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Research Article

ANTI-ARTHRITIC ACTIVITY OF *AMARANTHUS ROXBURGHIANUS* NEVSKI HYDROALCOHOLIC AREAL PLANT EXTRACT IN ACUTE AND CHRONIC MODELS IN ALBINO WISTAR RATS

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ABSTRACT

Objective: Evaluation of anti-arthritic activity of hydroalcoholic extract of *Amaranthus roxburghianus* Nevski aerial parts in albino Wistar rats.

Materials and Methods: *A. roxburghianus* dried aerial parts were extracted with ethyl alcohol: water (70:30) ratio, respectively, by the hot Soxhlet method. Hydroalcoholic *A. roxburghianus* extract (HARE) was further concentrated to obtain a semisolid residue. Two doses 200 and 400 mg/kg of HARE were tested against formaldehyde-induced acute non-immunological and Freund's complete adjuvant (FCA)-induced chronic immunological arthritis in albino Wistar rats. Arthritis assessment was done by morphological studies (paw volume, paw diameter, and body wt.) and hematological parameters radiological, histopathological, and organ wt. studies were also done on the 28th day after animals were sacrificed.

Results: Dose-dependent and significant inhibition of edema were observed in both acute as well as chronic models. The extract at dose 400 mg/kg showed most potent and significant ($p<0.05$) paw edema inhibition which is supported by the results of paw volume and diameter, hematological parameters, in FCA-induced arthritis model. Treatment with HARE also decreased the histopathological alterations induced by FCA model.

Conclusion: HARE protects synovial membrane by improving the health status exhibits promising anti-arthritic activity. This finding thus supports the traditional use of *A. roxburghianus* for arthritis. However, further studies are needed to carry out the isolation of active constituents of the fraction responsible for the activity.

Keywords: *Amaranthus roxburghianus*, Freund's complete adjuvant-induced arthritis, Formaldehyde-induced arthritis, Morphological, Hematological, Radiological, Histopathological and organ wt. studies.

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INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune multisystem disease characterized by pain, synovial membrane inflammation, peripheral joint inflammation, morning stiffness, destruction of articular tissues, and restricted joint movement [1-3]. The pathology and etiology of RA are complex and unknown. Destructive changes in the cartilage and bone and outgrowths of bones restrict the mobility of the joints [4]. Arthritis can cause severe disability and ultimately affects a person's ability to carry out everyday tasks, restricts the quality of life, and causes premature death [5]. RA is the most common inflammatory disorder affecting about 1% of the global adult population; females are three times more prone to RA than males [6-8]. Conventional treatment of RA with NSAIDs, corticosteroids, immune suppressants, and anti-rheumatic drugs tumor necrosis factor (TNF-a and monoclonal antibodies) have limitations [9-11]. Chronic treatment with above agents produces serious adverse effects such as GIT ulcers, cardiovascular, hematological, and renal toxicities [12,13]. Patients suffering from chronic autoimmune disorders are advised for alternative methods for symptomatic relief [14,15]. *Amaranthus roxburghianus* Nevski (Family: Amaranthaceae) is a wild plant whose leaves and tender shoots and can be edible and are commonly used as a leafy vegetable [16]. It is commonly known as chiri kura in Telugu language and is used as an abortifacient by tribes of Chittoor district, Andhra Pradesh state of India [17]. Used as iron tonic [18], its extracts or herbal formulations are rich in alkaloids, used in traditional Chinese and Ayurvedic medicine [19]. Its root extract in combination with piperine is used in the effective treatment of inflammatory bowel disease [20]. Further consumption of its leaves is reported for the treatment of sunstroke and urinary disorders [21]. Hence, we attempted to investigate the anti-arthritic effect of hydroalcoholic areal plant extract of *A. roxburghianus* in acute and chronic models in albino Wistar rats, to prove its importance in the treatment of rheumatism.

