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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.

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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 (Wilsdon 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 anti arthritic 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.

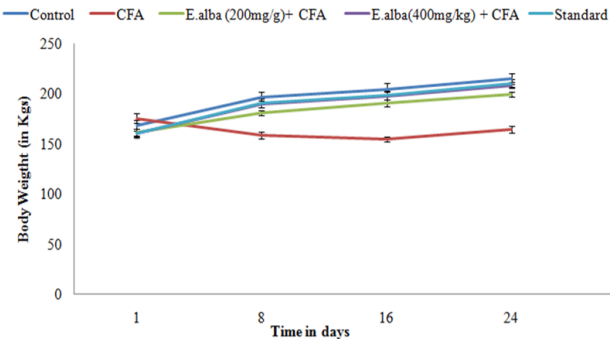

Figure 1: Effect of Eclipta alba on body weight in CFA induced arthritic rats

Experimental design

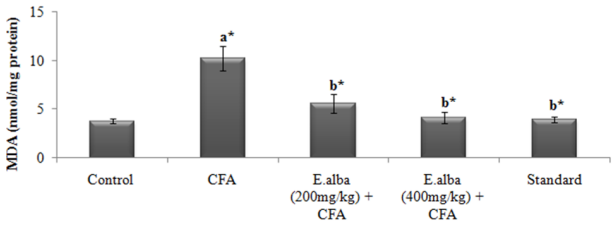

Figure 2: Effect of E.alba on lipid peroxidation alterationin CFA induced arthritis

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.

CFA induced arthritis

The animals were categorized into five groups (n=6)

Group I: Control animals were treated with 2% gum acacia through oral gavage for 28 days

Group II: To induce arthritis, animals were given single intradermal injection of 0.1 ml of CFA in hind paw.

Group III: Arthritic animals were orally administered with methanolic extract of E.alba suspended in 2% gum acacia (200 mg/kg) per day for 28 days.

Group IV: Arthritic animals were orally administered with methanolic extract of E.alba suspended in 2% gum acacia (400 mg/kg per day) for 28 days.

Group IV: Arthritic animals were orally administered with Diclofenac Sodium (5 mg/kg per day) for 28 days.

Assessment of body weight and hind paw volume

The rat paw volume was assessed at day 0 and before CFA injection and further measured various time points upto day 25. The hind paw volume was estimated by calculating the difference between final and initial paw volume. Further, the body weight was estimated from day 0 and at the end of the experimental period, the difference between initial and final weight was calculated (Kumar *et al.*, 2005).

Blood collection and tissue processing

After the final doses of extract and standard drugs the access to food was restricted overnight and on 29^{th} day the animals were anaesthetized using phenobarbital sodium (35mg/kg; i.p) and sacrificed by cervical decapitation. The blood was withdrawn from jugular vein in heparanized tubes and the serum was separated for the measurement of hematological parameters. The liver tissue was excised, cleaned from adherent tissues, rinsed in saline (4°C)

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*

Thedata are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparisonbetween 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	7.4 ± 1.25	3.0± 0.58	14.2 ± 2.25
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*

Thedata are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparisonbetween 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±0.45a*	32.45±2.56a*	85.76±6.45a*	1.28 ± 0.06a*
E.alba(200mg/kg) + CFA	5.24±0.35b*	45.65±3.12b*	106.54±5.56b*	2.12 ± 0.08b*
E.alba (400mg/kg)+ CFA	7.05±0.53b*	53.65±3.90b*	116.65±6.56b*	2.97 ± 0.09b*
Diclofenac Sodium (5mg/kg)	7.14±0.45b*	58.65±3.45b*	120.56±6.12b*	3.12 ± 0.08b*

Thedata are shown as mean ± SEM. Analysis was done by One-way ANOVA and comparisonbetween group 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 H_2O_2 decomposed /min / mg of protein); GPx (n moles of GSH oxidized / min / mg of protein); GSH(nmoles/g tissue)

and dried. Then tissue homogenate was prepared and used for the analyses of various biochemical markers.

