Anti-arthritic evaluation of Eclipta alba in a murine model Freund’s adjuvant provoked arthritis

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ABSTRACT

Eclipta alba (E. alba) is a medicinal plant with wide range of biological action encompassing antioxidant and anti-inflammatory. However, its anti arthritic activity is not reported till date. So we have evaluated the anti-arthritic property of E. alba methanolic extract in arthritis induced rats. The rats were made arthritic by single intradermal injection of complete freunds adjuvant (CFA) and E. alba (200 and 400mg/kg) were administered for 28 days. The assessment of arthritis was done by evaluating body weight, paw volume and alteration in hematological parameters (WBC, RBC, Hb and ESR). Further, to evaluate oxidative stress, malondialdehyde (MDA), a marker of lipid peroxidation and antioxidants (SOD, CAT, GPx and GSH) were measured. The arthritis induced rats showed significant decrease in body weight, elevated paw oedema, and changes in blood parameters. Treatment with E. alba significantly reduced the arthritic symptoms by its anti-inflammatory effect. Further, arthritic rats displayed elevated MDA and decreased antioxidant levels and treatment with E. alba inhibited the lipid peroxidation and restored the antioxidants to normal. The present study reveals that Eclipta alba showed effective anti-arthritic activity through its antioxidant and anti-inflammatory property. Further the anti-arthritic activity of E. alba might be due to the presence of various phytochemicals such as flavanoids and polyphenols.

INTRODUCTION

Rheumatoid arthritis (RA) is one of the autoimmune related diseases. Across the globe, it affects millions of individuals and highly prevalent among the age groups between 30-50 years (Lundkvist et al., 2008). The pathological feature of RA encompasses inflammation of joint, synovial tissue proliferation and articular cartilage damage leading to joint disability and depression (Dowman et al., 2012; Mcinnes and Schett, 2011). The prominent mechanism involved in the joint destruction during RA is oxidative stress and increased expression of variety of pro-inflammatory cytokines like TNF-α, IL-6 etc (Chimenti et al., 2015). Various pharmacological agents like steroids, immuno-suppressants and anti-inflammatory agents, particularly NSAIDs are used for the management of RA. However, these agents cause severe economic burden and elicit serious side effects (Wilson and Hill, 2017). Currently, intensifying researches have been carried out for the discovery of novel agents with minimal or no adverse effects for the treatment of RA. Intensifying studies have used Complete Freund’s adjuvant (CFA)
for the induction of arthritis in a murine model since it mimics the features of human rheumatoid arthritis (Geetha and Varalakshmi, 1999; Bihani et al., 2014). During CFA injection it shows rapid local inflammatory reactions and remains few days, followed by long period systemic inflammation and remains for many months (Neugebauer et al., 2007). Due to high treatment costs and adverse effects in allopathic medicines, arthritic patients are focusing towards herbal medicines for pain relief and reduction in joint swelling and damage (Rao et al., 1999).

_Eclipta alba_ (E.alba) belonging to the family Asteraceae is widely recommended for the management of many diseases like liver cirrhosis, jaundice, gallbladder problems and hepatitis (Singh et al., 2001). Previous study also showed significant inflammatory inhibiting property of E.alba in various inflammatory models (Kumar et al., 2005). So, we have evaluated the antiarthritic property of _E.alba_ of CFA induced arthritis in a murine model.

**MATERIALS AND METHODS**

Collection, Identification and extraction of _E.alba_

The entire plant of _E.alba_ was collected from the various gardens and nurseries of Palvancha, Bhadradri district, Telangana, India. Then the plant materials were dried under shade and coarsely powdered using pulverizer and packed in sealed containers. The powdered plant material was extracted using methanol by maceration method.

**Experimental design**

Male albino Wistar (160-180gms) were procured from Syanzyme health care business, Hyderabad and assimilated to lab condition and also provided with water and standard diet. The study was performed according to the CPCSEA regulations.
Table 1: Effect of *Eclipta alba* on paw volume in arthritis induced rats

| Groups                       | Paw Volume (in mm)  |
|------------------------------|---------------------|
|                              | Day 1               | Day 8               | Day 16              | Day 24              |
| Control                      | 4.3 ± 0.1           | 4.5 ± 0.2           | 4.4 ± 0.1           | 4.6 ± 0.1           |
| CFA                          | 7.6 ± 0.5 a*        | 16.6 ± 0.2 a*       | 22.5 ± 0.3 a*       | 25.7 ± 0.6 a*       |
| *E.alba + CFA (200mg/kg)*    | 5.8 ± 0.6 b*        | 7.5 ± 0.3 b*        | 6.7 ± 0.5 b*        | 5.5 ± 0.6 b*        |
| *E.alba + CFA (400mg/kg)*    | 5.7 ± 0.4 b*        | 6.9 ± 0.6 b*        | 5.9 ± 0.1 b*        | 4.8 ± 0.3 b*        |
| Diclofenac Sodium (5mg/kg)   | 5.9 ± 0.4 b*        | 7.1 ± 0.3 b*        | 6.2 ± 0.4 b*        | 4.9 ± 0.5 b*        |

The data are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparison between group was done by Tukey’s test. a-CFA vs Control; b-Extract and standard vs CFA. *p<0.05 denoted as significant.