MATERIALS AND METHODS

Plant collection and authentication

A. roxburghianus plants were purchased as leafy vegetables from the local market of Pileru, Chittoor District, Andhra Pradesh state of India in March 2017; photograph of which is shown in Fig. 1. Authentication of plant was carried out by Dr. K. Madava Chetty, Asst. Professor, Dept. of Botany, Shri Venkateswara University, Tirupati, A.P., India. A voucher specimen of the plant (Ref. No. 1656), dated 11/09/2017, has been preserved there for future references.

Extract preparation

The aerial plants were obtained by cutting the root portions and thoroughly washed with tap water, and air dried in the shade, powdered in grinder, and passed through # Sieve No. 40 (ASTM). The dried powder was defatted by petroleum ether and then successive extraction was done with ethyl alcohol:water with (70:30) ratio, respectively, by the hot Soxhlet method. The hydroalcoholic extract was concentrated in a rotary evaporator (Heidolph Instruments, Laborota 4000, Germany) under reduced pressure. The dried crude hydroalcoholic *A. roxburghianus* extract (HARE) was collected and preserved in an airtight glass container at 4–8°C until final use.

Experimental animals

Male rats of albino Wistar strain weighing between 180 and 200 g were used for the experiments. All the animals were obtained from Sree

Fig. 1: Photograph of *Amaranthus roxburghianus* Nevski

Venkateshwara Enterprises, Bengaluru, India. All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee of Sree Vidyanikethan College of Pharmacy, Tirupati, Chittoor Dist., A.P., India (Approval No.: SVCP/IAEC/I-001/2018-19 dated on 01/04/2019) in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Govt. of India, New Delhi. The animals were housed in polypropylene cages and maintained at 24°C±2°C under 12 h light/dark cycle and were feeded with standard pellet diet and had free access to water. The animals were given a standard diet supplied by Sree Venkateshwara Enterprises, Bengaluru, India. The composition of the diet is energy 3615 (kcal/kg), crude protein 22.05%, crude oil 4.5%, crude fiber 4.10%, ash 11.10%, and sand silica 0.75%.

Experimental design

A total of 30 male albino Wistar rats weighing 180–200 g were selected and allocated to five groups of six rats in each group (n=6). Group I was used as normal control. Group 2–5 were RA-induced and treated for Freund's complete adjuvant (FCA)-induced arthritis and similarly for formaldehyde-induced arthritis, as given in Table 1.

Dose selection

Acute toxicity studies were not conducted on the HARE as its safety up to 2000 mg/kg has been reported in an earlier study [19]. The two doses 200 and 400 mg/kg were selected based on earlier studies which have reported better response at doses above 400 mg/kg regarding analgesic and nephroprotective activities [20,21].

Evaluation of anti-arthritic activity

FCA-induced arthritic rats

FCA contains heat-killed dead Mycobacterium tuberculosis bacteria in liquid paraffin in a concentration of 10 mg/mL. All the rats, except those in the normal control group, were injected intradermally with 0.1 mL of FCA into the left hind paw on day "0." An interval of 7 days was given for arthritis to develop. All the animals developed the signs of arthritis such as swelling, redness, and restricted movement during this period [22]. The treatment was ended on day 28.

Morphological studies

Paw volume and diameter studies

Paw volume and paw thickness of rats were measured once in 7 days from day "0" to "28" by using Plethysmometer (UGO Basile, Italy 7140) and Vernier caliper respectively.

Body wt. studies

Body weight was measured by electronic balance (Shimadzu C054-E032S, Japan) before and after induction and the mean difference in the body wt. was reported.

Table 1: Experimental design

Group	Treatment
Group 1	Normal control
Group 2	Arthritis control
Group 3	Diclofenac 10 mg/kg
Group 4	HARE 200 mg/kg
Group 5	HARE 400 mg/kg

HARE: Hydroalcoholic *Amaranthus roxburghianus* extract

Hematological studies

Three milliliter blood samples was collected by retro-orbital puncture on 28th day; for the estimation of serum parameters (serum glutamate oxaloacetate transferase [SGOT], serum glutamic pyruvic transferase [SGPT], alkaline phosphatase [ALP], and total protein) and blood parameters (erythrocyte sedimentation rate [ESR] and %HB) by various diagnostic kits and standard procedures.

