

ANTIARTHRITIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF LEAVES OF *AMARANTHUS TRICOLOR* IN FREUND'S COMPLETE ADJUVANT- INDUCED ARTHRITIS IN WISTAR RATS

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ABSTRACT

The objective of the study was to evaluate antiarthritic activity of hydroalcoholic extract of *Amaranthus tricolor* L. (HAEAT) in Freund's complete adjuvant (FCA) induced arthritis in female Wistar rats. HAEAT was prepared by maceration and subjected to preliminary phytochemical screening and tested against FCA induced arthritis in rats. Arthritis assessment was done by measuring- paw volume, joint diameter, body weight, arthritis score, pain threshold, thermal hyperalgesia, haematological and biochemical parameters. The HAEAT was administered at the concentrations of 100, 200 and 400 mg/kg body weight. HAEAT (200 and 400 mg/kg) significantly ($p < 0.01$ and $p < 0.001$, respectively) decreased paw volume, joint diameter and increased pain threshold, body weight and paw withdrawal latency compared to arthritic control group. HAEAT (200

and 400 mg/kg) exhibits significant ($p < 0.01$ and $p < 0.001$, respectively) antiarthritic activity by increasing the haematological parameters like red blood cell (RBC), haemoglobin (Hb) and decreasing the white blood cell (WBC), platelets and serum C-reactive protein (CRP). The levels of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase were decreased and the level of total protein was increased by treatment with HAEAT (200 and 400 mg/kg). The present study suggests that HAEAT has antiarthritic potential, and it might be attributed to the phytoconstituents such as tannins, flavonoids and phenolic compounds present in the extract.

KEYWORDS: *Amaranthus tricolor* L, Rheumatoid arthritis, Freund's complete adjuvant, Antiarthritic.

I. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease.^[1] It is a common disease affecting millions of people.^[2] Although various drugs have been used to control RA, there are numerous reports regarding the side effects of these drugs.^[3] Non-steroidal anti-inflammatory drugs (NSAIDs) are used widely for the treatment of RA, although they have serious gastric and renal toxicities.^[4-5] Although the disease can start at any age, the peak onset is between 25 and 55 years. RA primarily affects the synovial joints of all extremities and is pathologically characterized by severe inflammation and progressive destruction of cartilage and subchondral bone.^[6] When chronic inflammation occurs in RA, it involves the actions of large numbers of lymphocytes, macrophages and polymorphonuclear cells in the inflamed joint.^[7] The inflammatory process of RA is reportedly associated with an increase of pro-inflammatory cytokines TNF- α and IL-1 β .^[8] The prevalence of RA in India subcontinent is 1.5-2 % of population. The epidemiological ratio of arthritis in female: male is 3:1 and the prevalence is 1% of the world population.^[9]

Freund's complete adjuvant (FCA) - induced arthritis in rats is a good laboratory model for studying RA.^[10-11] In this model the clinical and pathological changes are comparable to with those observed in human RA.^[12-13] The acute stage of arthritis is characterized by signs of hyperalgesia, decrease in body weight and increase in paw volume. In the later stages of disease (day 12+), rats with adjuvant arthritis are often relatively immobile due to the severity of paw swelling.^[14] Adjuvant induced arthritis model can be considered as a model of persistent or chronic pain stress.^[15] The FCA induced arthritis follows a biphasic time course, consisting of an acute local inflammatory reaction that subsides after 3–4 days and a chronic systemic reaction that shows a relapsing-remitting course after the initial two weeks and can persist for several months.^[16] It is not known why this biphasic pattern of activity is often seen but it may be due to an initial stimulus caused by the injection of FCA followed by the delayed hypersensitivity response known to be induced by FCA.^[17] Due to the increased frequency of intake of NSAIDs and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having lower side effects.^[18]

Amaranthus tricolor L. is a member of Amaranthaceae family. *Amaranthus tricolor* L. is a very closely related species to *Amaranthus spinosus*, *Amaranthus hybridus*, & *Amaranthus dubius*.^[19] The methanol extract of three plants (*Amaranthus viridis*, *Amaranthus caudatus* and *Amaranthus spinosus*) of Amaranthaceae family are reported to have analgesic activity at

the doses of 200 and 400 mg/kg body weight.^[20] *Amaranthus spinosus* is also reported for its anti-inflammatory activity.^[21] *Amaranthus tricolor* L. contains a great number of different betalains.^[22] Three galactosyldiacylglycerols (1-3) with potent cyclooxygenase and human tumor cell growth inhibitory activities have also been isolated from the leaves of *Amaranthus tricolor* L.^[23] Linolenic, palmitic acid and spinasterol are reported to be present in the leaves of the plant.^[24] *Amaranthus tricolor* also possesses antioxidant and hepatoprotective activity.^[25] Therefore the objective of the present investigation was to determine the efficacy of hydroalcoholic extract of leaves of *Amaranthus tricolor* L. (HAEAT) in FCA- induced arthritis in rat model.

