ANTI-INFLAMMATORY AND ANTI-ARTHRITIC POTENTIAL OF AQUEOUS AND ALCOHOLIC EXTRACTS OF EUPHORBIA ANTIQUORUM LINN.

Anand Nirmal Harpalani¹, Ashok Dundappa Taranalli¹, Kishor Vasant Otari³, Ravindra Veerayya Karadi², Rajkumar Virbhadrappa Shete³

¹Department of Pharmacology, KLES’s College of Pharmacy, Belgaum, Karnataka, India. ²Department of Pharmaceutical Biotechnology, KLES’s College of Pharmacy, Belgaum, Karnataka, India. ³Department of Pharmacology, RD’s College of Pharmacy, Bhor, Dist. Pune, Maharashtra, India.

*Corresponding author: Cell: +91 9970060776; Telefax: +91 2113 222710; E-mail: kvotari76@rediffmail.com

Summary

Euphorbia antiquorum Linn (EA) is traditionally used in inflammatory disorders such as rheumatism and gout. Hence, in present study aqueous (AEA) and alcoholic (EEA) extracts of EA were evaluated for anti-inflammatory and anti-arthritis activities. Dried and powdered whole plant material of EA was used for the extraction. The aqueous extract (AEA) was prepared by maceration using distilled water and the alcoholic extract (EEA) was prepared by Soxhlet extraction using ethanol (99 % v/v). Both the extracts were subjected to the preliminary phytochemical evaluation and acute oral toxicity study. The effect of the extracts was evaluated against acute inflammation using carrageenan induced rat paw edema and chronic inflammation using cotton pellet induced granuloma in rats and complete Freund’s adjuvant (CFA) induced arthritis in rats.

The phytochemical evaluation revealed the presence of carbohydrates and triterpenoids in AEA and triterpenoids and saponins in EEA. In acute oral toxicity study, EEA and AEA did not show any toxicity and mortality up to the dose of 2 g/kg. AEA and EEA at 200 and 400 mg/kg, po produced significant inhibition of carrageenan induced rat paw edema. AEA and EEA at 400 mg/kg, po showed significant inhibition of cotton pellet induced granuloma formation in rats. AEA 400 mg/kg, po effectively prevented the primary lesions and EEA 400 mg/kg, po effectively prevented both primary and secondary lesions of CFA induced arthritis in rats. The results revealed that the triterpenoids present in both the extracts of EA might be responsible for anti-inflammatory and anti-arthritic effects.

Key words: Euphorbia antiquorum; Anti-arthritis; Anti-inflammatory; Carrageenan, granuloma; Complete Freund’s adjuvant.
Introduction

The substantial risk is involved with the use of nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, methotrexate, or so-called disease-modifying drugs such as gold for the treatment of inflammation or arthritis [1,2]. It is now well established that some of these drugs cause gastrointestinal damage including lesions, ‘silent’ ulcers, and life threatening perforations and hemorrhage. The administration of NSAIDs to patients with congestive heart failure, renovascular hypertension, and cirrhosis of the liver may cause acute renal failure. Long-term NSAIDs may cause infertility in women and infertility and impotence in men [3,4]. Hence, there is an increasing demand for the development of newer agents with better pharmacological profile with less toxicity [1]. In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place by the way of having less or no side effects. Therefore, a systematic approach would be made to find out the efficacy of plant medicines against inflammation and arthritis [5].

Over the centuries, a number of medicinal plants have been exploited for the treatment of the disorders associated with the inflammatory conditions or for the control of inflammatory aspects of diseases. These medicinal plants owe their activities due to the phytoconstituents and may exert anti-inflammatory effect by interfering generally with the inflammatory pathways or specifically with certain components of the pathway, such as release of pro-inflammatory mediators and migration of leukocytes under inflammatory stimuli with consequent release of the cytoplasmic contents at inflammatory sites [6].

