RESULTS AND DISCUSSION
RESULTS AND DISCUSSION

*T.cordifolia* is widely used in Ayurvedic preparations for the treatment of various diseases viz. Inflammation, gout, arthritis, chronic fever, seminal weakness and jaundice. Eventhough its usage in ayurveda is numerous, a planned pharmacological and biochemical screening for its anti-inflammatory, antirheumatic, antioxidant and immunomodulatory activities were carried out in the present work.

3.1 TOXICITY STUDY OF TCE

There was no mortality in rats which received TCE upto a dose of 1600 mg/kg body weight orally. The animals were normal and did not exhibit symptoms of toxicity during the first 6 hr of continuous observations and subsequently for 10 days. This result indicates that TCE is non-toxic and safe.

3.1.1 Ulcerogenic Index

There was no sign of ulceration in the stomach of the rats which received TCE at the dose of 100, 200 and 400 mg/kg body weight orally for a period of six days.

It is currently accepted that NSAIDs cause gastrointestinal mucosal damage through inhibitory effect on prostaglandin synthesis. The reference drug used in this study was nimesulide. The reported ulcerogenic dose of nimesulide in rat is 20 mg/kg body weight (Ceserani et al., 1991).
In the present study, it is observed that TCE up to the dose of 400 mg/kg body weight orally for six days did not exhibit ulcerogenic effect when compared to the reference standard drug nimesulide and control rats. This study indicates TCE is not an ulcerogenic agent.

In an earlier study by Sarma et al. (1995a), T.cordifolia exhibited anti-ulcer activity against stress-induced gastric ulcer. This study correlates with the present finding, that TCE is not an ulcerogenic agent and TCE acts as a mucosal protective agent of the gastrointestinal tract.

3.2 PHARMACOLOGICAL ACTIVITY OF TCE

3.2.1 Anti-pyretic Activity of TCE

Figure 1 shows the antipyretic activity of the TCE. TCE exhibited a significant reduction of pyrexia at the dose of 100 and 200 mg/kg body weight orally in yeast induced pyretic rats. The anti-pyretic effect, after TCE treatment continued for 270 minutes and beyond, when compared to untreated rats. Reference drug nimesulide showed better anti-pyretic activity at the dose of 5 mg/kg body weight. It has been reported that ethanolic extract of whole plant of T.cordifolia at the dose of 500 mg/kg body weight showed significant anti-pyretic activity in rats (Vedavathy and Rao, 1990). This anti-pyretic action of T.cordifolia along with the anti-inflammatory and analgesic activity has made this drug a common prescription by Ayurvedic physicians for the treatment of rheumatic fever and rheumatoid arthritis (Sahastrayogam, 1986).
FIGURE 1. ANTI-PYRETIC STUDY IN TCE TREATED RATS

- Group I
- Group II
- Group III
- Group IV
3.2.2 Analgesic Activity of TCE

Figure 2 shows the analgesic activity of TCE in rats evaluated by the hot plate method. TCE showed a dose dependent (100 and 200 mg/kg body weight) analgesic activity in rats, comparable to that of standard NSAID drug nimesulide (5 mg/kg body weight). The analgesic activity of *T. cordifolia* has not been reported earlier. This analgesic activity of TCE has to play an important role in the treatment of RA. The pharmacological activity of *T. cordifolia* as an analgesic, antipyretic and anti-inflammatory agent with least ulcerogenic potential, justifies its importance as a single drug or in combination with other herbs in the treatment of rheumatic fever and RA by the practioners of Indian System of Medicine (Sahastrayogam, 1986).

3.3 IMMUNO - MODULATORY ACTIVITY OF TCE

3.3.1 Candida Phagocytosing Activity of TCE

Polymorphonuclear neutrophils (PMN) and mononuclear phagocytes may play a vital role, when a foreign antigen enter into our system. In inflammatory conditions reduced chemotaxis, adhesiveness, and intracellular bactericidal activity were seen. Many of the lipid peroxidation products, and particularly hydroperoxides and aldehydes derivatives, can block macrophage action and cause changes in chemotaxis and enzyme activity (Fridovich and Porter, 1981).

Table 1 shows a significant (p<0.001) decrease in the phagocytic index and a significant (p<0.01) decrease in avidity index was observed in
FIGURE 2. ANALGESIC STUDY IN TCE TREATED RATS
Table 1: Effect of TCE on candida phagocytosis of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytic index</td>
<td>70.17 ± 2.93</td>
<td>54.18 ± 2.04\textsuperscript{a***}</td>
<td>67.17 ± 2.86\textsuperscript{b***}</td>
<td>72.0 ± 3.74\textsuperscript{b***}</td>
</tr>
<tr>
<td>Avidity index</td>
<td>3.34 ± 0.58</td>
<td>2.38 ± 0.28\textsuperscript{a**}</td>
<td>3.51 ± 0.65\textsuperscript{b**}</td>
<td>3.81 ± 0.17\textsuperscript{b***}</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

One way analysis of variance and student Newmann Keul's test.

Treatment of groups are as follows : Group I - control; Group II - arthritis induced; Group III - arthritis induced + drug (100 mg/kg b.wt) for 14 days after 2 weeks from the day of adjuvant injection; Group IV - arthritis induced + drug (100 mg/kg b.wt) for 28 day from the day of adjuvant injection.

Comparisons are made with a - Group I; b - Group II and c - Group III

Values are statistically significant when \textsuperscript{***} p < 0.001; \textsuperscript{**} p < 0.01; \textsuperscript{*} p < 0.05
group II animals when compared with group I. Drug treatment significantly increased both phagocytic and avidity index of arthritic rats (group III and IV) when compared with untreated arthritic rats (group II).

Large quantities of phospholipase A$_2$ (PLA$_2$) with proinflammatory activity were found in synovial fluids of inflamed joints (Pruzanski et al., 1991). Purified human synovial fluid phospholipase A$_2$ markedly reduced chemotaxis, adhesiveness, and intracellular bactericidal activity of human PMN and it caused spontaneous superoxide generation followed by inhibition of phagocytosis-induced burst of energy. PLA$_2$ from sera of active RA patients caused spontaneous formation of the oxygen free radical superoxide and release of lysosomal enzymes and suppress conventional phagocytic activity of PMNs and monocytes (Pruzanski et al., 1991). Complete functional deficiency of phagocytic cell enzyme, myeloperoxidase is described in patient with rheumatoid arthritis. Measurement of oxygenation and free radical activity by blood monocytes and polymorphonuclear leukocytes shows gross reduction in myeloperoxidase-dependent chemiluminescence (Bell et al., 1993).

A healthy inflammatory response and the immune dysregulation frequently observed in inflammatory conditions due to altered Schiff base formation between ligands on antigen presenting cells and T cells is essential for antigen-induced T cell activation (Winrow et al., 1993).

Salin and Mc Cord (1975) demonstrated a protective effect of SOD, catalase and mannitol on the viability and integrity of phagocytosing PMN in vitro.
Immuno-modulating agents have been reported to act primarily on cellular rather than humoral immune response and to restore the immuno-competency of impaired hosts without hyperstimulating the normals. It augments macrophage chemotaxis, phagocytosis and promotes interaction with other immunoregulatory lymphoid cells.

*Tinospora cordifolia* possess anticomplementary and immunomodulatory activities. Active principles of *Tinospora cordifolia* showed reduced immunohaemolysis, and increased IgG antibodies in serum. Humoral and cell mediated immunity were also dose dependently enhanced. Macrophage activation was seen in mice treated with active principles of *Tinospora cordifolia* (cordioside, cordiofolioxide A and cordiol) (Kapil and Sharma, 1997).

Dhyley (1997) demonstrated the effect of some Indian herbs on macrophage functions in ochratoxin A treated mice, in which *Tinospora cordifolia* treated mice showed significantly increased macrophage chemotaxis. Chronic inflammatory diseases are fundamental disorders of immune regulation. The development of adjuvant arthritis in rats is thought to be a cell mediated delayed type hypersensitivity reaction to mycobacterial fragments (Kayashima *et al*., 1978). TCE activates the immune regulation and is capable of increasing the phagocytosing capacity of lymphocytes which is the most important beneficiary effect of the drug.
3.4 ANTI-INFLAMMATORY ACTIVITY OF TCE

3.4.1 Carrageenan - Induced Paw Oedema

Carrageenan - induced hind paw oedema (an in vivo model) has been useful for the study of mediators found in developing oedema associated with inflammation (Winter et al., 1962). The test drug used for experimental study has anti-inflammatory property, can be able to prevent this inflammatory oedema formation.

Figure 3 shows the anti-inflammatory activity of TCE (50, 100, 150, 200 and 250 mg/kg b.wt.) in carrageenan - induced hind paw oedema volume of rats. The volume of paw oedema was maximum at 2 hours after carrageenan administration and then there was a gradual decline.

TCE in doses from 50 to 250 mg/kg b.wt. administered orally showed a dose dependent anti-inflammatory activity in carrageenan - induced hind paw oedema in rats when compared with control rats. A significant reduction in the volume of oedema was observed in rats receiving 100mg and above per kg b.wt. The significance was less at the dose of 50mg/kg b.wt. when compared with the control rats.

Hence, further investigation on biochemical parameters related with inflammatory response was carried out with 100mg/kg b.wt. of TCE orally.

