Arthritis is a chronic inflammatory disease affecting the musculoskeletal system and caused by a multisystem autoimmune reaction. Some medicinal herbs work synergistically to reduce chronic joint inflammation in arthritis. The purpose of this study was to determine the antiarthritic activity of combination of red ginger rhizome and acalypha whole plant ethanolic extracts on complete Freund’s adjuvant induced arthritis. Adjuvant arthritis was induced in rats on day 0. Nine days after inoculation, the animals were selected and distributed into 5 groups. One group was given 0.5% CMC solution as vehicle-treated group, the others were given red ginger extract (280 mg/kg bw), acalypha extract (200 mg/kg), combination of red ginger and acalypha extracts (140 mg/kg bw:100 mg/kg bw), and triamcinolone (0.3 mg/kg bw) orally. The degree of inflammation was evaluated by hind paw swelling, supported by histopathology of ankle joints. Results suggested that oral dosage of acalypha extract, red ginger extract, as well as their combination reduced hind paw swelling, comparable to that’s of triamcinolone. Histopathology study revealed the significant reduction in mononuclear infiltration and increased the ankle joint space. The combination of red ginger rhizome and acalypha extracts showed protective effect against adjuvant induced arthritis.

**Keywords:** red ginger rhizome, acalypha, complete Freund’s adjuvant, antiarthritic, histopathology
INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socioeconomic impact. The world prevalence of rheumatoid arthritis is about 0.5-1% of the population. It usually occurs in the people between 25 and 55 years of age. Women are affected more often than men at ratio of 3 to 1 (Patwardhan et al., 2010).

Adjuvant induced arthritis (AIA) in rats is a chronic inflammatory disease characterized by infiltration of synovial membrane in association with destruction of joints resembles RA in humans. RA progress was divided in three stages. The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joints. Second is the rapid division and growth of cell, or pannus, which causes the synovium to thicken. In the third stage, the inflamed cell releases enzyme that may digest the bone and cartilage, often causing the joints to loses its shape and alignments, more pain and loss of movements. The most commonly prescribed medication for RA treatment is steroidal, non-steroidal anti-inflammatory, disease modifying antirheumatic and immunosuppressant drugs. Though the goal of these drugs have been to relieve pain and to decrease joint inflammation, to prevent joint destruction and to restore function of disabled joints, these drugs are known to produce various side effects including gastrointestinal disorders, immunodeiciency and humoral disturbances. Accordingly, reducing side effects should be considered while designing improved therapeutics for Rheumatoid arthritis, besides enhancing medicinal effectiveness. Traditional treatment is being increasingly recognized as an alternate approach to arthritic treatment (Bansod et al., 2010).

Red ginger (Zingiber officinale Rosc.) has been used for medicinal purposes since antiquity. In particular, it has been an important plant for the traditional Indonesian medicines. Although one of its indications has been historically to treat rheumatic disorders and although ginger extracts have shown the ability to inhibit arachidonic acid metabolism and have antiinflammatory action and/or antirheumatic properties, there are very limited published reports on the efficacy of this plant (Funk et al., 2009; Rehman et al., 2011; Shen et al., 2003). Acalypha indica belongs to Euphorbiaceae family and can be found in plains of Indonesia. It has been traditionally used as an anthelmintic, cathartic, scabies, and rheumatism. The extracts of A. indica have been reported to possess anti-inflammatory, analgesic and antimicrobial properties (Krishna et al., 2011).

The objective of our study was to determine the antiarthritic activity of combination of red ginger rhizome and acalypha whole plant ethanolic extracts on Complete Freund’s adjuvant (CFA) induced arthritis.

MATERIAL AND METHODS

Materials

The fresh plants of A. indica were collected from the local region and rhizomes of red ginger were purchased from local vendor. The plant materials were identified and authenticated taxonomically. CFA (Sigma) containing suspension of 0.1% w/v killed Mycobacterium tuberculosis bacteria homogenized in liquid paraffin.

Wistar rats were used for the study. The animals were acclimatized to animal house conditions. The rats were fed with commercial rat’s diet and water ad libitum.

Methods

Preparation of extracts

The rhizomes of red ginger were washed, cut into small pieces, and dried under shade. Coarse powder was made and extracted.
by maceration with 90% ethanol for 5 days at room temperature. Dried plants of *A. indica* were crushed into powder and extracted by maceration with 90% ethanol for 5 days at room temperature. The whole extract of individual plants was collected in conical flasks, filtered and the solvents were evaporated to dryness under reduced pressure.

**Drug treatment**

Animals were randomly divided into five groups of five animals each (n=5). Group I served as negative control (CMC Na 1% in double distilled water p.o). Group II was given reference standard, Triamcinolone (0.36 mg/kg p.o). Group III-V were administered red ginger extract (GE) 280 mg/kg bw, *A. indica* extract (AE) 200 mg/kg, and combination of GE-AE 140mg: 100 mg/kg bw, respectively. The prepared extract was administered once daily for 7 consecutive days.