Estimation of Hematological Parameters

The haematological parameters such as WBC, RBC, 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 methanolic extract (200 and 400mg/kg) signifi-

cantly 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 ($p<0.05$) 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 arthritis induced lipid peroxidation

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 $\pm$ 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 (Van Den 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 tis-

sue 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 significantly 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 arthritic 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, α and β-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-arthritic 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.

REFERENCES

Akira, S., Uematsu, S., Takeuchi, O. 2006. Pathogen Recognition and Innate Immunity. *Cell*, 124(4):783–801.

Baek, S., Lee, J., Lee, D., Park, M., Lee, J., Kwok, S., Park, S. 2014. Ursolic acid ameliorates autoimmune arthritis via suppression of Th17 and B cell differentiation. *Acta Pharmacologica Sinica*, 35(9):1177–1187.

Bax, M., Heemst, J. V., Huizinga, T. W. J., Toes, R. E. M. 2011. Genetics of rheumatoid arthritis: what have we learned? *Immunogenetics*, 63(8):459–466.

Bihani, G. V., Rojatkar, S. R., Bodhankar, S. L. 2014. Anti-arthritic activity of methanol extract of Cyathocline purpurea (whole plant) in Freund's complete adjuvant-induced arthritis in rats. *Biomedicine & Aging Pathology*, 4(3):197–206.

Chimenti, M. S., Triggianese, P., Conigliaro, P., Candi, E., Melino, G., Perricone, R. 2015. The interplay between inflammation and metabolism in rheumatoid arthritis. *Cell Death & Disease*, 6(9):1887–1887.

Deng, H., Fang, Y. 2012. Anti-Inflammatory Gallic Acid and Wedelolactone Are G Protein-Coupled Receptor-35 Agonists. *Pharmacology*, 89(3-4):211–219.

Dowman, B., Campbell, R. M., Zgaga, L., Adeloye, D., Chan, K. Y. 2012. Estimating the burden of rheumatoid arthritis in Africa: A systematic analysis. *Journal of Global Health*, 2(2):1–9.

Fahmy, R. 2011. Antioxidant effect of the Egyptian freshwater Procambarus clarkii extract in rat liver and erythrocytes. *African Journal of Pharmacy and Pharmacology*, 5(6):776–785.

Geetha, T., Varalakshmi, P. 1999. Anticomplement activity of triterpenes from Crataeva nurvala stem bark in adjuvant arthritis in rats. *General Pharmacology: The Vascular System*, 32(4):212–214.

Hochberg, M. C., Arnold, C. M., Hogans, B. B., Spivak, J. L. 1988. Serum immune reactive erythropoietin in rheumatoid arthritis: impaired response to anaemia. *Arthritis Rheum*, 31(10):1318–1321.

Hodge, G., Hodge, S., Han, P. 2002. Allium sativum (garlic) suppresses leukocyte inflammatory cytokine production in vitro: Potential therapeutic use in the treatment of inflammatory bowel disease. *Cytometry*, 48(4):209–215.

Karmakar, S., Kay, J., Gravallese, E. M. 2010. Bone Damage in Rheumatoid Arthritis: Mechanistic Insights and Approaches to Prevention. *Rheumatic Disease Clinics of North America*, 36:385–404.

Kumar, S. S., Sivakumar, T., Chandrasekar, M. J. N., Suresh, B. 2005. Evaluation of Anti-Inflammatory Activity of Eclipta alba in rats. *Ancient science of life*, 24:112–118.

Lee, D. Y., Choo, B. K., Yoon, T., Cheon, M. S., Lee, H. W., Lee, A. Y., Kim, H. K. 2009. Anti-inflammatory effects of Asparagus cochinchinensis extract in acute and chronic cutaneous inflammation. *Journal of Ethnopharmacology*, 121(1):28–34.