Carrageenan induced hind paw oedema has been useful for the evaluation of acute inflammatory conditions in experimental rats (Winter et al.,
FIGURE 3. EFFECT OF TCE ON PAW OEDEMA VOLUME OF CARRAGEENAM-INDUCED ARTHRITIC RATS
1962). In the present study, TCE exhibited anti-inflammatory activity by arresting the developments of oedema associated with acute inflammation. This anti-inflammatory activity of TCE may be attributed to a mechanism by which it inhibits the synthesis of mediator of inflammation like prostaglandin and/or anti-oxidant property against free radicals generated at the inflammatory tissues.

3.4.2 Adjuvant Induced Paw Oedema

Figure 4 shows the effect of TCE on hind paw oedema of rats with adjuvant induced arthritis. Administration of TCE (100mg/kg b.wt.) orally from the day 1 to 28 of the developing arthritis as well as the treatment started from 14th day to 28th day of established arthritic rats showed significant reduction in the volume of oedema when compared with the control rats.

TCE inhibits the oedema formation in both developing and established arthritis which provides the drug a wide therapeutic use in the treatment of RA as stated in the text of Ayurveda (Sahastrayogam, 1986).

3.4.3 Body Weight Changes

Figure 5 shows effect of TCE on the body weight changes in control and arthritic rats. Administration of TCE in normal and adjuvant induced arthritic rats showed a significant gain in body weight. Tinospora cordifolia is described as a "Rasayana" drug in Ayurvedic texts. Rasayana drug is prescribed to gain weight during the recovery from chronic illness
FIGURE 4. EFFECT OF TCE ON PAW OEDEMA VOLUME OF ADJUVANT-INDUCED ARTHRITIC RATS

Days

0 2 4 6 8 10 12 14 16 18 20 22 24 26 28

Oedema volume (ml)

0 1 2 3 4 5 6 7 8

Group I Group II Group III Group IV Group V
FIGURE 5. BODY WEIGHT CHANGES IN NORMAL AND ARTHRITIC RATS
(Shahastryogam, 1986). The mechanism of action of Rasayana drugs is not clearly understood.

Further studies conducted on certain routine biochemical and hematological parameters and other biochemical parameters like lysosomal enzymes, glycoproteins, lipid peroxidation, antioxidants, phagocytosing activity of leukocytes, membrane bound ATPases, in both developing and established arthritic rats showed TCE plays an important therapeutic role to bring about normalcy in the altered parameters and may be accounted for the anti-rheumatic activity.

### 3.5 ROUTINE BIOCHEMICAL PARAMETERS

Table 2 & 3 shows the effect of *Tinospora cardifolia* on routine biochemical parameters of control and arthritic rats.

Serum glucose and uric acid level of arthritic rats did not show any significant changes when compared with control. Blood urea nitrogen (Bun) and creatinine levels in serum were decreased significantly (p<0.001) in arthritic rats when compared with group I animals. After the treatment with the TCE the levels were brought back to near normal.

Levels of alanine transaminase (ALT) and aspartate transaminase (AST) did not show any significant change in arthritic conditions when compared with normal animals. Whereas serum alkaline phosphatase (ALP) showed significant (p<0.05) increase in group II rats when compared with
Table 2:  Effect of TCE on few serum biochemical parameters of control and adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</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>Glucose</td>
<td>79.83 ± 4.71</td>
<td>80.17 ± 4.62</td>
<td>78.5 ± 3.83</td>
<td>77.5 ± 3.02</td>
<td>75.61 ± 4.8</td>
</tr>
<tr>
<td>Bun</td>
<td>15.3 ± 1.6</td>
<td>11.05 ± 1.6***</td>
<td>10.08 ± 1.86b*</td>
<td>13.10 ± 0.28b*<strong>c</strong></td>
<td>15.07 ± 0.16b***</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.18 ± 0.30</td>
<td>0.68 ± 0.04***</td>
<td>0.83 ± 0.10a*</td>
<td>0.02 ± 0.19b**</td>
<td>1.20 ± 0.049b***</td>
</tr>
<tr>
<td>Uric acid</td>
<td>3.0 ± 0.4</td>
<td>2.62 ± 0.3</td>
<td>2.83 ± 0.27</td>
<td>3.1 ± 0.43</td>
<td>3.04 ± 0.22</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

One way analysis of variance and student Newmann Keul’s test.

Treatment of groups are as follows: Group I - control; Group II - arthritis induced; Group III - arthritis induced + drug (100 mg/kg b.wt) for 14 days after 2 weeks from the day of adjuvant injection; Group IV - arthritis induced + drug (100 mg/kg b.wt) for 28 day from the day of adjuvant injection; Group V - Control animal + drug (100 mg/kg b.wt) for 28days.

Comparisons are made with a - Group I; b - Group II and c - Group III; d - Group IV

Values are statistically significant when *** p < 0.001; ** p < 0.01; * p < 0.05
Table 3: Effect of TCE on few serum enzymes of adjuvant induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT IU/l</td>
<td>31.58 ± 5.7</td>
<td>33.70 ± 5.0</td>
<td>30.08 ± 1.50</td>
<td>34.97 ± 6.8</td>
</tr>
<tr>
<td>SGOT IU/l</td>
<td>24.17 ± 4.11</td>
<td>27.67 ± 3.84</td>
<td>25.75 ± 1.7&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>28.83 ± 1.44&lt;sup&gt;a*&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALP IU/l</td>
<td>47.2 ± 2.36</td>
<td>51.92 ± 3.29&lt;sup&gt;a*&lt;/sup&gt;</td>
<td>46.75 ± 4.80&lt;sup&gt;b*&lt;/sup&gt;</td>
<td>44.92 ± 1.36&lt;sup&gt;b**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

<sup>***</sup> p < 0.001; <sup>**</sup> p < 0.01; <sup>•</sup> p < 0.05
group I animals. Drug treatment revert back the changes in arthritic condition to near normal.

3.5.1 Haematological Studies

Table 4 depicts the haematological changes associated with arthritic conditions. Levels of hemoglobin, packed cell volume, RBC count were significantly decreased in group II animals with concomitant increase in WBC count and erythrocyte sedimentation rate when compared with control (group I) rats. The changes observed in arthritic rats were reverted back to near normal level in group III and group IV animals after the drug treatment. Group V animals did not show any significant change when compared with group I animals.

In rheumatoid arthritic patients an increased total leukocyte and platelet counts (Kjeldsen-Kragh et al., 1995), ESR (Kavanaghi et al., 1995; Aida, 1993) and a decreased haemoglobin (Vreugdenhil, 1992) and a reduction in serum creatinine (Mody et al., 1991), were reported.

Mody et al. (1991) demonstrated the most common biochemical abnormalities associated with arthritis were reduction in serum creatinine and raised serum alkaline phosphatase.

Hyperphosphatasaemia has been observed in patients with rheumatoid arthritis, and it has been suggested that the serum alkaline phosphatase (ALP) level is related to the activity of the disease. Studies have
Table 4: Effect of TCE on haematological parameters of control and adjuvant-induced arthritic 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>WBC (thousands/mm³)</td>
<td>6.0 ± 0.35</td>
<td>8.29 ± 0.48**</td>
<td>6.02 ± 0.39***</td>
<td>6.2 ± 0.32b***</td>
<td>6.65 ± 0.77b***</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.33 ± 1.22</td>
<td>12.33 ± 0.96a*</td>
<td>13.55 ± 1.2</td>
<td>14.94 ± 1.33b**</td>
<td>14.09 ± 0.23</td>
</tr>
<tr>
<td>RBC (millions/mm³)</td>
<td>5.02 ± 0.17</td>
<td>4.31 ± 0.45***</td>
<td>5.01 ± 0.16b**</td>
<td>5.2 ± 0.19b***</td>
<td>4.94 ± 0.09b**</td>
</tr>
<tr>
<td>PCV %</td>
<td>42.98 ± 3.6</td>
<td>37.0 ± 2.88a*</td>
<td>40.17 ± 3.71</td>
<td>44.83 ± 3.98b**</td>
<td>43.50 ± 0.89b**</td>
</tr>
<tr>
<td>ESR mm/1hr</td>
<td>303.00 ± 0.52</td>
<td>7.08 ± 0.59***</td>
<td>4.17 ± 0.52***</td>
<td>3.42 ± 0.74b***</td>
<td>3.17 ± 0.75b**<em>c</em></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 2

Comparisons are made as in table 2

*** p < 0.001; ** p < 0.01; * p < 0.05.
also showed that there is no significant difference or correlation was noted for either aspartate transaminase or alanine transaminase with RA (Aida, 1993).

The impaired activity of antioxidants level observed during arthritic condition may be due to the increased susceptibility to oxygen radical mediated lipid peroxidative damage, which in turn may be a contributing factor to the pathogenesis of anemia associated with rheumatoid arthritis (Richmond et al., 1961).

### 3.6 SERUM PROTEINS

Plasma total protein and albumin were reduced at the chronic phase of Freund's complete adjuvant induced arthritic rats (Fahim et al., 1995). Lowered A/G ratio was observed in chronic (21 and 29 days after adjuvant inoculation) arthritic rats (Eldin et al., 1992; Sugawara et al., 1992).

Table 5 depicts the effect of drug on the serum proteins level in arthritic rats. Serum levels of total protein, albumin and globulin were significantly altered in arthritic rats when compared with control animals. The drug treatment, however, did not show significant changes in serum proteins. In the present study, drug treatment did not reverse the altered A/G ratio in arthritic rats.

