group. They were kept in the polypropylene cages under standard environmental conditions of 12 hours dark-light cycle at a temperature of 24±1°C. Animals were fed with standard diet cakes prepared in the laboratory and allowed to freely access clean fresh water ad libitum. Prior to dosing, all animals were kept in the experimental conditions for about 7-10 days to allow them to be acclimatized. The sterilized paddy husk was used as the bedding material. All experimental protocols were approved by the institutional animal ethics committee (SBCP/2011-2012/1AEC/CPCSEA/3) of Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi. Once the inflammation was induced, food and water were served at the bottom of the cage as severely inflamed rats might have difficulty in obtaining feed from the top of the cage.

7.2.3. Standard Drugs and Chemicals Used

All chemicals used in the study were of analytical grade. Diclofenac and Indomethacin were obtained as a gift from the Micro Labs Pvt Ltd, Bangalore, India. Freund’s complete adjuvant was supplied by Sigma-Aldrich Chemical Co, USA. Polymerase chain reaction products were obtained from Helini Bio molecules, Chennai and Ray Bio® rat IL1 β and rat TNF α ELISA kits for serum samples were used to analyze serum concentration of IL1 β and rat TNF α in Wistar rats.

7.2.4. Experimental Set Up

The anti-arthritic study was performed in two stages, prophylactic and therapeutic. In each stage of study, animals were further divided into sixteen groups with six rats in
each group and were orally administered with the vehicle, standard drugs and different test extracts as tabulated in Table 31.

**Table 31: Experimental Groups in the Anti-arthritic Study**

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Orally administered with</th>
<th>Dose (mg/Kg Bwt p.o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Vehicle Control</td>
<td>Tween 80</td>
<td>2.5 (%)</td>
</tr>
<tr>
<td>II Arthritis Control</td>
<td>Tween 80</td>
<td>2.5 (%)</td>
</tr>
<tr>
<td>III Diclofenac</td>
<td>Diclofenac</td>
<td>200</td>
</tr>
<tr>
<td>IV Indomethacin</td>
<td>Indomethacin</td>
<td>400</td>
</tr>
<tr>
<td>V SOAE 200</td>
<td>Aqueous extract of <em>S. ovalifolia</em></td>
<td>200</td>
</tr>
<tr>
<td>VI SOAE 400</td>
<td>Aqueous extract of <em>S. ovalifolia</em></td>
<td>400</td>
</tr>
<tr>
<td>VII SOEE 200</td>
<td>Ethanol extract of <em>S. ovalifolia</em></td>
<td>200</td>
</tr>
<tr>
<td>VIII SOEE 400</td>
<td>Ethanol extract of <em>S. ovalifolia</em></td>
<td>400</td>
</tr>
<tr>
<td>IX SOCE 200</td>
<td>Chloroform extract of <em>S. ovalifolia</em></td>
<td>200</td>
</tr>
<tr>
<td>X SOCE 400</td>
<td>Chloroform extract of <em>S. ovalifolia</em></td>
<td>400</td>
</tr>
<tr>
<td>XI SZAE 200</td>
<td>Aqueous extract of <em>S. zeylanica</em></td>
<td>200</td>
</tr>
<tr>
<td>XII SZAE 400</td>
<td>Aqueous extract of <em>S. zeylanica</em></td>
<td>400</td>
</tr>
<tr>
<td>XIII SZEE 200</td>
<td>Ethanol extract of <em>S. zeylanica</em></td>
<td>200</td>
</tr>
<tr>
<td>XIV SZEE 400</td>
<td>Ethanol extract of <em>S. zeylanica</em></td>
<td>400</td>
</tr>
<tr>
<td>XV SZCE 200</td>
<td>Chloroform extract of <em>S. zeylanica</em></td>
<td>200</td>
</tr>
<tr>
<td>XVI SZCE 400</td>
<td>Chloroform extract of <em>S. zeylanica</em></td>
<td>400</td>
</tr>
</tbody>
</table>

**7.2.5. Induction of Adjuvant Arthritis in Wistar rats**

Group I served as normal vehicle control group which received 2.5% of Tween 80. Adjuvant induced arthritis was induced by intra dermal injection with 0.1ml Freund’s Complete Adjuvant (FCA) into the right hind paw of all rats in the rest sixteen groups. Group II was untreated and served as arthritis control which received 2.5% of Tween 80. Group III-XVI were of arthritic groups which received different test extracts of *S. zeylanica* and *S. ovalifolia* at two different dose levels of 200 mg/kgbw p.o and 200mg/kgbw p.o. In the prophylactic study, drug treatment was started fourteen days prior to adjuvant immunization until the completion of the experiment. In the therapeutic study, the drug treatment was commenced from 14th day of adjuvant induction and terminated on 28th day. Radiographic analysis was performed for all the animals.
periodically. The paw volume of the rats was measured by standard volumetric technique using a calibrated plethysmometer at 0, 7, 21 and 28 days of the study. Total body weight and food and water intake were recorded weekly.

**7.2.6. Erythrocyte Sedimentation Rate**

On 29th day, blood samples were collected by retro orbital puncture. Approximately 2 ml of blood samples were taken in sterilized Eppendorff tubes with sodium citrate for the determination of erythrocyte sedimentation rate.

Erythrocyte sedimentation rate (ESR) was determined by using Westergren method where the blood was drawn into a Westergren-Katz tube to the 200 mm mark. The tube was placed in a rack in a strictly vertical position for 1 hour at room temperature at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment was measured. The calculation was done by the measurement of distance of fall of RBC in 1 hour (Wolfe *et al.*, 1994).

**7.2.7. Histopathology of Joints**

Decalcified tissues were cut into 2 to 3mm thickness and were fixed in 10% buffered formalin prepared in normal saline. Tissues were dehydrated by immersing in alcohol from 50% to 100% concentration and the duration of dehydration in alcohol depended upon the size of the tissue. The dehydrated tissues were cleared in xylene for 0.5-1 hr for small piece of tissues and 2-4 hours for large thick tissues. The tissues were impregnated in paraffin wax at about 54-64°C for around 4hours and then placed in mould. Melted wax was poured in it and allowed to solidify and thin sections were made and fixed in microscopic slides.