Euphorbia antiquorum Linn (EA), family Euphorbiaceae, is commonly found in village shrubberies throughout the tropical and warm temperate regions of India and Ceylon. The plant is reported to be used traditionally in inflammatory disorders such as rheumatism and gout, to relieve pain in rheumatism and toothache (the juice, which flows from the branches), in nerve diseases, dropsy, palsy and deafness, and as a purgative [7]. It was reported that the medicinal properties of EA are similar to that of Euphorbia tirucalli which was proved for its anti-arthritic potential [8]. The phytochemicals, diterpenes and triterpenes, were isolated and characterized from the latex of EA [9,10]. Diterpenes and triterpenes were previously reported for anti-inflammatory and anti-arthritic activity [11,12,13,14,15]. However, the anti-inflammatory and anti-arthritic activity of EA was little investigated. Hence, the present study was undertaken to evaluate the anti-inflammatory and anti-arthritic potential of aqueous and alcoholic extracts of EA.

Materials and Methods

Collection and authentication:
The whole plant of EA was collected from Gokak district of Karnataka, India in the month of June. A voucher specimen of the plant material was deposited at and authenticated by Dr. Harsha Hegde, Regional Medical Research Center, Indian Council of Medical Research, Belgaum - 590010, Karnataka, India (3/12/2007).

Preparation of extracts:
The plant was completely washed with water to remove dirt and foreign material, dried in shade, and coarsely powdered. The powdered material was macerated in distilled water for a week and then macerate was filtered to obtain aqueous extract of EA (AEA). The alcoholic extract of EA (EEA) was prepared by Soxhlet extraction method using ethanol (99 % v/v). The extracts were evaporated in a China dish on water bath to obtain thick paste.
Drugs and chemicals:
Carrageenan (Himedia Ltd., Mumbai, India); complete Freund’s adjuvant (Sigma, USA); ibuprofen (Brufen® tablets, Abbot, India); and other chemicals were purchased from local vendors of Belgaum (Karnataka, India). All the chemicals used were of analytical grade.

Preparation of drug solution:
AEA, EEA, and Brufen® tablets were powder triturated and suspended in 0.5 % CMC in distilled water. All solutions were prepared freshly and stored in glass bottles. Vehicle (0.5 % CMC in distilled water), ibuprofen 50 mg/kg (used as reference standard) [16], AEA, and EEA were administered per orally (po).

Animals:
Healthy adult Swiss mice (20-30 g) of either sex or male Wistar rats (150-200 g) procured from Venkateshwara Enterprises, Bangalore (Karnataka, India) were used for the study. The animals were housed under standard conditions of temperature (25±3°C), relative humidity (55±10 %), and 12 h: 12 h light: dark cycle and fed with standard pellet rodent diet (Lipton India Ltd., Mumbai) and water ad libitum. The experimental protocols were approved by Institutional Animal Ethical Committee of KLES’s College of Pharmacy, Belgaum, Karnataka, India.

Preliminary phytochemical evaluation:
The AEA and EEA were subjected for the qualitative analysis by using the standard phytochemical tests to evaluate the presence of various phytoconstituents [17].

Acute oral toxicity (AOT) study:
Healthy adult Swiss mice (20-30 g) were subjected to AOT study as per Organization for Economic Co-operation and Development (OECD) guidelines 2001 (AOT-423). Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for total of 14 days. The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, and behaviour pattern were noted [18].

Carrageenan induced rat paw edema:
Male Wistar rats (150-180 g) were divided into six groups (n=6) and treated as follows. Control group: vehicle (10 ml/kg, po); Ibup 50: ibuprofen (50 mg/kg, po); AEA 200 and 400: AEA (200 and 400 mg/kg, po); and EEA 200 and 400: EEA (200 and 400 mg/kg, po). Thirty minutes after the respective treatments the animals were challenged with sub-planter injection of freshly prepared carrageenan (1 %, 0.1 ml) in right hind paw. The paw volume was measured immediately (0 h) and at the interval of 1 h upto 5 h using digital plethysmometer (UGO Basile, Italy). The change in paw volume was determined with respect to the left hind paw. The results were expressed as percentage edema calculated using the formula [19,20]:

\[
\text{\% Edema} = \frac{\text{Final paw volume (ml) – Initial paw volume (ml)}}{\text{Initial paw volume (ml)}} \times 100
\]