II. MATERIALS AND METHODS

Collection and authentication of plant

Amaranthus tricolor L. was collected during the month of august from the local market of Pune, Maharashtra, India. The plant was identified and authenticated at the Agharkar Research Institute, Pune, India and voucher specimen (WP-089) was deposited at that institute for future reference.

Chemicals

FCA (Sigma Aldrich, USA), diclofenac (gift sample from Emcure pharmaceuticals Ltd., Pune). All other chemicals and solvents used for study were of analytical grade and purchased from authentic vendors.

Experimental animals

Female Wistar rats weighing (180-220 g) were purchased from National Toxicology Centre, Pune, India. They were maintained at a temperature of 25 ± 1 °C and relative humidity of 45 to 55% under 12-h light : 12-h dark cycle. The animals had free access to food pellets (Manufactured by Pranav Agro Industries Ltd., Sangli India) and water was available *ad libitum*. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India (Approval No.: CPCSEA/22/12).

Preparation of *Amaranthus tricolor* L.

Amaranthus tricolor L. leaves (1 kg) were air dried at room temperature, powdered and macerated in water: methanol (50:50) for 7 days with intermittent shaking and filtered. The

filtrate was dried on tray dryer at 40 °C (yield – 11.3 % w/w). The dry extract was stored in a refrigerator and used for pharmacological studies.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening employing standard procedures and tests to reveal the presence of phytochemicals such as flavonoids, glycosides, tannins, alkaloids, saponins, phenolic compounds, etc.^[26-27]

Acute toxicity study

Acute toxicity study was performed as per OECD guideline No. 425. Five female Swiss albino mice were used for study. The animals were fasted overnight providing only water. The dry extract was administered orally at one dose level of 2000 mg/kg body weight. Animals were observed continuously for the first 4 h and then periodically up to 24 h for any toxic symptoms and mortality.

Freund's complete adjuvant (FCA) induced arthritis

FCA induced arthritis in rats exhibited many similarities to human RA. FCA injections in rats induced inflammation as primary lesion maximum after 3 to 4 days.

The animals were divided into six groups consisting of six animals each per group follows:

- Group I – Healthy Control, (non-arthritic);
- Group II – Arthritic control, FCA (0.1 ml)
- Group III– Arthritic animals treated with positive control, diclofenac 5 mg/kg, p.o.;
- Group IV– Arthritic animals treated with HAEAT 100 mg/kg, p.o.;
- Group V – Arthritic animals treated with HAEAT 200 mg/kg, p.o.;
- Group VI– Arthritic animals treated with HAEAT 400 mg/kg, p.o.

Arthritis was induced by the single sub-plantar injection of 0.1 ml of FCA containing 10 mg of heat killed *Mycobacterium tuberculosis* in 1 ml of paraffin oil (Sigma Aldrich) into the left hind paw of all the rats under light ether anesthesia.^[28-29] The dosing of all the groups started from day 12 once daily orally till day 28.

Antiarthritic activity of HAEAT was evaluated on the following parameters paw volume, joint diameter, pain threshold, thermal hyperalgesia, arthritic score and body weight on day 1, 4, 7, 10, 14, 17, 21, 24 and day 28.^[30] On day 28 the animals were anaesthetized with

anesthetic ether and the blood was withdrawn by retro-orbital puncture for the estimation of biochemical parameters, hematological parameters and serum CRP.

Measurement of Change in paw volume

Change in paw volume was measured using a Plethysmometer (UGO Basile, Italy) on day 0 before FCA injections and thereafter on day 1, 4, 7, 10, 14, 17, 21, 24 and day 28.^[31] The change in paw volume was calculated as the difference between the final and initial paw volume.

Measurement of Change in joint diameter

Change in joint diameter was measured using a digital Vernier caliper (Mitutoyo, Japan) on day 0 before FCA injections and thereafter on day 1, 4, 7, 10, 14, 17, 21, 24 and day 28.^[32] The change in joint diameter was calculated as the difference between the final and initial joint diameter.