Sections were kept in xylene for 3 minutes and transferred to absolute alcohol for 3 minutes which was transferred to 80% and 50% alcohol for 2 minutes. Slides were
washed in running tap water for 1 minute and kept in Harris’s Haematoxylin for 5-7 minutes. The slides were again washed in running tap water and excess dye was washed in 1% acid alcohol by continuous agitation for 15 seconds. After washing the slides in running tap water, the slides were dipped in ammonia water for 2-3 times to attain a blue colour. Slides were again washed for 30 seconds and counterstained with eosin for 3-5 minutes. Slides were washed in running tap water for 30 seconds and dehydrated by keeping in increasing concentration of alcohol (2-3 minutes in 50%, 70%, 95% and absolute alcohol). The sections were again cleared in xylene and mounted with DPX and observed under microscope.

7.2.8. Determination of Serum Concentration of TNF α and IL 1β by ELISA

Serum samples were prepared from the collected blood samples and were kept refrigerated until further use. Serum concentrations of TNF α and IL 1β were determined by Enzyme linked immune sorbent assay kits (Ray Bio® Rat TNF α and IL 1β kits.)

7.2.8. 1. Kit Reagents

The kit was provided with 96 well micro titre plates coated with Rat TNF α or IL 1β, (20X) wash buffer concentrate, Standards (recombinant rat TNF α or IL 1β), Assay Diluent A, Detection Antibody TNF-alpha or IL 1β, HRP-Streptavidin Concentrate, TMB5,5'-tetramethylbenzidine (TMB) in buffered solution and Stop Solution.

7.2.8. 2. Preparation of Standards

About 400 µl of assay buffer was added to the respective standards provided in the ELISA kits and mixed well. 100 µl of the fresh 400 µl of assay buffer prepared standard was mixed with 400 µl of assay buffer in a tube to prepare 20, 000 pg/ml stock standard solution. 400 µl of assay buffer was pipetted out into a series of tubes and the standard stock solution was serially diluted, whereas the assay buffer A alone served as the zero standard (0 pg/ml).
7.2.8. 3. Assay Procedure

Before starting the assay, all samples and kit reagents were brought to room temperature and mixed well. 100 µl of each standard and samples were pipetted out into appropriate wells and were covered and incubated for 2.5 hours at room temperature. The solution was then discarded from the wells after incubation and washed thrice with 1X washing solution prepared from the 10X wash buffer provided with the kit and the wells were inverted and blot dried by using paper towels. 100 µl of biotinylated antibody was added to each well and were incubated for 1 hour at room temperature. After incubation, the contents in the wells were discarded and the washing step was repeated. 100 µl of Streptavidin solution was added and incubated for 45 minutes. Following the incubation, the wells were washed again and were added with 100µl of TMB solution and incubated in dark for 30 minutes and 50 µl of stop solution was added after incubation and OD values were measured using ELISA reader at 450 nm.

7.2.8. 4. Calculation of Results

From the mean absorbance for each set of duplicate standards, controls and samples, the average zero standard optical density was subtracted. From the standard curve prepared by plotting the OD values against their concentration, the concentration of TNF α and IL1 β in the test samples were calculated.

7.2.9. Radiography

Inflamed paw of all animals were subjected for radiography by dental X-ray unit (Confident 70 Kvp & 8 MA, electrical specification AC 230 V) for about 1 minute. The light proof wrapper of the X-ray films were removed and kept in film holders and immersed in a developer solution at 68°F for 4.5 minutes by moving the film holder in the up-down position to avoid the formation of air bubbles. After developing, the films were
immersed in rinsing water (distilled water) for at least 20 seconds to get rid off the excess developing solution adhering to the film. After rinsing, the films were immersed in fixing solution for about 10 minutes for hardening. The hardened films were again rinsed in circulating distilled water for 20 minutes to remove the excess fixing solution and dried by hanging them on drying rack.

7.2.10. Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

7.2.10. 1. Extraction of Total RNA

At the end of the experiment, the rats were killed by cervical dislocation and knee joints were carefully removed and then total RNA of synovial cells were estimated as directed.

7.2.10. 2. Primers Used (Designed by Helini Biomolecules, Chennai.)

Ribosomal protein – Housekeeping genes

Forward Primer: 5’-TTTCTGGCCTGGCTTGTTTG-3’
Reverse Primer: 5’-CATTGCAGATGAGGCTTCCAA-3’

Tumour Necrosis Factor α (TNF α)

Forward Primer: 5’-GGGCTCCCTCTCAGTTC-3’
Reverse Primer: 5’-GTGGGCTACGGGCTTTCG-3’

Interleukin 1 Beta (IL1-β)

Forward Primer: 5’-CACAGCAGCATCTCGACAAGAG-3’
Reverse Primer: 5’-GCTCCACGGGCAAGACATAG-3’

7.2.10. 3. Isolation of Total RNA

About 25-50mg of tissue was transferred into 1.5ml centrifuge tube and 560μl of Lysis Buffer was added to it and grinded well using Micro Pestle. It was then mixed immediately by inverting centrifuge tubes and incubated at room temperature for 10
minutes. 560 μl of (100%) ethanol was added and mixed well by vortexing for 30 seconds. From the above mixture, 630μl of the sample was pipetted out into the PureFast® spin column and centrifuged for 1 min. The flow-through was discarded and the column was placed back into the same collection tube. The remaining 630μl of the sample was added and centrifuged for 1 minute and again the flow through was discarded. 500μl wash buffer-I was added to it and centrifuged again for 30-60 seconds and again the flow through was discarded. The column was placed back in the collection tube. The above step was repeated twice and again centrifuged for an additional 2 minutes to avoid residual ethanol. The PureFast® spin column was transferred into a fresh 1.5 ml micro-centrifuge tube and eluted with 60μl of Elution buffer.

7.2.10. 4. c DNA Synthesis Setup using Total RNA

Into a sterile, RNase free tube, 4μl of 5X RT buffer was added and to this mixture 1.5μl of enzyme mix, 5μl of Template RNA, 1.5μl Gene specific Primer Mix 5p/μl and 8μl of Nuclease-free Water were added. The total volume of the reaction mixture was 20 μl and was mixed gently and centrifuged. It was then incubated for 30 minutes at 50οC. The reaction was terminated by heating the mixture at 70οC for 5 minutes.