Microalbuminuria is frequently present in patients with rheumatoid arthritis. Treatment with gold and penicillamine seems to increase the risk of developing microalbuminuria. Previous studies have showed that the urinary
Table 5: Effect of TCE on serum proteins of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters (g/dl)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>5.970 ± 0.20</td>
<td>5.560 ± 0.14***</td>
<td>5.495 ± 0.12***</td>
<td>5.565 ± 0.09***</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.03 ± 0.12</td>
<td>3.18 ± 0.22***</td>
<td>3.15 ± 0.11***</td>
<td>3.28 ± 0.10***</td>
</tr>
<tr>
<td>Globulin</td>
<td>1.95 ± 0.24</td>
<td>2.37 ± 0.24**</td>
<td>2.35 ± 0.16*</td>
<td>2.28 ± 0.13*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1.

Comparisons are made as in table 1.

*** p < 0.001; ** p < 0.01; * p < 0.05.
albumin to creatinine ratio in patients with rheumatoid arthritis was significantly greater than in control. Urinary albumin measure is a simple and sensitive test to detect early subclinical renal dysfunction and drug induced renal damage in rheumatoid arthritis. Urinary albumin excretion was found to be significantly correlated with C-reactive protein and may be a sensitive indicator of disease activity in patients with rheumatoid arthritis (Pedersen et al., 1995).

The hepatic mRNA concentration of albumin was decreased in adjuvant arthritic rats, reaching their lowest level on day 11 to 15. As early as 4 days after arthritis induction, the hepatic mRNA level of albumin distinctly differed from control values (Geiger et al., 1992).

A case study by Mody et al. (1991) demonstrated, that reduction in serum albumin and raised serum globulins were found in a study of 256 rheumatoid arthritic patients of 104 coloured, 100 white and 52 black.

In the present study, however, there is no marked change in the serum protein level was observed during the course of treatment with TCE.

3.7 LIPID PROFILE

Development of paw inflammation after an injection with Freund's complete adjuvant, was significantly increased in male albino rats fed with high lipid diet for 5 consecutive weeks, than the rats not fed a high lipid diet (Yossif et al., 1995).
Table 6 shows the serum lipid level changes associated with adjuvant arthritis and its treatment with TCE. The serum concentration of total cholesterol, Triglycerides, LDL cholesterol and phospholipids were significantly (p<0.001) increased in Group II animals when compared with Group I animals. Free fatty acid level of serum, increased significantly in group II (p<0.01) animals when compared with control and a concomitantly decreased level (p<0.001) of HDL cholesterol was found in arthritic rats. Treatment with the TCE reversed the changes observed in arthritic condition.

In rheumatoid arthritic diseases oxygen free radicals (OFR) damage is thought to exert a pathogenic role. Polyunsaturated fatty acid (PUFA) are the main target of OFR lipoperoxidative injury (Azzini et al., 1994). Altered levels of serum lipid profile was found in RA patients (Munro et al., 1997).

Mishra and Pandey (1996) demonstrated that the serum levels of lipids and lipoproteins were significantly increased in rheumatoid arthritic patients when compared with normal control. Increased levels of serum triglycerides, total cholesterol and cholesterol content of LDL and VLDL were found in rheumatoid arthritis subjects. This suggests that arthritic subjects were relatively at higher risk of developing coronary heart disease. Furthermore hyper cholesterololaemia may aggravate the risk condition in arthritic patients by atherosclerosis.

Familial hypercholesterolemia is a disorder of lipoprotein metabolism characterised by elevated cholesterol, and LDL. These are the characteristic features of atherosclerosis. The same above observation was reported in RA by...
Table 6: Effect of TCE on serum lipid profile of control adjuvant induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/dL)</td>
<td>138.00 ± 21.66</td>
<td>174.83 ± 16.29***</td>
<td>150.67 ± 10.19b*</td>
<td>149.83 ± 4.07b*</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>177.30 ± 12.18</td>
<td>247.00 ± 20.85***</td>
<td>201.00 ± 23.0b***</td>
<td>192.67 ± 18.23b***</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>58.83 ± 3.39</td>
<td>45.92 ± 2.91***</td>
<td>50.25 ± 3.14***</td>
<td>56.25 ± 3.62b***</td>
</tr>
<tr>
<td>HDL - cholesterol</td>
<td>42.00 ± 8.56</td>
<td>72.67 ± 6.53***</td>
<td>60.33 ± 6.59***b</td>
<td>54.18 ± 5.85b<em><strong>b</strong></em></td>
</tr>
<tr>
<td>LDL - cholesterol</td>
<td>16.40 ± 2.10</td>
<td>24.22 ± 3.12***</td>
<td>22.15 ± 3.09**</td>
<td>21.15 ± 2.60a*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>97.50 ± 10.00</td>
<td>124.83 ± 14.5a**</td>
<td>106.00 ± 11.80</td>
<td>100.83 ± 17.30b*</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
Genel et al. (1997). Oxidatively modified low density lipoprotein has many biological activities which could contribute to the pathology of atherosclerotic lesion. James et al. (1998) demonstrated the presence of mild level of oxidised low density lipoprotein in synovial fluid of inflammatory joints.

Lower levels of the high density lipoprotein cholesterol (HDLc), apolipoprotein (A1) were found in RA patients (Seriolo et al., 1996) and juvenile RA patients (Tselepis et al., 1999). Serum triglyceride levels are significantly higher and serum high-density lipoprotein cholesterol levels are lower in patients with gout compared with healthy individuals (Weinberger, 1995).

The cholesterol content in the erythrocyte membrane of RA patients was significantly lowered as compared with that of control. The erythrocyte membrane of RA patients exhibit major abnormality in the poly unsaturated fatty acids of phospholipids with the level of eicosapentaenoic acid (Omega-3, 20:5) being significantly reduced (Masoom Yasinzai, 1996). This may be due to erythrocyte membrane disruption by inflammatory mediators which may finally lead to anemia. RA patients exhibited a number of disturbances in their lipoprotein composition which arise from the abnormal activity of lipid metabolising enzymes (Kitagawa et al., 1992).

All the above abnormalities associated with adjuvant arthritic rats were corrected by the administration of TCE and has showed the beneficial effect of the drug as an anti-inflammatory agent.
3.8 LYSOSOMAL ENZYMES

Lysosomal contents are held to be responsible for many of the local lesions of rheumatoid arthritis. Anderson (1970) reported that the increase in oedema of both hind paws of rats after adjuvant injection is paralleled by increases in the extracellular activities of lysosomal enzymes in the paw homogenates. The primary reason for implicating lysosomes in such changes is that each of the local lesions of human disease can be developed in laboratory animals injected with lysates of purified lysosomes. Moreover agents that disrupt biomembranes (especially those of lysosomes) produce changes in joints morphologically similar to lysosome-induced arthritis and to human disease (Weissmann et al., 1969). Disruption of the lysosomal membrane liberates lysosomal hydrolases that act upon the cellular proteins, nucleic acid and polypeptides.

Studies during past decades have indicated that lysosomes mediate at least in part, acute and chronic inflammation in joints. The enzymes present in the lysosomes can provoke inflammation, tissue injury and breakdown of connective tissues. The characteristic features of adjuvant induced arthritis is the extrusion of lysosomal hydrolases into the extra cellular compartment to provoke inflammation (Weissmann, 1972).

The local lesions of rheumatoid arthritis are characterised by the margination of leukocytes and their appearance in synovial fluid; hypertrophy and hyperplasia of synovial lining cells, many of which contain abnormal number and configurations of lysosomes; infiltration of the synovium by many
lymphocytes, frequently in cluster; the transformation of synovium into granulation tissue, which, as pannus, invades cartilage; and erosion of cartilage, initially of matrix and followed by chondrocyte death and attempts at regeneration (Weissmann, 1972).

Progressive destruction of cartilage occurs in chronic inflammatory joint diseases, such as rheumatoid arthritis, is probably brought about by the concerted action of proteinases derived from several types of cell. Because 90% of the cells found in RA synovial fluid (SF) are neutrophils (Barrett, 1978), these cells have been implicated as major mediators of tissue destruction observed through release of oxygen metabolites and proteinases, such as elastase cathepsin G and collagenase (Henson and Johnson, 1987).

Table 7,8 & 9 show the levels of lysosomal enzymes β-D-glucuronidase, β-D-galactosidase, acid phosphatase, cathepsin D and β-N-acetyl glucosaminidase of plasma, liver and kidney.

A significant (p<0.001) increase in the lysosomal enzymes such as β-D-glucuronidase, β-D-galactosidase, acid phosphatase, cathepsin-D and β-N-acetyl glucosaminidase of plasma, liver and kidney were seen in arthritic rats when compared with normal animals.