Measurement of Pain threshold

Pain threshold was measured using Randall-Selitto analgesiometer (UGO Basile, Italy) on day 0 just before FCA injections and thereafter at different time intervals till day 28. The pain threshold was expressed in grams. The hind paw was placed between the flat surface and blunt pointer and applied increasing pressure. The pain threshold was determined when rat attempted to remove the hind paw from the apparatus. The cut-off pressure was 450 g.^[33-34]

Measurement of Thermal hyperalgesia (Paw withdrawal latency)

Thermal hyperalgesia was measured using a radiant heat apparatus (UgoBasile, Italy) on day 0 just before FCA injections and thereafter at different time intervals till day 28. The paw withdrawal latency was expressed in sec. The paw was placed on the heat radiator with infrared intensity of lamp was set at 40. The radiant heat apparatus automatically tuned the heat source off when the reflected light beam was interrupted i.e. (when the animal withdraws its paw) and the withdrawal time (sec) of paw was recorded. The paw withdrawal latency of test group was compared with that of the control group. A cut of latency of 15 s was used to avoid tissue damage.^[35]

Measurement of Arthritis score

Arthritis score was monitored on day 0 just before FCA injections and thereafter at different time intervals till day 28. The arthritis score was evaluated by considering the morphological

features of arthritis like redness, swelling and erythema of hind paws. The visual criteria set was as follows, normal paw of the rat= 0 arthritis score, mild swelling and erythema of the rat paw = 1 arthritis score, swelling and erythema of the rat paw = 2 arthritis score, severe swelling and erythema of the rat paw = 3 arthritis score, gross deformity and inability to use the limb = 4 arthritis score.

Measurement of Body weight

Body weight was measured on day 0 just before FCA injections and thereafter at different time intervals till day 28.

Measurement of Haematological and serum parameters

On day 28, haematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb), and platelets were counted by usual standardized laboratory method. Serum C-reactive protein (CRP) was also measured by Immunospectrophotometry method.

Measurement of Biochemical parameters

On day 28, blood of the rats was withdrawn by retro-orbital puncture and serum was used for the estimation of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total protein levels by using standard marketed kits.^[36]

Histopathology of ankle joints

The animals were sacrificed on day 28 by cervical dislocation and ankle joints were separated from the hind paw and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5 μ thickness and subsequently stained with haematoxylin-eosin and evaluated under light microscope with 10X magnifications for the presence of inflammatory cells, hyperplasia of synovium, pannus formation and destruction of joint space.^[30]

Statistical analysis

Data was expressed as mean \pm SEM and statistical analysis was carried out by using GraphPad 5.0 software (GraphPad, San Diego, USA) by applying two-way ANOVA with Bonferroni test. $p < 0.05$ was considered to be significant.

III. RESULTS

Phytochemical investigation

Phytochemical investigation of *Amaranthus tricolor* L. extract showed the presence of flavonoids, tannins, alkaloids, carbohydrates and phenolic compounds.

Acute oral toxicity

HAEAT did not exhibit any toxic symptoms and mortality when given orally at dose of 2000 mg/kg b.w. Therefore three doses (100, 200 and 400 mg/kg b.w) were selected for pharmacological studies.

Effect of HAEAT on Change in paw volume

There was significant ($p < 0.001$) increase in paw volume of all the groups treated with FCA compared to healthy control. FCA administration in rats showed a biphasic response. HAEAT (200 and 400 mg/kg) significantly ($p < 0.01$ and $p < 0.001$, respectively) lowered the increase in paw volume as compared to FCA control group. The change in paw volume of HAEAT treated (400 mg/kg; 0.87 ± 0.28) and (200 mg/kg; 1.80 ± 0.27) was evident as compared to FCA control (3.60 ± 0.33) on day 28 (Figure 1).

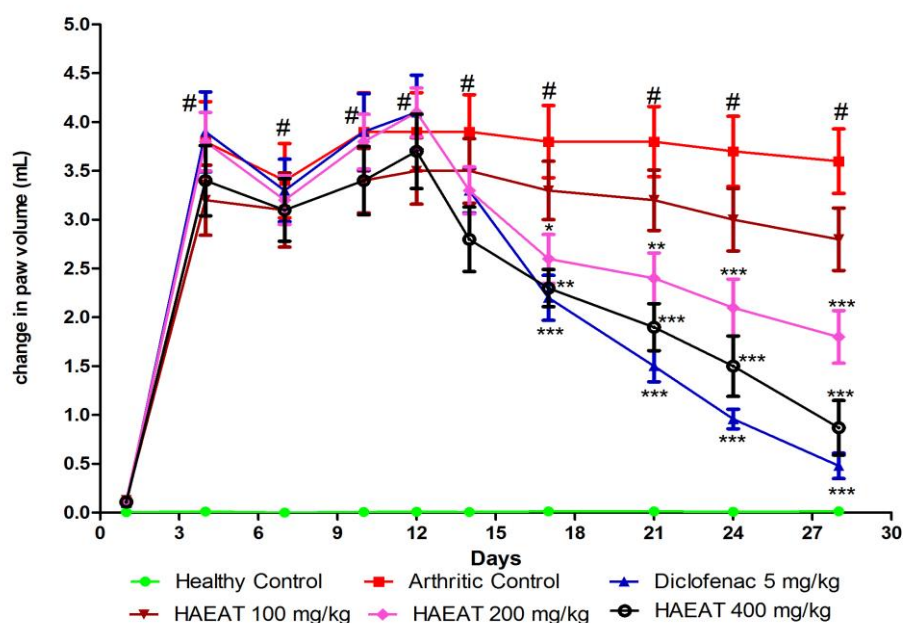


Figure (1): Effect of HAEAT on Change in paw volume in FCA- induced arthritis. Values are expressed as mean \pm SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.