Cotton pellet induced granuloma in rats:
Male Wistar rats with an average weight of 200 g were divided into four groups (n=6) and treated as follows for 7 days. Control group: vehicle (10 ml/kg, po); Ibup 50: ibuprofen (50 mg/kg, po); AEA 400: AEA 400 mg/kg, po; and EEA 400: EEA 400 mg/kg, po. The groin region of rat was shaved and disinfected with 70 % ethanol. Autoclaved cotton pellet (10±1 mg) was implanted subcutaneously by making incision in the axilla and groin region of each rat under ether
anesthesia. The respective treatments were initiated 30 min before the implantation of the cotton pellets and thereafter, for 7 consecutive days after the cotton pellet implantation. Animals were sacrificed on 8th day; pellets were dissected out; freed from extraneous tissue; dried in hot air oven at 60°C for 24 h; and weighed individually. The net dry weight i.e. after subtracting the initial weight from the final weight of the cotton pellet was determined for each animal. The mean net dry weight of the cotton pellets was calculated as weight of granuloma. The percentage inhibition of granuloma was determined using the formula: \( \frac{1}{W_2 - W_1} \times 100 \), where \( W_1 \) was mean net dry weight of the cotton pellets from the treated group and \( W_2 \) was mean net dry weight of the cotton pellets from the control group [21,22].

**Complete Freund's adjuvant (CFA) induced arthritis in rats:**
Male Wistar rats (150-200 g) were divided into four groups (n=6) and treated as follows for 21 days. Normal control group: vehicle (10 ml/kg, po); control group: vehicle (10 ml/kg, po); Ibup 50: ibuprofen (50 mg/kg, po); AEA 400: AEA 400 mg/kg, po; and EEA 400: EEA 400 mg/kg, po. The animals were made arthritic by subcutaneous injection of 0.1 ml 1% CFA into the sub-plantar side of the left hind paw of rats from all groups, except normal control group. Thirty minutes before CFA injection and thereafter, on each day animals from different groups were received respective treatments. On 21st day of CFA injection, animals were subjected to evaluation of change in paw volume and body weight; radiographic examination; change in spleen weight; and histopathological examination of spleen as described below [23].

**Change in paw volume:**
The volume of injected left paw and non-injected right paw of rats were measured on initial day immediately after CFA injection and thereafter, on 4th, 8th, 12th, 16th, and 21st day of CFA injection, using digital plethysmometer (UGO Basile, Italy). The percentage change in paw volume was determined with respect to the initial paw volume. The results were expressed as percentage edema calculated using formula given in section 3.

**Change in body weight:**
The change in body weight was determined with respect to initial body weight on the day before CFA injection. The mean change in body weight of the treated group was compared with the mean change in body weight of the arthritis control group.

**Spleen weight:**
On 21st day, animals were sacrificed by cervical dislocation, the spleen was dissected out, weighed, and the change in spleen weight was determined with respect to the spleen weight of animals from the normal control group.

**Histopathological examination of spleen:**
The isolated spleen tissues were sectioned and fixed in 10% formalin solution. The sections were stained with haematoxylin and eosin and examined microscopically (x400) for histopathological changes.

**Radiographic examination:**
Radiographic examinations were performed on 21st day of CFA injection by digital X-rays (Agfa – CR-30) to determine deformities in joints, bones, cartilages, and soft tissues of hind paws.

**Statistical analysis:**
The values were expressed as mean±SEM (n=6). The statistical significance was assessed using student t-test or one-way analysis of variance (ANOVA) followed by Dunnett’s test and \( P<0.05, P<0.01, \) and \( P<0.001 \) were considered to be statistically significant.
Results

Preliminary phytochemical evaluation:

The phytochemical evaluation revealed the presence of carbohydrates and triterpenoids in AEA and triterpenoids and saponins in EEA.

Acute oral toxicity study (AOT):

The animals treated with AEA and EEA did not show any signs of toxicity and mortality during the 24 hours up to the dose of 2 g/kg. Further, there was no any change in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, and behavior pattern of the animals.

Carrageenan induced rat paw edema:

The challenge with carrageenan (1% w/v, 0.1 ml) in the right hind paw of rats showed significant increase in the paw edema in the control group as compared to the contra-lateral (left) paw. The peak inflammation was observed at 3-4 h.