Lin, B., Zhao, Y., Han, P., Yue, W., Ma, X. Q., Rahman, K., Han, T. 2014. Anti-arthritic activity of Xanthium strumarium L. extract on complete Freund's adjuvant induced arthritis in rats. *Journal of Ethnopharmacology*, 155(1):248–255.

Liu, J. Y., Hou, Y. L., Cao, R., Qiu, H. X., Cheng, G. H., Tu, R., Liu, D. 2017. Protodioscin ameliorates oxidative stress, inflammation and histology outcome in

Complete Freund's adjuvant induced arthritis rats. *Apoptosis*, 22(11):1454–1460.

Lundkvist, J., Kastäng, F., Kobelt, G. 2008. The burden of rheumatoid arthritis and access to treatment: health burden and costs. *Eur J Health Econ*, 8(2):49–60.

Mcinnes, I. B., Schett, G. 2011. The Pathogenesis of Rheumatoid Arthritis. *New England Journal of Medicine*, 365(23):2205–2219.

Mondal, P., Das, S., Mahato, K., Borah, S., Junejo, J. A., Zaman, K. 2016. Evaluation of anti-arthritic potential of the hydro-alcoholic extract of the stem bark of Plumeria rubra in freund's complete adjuvant-induced arthritis in rats. *International Journal of Pharmaceutical Sciences and Research*, 7(9).

Neugebauer, V., Han, J. S., Adwanikar, H., Fu, Y., Ji, G. 2007. Techniques for Assessing Knee Joint Pain in Arthritis. *Molecular Pain*, 3:1744–8069.

Patel, M. G., Pundarikakshudu, K. 2016. Anti-arthritic activity of a classical Ayurvedic formulation Vatari Guggulu in rats. *Journal of Traditional and Complementary Medicine*, 6(4):389–394.

Rajendran, E. K. R. 2010. Anti-arthritic activity of Premna serratifolia Linn., wood against adjuvant induced arthritis. *Avicenna J Med Biotechnol*, 2(2):101–106.

Rao, J. K., Mihaliak, K., Kroenke, K., Bradley, J., Tierney, W. M., Weinberger, M. 1999. Use of Complementary Therapies for Arthritis among Patients of Rheumatologists. *Annals of Internal Medicine*, 131(6):409–416.

Shi, F., Zhou, D., Ji, Z., Xu, Z., Yang, H. 2015. Anti-arthritic activity of luteolin in Freund's complete adjuvant-induced arthritis in rats by suppressing P2X4 pathway. *Chemico-Biological Interactions*, 226:82–87.

Singh, B., Saxena, A. K., Chandan, B. K., Agarwal, S. G., Anand, K. K. 2001. In vivo hepatoprotective activity of active fraction from ethanolic extract of Eclipta alba leaves. *Indian J Physiol Pharmacol*, 45(4):435–441.

Tambe, R., Patil, A., Jain, P., Sancheti, J., Somani, G., Sathaye, S. 2017. Assessment of luteolin isolated from Eclipta alba leaves in animal models of epilepsy. *Pharmaceutical Biology*, 55(1):264–268.

Van Den Hoogen, H. M., Koes, B. W., Van Eijk, J. T., Bouter, L. M. 1995. On the accuracy of history, physical examination, and erythrocyte sedimentation rate in diagnosing low back pain in general practice. A criteria-based review of the literature. *Spine*, 20(3):318–327.

Vudara, S. R., Vedagiri, P. K. 2019. Pharmacological Study of the Methanolic Whole Plant Extract of Eclipta alba Against Ischemic Reperfusion Injury on Kidney of Sprague Dawley Rats. *International Journal of Pharmacognosy and Phytochemical Research*, 11(3):105–115.

Wilsdon, T. D., Hill, C. L. 2017. Managing the drug treatment of rheumatoid arthritis. *Australian Prescriber*, 40(2):51–58.

Zhang, X., Dong, Y., Dong, H., Zhang, W., Li, F. 2017. Investigation of the effect of phlomisoside F on complete Freund's adjuvant-induced arthritis. *Experimental and Therapeutic Medicine*, 13(2):710–716.