Later, rats were sacrificed with a high dose of halothane on 28th day and the ankle joints were used for further radiological, histological, and organ wt. studies.

Radiological studies

On the 28th day, X-ray images (Siemens, Heliophos D X-ray machine) of the hind limbs were taken and the changes at joints were assessed based on joint space and soft tissue swelling.

Histopathological studies

On the 28th day, ankle joints were removed and fixed in 10% buffered formalin. The bones were decalcified in 5% formic acid, processed for paraffin embedding, sectioned at 5 µm thickness, and subsequently stained with hematoxylin-eosin for examination under a light microscope for the presence of changes in synovium, cartilage, and joint space.

Organs wt. studies

On the 28th day, the spleen and thymus were removed, and weight of the organs were recorded and corrected for 100 g body wt.

Formaldehyde-induced arthritic rats

Rats were injected with 0.1 mL of 2% v/v of formaldehyde solution in the plantar surface of the left foot, on the 1st and 3rd day of the test. Drug treatment was started from the initial day, i.e., from the day of formaldehyde injection (0 day) and continued until the 10th day. The rat paw volume was recorded daily by using Plethysmometer (UGO Basile 7140, Italy) [23].

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

FCA-induced arthritic rats

Morphological studies

Paw volume and diameter studies

Effect of HARE on paw volume and diameter in FCA-induced arthritic rats was tabulated in Table 2. Challenge with FCA (0.1 mL) shows the development of paw edema which reached peak edema on the 21st day of injection. Diclofenac treated group shows significant inhibition of paw edema on day 7th ($p<0.05$), 14th ($p<0.01$), 21st ($p<0.001$), and day 28th ($p<0.001$). HARE (200 mg/kg) shows significant inhibition of paw edema on day 21st and day 28th with ($p<0.01$). Furthermore, rats treated with HARE (400 mg/kg) show significant inhibition of paw edema on day 7th ($p<0.05$), 14th ($p<0.05$), 21st ($p<0.01$), and day 28th ($p<0.01$). Paw diameter was increased up to 21st day of adjuvant induction and after that it slightly decreased. Diclofenac treated group shows significant

Table 2: Effect of HARE on paw volume and diameter in Freund's complete adjuvant-induced arthritic rats

Groups	Paw volume (mL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	0.16±0.03	0.16±0.02	0.16±0.04	0.17±0.03	0.17±0.01
Arthritis control	0.94±0.07	1.85±0.16	2.42±0.10	2.78±0.13	2.64±0.12
Diclofenac 10 mg/kg	0.93±0.06	1.94±0.10*	1.86±0.11**	1.58±0.11***	1.45±0.11***
HARE 200 mg/kg	0.90±0.07	1.41±0.10	2.18±0.12	1.92±0.17**	1.88±0.13**
HARE 400 mg/kg	0.92±0.06	1.98±0.11*	1.89±0.09*	1.77±0.19**	1.68±0.16**
Groups	**Paw diameter (mm)**				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal control	0.34±0.16	0.33±0.13	0.34±0.12	0.35±0.14	0.35±0.12
Arthritis control	8.00±0.11	15.00±0.24	20.00±0.33	23.00±0.10	21.00±0.51
Diclofenac 10 mg/kg	7.32±0.13	13.42±0.37	13.02±0.12**	12.86±0.23***	12.00±0.22***
HARE 200 mg/kg	9±0.15	13.47±0.45	18.67±0.74	17.12±0.09**	16±0.36**
HARE 400 mg/kg	8.43±0.20	13.09±0.13	17.02±0.56	16±0.10**	15±0.11**

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 as compared with control (one-way ANOVA followed by Dunnet's test). HARE: Hydroalcoholic *Amaranthus roxburghianus* extract

Table 3: Effect of HARE on body wt. in Freund's complete adjuvant-induced arthritic rats