The administration of AEA 200 mg/kg showed significant (P<0.05) inhibition of paw edema at 3-5 h as compared to the control group. The administration of AEA 400 mg/kg showed significant (P<0.05) inhibition of paw edema at 2-5 h as compared to the control group. The administration of EEA (200 and 400 mg/kg) showed significant (P<0.05) inhibition of paw edema at 2-5 h as compared to the control group. The administration of ibuprofen 50 mg/kg showed significant (P<0.05) inhibition of paw edema at 2-5 h as compared to the control group. (Table 1)

| Table 1. Effect of extracts of EA in carrageenan induced rats paw edema |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Groups         | 0 h             | 1 h             | 2 h             | 3 h             | 4 h             | 5 h             |
| Control        | 11.43±0.83      | 34.07±6.93      | 37.7±6.39       | 40.48±6.03      | 40.33±5.13      | 32.7±6.35       |
| Ibup 50        | 8.79±0.30       | 19.22±2.71      | 15.88±1.90*     | 13.77±3.58*     | 7.51±1.35*      | 12.47±1.85*     |
| AEA 200        | 9.17±0.76       | 25.52±2.47      | 29.12±5.26      | 21.52±4.10*     | 15.79±3.09*     | 15.47±2.19*     |
| AEA 400        | 10.14±0.28      | 20.39±1.81      | 19.91±2.36*     | 13.43±2.71*     | 13.47±4.58*     | 11.68±3.98*     |
| EEA 200        | 8.92±1.23       | 24.6±4.34       | 17.35±3.12*     | 14.46±3.49*     | 13.44±2.94*     | 12.48±2.49*     |
| EEA 400        | 8.75±0.62       | 20.31±3.36      | 13.13±4.14*     | 10.72±3.63*     | 11.08±3.72*     | 11.19±2.92*     |

The values were expressed as mean±SEM (n=6). *P<0.05 as compared with control group.

Cotton pellet induced granuloma in rats:

Implantation of cotton pellets in the groin region of the rats in the control group significantly provoked granuloma formation (proliferative inflammation), as evident from the increase in the net dry weight of cotton pellets. The administration of AEA 400 mg/kg and EEA 400 mg/kg showed significant (P<0.05) protection of granuloma formation, as evident from the decrease in the net dry weight of cotton pellets as compared to the control group. The administration of ibuprofen 50 mg/kg showed significant (P<0.05) prevention of the proliferative inflammation as compared to the control group. (Table 2)

| Table 2. Effect of extracts of EA in cotton pellet induced granuloma in rats |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Groups         | Weight of granuloma (g) | % Inhibition  |
| Control        | 44.33±2.98      | 0               |
| Ibup 50        | 29.83±2.49*     | 32.71           |
| AEA 400        | 24.50±3.45*     | 44.73           |
| EEA 400        | 24.33±2.06*     | 45.12           |

The values were expressed as mean±SEM (n=6). *P<0.05 as compared with control group.
Complete Freund's adjuvant induced arthritis in rats:

Injection of the CFA (1 % w/v, 0.1 ml) into the sub-plantar region of left hind paw of the rats in the arthritis control group induced arthritic changes, as evident from the evaluation of following parameters.

Change in paw volume:

a. Primary Lesions: Injection of the CFA (1 % w/v, 0.1 ml) into the sub-plantar region of left hind paw of the rats showed significant increase in percentage paw edema on 4th, 12th, 16th, and 21st day with peak increase on 12th day of CFA challenge in the arthritis control group. The administration of AEA 400 mg/kg showed significant (P<0.05) decrease in percentage paw edema on 12th and 16th day as compared to the arthritis control group. The administration of EEA 400 mg/kg showed significant (P<0.01 and P<0.05 respectively) decrease in percentage paw edema on 12th, 16th, and 21st day as compared to the arthritis control group. The administration of ibuprofen 50 mg/kg showed significant (P<0.05) decrease in percentage paw edema on 12th day as compared to the arthritis control group. (Table 3)

<table>
<thead>
<tr>
<th>Groups</th>
<th>4 day</th>
<th>8 day</th>
<th>12 day</th>
<th>16 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>62.37±4.85</td>
<td>62.17±4.25</td>
<td>102.30±7.63</td>
<td>64.35±7.47</td>
<td>89.35±5.97</td>
</tr>
<tr>
<td>Ibup 50</td>
<td>46.90±5.78</td>
<td>56.29±5.47</td>
<td>66.48±5.87</td>
<td>61.84±6.22</td>
<td>78.18±7.89</td>
</tr>
<tr>
<td>AEA 400</td>
<td>64.31±4.43</td>
<td>62.43±1.76</td>
<td>71.87±6.75</td>
<td>*38.88±3.09</td>
<td>77.97±6.07</td>
</tr>
<tr>
<td>EEA 400</td>
<td>61.90±4.86</td>
<td>60.83±5.97</td>
<td>33.7±3.17</td>
<td>*34.7±7.90</td>
<td>62.47±6.80</td>
</tr>
</tbody>
</table>