Effect of HAEAT on Change in joint diameter

There was significant ($p < 0.001$) increase in joint diameter of rats of all the groups treated with FCA compared to healthy control. HAEAT (200 and 400 mg/kg) significantly ($p < 0.01$ and $p < 0.001$, respectively) decreased the joint diameter as compared to FCA control. The change in joint diameter of HAEAT treated (400 mg/kg; 1.49 ± 0.27) and (200 mg/kg; 2.23 ± 0.20) was evident as compared to FCA control (3.63 ± 0.19) on day 28 (Figure 2).

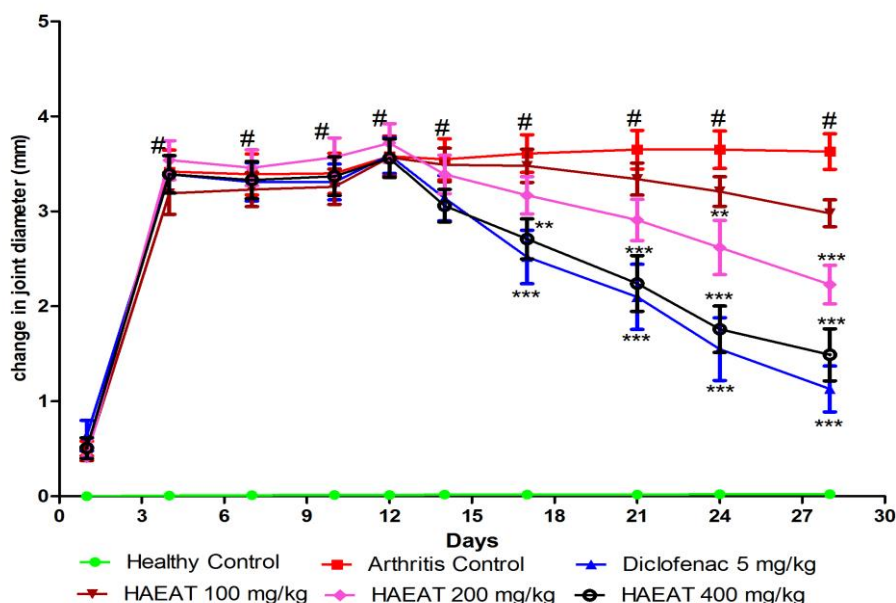


Figure (2): Effect of HAEAT on Change in joint diameter in FCA- induced arthritis. Values are expressed as mean \pm SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, ** $p < 0.01$, *** $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.

Effect of HAEAT on Pain threshold

HAEAT (400 and 200 mg/kg) significantly ($p < 0.001$) increased the pain threshold from day 17 and 24 respectively, whereas HAEAT (100 mg/kg) significantly ($p < 0.001$) increased the pain threshold on day 28. Diclofenac (5 mg/kg) also caused a significant ($p < 0.001$) increase in pain threshold from day 17. The pain threshold of HAEAT (400 mg/kg; 233 ± 5.6) and (200 mg/kg; 203 ± 4.8) was evident as compared to FCA control (141 ± 4.9) on day 28 (Figure 3).

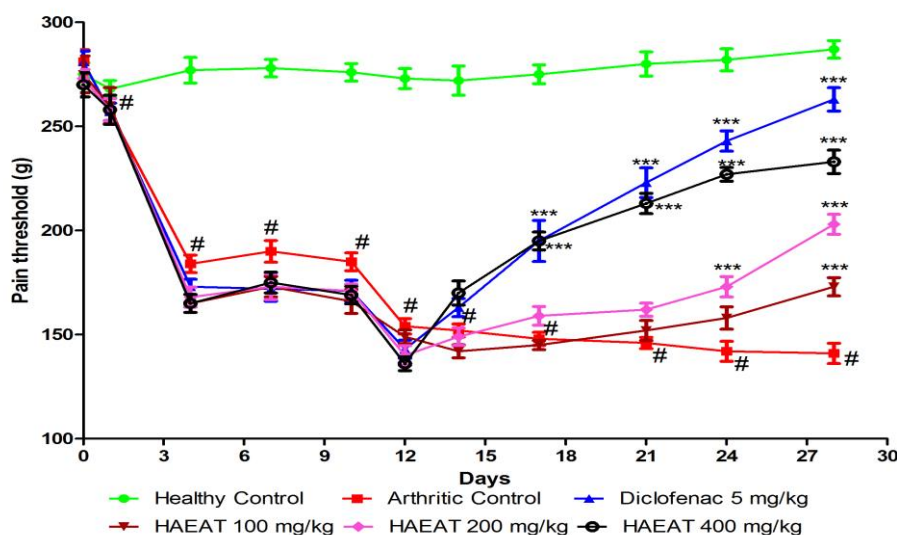


Figure (3): Effect of HAEAT on Pain threshold in FCA- induced arthritis. Values are expressed as mean \pm SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, *** $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.