Groups	Mean body wt. (g)		Mean difference in body wt
	Before induction	After induction	
Normal control	179±1.23	179±1.23	-
Arthritis control	165±3.13	176±2.4	11±1.26
Diclofenac 10 mg/kg	173±2.24	218±1.38	45±1.11**
HARE 200 mg/kg	170±1.12	202±3.21	32±2.03*
HARE 400 mg/kg	181.34±3.65	217±5.01	36±1.67*

Values are expressed as mean±SEM (n=6); *p<0.05, **p<0.01 as compared with control followed by student's t-test.
HARE: Hydroalcoholic *Amaranthus roxburghianus* extract

inhibition of paw diameter on day 14th (p<0.01), 21st (p<0.001), and day 28th (p<0.001). HARE (200 mg/kg) shows significant inhibition of paw diameter on day 21st and day 28th with (p<0.01). Furthermore, rats treated with HARE (400 mg/kg) show significant inhibition of paw diameter on day 21st and day 28th (p<0.01). Morphological observation of the rat paws was shown in Fig. 2.

Body wt. studies

Effect of HARE on body wt. in FCA-induced arthritic rats was tabulated in Table 3 indicates the increased body wt. during the treatment of standard drug and HARE.

Hematological studies

Effect of HARE on various serum and blood parameters in FCA-induced arthritic rats was tabulated in Table 4. Challenge with CFA (0.1 mL) shows an increased in level of SGOT, SGPT, ALP, and decreased in level of total protein in the control group. Diclofenac treated group shows decreased in level of SGOT (p<0.01), SGPT (p<0.01), ALP (p<0.001), and increased in level of total protein (p<0.01). HARE (200 mg/kg) shows significantly decreased in level of SGPT (p<0.05), ALP (p<0.05), and increased in level of total protein (p<0.05). HARE (400 mg/kg) treated group shows decreased in level of SGOT (p<0.05), SGPT (p<0.05), ALP (p<0.01), and increased in level of total protein (p<0.05).

Radiological studies

Radiological observations of the rat hind limbs were shown in Fig. 3. Arthritis control joint showed soft tissue swelling and narrowing of joint space. The joint space was intact and soft tissue swelling was reduced in rats treated with diclofenac (10 mg/kg), HARE (200 mg/kg), and HARE (400 mg/kg), as seen in Fig. 3.

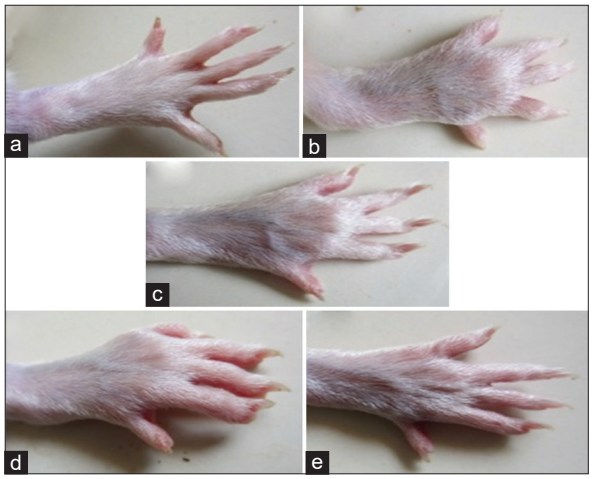

Fig. 2: Morphological observations of the rat paws (a) normal control (b) arthritis control (c) diclofenac (10 mg/kg) (d) hydroalcoholic *Amaranthus roxburghianus* extract (HARE) (200 mg/kg) (e) HARE (400 mg/kg) treated rats