Plasma level of lysosomal enzymes of drug treated (group III and IV) rats showed a significant (p<0.001) reduction in β-D. glucuronidase, Acid phosphatase, Cathepsin-D, and β.N. acetyl glucosaminidase levels when compared with arthritic (Group II) rats. β-D. galactosidase level of plasma also
Table 7: Effect of TCE on plasma lysosomal enzymes of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-D. Glucuronidase (10⁻² μmoles of p-nitrophenol liberated /hr/mg protein)</td>
<td>2.61 ± 0.20</td>
<td>14.96 ± 0.81***</td>
<td>10.03 ± 1.50a<em><strong>b</strong></em></td>
<td>7.57 ± 1.42a<em><strong>b</strong></em>c***</td>
</tr>
<tr>
<td>β-D. Galactosidase (10⁻² μmoles of p-nitrophenol liberated /hr/mg protein)</td>
<td>1.33 ± 0.26</td>
<td>2.03 ± 0.204***</td>
<td>1.55 ± 0.09a*b**</td>
<td>1.47 ± 0.22b***</td>
</tr>
<tr>
<td>Acid phosphatase (10⁻² μmoles of phenol liberated/min/mg protein)</td>
<td>1.94 ± 0.22</td>
<td>3.69 ± 0.24***</td>
<td>2.89 ± 0.17a<em><strong>b</strong></em></td>
<td>2.57 ± 0.17a<em><strong>b</strong></em>c***</td>
</tr>
<tr>
<td>Cathepsin - D (10⁻³ μmoles of tyrosine liberated/hr/mg protein)</td>
<td>6.96 ± 0.45</td>
<td>17.22 ± 0.97***</td>
<td>12.14 ± 2.14a<em><strong>b</strong></em></td>
<td>9.99 ± 0.85a<em><strong>b</strong></em>c***</td>
</tr>
<tr>
<td>β-N. Acetyl glucosaminidase (10⁻² μmoles of p.nitrophenol liberated /hr/mg protein)</td>
<td>2.06 ± 0.13</td>
<td>3.03 ± 0.18***</td>
<td>2.35 ± 0.18a<em><strong>b</strong></em></td>
<td>2.22 ± 0.10b***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1.

Comparisons are made as in table 1.

*** p < 0.001; ** p < 0.01; * p < 0.05.
Table 8: Effect of TCE on liver lysosomal enzymes of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-D. Glucuronidase (10^{-2} μmoles of p-nitrophenol liberated/hr/mg protein)</td>
<td>29.63 ± 1.35</td>
<td>41.48 ± 1.43***</td>
<td>34.72 ± 2.85***</td>
<td>34.9 ± 0.49***</td>
</tr>
<tr>
<td>β-D. Galactosidase (10^{-2} μmoles of p-nitrophenol liberated/hr/mg protein)</td>
<td>12.48 ± 1.56</td>
<td>18.50 ± 1.75***</td>
<td>16.39 ± 0.39***</td>
<td>14.35 ± 0.27***</td>
</tr>
<tr>
<td>ACP (10^{-2} μmoles of phenol liberated/min/mg protein)</td>
<td>8.30 ± 0.47</td>
<td>13.08 ± 1.70***</td>
<td>11.72 ± 0.64***</td>
<td>10.37 ± 0.85***</td>
</tr>
<tr>
<td>Cathepsin - D (10^{-3} μmoles of tyrosine liberated/hr/mg protein)</td>
<td>37.42 ± 3.09</td>
<td>64.67 ± 2.96***</td>
<td>51.58 ± 1.16***</td>
<td>48.12 ± 0.45***</td>
</tr>
<tr>
<td>β-N.Acetyl glucosaminidase 10^{-2} μmoles of p.nitrophenol liberated /hr/mg protein</td>
<td>27.68 ± 1.74</td>
<td>42.71 ± 2.11***</td>
<td>37.68 ± 2.72***</td>
<td>36.98 ± 1.06***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
Table 9: Effect of TCE on kidney lysosomal enzymes of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-D. Glucuronidase (10⁻² μmoles of p-nitrophenol liberated /hr/mg protein)</td>
<td>31.84 ± 0.41</td>
<td>44.20 ± 1.05***</td>
<td>39.66 ± 1.83a<em><strong>b</strong></em></td>
<td>36.69 ± 1.03a<em><strong>b</strong></em>c***</td>
</tr>
<tr>
<td>β-D. Galactosidase (10⁻² μmoles of p-nitrophenol liberated/hr/mg protein)</td>
<td>10.37 ± 1.26</td>
<td>16.05 ± 0.62a***</td>
<td>14.28 ± 0.41a*<strong>b</strong></td>
<td>13.16 ± 0.66a<em><strong>b</strong></em> ***</td>
</tr>
<tr>
<td>ACP (10⁻² μmoles of phenol liberated/ min/mg protein)</td>
<td>5.90 ± 0.23</td>
<td>7.75 ± 0.78a***</td>
<td>6.88 ± 0.21a**<em>b</em></td>
<td>6.30 ± 0.46b***</td>
</tr>
<tr>
<td>Cathepsin - D (10⁻³ μmoles of tyrosine liberated/hr/mg protein)</td>
<td>45.01 ± 2.36</td>
<td>66.89 ± 2.77a***</td>
<td>55.39 ± 2.92a<em><strong>b</strong></em></td>
<td>48.90 ± 2.16a<em><strong>b</strong></em>c***</td>
</tr>
<tr>
<td>β.N.Acetyl glucosaminidase (10⁻² μmoles of p.nitrophenol liberated /hr/mg protein)</td>
<td>34.12 ± 2.83</td>
<td>46.89 ± 1.10a***</td>
<td>39.65 ± 2.10a<em><strong>b</strong></em></td>
<td>39.28 ± 2.20a<em><strong>b</strong></em>c***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
showed a significant reduction in group III (p<0.01) and group IV (p<0.001) animals when compared to group II animals.

In liver and kidney the enzyme content of β-D glucosamidase, Cathepsin-D and β-N acetyl glucosaminidase were found to be significantly (p<0.001) decreased in drug treated (group III and IV) rats, whereas β-D galactosidase level in both liver and kidney of the group III animals showed lesser significant decrement (p<0.5; p<0.01, respectively). However, Group IV animals showed a greater significant (p<0.001) decrement of β-D galactosidase in both liver and kidney when compared to arthritic rats.

In liver, acid phosphatase level was unaltered in drug treated arthritic group III, but group IV showed significantly (p<0.001) reduced level. Whereas in kidney acid phosphatase level of Group III (p<0.05) and Group IV (p<0.001) animals was significantly reduced when compared to group II animals.

Histochemical and ultrastructural studies have shown that lysosomes of synovial lining cells from patients with rheumatoid arthritis have an abnormal appearance and may be more permeable to substrate (Hamerman, 1968).

An increased level of β-glucuronidase and β-N acetyl glucosaminidase in synovial fluid of rats with adjuvant induced arthritis was reported by Carevic and Djokic (1988). Weissmann (1972) demonstrated an increased level
of lysosomal enzymes like acid phosphatase, β-glucuronidase, cathepsin-D and lysozyme in synovia and synovial fluid of patients with rheumatoid arthritis.

Cathepsin D-type enzyme is present in 2-3 times greater amount in early osteoarthritis and discoloured human articular cartilage than in apparently normal cartilage (Sapolsky, 1973). Antiserum to the purified cathepsin enzyme was capable of inhibiting cartilage degradation (Barret and Dingle, 1971). Elevated levels of N-acetyl β-D-glucosaminidase activity was observed in serum of experimental arthritic rats (Agha and Gad, 1995).

Increased level of lysosomal enzymes such as β-glucuronidase, cathepsin-D, β-galactosidase and acid phosphatase in liver and kidney of arthritic rats was demonstrated by Vijayalakshmi et al. (1995).

Since extracellular release of lysosomal enzymes may be crucial to the pathogenesis of tissue injury and inflammation, it is likely that reduction of such enzyme release may be attributed to the anti-inflammatory activity of the test drug T.cordifolia extract. It may inhibit fusion between lysosomal and plasma membrane by increasing intercellular level of cAMP or promote disassembly of cytoplasmic microtubules, as was observed for some other anti-inflammatory agents. (Weissmann et al., 1971) or by influencing contact between immunologic reactants and the cell surface which generate "signals" that provoke exocytosis of lysosomal enzymes (Ringerose et al., 1975).
The exact mechanism of TCE action on the reduction of lysosomal enzymes is not known. It may be acted through stabilizing the lysosomal membrane and inhibits the release of lysosomal enzymes.

3.9 GLYCOPROTEINS

The inflammatory process of the adjuvant arthritis is a systematic disease and causes alterations in the metabolism of connective tissue macromolecular components involving many organs. The changes occurring in the metabolism of connective tissues macromolecules such as glycoproteins, proteoglycans, glycosaminoglycans and collagen in arthritic disease are of considerable importance from the point of view of the incidence of rheumatoid arthritis (Kesava Reddy and Dhar, 1988).

Tables 10, 11 & 12 show the level of protein bound carbohydrates in plasma, liver and kidney.

Hexose, hexosaminase and sialic acid concentrations of plasma, liver and kidney were found to be significantly (p<0.001) increased in arthritic rats when compared to Group I animals.