Effect of HAEAT on Thermal hyperalgesia (Paw withdrawal latency)

The paw withdrawal latency in FCA administered rats decreased progressively till day 12. HAEAT (400 and 200 mg/kg) significantly ($p < 0.001$) increased the paw withdrawal latency from day 17, where as HAEAT (100 mg/kg) significantly ($p < 0.05$) increased the paw withdrawal latency from day 21. Diclofenac (5 mg/kg) also caused a significant ($p < 0.001$) increase in paw withdrawal latency. The paw withdrawal latency of HAEAT (400 mg/kg; 8.6 ± 0.38) and (200 mg/kg; 6.5 ± 0.23) was evident as compared to FCA control (2.5 ± 0.15) on day 28 (Figure 4).

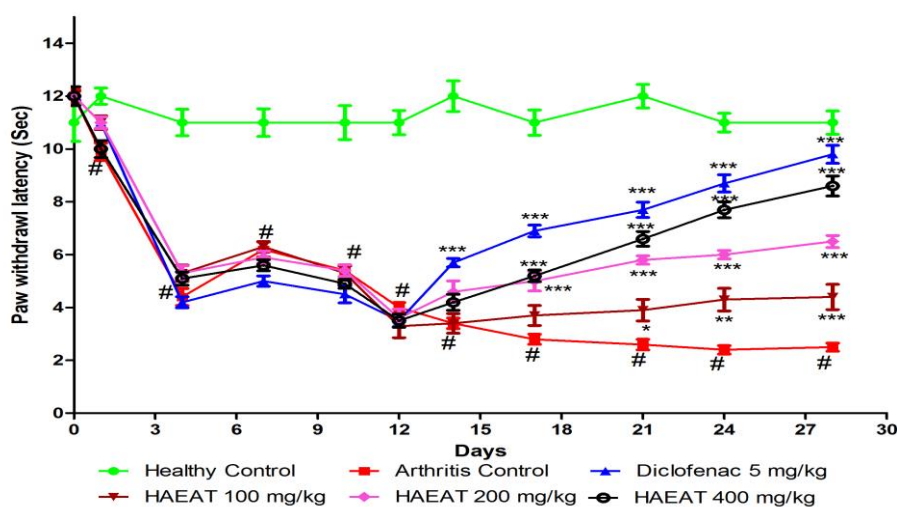


Figure (4): Effect of HAEAT on Paw withdrawal latency in FCA- induced arthritis. Values are expressed as mean \pm SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.

Effect of HAEAT on Arthritis score

The severity of arthritis was expressed as the arthritis score for each individual rat. HAEAT treatment altered the arthritis score which was significantly ($p < 0.001$) increased in FCA control group, representing a significant decrease in pain. The arthritis score of HAEAT (400 mg/kg; 1.30 ± 0.33) and (200 mg/kg; 2.00 ± 0.37) was evident as compared to FCA control (3.80 ± 0.17) on day 28 (Figure 5).