The values were expressed as mean±SEM (n=6). *P<0.05 and **P<0.01 as compared with control group.

b. Secondary Lesions: The CFA challenge in left hind paw of the rats showed significant increase in percentage paw edema in non-injected right paw on 12th, 16th, and 21st day of CFA challenge in the arthritis control group. The administration of AEA 400 mg/kg showed significant (P<0.01) decrease in percentage paw edema in non-injected right paw on 12th and 16th day as compared to the arthritis control group. The administration of EEA 400 mg/kg showed significant (P<0.01) decrease in percentage paw edema in non-injected right paw on 12th, 16th, and 21st day as compared to the arthritis control group. The administration of ibuprofen 50 mg/kg showed insignificant effects in this regard. (Table 4)

<table>
<thead>
<tr>
<th>Groups</th>
<th>4 day</th>
<th>8 day</th>
<th>12 day</th>
<th>16 day</th>
<th>21 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>10.02±3.03</td>
<td>16.63±2.82</td>
<td>45.93±5.26</td>
<td>31.37±4.38</td>
<td>28.15±3.21</td>
</tr>
<tr>
<td>Ibup 50</td>
<td>1.87±2.68</td>
<td>5.30±5.39</td>
<td>27.09±5.49</td>
<td>20.48±5.45</td>
<td>21.33±9.04</td>
</tr>
<tr>
<td>AEA 400</td>
<td>1.83±1.17</td>
<td>15.46±1.61</td>
<td>7.54±3.09</td>
<td>*17.39±1.66</td>
<td>20.84±3.57</td>
</tr>
<tr>
<td>EEA 400</td>
<td>6.11±3.51</td>
<td>6.59±2.78</td>
<td>5.88±1.53</td>
<td>*4.17±1.54</td>
<td>5.05±1.29</td>
</tr>
</tbody>
</table>

The values were expressed as mean±SEM (n=6). *P<0.05 and **P<0.01 as compared with control group.

Change in body weight:

The animals challenged with CFA in the arthritis control group showed significant (P<0.05) decrease in the change in body weight. The administration of EEA 400 mg/kg showed significant (P<0.05) increase in the change in body weight as compared to the arthritis control group, whereas, AEA 400 mg/kg and ibuprofen 50 mg/kg showed insignificant effects in this regard. (Table 5)
Spleen weight:
The animals challenged with CFA in the arthritis control group showed significant (P<0.05) increase in the spleen weight as compared to the normal control group. The administration of AEA 400 mg/kg and EEA 400 mg/kg showed significant (P<0.05) decrease in the spleen weight as compared to the arthritis control group. Whereas, ibuprofen 50 mg/kg showed insignificant effects in this regard. (Table 5)

Table 5. Effect of extracts of EA on body weight and spleen weight in CFA induced arthritis in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in body weight (g)</th>
<th>Change in spleen weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis control</td>
<td>21.42±1.18</td>
<td>0.230±0.004</td>
</tr>
<tr>
<td>Ibup 50</td>
<td>28.42±2.58</td>
<td>0.218±0.002</td>
</tr>
<tr>
<td>AEA 400</td>
<td>26.75±2.76</td>
<td>0.217±0.003*</td>
</tr>
<tr>
<td>EEA 400</td>
<td>31.17±1.82</td>
<td>0.212±0.006*</td>
</tr>
</tbody>
</table>

The values were expressed as mean±SEM (n=6). *P<0.05 as compared with control group.