7.2.10. 5. Gene Amplification Using c DNA

Into a sterile, RNase free PCR tube, 10μl of 2X SYBR Green Master mix, 1.5μl of Gene specific Primer Mix (5pmoles/μl), 2μl of c DNA and 6.5μl of nuclease free Water were added, mixed gently and spinned down briefly and programmed. The relative content of each sample was computed and compared with internal consulting. The average OD values of the strap of amplified products were determined.
Table 32: RT-PCR Reaction Protocol

<table>
<thead>
<tr>
<th>Programme</th>
<th>Description</th>
<th>Temperature and Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Initial Denaturation (Taq activation-40 cycles)</td>
<td>94°C for 15 min</td>
</tr>
<tr>
<td>2</td>
<td>Denaturation</td>
<td>94°C for 30sec</td>
</tr>
<tr>
<td>3</td>
<td>Annealing</td>
<td>55°C for 30sec</td>
</tr>
</tbody>
</table>

7.2.11. In vivo Anti Oxidant Activity

7.2.11. 1. Estimation of Total Proteins and Peroxidase

The isolated liver from the experimental animal was divided into two parts. First part was macerated with 0.15 M potassium chloride and the resultant homogenate (10 %w/v) was centrifuged at 8000 rpm for 10 minutes. The supernatant was collected in clean tubes and was used for the estimation of anti oxidant activity such as total proteins (Lowry et al., 1951; Sapan et al., 1999), peroxidise and catalase by commercially available kits.

7.2.11. 2. Estimation of Catalase (Sinha, 1972)

About 0.1ml of tissue homogenate was mixed with 0.4 ml of 2 M H$_2$O$_2$ and 1.0 ml of 0.01 M phosphate buffer (pH 7). The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in the ratio of 1:3). The Optical density of the reaction mixture was read at 620 nm and was expressed as micromoles of H$_2$O$_2$ consumed per minute per mg of protein.

7.2.11. 3. Reduced Glutathione (GSH)

One ml of liver homogenate supernatant was mixed thoroughly with 0.5 ml of Ellman’s reagent and 3 ml of 0.2 M phosphate buffer (pH 8.0). The absorbance was read at 412 nm. The activity of GSH was expressed as mM GSH formed/g tissue (Ellman, 1959).
7.2.11. 4. Estimation of Superoxide Dismutase (SOD)

The assay for SOD was based on SOD mediated inhibition of the reduction of nitro blue tetrazolium to blue formation by superoxide anions as described by Beauchamp and Fridovich (1971). Units of SOD activity determined were expressed in terms of milligrams of total protein (TP).

7.3. Results and Discussion

7.3. 1. Inhibition of Paw Oedema

In the therapeutic model of anti arthritic study, animals were injected with the adjuvant on the 0\textsuperscript{th} day and were left untreated until 14\textsuperscript{th} day of the study. Similar patterns of increase in the paw volume in all the animal groups were observed. The standard drugs, Diclofenac and Indomethacin significantly reduced the paw volume from the 14\textsuperscript{th} day of the study, \textit{i.e.} soon after their administration till the end of the study (Plates 11&12). At both dose levels, the ethanol extracts of \textit{S. zeylanica} exerted a maximum reduction (31.52\%) in the paw volume followed by the aqueous extract (Figures 14 &15). The chloroform extract also exerted a significant reduction in the paw volume when compared to the positive control group when tested on the 21\textsuperscript{st} day of experiment. Similar pattern of suppression of disease progression was noted with \textit{S. ovalifolia} (Figures 16 & 17), where the ethanol extracts suppressed the paw volume increase significantly from the day of administration of the extract followed by aqueous and chloroform extracts. Twelve hours after administration of drugs on the 14\textsuperscript{th} day, only standard drugs exerted significant protection against paw oedema (p<0.05). On the 21\textsuperscript{st} day, Diclofenac exerted 21\% (p<0.001) inhibition followed by SOEE 400 (19.51\%, p<0.001), Indomethacin (18.93\%, p<0.001) and SZEE (18.46\%, P<0.001). On the 28\textsuperscript{th} day, the standard drug Diclofenac could achieve 39.97\% (p<0.001) reduction in the paw volume followed by Indomethacin (37.29\%, p<0.001). After 14 days of treatment with the test extracts, SOEE 400 achieved
36.2% (p<0.001) protection. Next to the SOEE 400 group, SOAE inhibited the disease progression to 32.58% (p<0.001). Ethanol as well as the aqueous extracts of *S. zeylanica* also exerted significant (p<0.001) protection (SZEE-31.52%, SZAE-28.12%) against arthritis. A dose dependant anti-arthritic activity in all of the extracts tested was observed. Both the ethanol as well as the aqueous extracts exerted significant anti arthritic activity in the therapeutic model of study (Figures 18 &19).

A significant prevention of increase in the paw volume by *S. zeylanica* as well as *S. ovalifolia* was noted in the prophylactic study (Plates 13 &14). Paw volume rapidly increased in the arthritic control when compared to the negative control group. The ethanol extracts of *S. zeylanica* and *S. ovalifolia* exerted its maximum protection (50.42% and 50.01%) at their highest concentrations. Figures 20 & 21 indicate the reduction in the paw oedema achieved by the pre treatment with *S. zeylanica* and Figures 22 & 23 that of *S. ovalifolia*. The pre treatment with ethanol and aqueous extracts of both plants rendered significant protection when compared to the arthritic control as well as the chloroform extract treated groups. Among the tested extracts, the SZEE 400 exerted highest protection of 50.41% (p<0.001) followed by SOEE400 (50.02%, p<0.001), SOEE200 (46.81%, p<0.001), SZEE 200 (46.18%, p<0.001), SOAE 400 (42.77%, p<0.001), SZAE 400 (37.48%, p<0.001) SZAE 200 (33.13%, p<0.001) and SOAE 200 (32.63%, p<0.001) (Figures 24 & 25).
Figure 14: Therapeutic activity of *S. zeylanica* at 200 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.

Figure 15: Therapeutic activity of *S. zeylanica* at 400 mg/kg dose level in adjuvant induced paw oedema.

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 16: Therapeutic activity of *S. ovalifolia* at 200 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.