**Freund's adjuvant induced arthritis**

Arthritis was induced by injecting a 0.2 ml of CFA into the left hind paw. Drug treatment was started from 5th day after adjuvant injection and continued till 7 days. Paw volume was measured daily from first day after adjuvant induction using plethysmometer. The mean changes in injected paw edema with respect to initial paw volume, were calculated on respective days and % inhibition of paw edema with respect to untreated group was calculated (Bendele, 2001).

**RESULTS AND DISCUSSION**

RA is a chronic inflammatory disease characterized by fibroblastic proliferation, infiltration of the synovial lining by inflammatory cells which leads to expression of proinflammatory cytokines and a paucity of apoptosis resulting in bone and joint destruction (Ghildiyal *et al.*, 2013). Despite widely research being carried out for immune disease, it still remains a challenge since no satisfactory treatment is available in clinical practices.

Hence, there is enormous interest worldwide for the use of an alternative medicine. The research has been focused on formulations used in traditional medicine for treatment of RA (Long *et al.*, 2001; Ram *et al.*, 2012).

CFA is used to initiate induction of arthritis. This model is the original model of RA, has been extensively used to preclinical screening of new antiarthritis compounds. After a single injection of the adjuvant, a rapid, reliable, robust, and easily measurable polyarthritis develops. The joint pathology seen in this animal model shares the cartilage degradation, bone reportion, and cellular influx seen in human RA (Bendele *et al.*, 1999). CFA-induced arthritic animals produced increase in paw volume and paw thickness in disease control group. Treatment with *A. indica* extracts and triamcinolone to diseased animal showed statistically significant (P<0.05) reduction in oedema inhibitory activity compared to disease control group. Extract of red ginger rhizome produced lower effect than that’s of triamcinolone (Figure 1). Based on area under curve (AUC) data of oedema inhibitory activity, AE and combination of GE-AE showed comparable antiarthritis activity to triamcinolone, however GE showed the less AUC (Figure 2).
Figure 1. Curve of oedema inhibitory percentage vs days of treatment

Figure 2. AUC of oedema inhibitory percentage

Normat rat’s ankle showed clear and wide joint space, but arthritis rat’s ankle showed narrow joint space. Histopathology of ankle joint in CFA treated rats, revealed enhanced neutrophil infiltration, whereas in triamcinolone as well as GE treated rats there were significant reduction in neutrophil infiltration (Figure 3).
Table 1 presented the average length of joint space on histopathology study. On normal rat group, the joint space length was wide, contrary to the narrow joint space on arthritis group. Therapy with triamcinolone for 7 days significantly increased the length of joint space, and comparable effect was showed by AE group. The effect of AE group was much better than that’s of GE and combination of GE-AE. It was in accordance with the reasearch of Krishna et al. (2011), which reported the widen of joint space length after AE administration, due to decreasing of capillary permeability. Both of GE and the combination of GE-AE showed less of joint space length, but the joint space was clear from infiltration.

Table 2. Length of joint space on histopathology study

<table>
<thead>
<tr>
<th>Group</th>
<th>Length of joint space (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GE 280mg/kg</td>
<td>121.56 ± 56.56</td>
</tr>
<tr>
<td>AE 200mg/kg</td>
<td>173.76 ± 63.17</td>
</tr>
<tr>
<td>GE:AE 140mg:100mg/kg</td>
<td>111.1 ± 26.02</td>
</tr>
<tr>
<td>Triamcinolone 0.36mg/kg</td>
<td>179.33 ± 64.10</td>
</tr>
<tr>
<td>Negative control</td>
<td>104.13 ± 17.06</td>
</tr>
<tr>
<td>Normal rat</td>
<td>229.63 ± 35.61</td>
</tr>
</tbody>
</table>
Rehman et al. (2011) reviewed that ginger constituents inhibit arachidonic acid metabolism and thus prostaglandin synthesis. This may play a role in its anti-inflammatory properties. Ginger may be a stronger inhibitor of prostaglandin synthesis than indomethacin. It can be used either for rheumatoid or osteoarthritis (Shen et al., 2003). Our phytochemical investigation showed the presence of alkaloids, flavonoids, terpenes, and saponin in the Acalypha extract, that could be suggested to contribute to the antiarthritis activity of the extract. Each of ginger extract and Acalypha extract showed the preference as antiarthritis activity. Acalypha extract showed oedema inhibitory activity and enhancement of joint space length comparable to triamcinolone, while ginger extract produced clear joint space. Hence the combination of Acalypha extract and ginger extract has an advantage compared to each single extract.

**CONCLUSION**

Oral dosage of acalypha extract, red ginger extract, as well as their combination reduced hind paw swelling, comparable to that’s of triamcinolone. Histopathology study revealed the significant reduction in mononuclear infiltration and increased the ankle joint space. The combination of red ginger rhizome and acalypha extracts showed protective effect against adjuvant induced arthritis.

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