Hexose concentration in both plasma and kidney was found to be significantly (p<0.001) reduced in drug treated (group III and group IV) rats, whereas the concentration of hexose in liver showed a significant decrement (group III (p<0.01) and group IV (p<0.001), respectively) when compared with arthritic (group II) rats. Hexosamine concentration of plasma showed a
Table 10: Effect of TCE on plasma glycoproteins of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose</td>
<td>109.42 ± 9.3</td>
<td>206.00 ± 13.2***</td>
<td>165.25 ± 11.93***</td>
<td>144.6 ± 9.37<em><strong>b</strong></em>c***</td>
</tr>
<tr>
<td>Hexoxamine</td>
<td>25.17 ± 2.89</td>
<td>39.62 ± 3.0***</td>
<td>35.27 ± 1.5<em><strong>b</strong></em></td>
<td>30.63 ± 1.66<em><strong>b</strong></em></td>
</tr>
<tr>
<td>Sialic acid</td>
<td>41.77 ± 1.03</td>
<td>84.86 ± 4.0***</td>
<td>60.68 ± 3.55<em><strong>b</strong></em></td>
<td>62.23 ± 4.41<em><strong>b</strong></em></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
Table 11: Effect of TCE on liver glycoproteins of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose (mg/100 mg of defatted tissue)</td>
<td>20.93 ± 1.28</td>
<td>29.47 ± 1.44a***</td>
<td>26.44 ± 1.18a*<strong>b</strong></td>
<td>25.37 ± 2.13a<em><strong>b</strong></em></td>
</tr>
<tr>
<td>Hexoxamine (mg/100 mg of defatted tissue)</td>
<td>6.00 ± 0.20</td>
<td>9.80 ± 0.09a***</td>
<td>6.77 ± 0.54a<em><strong>b</strong></em></td>
<td>6.45 ± 0.09a<em><strong>b</strong></em></td>
</tr>
<tr>
<td>Sialic acid (µg/100 mg of defatted tissue)</td>
<td>0.45 ± 0.03</td>
<td>0.69 ± 0.01a***</td>
<td>0.50 ± 0.02a<em><strong>b</strong></em></td>
<td>0.44 ± 0.02b<em><strong>c</strong></em></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
Table 12: Effect of TCE on kidney glycoproteins of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n moles of Pi liberated/min/mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein)</td>
<td>16.73 ± 0.29</td>
<td>26.37 ± 1.45*</td>
<td>19.78 ± 0.60***</td>
<td>18.75 ± 0.42***</td>
</tr>
<tr>
<td>Hexose (mg/100 mg of defatted</td>
<td>6.04 ± 0.50</td>
<td>8.54 ± 0.57*</td>
<td>7.23 ± 0.44***</td>
<td>6.35 ± 0.40***</td>
</tr>
<tr>
<td>tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexoxamine (mg/100 mg of</td>
<td>0.40 ± 0.012</td>
<td>0.752 ± 0.079***</td>
<td>0.566 ± 0.011***</td>
<td>0.44 ± 0.052***</td>
</tr>
<tr>
<td>defatted tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sialic acid (µg/100 mg of defatted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tissue)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
significant reduction in drug treated group III (p<0.05) and group IV (p<0.001) when compared with arthritic (Group II) rats. A significantly lower concentration (p<0.001) of Hexosamine was found in liver and kidney of drug treated groups (III & IV) when compared with arthritic rats. Sialic acid concentration was significantly (p<0.001) reduced in plasma, liver and kidney of group III and group IV animals when compared with untreated arthritic (group II) rats.

The physiological function of the glycoprotein in the connective tissue remains unclear. It has been suggested that the glycoproteins have some function in stabilizing the tissue and that may be involved in maintaining the structural stability of collagen fibrile (Jackson and Bentley, 1968).

Previous studies have established that the total protein bound carbohydrates in plasma increase in response to injury caused by inflammatory agents. Injection of turpentine and other chemical inflammatory agents, the growth of tumors and various other pathological conditions have long been known to cause an increase in plasma total protein bound carbohydrates in mammal

Turpentine-induced inflammation in the rats caused 1.6 to 2.3 fold increase in liver homogenate sialyl-, galactosyl-and N-acetylglucosaminyl transferases total and specific enzyme activities. These enzymes increased the glycosylation potential of the liver. The potential increase of glycosylation of liver probably play an important role in the increased synthesis of acute-phase
glycoproteins. These acute-phase reactants play an important roles in development of inflammation (Lombart et al., 1980)

Sialyltransferase activity in rat serum increases 4 fold in 48 hours in response to Turpentine-induced inflammation (Fraser et al., 1984).

Exer et al. (1976) have reported an increase in metabolic turnover of proteoglycan in the ligament and cartilage during the chronic inflammatory process of adjuvant arthritis. Arumugam and Bose (1980) have reported an elevated levels of glycohydrolases in adjuvant arthritic rats. These enzymes are involved in the degradation of the structural macromolecules of connective tissue and cartilage proteoglycans. Coolbear and Mookerjea (1984) have reported an increased dolichol phosphokinase activity and dolichol phosphate linked glycosylation of mannose in the liver of inflamed rats.

In both acute and chronic phase of adjuvant induced arthritis the content of total hexose, sialic acid and hexosamine were found to be increased significantly in the inflamed tissues such as bone, cartilage, skin, kidney, spleen and serum (Kesava Reddy and Dhar, 1987 and 1988).

The results of the present investigation have established an increased metabolic turnover of glycoproteins in the adjuvant induced arthritis. The decrement in the levels of glycoproteins in plasma, liver and kidney of TCE treated rats may be attributed to the inhibition of total and specific transferases enzymes activity in the inflamed tissues and also to a decrease
turnover of proteoglycans by decreasing the acid hydrolases release from lysosomes.

### 3.10 COLLAGEN

Table 13 showed the effect of TCE on cartilage collagen of AIA rats. A significant \((p<0.001)\) decreased level of collagen was observed in group II rats when compared with group I rats. Group IV animal showed a significantly increased level of collagen content when compared with group II \((p<0.001)\) rats. However, no significant change was observed between group II and III.

The destruction of articular cartilage is a major pathological event in RA, leading ultimately to the loss of joint function. The extra cellular matrix of cartilage consists predominantly of a gel like solution of polyanionic proteoglycans embedded in a meshwork of type III collagen fibres (Mow et al., 1991). The precise mechanisms by which articular cartilage is degraded in joint disease have yet to be elucidated, but the matrix metallo proteinase (MMPs) family (the stromelysins, gelatinases and collagenases) is strongly implicated. Between them, these proteinases have the capacity to degrade all the proteinaceous components of articular cartilage. In the family of MMPs, collagenase likely to be a key proteinase in mediating irreversible damage to the collagen network of articular cartilage, because it is the only proteinase that is able to cleave native fibrillar collagen in its helical region (Karran et al., 1995).
Table 13: Effect of TCE on cartilage collagen of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen (mg/100mg cartilage)</td>
<td>58.08 ± 3.15</td>
<td>41.92 ± 4.95&lt;sup&gt;***&lt;/sup&gt;</td>
<td>45.17 ± 3.20&lt;sup&gt;***&lt;/sup&gt;</td>
<td>53.33 ± 4.42&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

<sup>***</sup> p < 0.001; <sup>**</sup> p < 0.01; <sup>*</sup> p < 0.05
Biochemical evidence of increased collagen degradation can be readily observed in RA patients (St.Clair et al., 1998). Antioxidant like anti-inflammatory agent, ebselen blocks the cartilage proteoglycan breakdown in cartilage organ culture, stimulated with IL-1 was reported by Pratta et al. (1998).

Previous reports show an increase degradation of cartilage in both AIA rats (Del Pozo et al., 1990) and RA patients (Mansson et al., 1995). In the present study TCE has decreased collagen degradation form the cartilage matrix in developing arthritis, without any effect on the established arthritis.

3.11 LIPID PEROXIDATION AND INFLAMMATION

In recent years it has become increasingly evident that in man and animals free radicals play a variety of role in normal regulation systems, the deregulation of which may play an important role in inflammation.

Isolated human polymorphonuclear leukocytes (PMN) engaged in phagocytosis liberate superoxide radicals and hydrogen peroxide into the surroundings. These two chemical species react to produce the hydroxy radical, which attacks the leukocytes and leads to premature death of the cell. The hydroxy radical may be scavenged by mannitol, or its formation can be prevented by the addition of superoxide dismutase or catalase to the medium, thereby eliminating the premature death of the cells (Salin and McCord 1975).
Oxygen free radicals have been implicated as mediators of tissue damage in patients with rheumatoid arthritis. Thus it is possible that several micronutrients acting as antioxidants and free radical scavengers provide protection against RA. Elevated risk of RA was observed at low level of antioxidants like alpha-tocopherol, beta-carotene and selenium (Heliovaara et al., 1994).

Free radicals formed during several pathological conditions were scavenged by enzymatic and non-enzymatic antioxidant system. The main target of free radicals are the membrane lipids and liberate arachidonic acid. Eicosanoids are formed by enzymatic oxidation of arachidonic acid. Research work of the last two decades has identified eicosanoids, as mediators of inflammatory rheumatic diseases; an increased formation of these compounds correlates with flares of the disease. Research work of the past decade has revealed the importance of antioxidants in deregulating the oxygenation of arachidonic acid for eicosanoid formation. The proper function of the redox cycle, in which vitamin E and C as well as selenium; copper, zinc and iron containing metalloenzymes are active, is necessary to transform free radicals to $O_2$ and water (Adam, 1995).

Reactive oxygen species are capable of producing reversible or irreversible damage to molecules of all biochemical classes, their most damaging effect is the induction of lipid peroxidation (Morris et al., 1995). The cell membrane composed of poly-unsaturated fatty acids, is a primary target for reactive oxygen attack leading to cell membrane damage (Wills, 1966).
Lipid peroxidation is a chain reaction in which three stages are involved, these are initiation, propagation and termination. Initiation of a peroxidation sequence is a membrane or polyunsaturated fatty acid is due to attack by any species with sufficient reactivity to abstract a hydrogen atom (H•) from a methylene group (CH₂). Such species include the •OH radical, alkoxy radical (•RO) and peroxy radical (ROO•), but not O₂• or H₂O₂ (Dixit et al., 1983).

Most membrane poly unsaturated fatty acids have their double bonds unconjugated and separated by methylene groups. The presence of a double bond adjacent to methylene group makes the methylene C-H bond weaker and therefore the hydrogen is more susceptible to abstraction. This leaves behind an unpaired electron on the carbon. The carbon-centered radical stabilizes by undergoing a molecular rearrangement of the double bonds to form a conjugated diene which then combines with O₂ to form a peroxy radical. The peroxy radical is itself capable of abstracting a hydrogen atom from another poly unsaturated fatty acid and so starts a chain reaction.