Figure 17: Therapeutic activity of *S. ovalifolia* at 400 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 18: Rate of protection exerted by different extracts of *S. zeylanica* in therapeutic model of adjuvant induced arthritis

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.

Figure 19: Rate of protection exerted by different extracts of *S. ovalifolia* in therapeutic model of adjuvant induced arthritis

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 20: Prophylactic activity of *S. zeylanica* at 200 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.

Figure 21: Prophylactic activity of *S. zeylanica* at 400 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 22: Prophylactic activity of *S. ovalifolia* at 200 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.

Figure 23: Prophylactic activity of *S. ovalifolia* at 400 mg/kg dose level in adjuvant induced paw oedema

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 24: Rate of protection exerted by different extracts of *S. zeylanica* in prophylactic model of adjuvant induced arthritis

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.

Figure 25: Rate of protection exerted by different extracts of *S. ovalifolia* in prophylactic model of adjuvant induced arthritis

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Plate 11: Protection exerted by different extracts of *S. zeylanica* and *S. ovalifolia* in therapeutic model of adjuvant induced arthritis*

Plate 12: Radiography of inflammed paws after therapeutic anti-arthritic study*

Plate 13: Protection exerted by different extracts of *S. zeylanica* and *S. ovalifolia* in prophylactic model of adjuvant induced arthritis*

Plate 14: Radiography of Inflamed Paws after Prophylactic Anti-arthritic Study*

7.3.2. Radiography

Radiography helps to assess the course of arthritis very clearly. Radiography of paw of experimental animals advocates the anti arthritic activities of ethanol as well as the aqueous extracts of both plants. Radiography clearly detected the soft tissue swelling as well the joint space reduction in the arthritic rats (Plates 12 &14).

7.3.3. Body Weight and Food and Water Consumption

Along with the potency of different extracts of *S. zeylanica* and *S. ovalifolia* in reducing the arthritic score, their effect in the food consumption, weight gain and water consumption were evaluated by therapeutic as well as prophylactic model of anti arthritic study. All animals were challenged with the antigen from the 0\textsuperscript{th} day and treatment started from the day of 14 and continued till 28\textsuperscript{th} day. In the therapeutic model of arthritic study, it was noted that the animals in the vehicle control group attained an increase in the body weight during the experimental period. Adjuvant challenge retarded the weight gain in all the animals. The body weight of arthritis control group drastically reduced periodically. Same pattern of weight reduction was noted in chloroform extract treated groups of both plants whereas dose dependent difference could not be noticed (Figures 26 & 27). Rest of the test extracts as well as the control drugs, Diclofenac and Indomethacin exerted weight gain after 14\textsuperscript{th} day of adjuvant challenge. Ethanol extracts of both plants achieved good weight gain after 14\textsuperscript{th} day when compared to the aqueous as well as the chloroform extracts. Similar results were reported when adjuvant induced rats were treated with different extracts of *H. enneaspermus* (Tripathy *et al*., 2009).

Adjuvant challenge also affected the food consumption of all animals. There observed a sudden reduction in the amount of food consumed was observed on the 7\textsuperscript{th} day after the adjuvant challenge. The food consumption of arthritic control group reduced gradually from 0\textsuperscript{th} day to 28\textsuperscript{th} day. After first week of antigen challenge, the food
consumption started to increase gradually and after the treatment with plant extracts and control drugs. The food consumption of aqueous and ethanol extract groups of both plants followed similar pattern as that of the standard drugs and vehicle control groups. Water consumption of test animals did not show any kind of relation with food consumption and disease progression. A slight increase in the consumption of water was noticed in all groups except in the arthritis control group (Figures 26 & 27) in the therapeutic model of anti-arthritic study. Water consumption of all the experimental groups followed a similar pattern as that of the vehicle control group.

The potency of the test extracts to prevent the development of arthritis was studied using prophylactic anti arthritic study using Wistar Albino rats. In this model of arthritic study, test extracts as well as the control drugs were administered using oral gavage from the 0\textsuperscript{th} day of the experiment and continued till the end of the experiment. The adjuvant challenge was done on the 14\textsuperscript{th} day and the development of arthritis was monitored regularly. The total body weight of the animal directly indicates the health condition and stress of any animal and hence the body weight as well as the food and water consumption was regularly monitored. In the present study, a gradual increase in the body weight of all the animals involved in the experiment was noted till the antigen challenge on the 14\textsuperscript{th} day. In all the experimental animals, a slight reduction in the body weight was noted whereas in the positive control group, the body weight significantly reduced when checked seventh day after the antigen challenge. On the 28\textsuperscript{th} day of the experiment, it was noted that all animals got an increase in weight where as the arthritic control group continued to lose weight. The ethanol extracts of both plants at higher dose levels prevented the fall in the body weight and was comparable to the effect of the standard drug Diclofenac. Among the test drug treated groups, a reduction in the body weight was
noted with the chloroform extracts treated groups next to the positive control group (Figures 28 & 29).

The food consumption pattern in the experimental groups almost correlates to the weight loss pattern in the prophylactic study. The food consumption of the animals increased gradually from the 0th day till adjuvant administration in all the groups. Except in the vehicle control and standard drug treated groups, food consumption got declined after adjuvant challenge in all the experimental groups, but at the end of the experiment there was observed an increase in the food consumption except in the positive control group. Hence, it is concluded that the reduced food consumption might be due to the pain and stress induced after adjuvant challenge which might led to the reduction in the body mass of animals. The lowest food consumption in the arthritic control rats correlated to the progression of the disease and there by caused a marked weight loss. Hence, it is assumed that the pain and inflammation of paw might have prevented from proper feeding and drinking even after the food and water were served at the bottom of the cage after adjuvant administration (Figures 28 & 29). The adjuvant arthritis also induces catexia (Walsmith et al., 2004.) where lipolysis cause the loss of adipose tissue mass which in turn effect the reduction in the weight of arthritic animals (Granado et al., 2006). In the chronic arthritic conditions, there occurred a reduction in the lipogenesis which might have led to the reduction in the body mass. Hence, the reduced weight of the arthritic animals might be due to reduced food consumption along with the reduction in the weight of white adipose tissue (Martin et al., 2007). Ethanol and aqueous extracts of both plants effectively balanced the weight loss after adjuvant challenge when compared to the positive control group and chloroform extract treated groups.
Figure 26: Therapeutic effect of different extracts of *S. ovalifolia* on body weight, food and water consumption of adjuvant induced arthritic rats

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 27: Therapeutic effect of different extracts of *S. zeylanica* on body weight, food and water consumption of adjuvant induced arthritic rats.