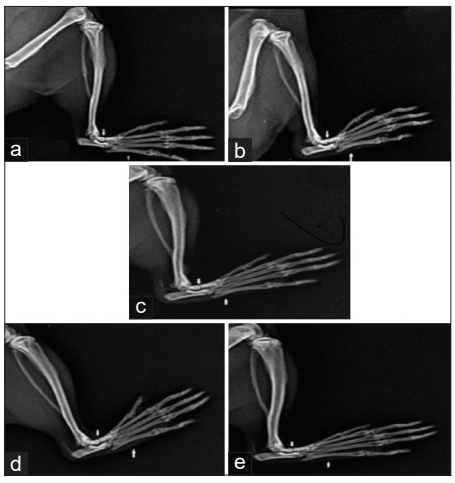

Fig. 3: Radiological observation of the rat hind limbs (a) normal control (b) arthritis control (c) diclofenac (10 mg/kg) (d) hydroalcoholic *Amaranthus roxburghianus* extract (HARE) (200 mg/kg) (e) HARE (400 mg/kg) treated rats

Table 4: Effect of HARE on hematological parameters in Freund's complete adjuvant-induced arthritic rats

Groups	Serum parameters				Blood parameters	
	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total protein (g/dl)	ESR (mm/hr)	HB (g %)
Normal control	126.50±1.38	62.75±1.70	142.35±1.25	7.95±0.11	13.17±1.35	17.33±0.76
Arthritis control	100.27±13.01	70.26±1.87	268.36±20.13	3.02±0.22	35.3±5.4	7.5±0.6
Diclofenac 10 mg/kg	51.32±6.55**	47.08± 2.14**	140.65±16.27***	7.93±0.10**	16.47±.6.04**	13.3±1.01**
HARE 200 mg/kg	75±8.81	53.48±1.30*	198.81±13.44*	6.84±0.45*	25.36±7.12	11.2±0.9**
HARE 400 mg/kg	67.93±4.41*	51.63±4.35*	172.18±10.68**	6.96±.11*	19.2±10.21*	12.4±1.11**

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 as compared with control (one-way ANOVA followed by Dunnet's test). HARE: Hydroalcoholic *Amaranthus roxburghianus* extract

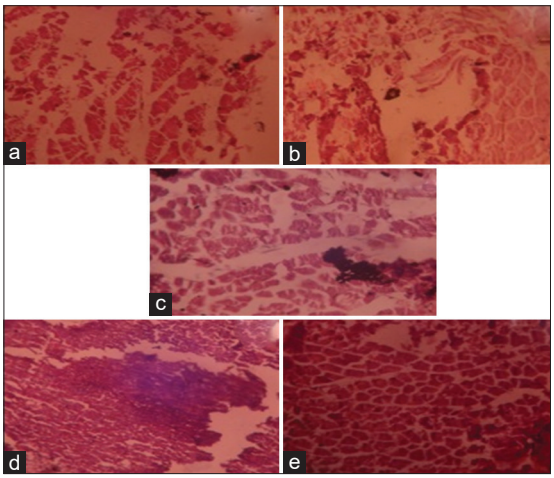

Fig. 4: Histopathological observation of the rat ankle tissues (a) normal control (b) arthritis control (c) diclofenac (10 mg/kg) (d) hydroalcoholic *Amaranthus roxburghianus* extract (HARE) (200 mg/kg) (e) HARE (400 mg/kg) treated rats. Magnification: ×100; thickness: 5 µm

Histopathological studies

Histopathological observations of the rat ankle joint tissues were shown in Fig. 4 indicate that show destructive lesions in connective tissue, vascularity into joint space, and granuloma formation in the arthritis control group. There was normal connective tissue structure with the absence of necrosis in the ankle joint of the normal control group. Diclofenac treatment showed normal connective tissue of ankle joint with the presence of lesser edema and absence of necrosis. HARE-treated rats produced knee joint protection compared to arthritis control group rats by reducing the inflammation and necrosis. Rats treated with HARE (200 mg/kg) showed granuloma formation along with edema and necrosis with few inflammatory cells. Rats treated with EABP (400 mg/kg) showed mild necrosis with edema, but granuloma was absent in ankle joint.

Organs wt. studies

There was a decrease in thymus wt. whereas the mean spleen wt. was increased in the FCA treated rats as compared to the NC group (Table 5). A rise in spleen wt. was significantly (p<0.01) inhibited in rats treated with HARE (200 and 400 mg/kg) and diclofenac (10 mg/kg) as compared to FCA treated rats. Only treatment with dose 400 mg/kg of HARE and diclofenac attenuated the decreased wt. of the thymus, significantly (p<0.01).