The free radical chain reaction propagates until two free radicals conjugate each other to terminate the chain (Blake et al., 1987). The reaction can also be terminated in the presence of a chain-breaking anti-oxidant such as vitamin E (Dixit et al., 1983; Eldin et al., 1992).

Table 14 shows the level of lipid peroxidation concentration in plasma, erythrocyte membrane, liver and kidney of control and experimental groups.
Table 14: Effect of TCE on lipids peroxidation level of plasma, erythrocyte membrane, liver and kidney of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters MDA (nmoles of MDA liberated /min/mg protein)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma LPO</td>
<td>0.08 ± 0.007</td>
<td>0.21 ± 0.026***</td>
<td>0.12 ± 0.021a<em><strong>b</strong></em></td>
<td>0.093 ± 0.006b***</td>
</tr>
<tr>
<td>Erythrocyte membrane LPO</td>
<td>1.24 ± 0.086</td>
<td>2.08 ± 0.17a***</td>
<td>1.72 ± 0.10a<em><strong>b</strong></em></td>
<td>1.32 ± 0.06b<em><strong>c</strong></em></td>
</tr>
<tr>
<td>Liver LPO</td>
<td>0.98 ± 0.10</td>
<td>1.89 ± 0.14a***</td>
<td>1.16 ± 0.09a<em><strong>b</strong></em></td>
<td>0.96 ± 0.04b<em><strong>c</strong></em></td>
</tr>
<tr>
<td>Kidney LPO</td>
<td>0.87 ± 0.068</td>
<td>1.35 ± 0.051a***</td>
<td>1.08 ± 0.263b*</td>
<td>0.90 ± 0.174b***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
The experimental arthritic (group II) rats exhibited significantly (p<0.001) increased level of lipid peroxidation in plasma, erythrocyte membrane, liver and kidney.

Lipid peroxidation level was significantly (p<0.001) decreased in plasma, erythrocyte membrane and liver of group III and IV rats. A significant reduction of LPO in kidney of group III (p<0.05) and group IV (p<0.001)) drug treated rats was also observed when compared with control rats.

Extensive studies have been undertaken to elucidate the etiology of arthritic disease but there is still much to be clarified. In the past decade, formation of lipid peroxide in the target tissues of inflammation is considered as most commonly promoting factor in rheumatoid arthritis.

Increased lipid peroxidation in body tissue has been observed in a wide range of tissue injury such as CCl₄ toxicity, atherosclerosis, and carrageenan induced inflammation (Bardhan and Sharma, 1983). Increased level of peroxidation products in blood of RA patients (Taraza et al., 1997; Gambhir et al., 1997) and in plasma of arthritic rats (Geetha et al., 1998) was reported. Vijayalakshmi et al. (1997) demonstrated an increased level of LPO in plasma, liver, kidney and heart of adjuvant induced arthritic rats. The increased lipid peroxide level in erythrocyte membrane may be due to enhanced free radical inflicting damage (Hailliwell and Gutteriide, 1989).

Documentary evidence exists for the increased generation of oxidants in vivo in patients with active RA, (Dia et al., 1997; Heliovaara et al., 1994) but
the contribution of these oxidants to the disease process is still uncertain. Granulocytes which accumulate in the rheumatoid joints are known to produce oxygen derived free radicals during phagocytosis of bacteria and immune complexes (Babior, 1981). Increased lipid peroxides may be due to the release from neutrophils and monocytes at the site of inflammation (Green Wald, 1981).

Lipid peroxidation causes a decrease in membrane fluidity and barrier function of membranes. The many products of lipid peroxidation such as hydroperoxides or their aldehyde derivatives can inhibit protein synthesis, block macrophage actions and alter chemotactic and enzyme activity (Fridovich and Porter, 1981).

A statistically significant increase of TBARS and lowered vitamin E was found in the plasma of the children with juvenile rheumatoid arthritis (Sklodowska et al., 1996).

Results obtained in the present study demonstrated that the drug significantly inhibits lipid peroxidation in the plasma and erythrocyte membrane, liver and kidney of the adjuvant induced arthritic rats. This result establishes that TCE is an antioxidant and it acts as a scavenger of free radicals which is responsible for the pathogenesis of inflammation.
3.12 ANTIOXIDANTS AND INFLAMMATIONS

Now the word antioxidation has become a general term and every day we are bombarded with claims of antioxidant protection against a host of diseases. Rheumatoid arthritis, atherosclerosis, cancer, gastric ulcer, hypertension, stroke and a host of other diseases have been suggested to be induced by oxidative stress, and antioxidants have been suggested to be beneficial in the prevention and treatment of these diseases (Parthasarathy et al., 1998).

Kucera et al. (1996) assessed FRS in patients with rheumatoid arthritis, other inflammatory articular diseases and degenerative diseases of the joints and spine. Their results showed that subjects with inflammation of the synovial membrane respond frequently by a rise of indicators of the antioxidant system. Obviously a defense reaction of the organism is involved to the increased formation and action of oxygen free radicals in the inflamed articular linings.

Eldin et al. (1996) demonstrated a significant decrease in serum SH group accompanied by a significant increase in blood GSH level and erythrocyte, SOD activity in both acute and chronic phase of adjuvant arthritis. Administration of vitamin E before and after adjuvant inoculation increased the lowered serum SH group in arthritic rats so that their level was restored to the prearthritic values especially in the chronic treated groups. These treatment produced no changes in blood GSH. However, its administration after adjuvant inoculation decreased slightly the activity of erythrocyte SOD in arthritic rats.
Several defence mechanisms act to protect the organism from the toxic effect of oxygen free radicals. Among them are vitamin E (Eldin et al., 1996), vitamin C (Eldin et al., 1992) serum sulfhydryl group (SH) (Lorber et al., 1975), ceruloplasmin (CP), blood glutathione (GSH) (Fukuda et al., 1994; Shingu et al., 1994) and the intracellular superoxide dismutase (SOD) as well as the acute phase glycoprotein \( \alpha_2 \)-macroglobulin (\( \alpha_2 \)-M) (Flower and Thomson, 1979).

Vijayalakshmi et al., 1997 demonstrated that the role of free radicals in adjuvant-induced arthritic rats. In which increased lipid peroxides levels in both plasma and tissues (liver kidney & heart) were observed, as well as decreased levels of catalase and increased level of SOD were observed in plasma, liver and kidney of adjuvant arthritic rats.

A balanced action between the antioxidants are important for the effective removal of oxygen radicals. The observed alteration in antioxidant system of arthritic animals is the result of the increased level of oxygen radicals due to inflammation.

Table 15 and 16 show the levels of enzymatic and non-enzymatic antioxidants such as SOD, catalase, GPx, GSH and TSH in liver and kidney of control and experimental groups.
Table 15: Effect of TCE on liver enzymatic and non-enzymatic antioxidant of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide Dismutase (units/min/mg protein)</td>
<td>3.02 ± 0.31</td>
<td>4.16 ± 0.66&lt;sup&gt;***&lt;/sup&gt;</td>
<td>3.85 ± 0.3&lt;sup&gt;†&lt;/sup&gt;</td>
<td>3.40 ± 0.38&lt;sup&gt;b‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Catalase (µmoles of H₂O₂ consumed/min/mg protein)</td>
<td>213.17 ± 26.54</td>
<td>131.5 ± 15.80&lt;sup&gt;***&lt;/sup&gt;</td>
<td>189.83 ± 15.87&lt;sup&gt;b***&lt;/sup&gt;</td>
<td>217.00 ± 10.88&lt;sup&gt;b***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glutathione peroxidase (µg of GSH utilized/min/mg protein)</td>
<td>15.18 ± 3.34</td>
<td>13.77 ± 7.68</td>
<td>14.92 ± 2.48</td>
<td>15.38 ± 2.08</td>
</tr>
<tr>
<td>Reduced glutathione (µg/mg protein)</td>
<td>3.39 ± 0.48</td>
<td>3.21 ± 0.51</td>
<td>3.46 ± 0.31</td>
<td>3.45 ± 0.18</td>
</tr>
<tr>
<td>Total thiols (µg/mg protein)</td>
<td>2.47 ± 0.249</td>
<td>1.59 ± 0.17&lt;sup&gt;††&lt;/sup&gt;</td>
<td>2.07 ± 0.174&lt;sup&gt;b‡‡&lt;/sup&gt;</td>
<td>2.12 ± 0.19&lt;sup&gt;‡‡&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

<sup>***</sup> p < 0.001;  <sup>**</sup> p < 0.01;  <sup>†</sup> p < 0.05
Table 16: Effect of TCE on kidney enzymatic and non-enzymatic antioxidant of arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide Dismutase (units/min/mg protein)</td>
<td>2.65 ± 0.33</td>
<td>3.20 ± 0.42**</td>
<td>2.69 ± 0.17b*</td>
<td>2.62 ± 0.45b**</td>
</tr>
<tr>
<td>Catalase (μmoles of H₂O₂ consumed/min/mg protein)</td>
<td>161.0 ± 17.48</td>
<td>139.83 ± 6.82a*</td>
<td>158.5 ± 14.92</td>
<td>164.67 ± 13.71b*</td>
</tr>
<tr>
<td>Glutathione peroxidase (μg of GSH utilized/min/mg protein)</td>
<td>11.02 ± 6.23</td>
<td>10.55 ± 7.07</td>
<td>10.78 ± 2.79</td>
<td>11.86 ± 3.39</td>
</tr>
<tr>
<td>Reduced glutathione (μg/mg protein)</td>
<td>3.39 ± 0.59</td>
<td>3.21 ± 0.28</td>
<td>3.46 ± 0.31</td>
<td>3.45 ± 0.98</td>
</tr>
<tr>
<td>Total thiols (μg/mg protein)</td>
<td>2.14 ± 0.22</td>
<td>1.78 ± 0.15**</td>
<td>2.11 ± 0.18b**</td>
<td>2.28 ± 0.10b***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
3.12.1 *Superoxide Dismutase (SOD)*

Superoxide dismutase catalyzes the dismutation of the $O_2^-$ into oxygen and hydrogen peroxide, emerged during several pathological conditions and is thought to be protective of tissue, cell and proteins against damage by oxygen radicals.