One way ANOVA followed by Dunnett’s test, Values are expressed as mean± SD, n=6.
Figure 28: Prophylactic effect of different extracts of *S. ovalifolia* on body weight, food and water consumption of adjuvant induced arthritic rats

One way ANOVA followed by Dunnett’s t test Values are expressed as mean± SD, n=6.
Figure 29: Prophylactic effect of different extracts of *S. zeylanica* on body weight, food and water consumption of adjuvant induced arthritic rats

One way ANOVA followed by Dunnett’ test Values are expressed as mean± SD, n=6.
7.3.4. Histopathology of Joints

In agreement with reduction of paw volume, histopathological findings of joints advocate that the treatment with the test extracts markedly ameliorated disease. As compared with the normal joint structure of control rat, the arthritic control joint exhibited widening of synovial cavity, expansion of pannus, heavy infiltration of the mono nuclear cell in the synovial membrane, thickening of synovial membrane and destruction of cartilage tissue were noticed. The left tibiotarsal joint of arthritic control produced marked increase in the paw volume when compared to the normal control rats (Plates 15 &16).

In rats treated with the standard drugs, Diclofenac and Indomethacin, a pronounced reduction in the paw volume was observed when compared to the arthritic group. Very mild lympho plasmacytic infiltration was observed in the synovial membrane. Slightly hyper plastic and moderate infiltration in the dermal area was also noted and massive cartilage destruction and bone erosion were absent. In rats treated with the aqueous and ethanol extracts of S. zeylanica as well as the S. ovalifolia, marked increase in the synovial inflammation was reduced when compared to the positive control rats. Mild lympho plasmacytic infiltration was noted in these groups where as cartilage erosion as well as the bone destruction was not seen. Histopathology of the tibio tarsal joints of the rats indicate that the aqueous as well as the ethanol extracts of both plants reduced cartilage destruction, bone erosion and mononuclear cell infiltration in the synovial membrane. The paws of rats treated with the ethanol and aqueous extracts were significantly less inflammed and the histopathology results coincides with plethysmometer readings of the paw volume. Thickening of synovium and cartilage destruction were not present in these cases as compared to the chloroform extract treated
groups and positive control groups. Chloroform extract treated animals had a lower degree of synovial inflammation that was still above that found in non-arthritic animals.

The histology of arthritic control rats of prophylactic study (Plate 16) exhibited dense infiltration of mononuclear cells in the synovial membrane and in the extensively expanded synovial pannus. Marked cartilage destruction and slight bone destruction by invading synovium was also noted in the arthritic control groups. The rats pre-treated with standard drugs, ethanol and aqueous extracts of both plants developed mild to moderate lympho plasmacytic infiltration in the synovial membrane. Cartilage and bone destruction were not observed in these groups. In the groups pre-treated with chloroform extracts, a heavy infiltration of mononuclear cells in the synovial pannus along with slight destruction of cartilage tissue was noticed. Marked bone destruction was not observed in the experimental groups when compared to the positive control group. In the therapeutic model of anti arthritic study (Plate 15), histology of the tibiotarsal joints of inflammed paw highly correlated with the rate of inflammation in different groups as well as the protection rendered by the standard drugs and the test extracts.
a: Normal control, b: arthritis control, c: standard drug Diclofenac d: standard drug Indomethacin, e-g: aqueous ethanol and chloroform extracts of S. zeylanica and h-j: aqueous ethanol and chloroform extracts of S. ovalifolia. Photomicrographs were taken at 200X, unless mentioned.
Plate 16:Histological observation of left tibiotarsal joints of rats under prophylactic model of anti-arthritic study

a: Normal control, b: arthritis control, c: standard drug Diclofenac d: standard drug Indomethacin, e-g: aqueous, ethanol and chloroform extracts of S. zeylanica and h-j: aqueous, ethanol and chloroform extracts of S. ovalifolia. Photomicrographs were taken at 200X, unless mentioned.
7.3.5. Erythrocyte Sedimentation Rate

In both prophylactic as well as therapeutic studies, Erythrocyte sedimentation rate (ESR) was found to be very higher in arthritic control groups when compared to the respective treatment groups (Table 33). The Erythrocyte sedimentation is a test used to measure the arthritis indirectly. Proteins are assumed to affect the repellent surface charges on red blood cells which cause them to aggregate into orderly stacks or rouleaux (Grant et al., 1970). This led to the increased sedimentation rate of red blood cells and hence ESR level got increased in the arthritic rats.

Table 33: Effect of different extracts of S. zeylanica and S. ovalifolia on erythrocyte sedimentation rate of adjuvant induced arthritis

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Therapeutic Study</th>
<th>Prophylactic Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>4±0.00</td>
<td>2.35±0.024</td>
</tr>
<tr>
<td>Arthritic control</td>
<td>11.33±0.46</td>
<td>10.78±0.13</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>4.33±0.16</td>
<td>2.67±0.07</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5±0.04</td>
<td>3.33±0.32</td>
</tr>
<tr>
<td>SZAE 200</td>
<td>6.3±0.46</td>
<td>4±0.00</td>
</tr>
<tr>
<td>SZEE 200</td>
<td>5.7±0.55</td>
<td>3.35±0.025</td>
</tr>
<tr>
<td>SZCE 200</td>
<td>10.13±0.05</td>
<td>9±0.25</td>
</tr>
<tr>
<td>SZAE400</td>
<td>6±0.00</td>
<td>3±0.42</td>
</tr>
<tr>
<td>SZEE400</td>
<td>4.8±0.26</td>
<td>3.25±0.16</td>
</tr>
<tr>
<td>SZCE 400</td>
<td>9±0.17</td>
<td>8.67±0.13</td>
</tr>
<tr>
<td>SOAE 200</td>
<td>5.6±0.156</td>
<td>3.5±0.43</td>
</tr>
<tr>
<td>SOEE 200</td>
<td>5±0.47</td>
<td>3.33±0.56</td>
</tr>
<tr>
<td>SOCE 200</td>
<td>11±0.33</td>
<td>7.67±0.25</td>
</tr>
<tr>
<td>SOAE 400</td>
<td>5.6±0.45</td>
<td>3.00±0.56</td>
</tr>
<tr>
<td>SOEE 400</td>
<td>4.6±0.05</td>
<td>2.95±0.45</td>
</tr>
<tr>
<td>SOCE 400</td>
<td>9.33±0.015</td>
<td>6.33±0.5</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SD, One way ANOVA followed by Dunnett’s t’ test, *P<0.01, n=6)