Histopathology of spleen:
Histopathology of spleen of animals from the arthritic control group revealed the marked follicular hyperplasia and inter-follicular (red pulp) cytosis mainly around penicilary arteries (Fig 1b and 1c) as compared to the spleen of animals from the normal control group (Fig 1a). The spleen of AEA 400 mg/kg and ibuprofen 50 mg/kg treated animals was observed same as that of animals from the arthritis control group. Whereas, the spleen of EEA 400 mg/kg treated animals showed congestion, reduced follicles leading to lymphoid depletion, and atrophic germinal follicles with expanded red pulp indicating decreased immune response (Fig 1d).
Figure 1: Effect of AEA and EEA on histopathology of spleen in CFA induced arthritis in rats (x400): a) Normal control group; b) Arthritis control group (increased white pulp, follicular hyperplasia); c) Arthritis control group (increased red pulp, inter-follicular cytosis around penicilary arteries); d) EEA 400 mg/kg (reduced follicles, atrophic germinal follicles, expanded red pulp).

Radiographic examinations:
The results of radiographic examination were discussed with two orthopedics and two radiologists. The results of radiographic examination of rats from the arthritis control group revealed the severe soft tissue swelling, narrowing of the joint spaces, and the subsequent destruction of the bones and cartilages in the knee joint (Fig 2b) as compared to the normal control group (Fig 2a). The administration of EEA 400 mg/kg, but not AEA 400 mg/kg and ibuprofen 50 mg/kg, was markedly prevented the soft tissue swelling and the destruction of the knee joints (Fig 2c) as compared to the arthritis control group.

Figure 2: Effect of AEA and EEA on radiographic examinations of spleen in CFA induced arthritis in rats: a) Normal control group; b) Arthritis control group; c) EEA 400 mg/kg.

Discussion
The carrageenan induced paw inflammation has been accepted as a useful phlogistic tool for investigating systemic anti-inflammatory agent [24]. The edema induced by carrageenan is highly sensitive to NSAIDs and has been accepted as a useful indicator for identifying the new anti-inflammatory molecules [25]. Local injection of carrageenan into rat hind paw induces acute inflammatory responses such as edema [26]. The carrageenan induced edema has been described
as a biphasic event, a rapid early phase (up to 2 h) triggered by the concerted release of histamine, bradykinin, 5-hydroxytryptamine, or cyclooxygenase products and a more sustained late phase (2 to 5 h) regulated by neutrophil infiltration and sustained production of arachidonic metabolites (prostanoids) (primarily by cyclooxygenase) or nitric oxide from inducible nitric oxide synthase [27,28,29]. Hence, in the present study the effect of AEA and EEA on carrageenan induced paw inflammation was evaluated up to 5 h. The results of the carrageenan induced rat paw edema revealed that AEA (200 and 400 mg/kg) and EEA (200 and 400 mg/kg) showed significant inhibition of paw edema at 2-5 h of carrageenan challenge as compared to the control group. This revealed that both the extracts were effective in preventing more sustained late phase (2-5 h) of edema.

Inflammatory responses occur in three distinct phases, each apparently mediated by different mechanisms. An acute, transient phase, characterized by local vasodilatation and increased capillary permeability; a sub-acute phase, characterized by infiltration of leukocytes and phagocytic cells; and a chronic proliferative phase, in which tissue degeneration and fibrosis occur. The granuloma formation is a widely used method for assessment of anti-inflammatory activity of compounds against chronic (proliferative phase) inflammation. This method is useful for evaluation of steroidal and NSAIDs that shows higher activity in this model [22, 30]. In present study, implantation of cotton pellets in the groin region of the rats in the control group were significantly provoked granuloma formation, as evident from the increase in the net dry weight of cotton pellets. The AEA and EEA were found to be effective in preventing the granuloma formation at 400 mg/kg.

Adjuvant-induced arthritis in rats is a well established experimental model that has features similar to the human rheumatoid arthritis. In addition, it is a good chronic inflammatory model for development of potential analgesic and/or anti-inflammatory drugs useful for the treatment of arthritis [31,32]. An injection of CFA into the rat paw induces inflammation as primary lesion with a maximum after 3 to 5 days. Secondary lesions occur after a delay of approximately 11 to 12 days which are characterized by inflammation of non-injected sites (hind-leg, forepaws, ears, nose, and tail); decrease in body weight; and cell-mediated immunity. The suppression of these effects suggests immunosuppressive activity [8,22].