Three types of SOD have been found in mammalian tissues and designated Cu Zn SOD, Mn SOD and extracellular SOD. The Cu Zn SOD and extracellular SOD are localized in the cytosol and extracellular fluid, respectively. Whereas Mn SOD is localized in the mitochondrial matrix (Shingu et al., 1994).

SOD activity was found to be significantly increased in both liver and kidney of group II animals ($p<0.001; p<0.01$, respectively) when compared with normal control (group I) animals. Group III animals showed a significant ($p<0.05$) reduction of SOD activity in kidney but not in liver. A significant reduction of SOD activity in both liver and kidney was also observed in group IV animals ($p<0.05; p<0.001$, respectively).

The level of Cu/Zn SOD was found to be increased in rheumatoid arthritic patients (Mazzetti et al., 1996). These results support the hypothesis that the increased amount of copper/zinc superoxide dismutase is probably inadequate to exert an effective antioxidant protection but can result in a pro-inflammatory, pathogenic effect enhancing tissue damage. Cu/Zn SOD might be used as a marker of inflammatory activity in rheumatoid arthritis.
Moreover, a significant increase in erythrocyte SOD activity has been observed in acute and chronic phases of arthritis. The increase appear to be a reflex mechanism to guard against extra cellular oxygen-free radicals (Fridovich, 1975; Marklund et al., 1987). The increased delivery of NADPH from the stimulated HMP shunt during inflammation (Roos and Weening, 1979) may account for the activation of SOD in arthritic rats. Treatment with vitamin E decreased the elevated SOD activity of arthritic rats (Eldin, 1992).

The role of oxygen free radicals in experimental collagen induced arthritis was demonstrated by Kakimoto et al. (1993) by administering SOD to see the effect on the development of the disease. Gelatin SOD, which had a long in vivo half life showed significant reduction of the disease with out affecting the anti-CII immune response. The rate of glycoseaminoglycon (GAG) released from articular cartilage organ culture system was increased, in the presence of IL-1 (10 ng), when human recombinant SOD (150 μg ml) was added along with IL-1, it completely blocked the IL-1 mediated increase in GAG release (Fukuda et al., 1994).

3.12.2 Catalase

Catalase activity was found to be significantly decreased in both liver and kidney of group II animals (p<0.001; p<0.05 respectively) when compared with control animals. Catalase activity in liver of group III animals showed a significant (p<0.001) increase but not in kidney. Whereas group IV animals showed significantly (p<0.001; p<0.05 respectively) increased activity in both liver and kidney when compared with group II animals.
Catalase, a heme containing protein, form a second line of defense along with glutathione peroxidase, while they efficiently share the task of reducing $\text{H}_2\text{O}_2$ to water. At low concentration of $\text{H}_2\text{O}_2$, catalase acts as a peroxidase.

Human serum catalase can protect alpha-1-proteinase inhibitor from inactivation by $\text{H}_2\text{O}_2$. The primary source of serum catalase is probably erythrocytic. The enzyme correlates with haemoglobin concentration in sera from control subjects but not in sera from patients with rheumatoid arthritis. Catalase is inactivated by oxidants, such as $\text{H}_2\text{O}_2$ and hypochlorous acid and it is suggested that the decrease in catalase/haemoglobin ratio observed in rheumatoid serum is due to oxidant stress associated with inflammation (Kokkaliari et al., 1992) and is also observed from the increased dismutation of oxygen free radicals by SOD.

3.12.3 Reduced Glutathione and Glutathione Peroxidase

Generally tissue glutathione depletion seems to be responsible for the induction of lipid peroxidation. The increase in glutathione content is frequently accompanied by the decrease in lipid peroxidation (Chaurasia et al., 1995).

The result obtained by this study showed that there is no significant alteration in the reduced glutathione as well as the glutathione peroxidase level. Earlier studies do not agree on the activity level of GSH-Px among RA
patients, the activity levels have been reported to range from low to high (Tarp, 1994).

Banford et al. (1982) demonstrated a significant increase in blood GSH level in acute and chronic phases of inflammation of adjuvant induced arthritic rats. However, it disagreed with those of Koster et al. (1986) who found no changes in the level of blood GSH in arthritic patients.

Previous reports demonstrated that the plasma GSH-Px activity was not increased in RA patients in general (Honkanen, 1991). No significant difference was found in whole blood GPx activity between 28 RA patients and 36 control (Chorazy et al., 1992). The present result is in agreement with the earlier findings of Koster et al., 1986.

3.12.4 Total Thiols (TSH)

A decreased level of TSH was observed in both liver and kidney of group II animals (p<0.001; p<0.01 respectively) when compared with normal control animals. Drug treated animals (group III and IV) showed a significant (p<0.01; p<0.001 respectively) increase in both liver and kidney thiols when compared with arthritic animals.

Since the greatest majority of serum SH group (85 to 90%) are found in albumin, the reduced level of serum albumin was observed in arthritic rats (Eldin et al., 1992). The result of the present study is also in agreement with Eldin et al. (1992).
Moreover, several Reactive oxygens species (ROS) such as hydrogen peroxide and lipid peroxide are released from neutrophils and monocytes in inflammation as well as the elevated level of serum oxidase activity, all of them might be responsible for the decreased serum thiols group level by oxidation (Hall et al., 1984). Administration of non-enzymatic antioxidant vitamin E increased the lowered level of serum thiols (Herman, 1982).

Serum sulfhydryls (SH) are the most critical targets for free radicals attack. This vulnerability of cysteine residues is due to radical tunneling the neighbouring radical centers. Once a free radical hits specific amino acyl site within a protein, it will be rapidly transferred to cysteine residue via methionines, tryptophanes and tyrosines, subsequently forming cysteine radicals (Butler et al., 1988). The consequence of oxidative modification of proteins are altered enzyme activities and increased proteolysis, eventually leading to pathological tissue degradation.

Sulfhydryls are also intrinsically involved in the regulation of the intra and extracellular redox potential, serves as cofactor for several enzymes, and are required for the synthesis of DNA precursors, and regulate T-cell function both *in vitro* and *in vivo* (Staal et al., 1990).

After the TCE treatment, the antioxidant levels were brought back to near normal levels, which explain the free radical scavenging role of *T. cordifolia* and the inhibitory effect on the formation of lipid peroxide.
Administration of TCE exert its antiarthritic effect by retarding lipid peroxidation and causing a modulation in cellular antioxidant defence system.

3.13 MEMBRANE BOUND ATPases

The three membrane bound adenosine triphosphatases are Na\(^+\), K\(^+\)-ATPases, Mg\(^{2+}\)-ATPases and Ca\(^{2+}\)-ATPases, which are integral part of the membranes, any alteration in the structural integrity of the membrane will affect the activities of these ATPases. These enzymes are responsible for the transport of Na\(^+\), K\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) ions. The activity of these enzymes are found to be affected by ionic concentration (Jain and Shohet, 1981) and lipid peroxidation (Ohta et al., 1989), membrane fluidity (Kimelber, 1975) and hormones (Cohen et al., 1986).

Table 17,18 & 19 show the activity of Na\(^+\), K\(^+\)-ATPase Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase in erythrocyte membrane, liver and kidney.

A significant (p<0.001) decrease in the level of Na\(^+\), K\(^+\)-ATPase was observed in the erythrocyte membrane, liver and kidney of group II animals when compared with group I animals. Drug treatment increased the enzyme activity significantly (p<0.01) in erythrocyte membrane of group IV animals but not in group III animals when compared to group II animals. No significant change was observed in the liver of group III and IV animals when compared with arthritic group. Kidney of group III & IV animals showed significant (p<0.001) increase of Na\(^+\), K\(^+\)-ATPase activity when compared to arthritic animals (group II).
Table 17: Effect of TCE on Erythrocyte membrane ATPases of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µ moles of Pi liberated/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺, K⁺ - ATPase</td>
<td>0.319 ± 0.034</td>
<td>0.251 ± 0.025**</td>
<td>0.275 ± 0.025**</td>
<td>0.301 ± 0.012***</td>
</tr>
<tr>
<td>Mg²⁺ - ATPase</td>
<td>0.367 ± 0.016</td>
<td>0.289 ± 0.019***</td>
<td>0.340 ± 0.016**</td>
<td>0.355 ± 0.033***</td>
</tr>
<tr>
<td>Ca²⁺ - ATPase</td>
<td>0.310 ± 0.026</td>
<td>0.274 ± 0.033**</td>
<td>0.297 ± 0.017</td>
<td>0.299 ± 0.018</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
Table 18: Effect of TCE on liver ATPases of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters (µ moles of Pi liberated/min/mg protein)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺, K⁺ - ATPase</td>
<td>0.242 ± 0.038</td>
<td>0.178 ± 0.008&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.190 ± 0.014&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.201 ± 0.020&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg&lt;sup&gt;2+&lt;/sup&gt; - ATPase</td>
<td>0.20 ± 0.016</td>
<td>0.165 ± 0.015&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.190 ± 0.007&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.197 ± 0.024&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt; - ATPase</td>
<td>0.187 ± 0.016</td>
<td>0.150 ± 0.011&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.173 ± 0.015&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.189 ± 0.015&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1.