The rates of sedimentation of RBCs were significantly reduced in the experimental groups treated with ethanol and aqueous extracts of both plants. The chloroform extract treated groups did not suppress the increase in the ESR level when compared to the positive control groups. The ethanol and aqueous extracts exerted their
maximum activity when rats were pre-fed with the extracts than as the therapeutic agents. It was reported that the flavanoids in the plants can neutralize the surface charges of RBCs (Mishra et al., 2009) and thereby reduce the ESR level. In conclusion, the flavanoids present in the ethanol and aqueous extracts account for their anti-arthritic activity by reducing the ESR level of red blood cells.

7.3.6. Serum Concentration of Tumour Necrosis Factor α and Interleukin 1β

Quantification of serum concentrations of TNF α and Interleukin 1β by Enzyme linked Immunosorbant Assay set light to the anti TNF α and Interleukin 1β activities of ethanol extracts of S. zeylanica and S. ovalifolia in adjuvant induced arthritis.

Figures 30 & 31 represents the significant increase in the concentrations of pro-inflammatory cytokines such as IL 1 β and TNF α in the serum of arthritic control rats (158.4 ± 4.3 & 86±2.1 pg/ml respectively) when compared to the negative (35.2±2.45 pg/ml and 27.6± 4.2 pg/ml respectively) control group. The ethanol extracts of both plants achieved a highly significant reduction in the serum concentration of IL 1 β (46±0.24 pg/ml for SZEE and 37±2.3 pg/ml for SOEE ) and TNF α (63±1.24 pg/ml for SZEE and 48± 1.23 pg/ml for SOEE) followed by the aqueous extracts (Figures 30-33). Synovial IL 1 β and TNF α expression was also highly elevated in the model group (arthritic control rats) as compared to the negative control group. The ethanol extracts of S. zeylanica as well as S. ovalifolia significantly reduced the synovial expression of IL 1 β and TNF α whereas the aqueous and chloroform extracts achieved minimal effect.

The role of pro-inflammatory cytokines such as TNF α and IL1 β in rheumatoid arthritis has been extensively studied and validated in preclinical animal models and in humans (Dinarello, 2002). TNF α is produced mainly by monocytes and macrophages and has an extremely broad spectrum of biological activities. It is one of the key cytokine
molecules that cause inflammation in rheumatoid arthritis. Apart from its autocrine stimulating role, it also acts as paracrine inducer of other inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8 and granulocyte monocyte-colony stimulating factor (Butler et al., 1995; Haworth et al., 1991 and Chen et al., 1999) and stimulate fibroblasts to express adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1) (Tilders, 1994). TNF α also stimulate synoviocyte and cartilage cells to synthesize PGE2 and collagenase causing synovium and cartilage destruction (Choy and Panayi, 2001).

The over expression of IL1 β in the lining layer and sub lining cells of inflammed synovium of rheumatoid arthritis patients were reported. Apart from this, an upregulation of IL1 β mRNA in the cartilage of the arthritis patients were noticed when compared to the normal control people (Lipsky et al., 2000; Olszewski et al., 2001; Nadiv et al., 2007). An increased production of IL1 β in the synoviocytes may lead to higher risk of severe joint damage even in the absence of systemic inflammation. Therefore according to the earlier studies, it is clear that the early phases of the disease seemed to be characterised by a systemic increase in the IL1 β and TNF α causing early joint swelling and the IL1 β combined immune complexes leading to cartilage and bone destruction. In majority of arthritic models, the key driver seems to be IL1 β and TNF α and along with IL6 involve in the early phases of inflammation and transition from the acute to the chronic phases of inflammatory process and hence there cytokines remain key target for an early intervention in human arthritis (Ferraccioli et al., 2010).

The present study revealed that the serum level of TNF α and IL1 β of rats in the model group (158.4 & 86 pg/ml respectively) were significantly higher than that of the normal control rats. The synovial expression of TNF α and IL1 β mRNA were significantly reduced in the ethanol extracts followed by the aqueous extracts (Figures 30-33) of the tested plants than the arthritic control rats. These observations correlated
well with the paw volume measurements in the experimental groups. The results of the present study suggested that the pro inflammatory cytokines such as TNF α and IL1 β are expressed in almost all types of arthritic animal models and human arthritic joints and blockade of these cytokines cause amelioration of the disease. Duan et al., (2001) reported that sesquiterpene, pyridine alkaloids from T. wilfordii, significantly inhibited the production of pro-inflammatory cytokines including TNFα, IL-1β, IL-4, IL-2 and IFN-γ in human peripheral mononuclear cells.

Lu et al., (2003) reported that the decoction of S. china (90, 180 g.kg-1) intra gastric injection could significantly inhibit adjuvant arthritis in mouse's secondary inflammatory swelling, reduced thymus and spleen weights, decreased CD4/CD8, but had little influence on B Cell. Luo et al., (2014) studied the therapeutic effect of the bioactive fraction of S. china on chronic pelvic inflammatory disease (C PID), where all the test doses of the bioactive fraction of S. china obviously decreased uterine TNFα content (P<0.01) and significantly increased uterine IL-4 expression level (P<0.05) and suggested that this might be a pharmacological mechanism underlying its therapeutic effect on CPID and cervical adhesion.