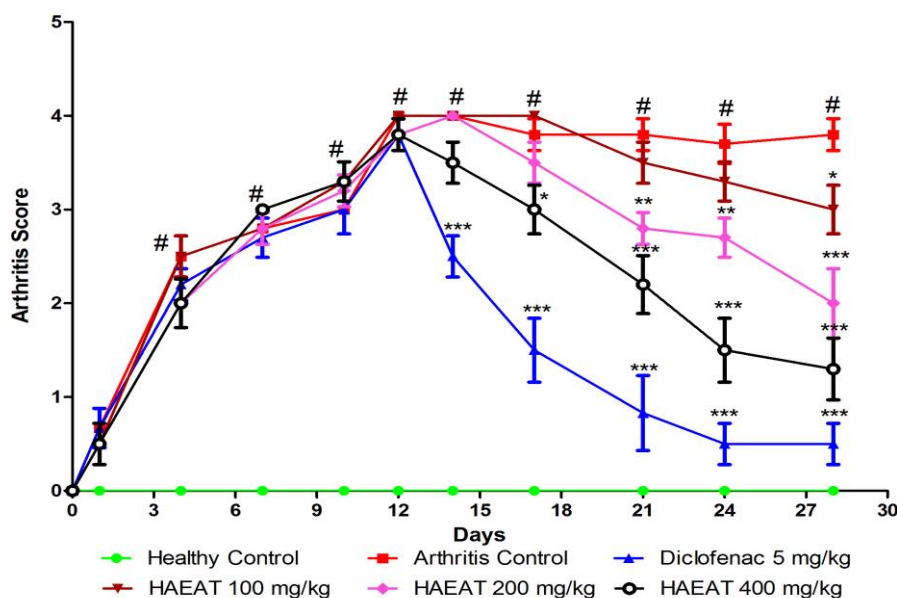


Figure (5): Effect of HAEAT on Arthritis score in FCA- induced arthritis. Values are expressed as mean \pm SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.**

Effect of HAEAT on Body weight

The rats in the arthritic control group lost body weight as compared with the HAEAT and diclofenac treated group. The body weight of HAEAT (400 mg/kg; 186 ± 5.7) and (200 mg/kg; 171 ± 2.1) was evident as compared to FCA control group (141 ± 4.1) on day 28. HAEAT (400 and 200 mg/kg) showed significant ($p < 0.001$) increase in body weight from day 21 and 24 respectively (Figure 6). The results indicate that HAEAT (400 and 200 mg/kg) increased the body weight by 30.66 % and 21.67 % respectively on day 28 while diclofenac (5 mg/kg) increased the body weight by 37.76 % as compared to arthritic control group.

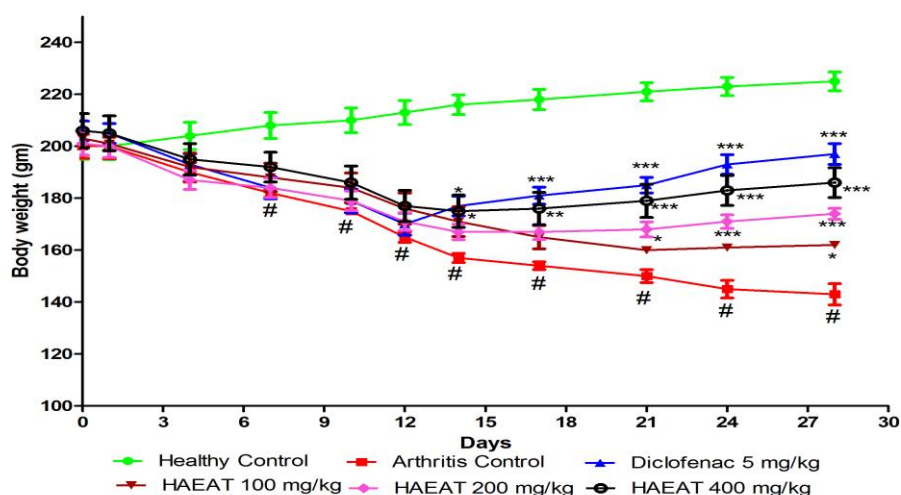


Figure (6): Effect of HAEAT on Body weight in FCA- induced arthritis. Values are expressed as mean \pm SEM for six animals and analysed by Two way ANOVA followed by Bonferroni post-hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.

Effect of HAEAT on Haematological and Serum parameters

The decreased levels of RBC and Hb and increased levels of WBC and platelets were observed in arthritic control group. These conditions were significantly altered with by treatment with HAEAT and diclofenac. The increased level of serum C-reactive protein observed in arthritic control group was also significantly decreased by treatment with HAEAT and diclofenac. The effects observed by HAEAT were dose dependent (Table 1).

Table (1): Effect of HAEAT on Haematological and Serum parameters in FCA- induced arthritis in rats

Treatment groups	RBC (* 10^6 cells/mm 3)	WBC (* 10^3 cells/mm 3)	Hb (g/dl)	Platelets (* 10^3 cells/mm 3)	CRP (mg/lit)
Healthy control	7.0 \pm 0.07	7.16 \pm 0.20	14.8 \pm 0.42	907 \pm 37	1.3 \pm 0.05
Arthritis control	3.4 \pm 0.15 [#]	13.6 \pm 0.39 [#]	9.87 \pm 0.30 [#]	1731 \pm 40 [#]	7.75 \pm 0.22 [#]
Diclofenac (5 mg/kg, p.o)	6.5 \pm 0.17***	7.74 \pm 0.25***	14.2 \pm 0.27***	1126 \pm 48***	3.03 \pm 0.19***
HAEAT (100 mg/kg, p.o)	3.7 \pm 0.14	13.1 \pm 0.32	10.9 \pm 0.35	1608 \pm 31	7.33 \pm 0.15
HAEAT (200 mg/kg, p.o)	4.3 \pm 0.18**	11.6 \pm 0.32***	11.9 \pm 0.40**	1449 \pm 41***	5.72 \pm 0.20***
HAEAT (400 mg/kg, p.o)	5.9 \pm 0.20***	9.71 \pm 0.30***	12.5 \pm 0.60***	1353 \pm 29***	3.98 \pm 0.25***

Values are expressed as mean \pm SEM for six animals and analysed by One way ANOVA followed by Dunnett's test, ** $p < 0.01$, *** $p < 0.001$ when compared to arthritic control # $p < 0.001$ when compared to healthy control.