The spleen is an important lymphoid organ involved in immune responses against all types of antigens that appears in the circulation and it provides a readily available source of cells known to be involved in adjuvant arthritis. Increased cellularity in the spleen of CFA injected rats provoked the interest as a potential for concomitant classical antibody formation where the increased antibody titer in arthritic animals further supports the hyper-immune status by humoral immunity. Further, the increase in spleen weight of the CFA induced arthritic rats has been reported to be associated with splenomegaly, generalized lymphadenopathy, and altered hepatic function [33]. In present study, injection of the CFA (1 % w/v, 0.1 ml) into the sub-plantar region of left hind paw of the rats in the arthritis control group significantly induced the primary and secondary lesions with decreased change in body weight, increased spleen weight, and histological injury to spleen.

The results of the CFA induced arthritis revealed the significant prevention of injected paw inflammation by AEA 400 mg/kg on 12th and 16th day and that by EEA 400 mg/kg on 12th, 16th, and 21st day. In addition, the significant prevention of non-injected paw inflammation was evident after the treatment with AEA 400 mg/kg on 12th and 16th day and that with EEA 400 mg/kg on 12th, 16th, and 21st. Further, treatment with EEA 400 mg/kg was significantly increased the change in body weight and prevented the histological injury to spleen. Whereas, AEA 400 mg/kg, EEA 400 mg/kg were significantly decreased the spleen weight. These results indicated that AEA 400 mg/kg was most effective in preventing the primary lesions and EEA 400 mg/kg was effective in preventing both primary as well as secondary lesions of CFA induced arthritis.
Eighty percent of patients with rheumatoid arthritis have a circulating antibody directed against antigens specific for Epstein-Barr virus and the autoantibody response in rheumatoid arthritis enhances the response to these antigens. Epstein-Barr virus is well established as a polyclonal activator of B-lymphocytes, resulting in the overproduction of immunoglobulins including rheumatoid factor [34]. Eupha-7,9(11),24-trien-3beta-ol (Antiquo C) and other triterpenes from EA latex possess inhibitory effects on Epstein-Barr virus activation [35]. In present study, the histopathology of spleen of rats treated with EEA revealed atrophic germinal follicles. Thus, the suppression in activation of B-lymphocytes and decreased immunological response may be responsible for the anti-arthritic potential of EA.

Hence, the findings of the present study exhibited the anti-inflammatory and anti-arthritic potential of aqueous and alcoholic extracts of EA. The previous phytochemical investigations on EA showed the presence of triterpenoids in the stem [9] and diterpenoids in the latex [10]. It was reported that the anti-inflammatory activity is a common property of many triterpenoids [36]. The anti-inflammatory effects of triterpenes have been attributed to various mechanisms including inhibition of lipooxygenase and cyclooxygenase activities [37]. Additionally, the antioxidant activity has been described for several triterpenes, such as α- and β- amyrins, oleanolic acid, ursolic acid, lupeol and glycirretinic acid [38]. In present study, the phytochemical evaluation revealed the presence of carbohydrates and triterpenoids in AEA and triterpenoids and saponin glycosides in EEA, which was consistent with the previous reports. Furthermore, Jyothi et al., 2008 has been reported that the aqueous extract of the aerial parts of EA produced significant antioxidant activity by scavenging off the free radicals but not by interfering with the generation of the free radicals. The antioxidant potential of EA may be attributed to the presence of polyphenolic compounds [39].

From the findings of the present study and the previous reports, it was revealed that the triterpenoids present in both aqueous and alcoholic extracts of EA might be responsible for the anti-inflammatory and anti-arthritic effect. The anti-inflammatory and anti-arthritic effects might be attributed, directly or indirectly, to relative antioxidant effect; inhibition of the arachidonic metabolites; suppression of cell-mediated immunity thereby suggesting the immunosuppressive activity; or prevention of tissue degeneration and fibrosis.

Further studies like isolation and characterization of the active principal(s) responsible for such activity are needed to confirm. However, the present study justifies the traditional claims that the plant is useful in treating the conditions associated with rheumatism. Thus Euphorbia antiquorum Linn. would be a good candidate for further development as a new phytotherapeutic medicine.

Acknowledgement

The authors are thankful to Dr. F. V. Manvi, Principal and Head, Department of Pharmaceutics, KLES’s College of Pharmacy, Belgaum, Karnataka (India) for providing the facilities necessary to carry out the study.

References