Comparisons are made as in table 1.

<sup>***</sup> p < 0.001; <sup>**</sup> p < 0.01; <sup>*</sup> p < 0.05
Table 19: Effect of TCE on kidney ATPases of adjuvant-induced arthritic rats.

<table>
<thead>
<tr>
<th>Parameters (μ moles of Pi liberated/min/mg protein)</th>
<th>Group I (mean ± S.D.)</th>
<th>Group II (mean ± S.D.)</th>
<th>Group III (mean ± S.D.)</th>
<th>Group IV (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+), K(^+) - ATPase</td>
<td>0.556 ± 0.025</td>
<td>0.348 ± 0.046(^{***})</td>
<td>0.482 ± 0.030(^{<strong>;</strong>*})</td>
<td>0.486 ± 0.018(^{<strong>;</strong>*})</td>
</tr>
<tr>
<td>Mg(^{2+}) - ATPase</td>
<td>0.501 ± 0.019</td>
<td>0.329 ± 0.028(^{***})</td>
<td>0.417 ± 0.05(^{<strong>;</strong>*})</td>
<td>0.415 ± 0.025(^{<strong>;</strong>*})</td>
</tr>
<tr>
<td>Ca(^{2+}) - ATPase</td>
<td>0.335 ± 0.039</td>
<td>0.254 ± 0.028(^{***})</td>
<td>0.275 ± 0.023(^{**})</td>
<td>0.288 ± 0.031(^*)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of six animals.

Treatment of groups are as in table 1

Comparisons are made as in table 1

*** p < 0.001; ** p < 0.01; * p < 0.05
A significant decrease in the level of Mg$^{2+}$-ATPase was observed in both erythrocyte membrane and kidney ($p<0.001$) of group II animals when compared with group I animals. However, the liver Mg$^{2+}$-ATPase activity was less significant ($p<0.05$) in group II animals when compared with group I animals. Drug treatment increased the enzyme activity in erythrocyte membrane ($p<0.01$), liver ($p<0.05$) and kidney ($p<0.001$) of group III animals whereas group IV animals showed significantly increased Mg$^{2+}$-ATPase activity in erythrocyte membrane ($p<0.001$) liver ($p<0.05$) and kidney ($p<0.001$) when compared to group II animals.

The level of Ca$^{2+}$-ATPase enzyme activity was significantly decreased in erythrocyte membrane ($p<0.05$), liver ($p<0.01$) and kidney ($p<0.001$) of group II animals when compared with control (group I) animals. A significant increase in the enzyme activity in liver was observed in TCE treated group III ($p<0.05$) and group IV ($p<0.001$) animals. However, there is no significant change in the enzyme activity of erythrocyte and kidney of TCE treated groups (Group III & IV) of animals when compared with untreated arthritic rats (Group II).

An altered Na$^+$, K$^+$-ATPase activity in erythrocyte membrane of RA patients was reported by Masoom Yosinzai (1996). An increased intra-erythrocyte Na$^+$ concentration and decreased Na$^+$, K$^+$-ATPase activity in erythrocyte membrane of RA was previously reported by Rabini et al. (1990). Mardh et al. (1991) found that the highest titres of H$^+$, K$^+$ ATPase autoantibodies were in rheumatoid arthritis.
The activity of Na⁺, K⁺-ATPase was inactivated by thiol blocking reagent has been studied by preincubation of MNC with an impairment SH blocker p-hydroxymercuriphenyl sulphonate (PHMPSH) (Maubach, 1993). In the present study, TCE treatment restores the thiols level (Tables 15 and 16) and this may indirectly activate the Na⁺, K⁺-ATPases.

An impaired activity of Na⁺, K⁺-ATPase has been seen in rheumatoid arthritic patients. Foey et al. (1997) demonstrated an impaired Na⁺, K⁺-ATPase activity on rheumatoid mononuclear cells that might promote pro-inflammatory cytokine secretion in patients with RA. Cytokines, including TNFα and IL-1β, are essential to the chronic inflammatory process and tissue damage that are characteristic features for the diseases of RA.

Decreased activity of both Na⁺, K⁺-ATPase and Ca²⁺-ATPase was observed in arthritic rats, caused by oxygen free radicals leads to the accumulation of Ca²⁺ in cytoplasm and cell injury (Farber, 1990).

Administration of TCE reduced lipid peroxidation of membrane and protected the membrane integrity, there by the membrane bound ATPases. The normalisation of the ionic concentration by the drug helps the system to be relived from several pathological events.
Annexure II Photograph shows characteristics active principles from *T. cordifolia* stem extract and authentic samples on thin layer chromatogram observed under UV light (365 nm)

<table>
<thead>
<tr>
<th>TCE</th>
<th>Tinospora cordifolia extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>Tinosporin</td>
</tr>
<tr>
<td>T₂</td>
<td>Tembetarine</td>
</tr>
<tr>
<td>T₃</td>
<td>Berberine</td>
</tr>
<tr>
<td>T₄</td>
<td>Magnoflorine</td>
</tr>
<tr>
<td>T₅</td>
<td>Choline</td>
</tr>
<tr>
<td>T₆</td>
<td>Palmatine</td>
</tr>
</tbody>
</table>
ANNEXURE - II

CHROMATOGRAPHIC SEPARATION OF THE WATER EXTRACT OF TINOSPORA CORDIFOLIA.
PLATE 2

The hind paw of normal rat before inflammation is shown in (Fig.A). During adjuvant induced arthritis the hind paw oedema was noted (Fig.B). During treatment the oedema subsided in group III (Fig.C) and group IV (Fig.D) animals.
PLATE 3

The hind portion of the normal rat including tail is shown in (Fig.A). The presence of nodules were noted distinctly in adjuvant-induced arthritic (Fig.B) animals.
PLATE - 3

FIG. A

FIG. B
Radiological appearance of the normal knee joints with no narrowing of joint space was noted. Ankle joints show normal architecture with no evidence of soft tissue swelling of osteophyte formation. Vertebra appear normal with no inter vertebral narrowing (Fig.A).

In the adjuvant-induced arthritic animals (group II) osteophyte formation with narrowing of joint space associated with mild soft tissue swelling in the knees was noted. Ankle joints also show mild soft tissue swelling. Intervertebral disc showed narrowing. Loss of bone density was noticed femur, fibula and in lumbosacral region (Fig.B).

In group III animals mild osteophyte formation and narrowing of joint space in both knee joints with soft tissue swelling; ankle joints appearing normal. Intervertebral disc show mild narrowing in the lumbosacral region (Fig.C).

In group IV animals normal knee joints with no narrowing of joint space and ankle joints show normal architecture with no evidence of soft tissue swelling of osteophyte formation. Vertebra appear normal with no inter vertebral narrowing (Fig.D).
Section of a group I rat liver showing normal architecture with lobular arrangement (Fig.A).

The arrangement of hepatocytes within the parenchyma was found to be altered in the adjuvant induced arthritic animals (group II). Focal necrosis were also noticed in the liver (Fig.B). This micrograph shows extensive lymphocytic infiltration involving the area around the portal triad with numerous apoptotic bodies; extensive periportal infiltration of cells was found extending upto the hepatocytes (Fig.C). This periportal chronic inflammatory infiltrate extends even beyond its own limiting plate which is predominantly composed of lymphocytes.

The arthritic animals (group III) under treatment exhibits partial revival with mild sinusoidal dilatation (Fig.D).

The group IV animals showed normal hepatocytes within the liver parenchyma. The hepatocytes form flat and anastamosing plates with sparse apoptotic bodies (Fig.E).

All the section were stained with H & E. Photographs were taken under uniform magnification (H & E x 100).
Group I animals showed normal histological pattern of kidney as seen in Fig.A.

The kidney of adjuvant induced arthritic animal (group II) exhibited marked renal lesions showing extensive sclerosis and fibrosis of glomeruli with degeneration and irregular desquamation of the epithelium of tubules (Fig.B).

The group III animals showed renal lesion effects with mild scattered inflammatory infiltrate in glomeruli and tubules possessing mononuclear cells and lymphocytes and intraluminal casts (Fig.C).

The kidney in treated animals (group IV) showed intact glomeruli with adjacent normal tubular cells.

All the section were stained with H & E. Photographs were taken under uniform magnification (H & E x 100).
The micrograph focuses on the normal articular cartilage of the synovial joint in group I animals (Fig.A). The cells are small and flattened parallel to the surface in the superficial tangential zone. Superficially there is another layer called transitional layer; the cells are larger and rounded. They may lie in single or in groups. These cell are found embedded in the homogenous matrix. On proceedings to the deep surface of the cartilage, the cells are still larger and contain a calcified matrix.

In group II the cartilage has lost its normal architecture and there is partial disarray of the chondrocytes in irregular matrix (Fig.B).

In the group III animals the cartilage cells are well formed and there is focal scattering of lymphocytes (Fig.C).

In the group IV animals there is dense network of collagenous tissue forming the homogenous matrix and the presence of normal cartilagenous cells (Fig.D).

All the sections were stained with H & E. Photographs were taken under uniform magnification (H & E x 100).