Effect of HAEAT on Biochemical parameters

As a result of FCA-induced arthritis, the serum levels of AST, ALT and ALP were significantly increased and total protein level was significantly decreased in arthritic group. These enzyme levels were dose dependently altered by treatment with HAEAT and diclofenac. The level of AST, ALT and ALP were significantly decreased by treatment with HAEAT and diclofenac and the level of total protein was significantly increased by treatment with HAEAT and diclofenac (Table 2).

Table (2): Effect of HAEAT on Biochemical parameters in FCA- induced arthritis in rats

Treatment groups	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/dl)
Healthy control	40 ± 1.3	44 ± 4.1	70.3 ± 3.24	6.53 ± 0.20
Arthritis control	119 ± 3.2 [#]	175 ± 5.5 [#]	447 ± 3.82 [#]	5.07 ± 0.19 [#]
Diclofenac (5 mg/kg, p.o)	55 ± 2.6***	57 ± 4.9***	126 ± 4.39***	6.42 ± 0.24***
HAEAT (100 mg/kg, p.o)	107 ± 1.6*	162 ± 3.3	429 ± 4.25*	5.22 ± 0.17
HAEAT (200 mg/kg, p.o)	91 ± 2.5***	121 ± 4.8***	347 ± 4.62***	5.85 ± 0.07*
HAEAT (400 mg/kg, p.o)	66 ± 3.5***	64 ± 3.7***	200 ± 4.28***	6.15 ± 0.18**

Values are expressed as mean ± SEM for six animals and analysed by One way ANOVA followed by Dunnett's test, *p<0.05, **p<0.01, ***p<0.001 when compared to arthritic control #p<0.001 when compared to healthy control.

Effect of HAEAT on histopathology of ankle joints

Histopathology of healthy control rats showed intact morphology of synovium and synovial lining of synovial joint. No inflammation and necrosis of bone was observed (Figure A). FCA treated rats showed necrosis of bone, influx of inflammatory cells, pannus formation, chronic inflammation and disturbed synovial lining (Figure B). Diclofenac (5 mg/kg) treated rats showed significant protection against necrosis of bone, low influx of inflammatory cells and pannus formation (Figure C). HAEAT (100 mg/kg) treated rats showed influx of inflammatory cells with evidence of disturbed synovial lining and pannus formation (Figure D). HAEAT (200 mg/kg) treated rats showed moderate necrosis of bone, with low influx of inflammatory cells (Figure E). HAEAT (400 mg/kg) treated rats showed significant lesser pannus formation, no necrosis of bone and minimal inflammatory cells (Figure F).