Table 2: Effect of *E.alba* on hematological alteration in CFA induced arthritis

| Groups                       | WBC(×103/L)  | RBC(×106/L) | ESR(mm/Hr) | Hb(gm/dl) |
|------------------------------|--------------|-------------|------------|-----------|
| Control                      | 5.2 ± 0.98   | 0.98 3.0    | 7.4 ± 1.25 | 1.25 3.0  |
| CFA                          | 9.4 ± 1.29 a*| 4.9 ± 0.78 a*| 20.3 ± 3.56 a*| 9.3 ± 1.12 a*|
| *E.alba (200mg/kg) + CFA*    | 7.7 ± 1.12 b*| 6.7 ± 0.95 b*| 13.1 ± 2.56 b*| 12.6 ± 3.12 b*|
| *E.alba (400mg/kg) + CFA*    | 6.6 ± 1.05 b*| 7.1 ± 1.35 b*| 10.8 ± 1.14 b*| 13.5 ± 3.12 b*|
| Diclofenac Sodium (5mg/kg)   | 5.56 ± 1.45 b*| 7.2 ± 1.12 b*| 9.8 ± 1.78 b*| 13.8 ± 2.89 b*|

The data are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparison between groups was done by Tukey’s test. a-CFA vs Control; b-Extract and standard vs CFA. *p<0.05 denoted as significant.

Table 3: Effect of *E.alba* on antioxidant status in CFA induced arthritis

| Groups                       | SOD | CAT | GPx | GSH |
|------------------------------|-----|-----|-----|-----|
| Control                      | 7.28±0.76 | 65.75±4.12 | 122.45±6.76 | 3.45±0.05 |
| CFA                          | 3.56±0.45 a* | 32.45±2.56 a* | 85.76±6.45 a* | 1.28±0.06 a* |
| *E.alba (200mg/kg) + CFA*    | 5.24±0.35 b* | 45.65±3.12 b* | 106.54±5.56 b* | 2.12±0.08 b* |
| *E.alba (400mg/kg) + CFA*    | 7.05±0.53 b* | 53.65±3.90 b* | 116.65±6.56 b* | 2.97±0.09 b* |
| Diclofenac Sodium (5mg/kg)   | 7.14±0.45 b* | 58.65±3.45 b* | 120.56±6.12 b* | 3.12±0.08 b* |

The data are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparison between groups was done by Tukey’s test. a-CFA vs Control; b-Extract and standard vs CFA. *p<0.05 denoted as significant. SOD (units/mg of protein); CAT (n moles of H2O2 decomposed /min / mg of protein); GPx (n moles of GSH oxidized / min / mg of protein); GSH (nmoles/g tissue).

hemoglobin (Hb) and ESR were measured using Sysmex XE-2100, India autoanalyser

**Measurement of Lipid peroxidation**

The lipid peroxidation (LPO) marker, malondialdehyde (MDA) was measured as per the information mentioned in the kit procured from Kamineni Life Sciences Pvt. Ltd. Hyderabad, India

**Measurement of antioxidants**

The hepatic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) were measured according to the informational instructions mentioned in the kit procured from Kamineni Life Sciences Pvt. Ltd. Hyderabad, India.

**Statistical analysis**

The data are shown as Mean ± SEM. Analysis were done by one way ANOVA and T'Tukey's comparison test was used to measure the significance between groups. p<0.05 was noted as statistically significant.

**RESULTS AND DISCUSSION**

**Effect of *E.alba* on body weight and paw swelling in arthritic rats**

In this study, body weight was significantly (p<0.05) decreased in arthritic rats at day 8, 16 and 24 when compared to control. Administration of *E.alba* methanol extract (200 and 400mg/kg) significantly increased the body weight when compared to the arthritic group. The data are shown in Figure 1.

Further, after FCA induction, the animals showed arthritis development as seen by the significant
increase in paw volume from day 1 as that of the control group. Observation of paw volume was recorded at regular interval from day 1 of adjuvant injection. The rats treated with E. alba methanolic extract (200 and 400mg/kg) showed effective decrease in paw volume as that of the arthritic rats. The results are shown in Table 1.

**Effect of E. alba on hematological changes in arthritis challenged rats**

During arthritis the animals showed significant (p<0.05) increase of WBC and ESR as that of the control group. Meanwhile, the arthritic animals also displayed significant (p<0.05) decrease in RBC and Hb as that of the control group. Treatment with E. alba methanolic extract (200 and 400mg/kg) significantly (p<0.05) decreased the WBC and ESR and increases the RBC and Hb as that of the arthritic rats. The results are shown in Table 2.