An in vivo study addressed the modulation of inflammatory mediators by coumarinolignoids in female Swiss albino mice by Bawankule et al., (2008) proved that the oral administration of coumarinolignoids significantly decreased the expression of pro inflammatory mediators such as IL-6, TNF-α and nitric oxide and increased the anti inflammatory IL-4 cytokine production from macrophages and spleenocytes in a dose dependant manner. A modest reduction in the LPS-induced pro-IL-1mRNA was reported by in vitro administration of Gammalinolenic acid which markedly reduced the auto-induction of IL-1induced pro-IL-1gene expression Furse et al., (2002). Cho et al., (2000) demonstrated that Paenol (2’-hydroxy,4’ methoxy acetophenon) exhibited
analgesic and anti-inflammatory effect mediated by the inhibition of the formation of several pro-inflammatory cytokines such as TNF α, IL-1β and IL-6 and over expression of NO and PGE2. Okabe et al., (2001) reported the prevention of TNF α release in BALB13T3 cells stimulated with okaic acid by Epigallochatechin gallate (EGCG), the main constituent of green tea polyphenols and aqueous extract of leaves of *Acer nickoense* Maxim. Aqueous extracts of bark of *Cinnamomum cassia* (560 mg/kg) and rhizomes of *Cnidium monnieri* (560 mg/kg) are also reported to decrease the TNF α and IL1 α release individually after two weeks administration in murine model in vivo studies (Haranaka et al., 1985).

The results of the present study suggests that the ethanol extracts of both plants *viz.*, *S. zeylanica* and *S. ovalifolia* reduced the serum level of TNF and IL1 β α (Figures 30-33) as well as the expression of TNF α mRNA (Figures 34 & 35) and IL1 β mRNA in the synovium of the arthritic rats when compared to the model group both in the prophylactic as well as therapeutic model of arthritic study (Plate 17 & Figures 36 & 37). The findings of the present study well correlated with the result of Perera et al.,(2010) where the ploy herbal formulation Yi Shen Juan Bi (YJB) could significantly reduced the level of TNF α and IL1 β in serum and TNF α and IL1 β mRNA expression in synovial cells. Hence, it could be therefore advocated that this might be one of the mechanisms by which the extracts ameliorate the arthritis.
Figure 30: Therapeutic effect of different extracts of *S. zeylanica* on TNF $\alpha$ and IL1 $\beta$ in serum of adjuvant induced arthritic rats

One way ANOVA followed by Dunnett’s test. Values are expressed as mean± SD, n=6.

Figure 31: Therapeutic effect of different extracts of *S. ovalifolia* on TNF $\alpha$ and IL1 $\beta$ in serum of adjuvant induced arthritic rats

One way ANOVA followed by Dunnett’s test. Values are expressed as mean± SD, n=6.
Figure 32: Serum concentrations of TNFα and IL1β in adjuvant induced arthritic rats pre-fed with different extracts of *S. zeylanica*

One way ANOVA followed by Dunnett’s test Values are expressed as mean± SD, n=6.

Figure 33: Serum concentrations of TNFα and IL1β in adjuvant induced arthritic rats pre-fed with different extracts of *S. ovalifolia*

One way ANOVA followed by Dunnett’s test Values are expressed as mean± SD, n=6.
Plate 17: Therapeutic effect of different extracts of *S. ovalifolia* and *S. zeylanica* on expression of TNF α and IL 1 β in the synovium of adjuvant induced arthritic rats

Lane 1: Control, Lane 2: Arthritic control, Lane 3: Diclofenac sodium, Lane 4: Aqueous extract of *S. zeylanica*, Lane 5: Ethanol extract of *S. zeylanica*, Lane 6: Aqueous extract of *S. ovalifolia* and Lane 7: Ethanol extract of *S. ovalifolia*.

Plate 18: Prophylactic effect of different extracts of *S. ovalifolia* and *S. zeylanica* on expression of TNF α and IL 1 β in the synovium of adjuvant induced arthritic rats

Lane 1: Control, Lane 2: Arthritic control, Lane 3: Diclofenac sodium, Lane 4: Aqueous extract of *S. zeylanica*, Lane 5: Ethanol extract of *S. zeylanica*, Lane 6: Aqueous extract of *S. ovalifolia* and Lane 7: Ethanol extract of *S. ovalifolia*.
Figure 34: Relative quantification chart of TNF α expression in Therapeutic model of Anti-arthritic study

![Diagram showing relative quantification chart of TNF α expression in Therapeutic model.](image)

Values are expressed as Mean ±standard deviation, n=3; *: compared to arthritic control; #: p<0.05 compared to normal control.

Figure 35: Relative quantification chart of TNF α expression in Prophylactic model of Anti-arthritic study

![Diagram showing relative quantification chart of TNF α expression in Prophylactic model.](image)

Values are expressed as Mean ±standard deviation, n=3; *: compared to arthritic control; #: p<0.05 compared to normal control.
Figure 36: Relative quantification chart of IL 1β expression in Therapeutic model of Anti-arthritic study

Values are expressed as Mean ± standard deviation, n=3; *: compared to arthritic control; #: p<0.05 compared to normal control

Figure 37: Relative quantification chart of IL 1β expression in Prophylactic model of Anti-arthritic study

Values are expressed as Mean ± standard deviation, n=3; *: compared to arthritic control; #: p<0.05 compared to normal control
7.3.7. In Vivo Anti Oxidant Activity

Reactive oxygen species plays a major role in the pathogenesis of rheumatoid arthritis. Superoxide radicals, hydrogen peroxide and the highly reactive hydroxyl radicals are generated by monocytes, neutrophils and macrophages during phagocytosis. In arthritic condition, the granulocytes and macrophages accumulate in the affected area and produce large amounts of superoxide and hydrogen peroxide radicals (Halley and Cheeseman, 1993). Oxygen free radicals have been implicated as mediators of tissue damage in patients with rheumatoid arthritis.