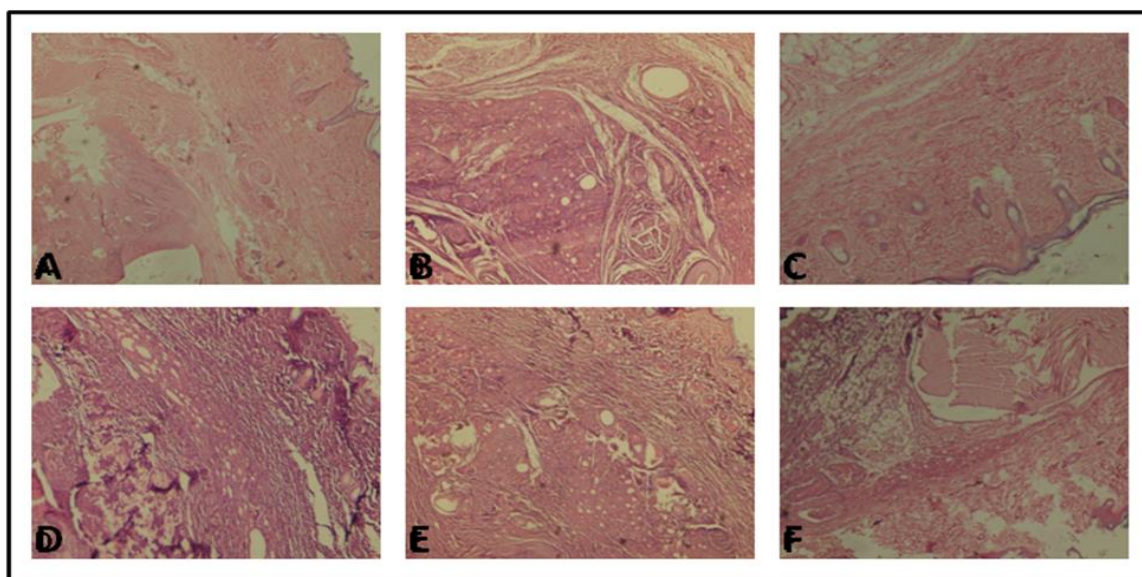


Figure (7): Histopathology of ankle joint. (A) Healthy control, (B) Arthritic control, (C) Diclofenac 5 mg/kg treated, (D) HAEAT 100 mg/kg treated, (E) HAEAT 200 mg/kg treated, (F) HAEAT 400 mg/kg treated.

IV. DISCUSSION

Alternative medicines for the treatment of RA are becoming more popular. Many medicinal plants provide relief of symptoms in RA whose effects are comparable to that of available conventional medicinal agents. Acute toxicity studies revealed the non-toxic nature of extract at the dose of 2000 mg/kg. There were no toxic symptoms or mortality found at selected doses until the end of study period.

In the present investigation the swelling of the paw was observed between 1 to 4 days after FCA administration. The inflammatory reaction increased during the next 8-10 days and was maximally increased at the time when disseminated arthritis appeared.^[37] The present study demonstrated that HAEAT (400 mg/kg) significantly reduced adjuvant- induced arthritis as shown by the decrease in paw edema and also by decrease in arthritis score. One of the important determinants during RA is the change in joint diameter.^[38] Treatment with HAEAT (200 and 400 mg/kg) significantly decreased the joint size as compared to the arthritic control animals. Pain is the predominant clinical feature of RA. The treatment with HAEAT at all the three doses increased the paw withdrawal latency, indicating a protective effect against the pain induced by thermal stimulus (Figure 4). Also the pain threshold was significantly increased by treatment with HAEAT and diclofenac, indicating a reduction in pain.

Other information relating to the antiarthritic activity of HAEAT that has been obtained during the study includes alterations in haematological parameters, CRP level, and changes in body weight. It has been reported that a moderate rise in WBC count occurs in arthritis due to an IL-1 β mediated rise in respective colony stimulating factors.^[39] The present study reveals that HAEAT and diclofenac treatment significantly decreased the WBC count. In addition to this other characteristic alterations in haematological parameters such as reduction in RBC count and reduction in Hb count in arthritic rat is due to decreased response of the bone marrow erythropoietin which was dose dependently increased by treatment with HAEAT. The increased platelet count in arthritic rat was also decreased by HAEAT treatment (Table 1). In the present study it was found that HAEAT and diclofenac treatment increased the body weight as compared to the arthritic control animals (Figure 6). The decrease in body weight in arthritic control group may be due to reduced absorption of nutrients through the intestine. Therefore the restoration of the body weight in rats by HAEAT may involve improvement in the absorption of the nutrients through the intestine of rats. CRP is a marker of inflammation and its level dramatically rises in RA.^[40] It has been recognized as a strong indicator of RA. The treatment with the HAEAT (200 and 400 mg/kg) significantly ($p < 0.001$) reduced the level of CRP as compared to arthritic control group which provided evidence for its antiarthritic effect (Table 1).

Measurement of serum AST, ALT, ALP and total protein provides an excellent and simple tool to measure the antiarthritic activity of the drug. The serum AST, ALT and ALP were significantly increased in arthritic control animals, since these are good indices of liver and kidney impairment, which is considered as a feature of adjuvant arthritis. The serum AST, ALT, and ALP levels were significantly decreased by treatment with HAEAT and diclofenac when compared to arthritic control animals which confirm the antiarthritic activity of the HAEAT and also the safety of the drug for long-term administration in the treatment of arthritis (Table 2). The serum total protein level was significantly increased by treatment with the HAEAT, which was reported to decrease in RA.^[41]

Phytochemical analysis of the extract showed the presence of flavonoids, alkaloids, sterols and phenolic compounds. These secondary plant metabolites present in the extract may be responsible for the antiarthritic activity.^[42] As such the exact mechanism of the HAEAT for its antiarthritic effect is not known but it can be speculated that one or more of these secondary plant metabolites may be responsible for its antiarthritic effect.

V. CONCLUSION

To conclude, the results of the present study suggest that HAEAT was effective in FCA-induced arthritis in rats. Since sterols, flavonoids and phenolic compounds are the major phytochemicals present in the extract it can be assumed that they might be playing an active role in this activity. Further studies will provide deeper insight into the antiarthritic activity of *Amaranthus tricolor* L. and eventually lead to the development of a new class of antiarthritic agent.

VI. ACKNOWLEDGEMENTS

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