**Efficacy of E. alba on antioxidant status in arthritic rats**

In this study, arthritic rat displayed significant (p<0.05) elevation in malondialdehyde (MDA) levels as that of the control group. Treatment with E.alba methanolic extract (200 and 400mg/kg) reduced the MDA level when compared to arthritic group (p<0.05). The results are shown in Figure 2.

In Figure 2 shows, MDA: Malondialdehyde. The data are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparison between group was done by Tukey’s test. a- CFA vs Control; b- Extract and standard vs CFA.

* p<0.05 denoted as significant.

**Effect of E. alba on antioxidant status in arthritic rats**

Arthritic rats displayed significant (p<0.05) reduction in antioxidants such as SOD, CAT, GPx and GSH. E.alba methanolic extract (200 and 400mg/kg) supplementation significantly (p<0.05) increased the antioxidant level as that of the arthritic rats. The data are shown in Table 3.

RA is an inflammatory joint disease and the etiological factors linked with this disease is still obscure (Lee et al., 2009; Karmakar et al., 2010). Further, it also affects the immune system and other vital organs. Due to this, development of novel drugs in reducing the symptoms of arthritis is on high need (Bax et al., 2011). In preclinical models, the arthritis is induced by complete freunds adjuvant (CFA) and it is a valid therapeutic model resembling human arthritis for the evaluation of new drugs.

Intradermal injection of CFA into the rat’s hind paw elicits local inflammation and extended systemic effects and elicits noxious response to stress proteins and proteoglycans content present in cartilage (Lin et al., 2014). The local effects of CFA slow down in 2-3 days, but the chronic phase persists for more than a week to many months (Akira et al., 2006). During the chronic phase, the CFA triggers secretion of inflammatory proteins such as cytokines, prostaglandins and lysosomal enzymes.

Body weight changes is due to the inflammation response induced by CFA and in the present CFA induced arthritis rats showed marked decrease in body weight as a result of prolonged inflammation as per previous reports (Mondal et al, 2016). However treatment with E.alba significantly increased body weight mediated through its anti-inflammatory property.

In our study, CFA induced arthritic rats showed marked elevation in paw swelling as compared to the control rats (Zhang et al., 2017). Paw swelling during CFA injection shows the intensity of inflammation and it is primarily due to the elevated concentration of monocytes and granulocytes during the starting phase of inflammation (Rajendran, 2010). Treatment with E.alba extract elicited marked reduction in paw volume which might be due to the inhibition of inflammatory mediators secretion.

The characteristic pathological feature during RA is hematological disturbances with reduced RBC count and Hb level with a substantial elevation in ESR which reflects the severity of conditions (Hochberg et al., 1988; Patel and Pundarikakshudu, 2016). ESR is used to evaluate inflammation since RBC settles faster due to the proteins generated during inflammation and erythrocytes sediments faster leading to increased erythrocyte sedimentation rate (Hoogen et al., 1995). In our study, CFA induced arthritis rats displayed significant reduction in RBC and Hb and elevation in WBC and ESR. Treatment with E.alba extract significantly restored the hematological alteration to normalcy. Previous study reports that E.alba significantly restored the altered hematologic parameters to normal in ischemic/reperfusion stress conditions (Vudara and Vedagiri, 2019).

Lipid peroxidation is the process of oxidative deterioration of lipid membrane, which releases a toxic adduct malondialdehyde (MDA) (Hodge et al., 2002). In this study, CFA induced arthritic rats displayed elevated levels of MDA in hepatic tissue due to the free radicals generated by CFA (Liu et al., 2017). Treatment with E.alba effectively reduced the MDA level as evident in the current report. Previous reports show that E.alba signifi-
cantly inhibited the lipid peroxidation in epilepsy model (Fahmy, 2011). Further, CFA intoxicated rats showed decreased antioxidants such as SOD, CAT, GPx and GSH. The decreased level of antioxidants might be due to the accumulation of lipid peroxidation end products, precisely MDA through inhibition of protein synthesis [28]. The reduced level of GSH is as a result of over utilization to counter attack the free radicals generated by CFA. E.alba treatment significantly increased the antioxidant levels thus authentifying (validating), E.alba as a potent antioxidant in prevention of arthritis. Thus the anti-arthritis activity of E.alba is due to its anti-inflammatory and antioxidant potential exhibited by the presence of various phyto-constituents in the extract. Previous reports show the presence of luteolin in E.alba which has significant antioxidant and anti-inflammatory effects (Tambe et al., 2017; Shi et al., 2015). Further, E.alba also posses various compounds like wedelolactone, Eclalbasaponins, \( \alpha \) and \( \beta \)-amyrin, Oleanolic and ursolic acids which shows effective antioxidant and anti-inflammatory properties (Deng and Fang, 2012; Baek et al., 2014).

**CONCLUSIONS**

Thus the outcome of the study showed that E.alba displayed potent anti-artheritic activity in CFA challenged arthritic rats. The effect of E.alba is due to the presence of various phyto-constituents which elicits significant anti-inflammatory and antioxidant potential. Future studies aiming isolation of active new compounds are highly warranted.

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