Table 34: In vivo anti oxidant activity of different extracts of S. zeylanica and S. ovalifolia in adjuvant induced arthritis

<table>
<thead>
<tr>
<th></th>
<th>Total proteins mg/dl</th>
<th>Peroxidase nm/100mg tissue</th>
<th>SOD U/mg tissue</th>
<th>Catalase U/mg tissue</th>
<th>Glutathione nm/100mg tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>10.31±0.22</td>
<td>27.17±2.05</td>
<td>3.18±0.80</td>
<td>6.83±0.64</td>
<td>49.66±2.48</td>
</tr>
<tr>
<td>Arthritic Control</td>
<td>4.39±0.149</td>
<td>15.98±0.121</td>
<td>1.57±0.32</td>
<td>1.78±0.03</td>
<td>31.96±1.60</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>11.61±0.46*</td>
<td>24.71±1.30*</td>
<td>2.83±0.07*</td>
<td>3.41±0.23*</td>
<td>49.18±1.34*</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10.7±0.33*</td>
<td>23.65±0.67*</td>
<td>2.51±0.15*</td>
<td>3.13±0.25</td>
<td>49.5±3.03*</td>
</tr>
<tr>
<td>SZAE 200</td>
<td>6.89±0.64*</td>
<td>24.75±3.30*</td>
<td>2.03±0.58*</td>
<td>2.22±0.22</td>
<td>50.53±3.81*</td>
</tr>
<tr>
<td>SZEE 200</td>
<td>10.33±0.11*</td>
<td>28.01±0.51*</td>
<td>3.39±0.37*</td>
<td>3.39±0.17*</td>
<td>52.55±2.06*</td>
</tr>
<tr>
<td>SZCE 200</td>
<td>3.61±0.46</td>
<td>18.04±1.12</td>
<td>1.62±0.25</td>
<td>1.78±0.08</td>
<td>30.52±0.65</td>
</tr>
<tr>
<td>SZAEE400</td>
<td>9.52±0.37*</td>
<td>28.13±2.00*</td>
<td>3.17±0.32*</td>
<td>3.53±0.32*</td>
<td>50.87±2.56*</td>
</tr>
<tr>
<td>SZEE 400</td>
<td>11.76±0.461*</td>
<td>29.28±1.15*</td>
<td>3.91±0.05*</td>
<td>4.33±0.57*</td>
<td>56.04±1.19*</td>
</tr>
<tr>
<td>SZCE 400</td>
<td>4.63±0.205</td>
<td>19.22±0.31</td>
<td>1.64±0.34</td>
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<td>SOAE 200</td>
<td>8.61±0.41*</td>
<td>23.47±0.56*</td>
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<td>51.79±1.47*</td>
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<td>SOEE 200</td>
<td>11.5±0.68*</td>
<td>27.98±0.56*</td>
<td>3.43±0.21*</td>
<td>4.16±0.63*</td>
<td>54.72±5.11*</td>
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<td>SOCE 200</td>
<td>3.89±0.57*</td>
<td>15.48±0.61</td>
<td>2.14±0.55</td>
<td>1.83±0.16</td>
<td>38.68±0.82</td>
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<td>SOAE 400</td>
<td>11.19±0.99*</td>
<td>28.68±0.95*</td>
<td>3.51±0.22*</td>
<td>3.76±0.15*</td>
<td>54.22±1.48*</td>
</tr>
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<td>SOEE 400</td>
<td>12.88±0.64*</td>
<td>30.12±0.92*</td>
<td>3.9±.10*</td>
<td>5.03±0.30*</td>
<td>57.43±0.15*</td>
</tr>
<tr>
<td>SOCE 400</td>
<td>4.34±1.11</td>
<td>16.15±0.19</td>
<td>2.01±0.08</td>
<td>1.99±0.01</td>
<td>39.34±0.30</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM. One way ANOVA followed by Turkey Kramer multiple comparison test *p<0.01 when compared with the arthritic control.
Reactive nitrogen intermediate like NO activates matrix metallo proteinases which inhibits collagen and proteoglycan synthesis by chondrocytes and promotes vasodilation which sequentially leads to fluid and cellular influx into an inflammatory site. In combination with ROS, NO produces peroxo nitrite which promotes chondrocyte apoptosis (Lotz et al., 1999). The anti oxidant enzymes like super oxide dismutase, catalase, peroxidase etc. compose a defence mechanism against the free radical produced in our body. Oxidative stresses are developed through a series of events and develop different diseases when this oxidative balance collapses. Catalase is an enzymatic haemoprotein localized in the peroxisomes and micro-peroxisomes which decomposes hydrogen peroxide to water and oxygen protects tissues from highly reactive hydroxyl radicals (Chance et al., 1952). Reduction in the anti oxidant enzymes in the body results in the accumulation of free radicals and hydrogen peroxide (Gupta et al., 2003). Glutathione is intra-cellular thiol rich tripeptide which plays a major role in the protection of cells and tissue structures (Anderson, 1998). Glutathione (GSH) is a tripeptide non-enzymatic biological antioxidants abundantly present in liver (Anderson, 1998) which acts as a substrate for the H2O2 removing enzyme glutathione peroxidase and for dehydroascorbate reductase (Ahmad, et al., 2001). Murali et al., (2010) demonstrated that the methanol extract of S. zeylanica (p<0.001) increased the hepatic levels of glutathione (200 mg/kg, 400 mg/kg and 600 mg/kg dose levels) when compared to the control in carbon tetra chloride induced liver toxicity in Wistar albino rats. Similarly low concentration of glutathione has been implicated in rheumatoid arthritis also. Ahemad et al., (2012) also demonstrated the anti cataleptic and antioxidant effects of ethanol root extract of S. zeylanica in haloperidol-induced catalepsy and oxidative stress in rats.

In the present study, the quantity of the anti oxidant enzymes such as peroxidase, super oxide dismutase, catalase and glutathione were significantly reduced in the arthritic
control group. A significant increase in the anti oxidant enzymes were noted in animals treated with ethanol and aqueous extracts of both plants. But the amounts of anti oxidant enzymes in the chloroform extract treated groups were drastically reduced as seen in the model group (Table 34). It is clear from the present study that the oral administration of ethanol extracts achieved a maximum anti oxidant activity which complement in their anti inflammatory and anti oxidant properties.

The ethanol extracts of both plants exerted a maximum reduction in the paw oedema in the adjuvant induced chronic arthritic model in Wistar Albino rats. The radiological examination and histopathology of paw joints support the efficacy of these extracts both in the therapeutic and prophylactic models. The aqueous extracts also produced a significant anti-arthritic activity next to the ethanol extracts. These extracts significantly reduced the expression of pro inflammatory cytokines such as TNF α and IL1β in the rat synovial tissue and their level in the circulating blood. A significant anti oxidant activity was also noted with these enzymes which account for their anti-arthritic activity.