Formaldehyde-induced arthritic rats

Effect of HARE on paw volume in formaldehyde-induced arthritic rats was tabulated in Table 6. Sub-planter injection of 0.1 mL of 2% v/v formaldehyde shows increased in paw edema which reaches a peak on day 6th and after that it slightly decreased on day 10th. Diclofenac (10 mg/kg) treated rat shows significant changes in paw edema on the day 3rd (p<0.05), day 6th (p<0.001), and day 10th (p<0.001).

Table 5: Effect of HARE on thymus and spleen wt. in Freund's complete adjuvant-induced arthritic rats

Groups	Spleen wt. (mg/100 g b.wt.)	Thymus wt. (mg/100 g b.wt.)
Normal control	185.5±4.09	103.5±2.01
Arthritis control	249.16±2.71	81.00±0.81
Diclofenac 10 mg/kg	209.83±4.20**	91.00±0.96**
HARE 200 mg/kg	231.50±1.56**	84.50±0.76
HARE 400 mg/kg	212.00±1.06**	89.50±1.33**

Values are expressed as the mean±SEM (n=6); *p<0.05, **p<0.01 as compared with control (one-way ANOVA followed by Dunnet's test). HARE: Hydroalcoholic *Amaranthus roxburghianus* extract

Table 6: Effect of HARE on paw volume in formaldehyde-induced arthritic rats

Groups	Paw volume (mL)			
	Day 0	Day 3	Day 6	Day 10
Normal control	0.16±0.03	0.16±0.02	0.16±0.04	0.17±0.03
Arthritis control	0.92±0.09	1.48±0.32	1.97±0.44	1.7 5± 0.21
Diclofenac 10 mg/kg	0.90±0.03	1.22±0.24***	1.19±0.13*	1.09±0.27***
HARE 200 mg/kg	0.94±0.11	1.59±0.21*	1.36±0.20	1.23±0.42**
HARE 400 mg/kg	0.91±0.09	1.43±0.19**	1.41±0.31	1.21±0.22**

Values are expressed as mean±SEM (n=6). *p<0.05, **p<0.01, ***p<0.001 as compared with control (one-way ANOVA followed by Dunnet's test). HARE: Hydroalcoholic *Amaranthus roxburghianus* extract

HARE (200 mg/kg) shows significant changes in paw edema on day 6th (p<0.05) and day 10th (p<0.01). Furthermore, rats treated with HARE (400 mg/kg) show significant changes in paw edema on day 6th (p<0.01) and day 10th (p<0.001).

DISCUSSION

The FCA-induced arthritis model in rats is the most common model. This preclinical model predicted the activities of a number of compounds that are currently used in the treatment of RA are being tested in clinical trials. The basis on biochemical parameters, the 4 phases of arthritis, Phase 1: Acute local inflammation of liver and systemic effects (1–4 days); Phase 2: Remission of acute inflammation and periarthritis (7–12 days); Phase 3: Chronic inflammation, periarthritis and osteogenic activity (12–28 days); and Phase 4: Permanent articular deformity and minimal inflammation (35 days onward) [9]. The present study was carried out to see the efficiency of hydroalcoholic extract of aerial parts of indigenous herb, *A. roxburghianus* against arthritis. In the present study, male Wistar strain rats were selected to induce arthritis because they develop a chronic swelling in multiple joints due to the accumulation of inflammatory cells, erosion of joint cartilage, and bone destruction.

It has close similarities to human rheumatoid diseases [1]. The determination of paw swelling is apparently simple, sensitive, and quick procedure for evaluating the degree of inflammation and the therapeutic effects of drugs. The Freund's adjuvant model is chosen as it develops chronic swelling in multiple joints with the influence of inflammatory cells with erosion of joint cartilage and bone destruction. Chronic inflammation involves the release of the number of mediators such as cytokines (interleukin-1B and TNF-alpha), granulocyte macrophage-colony stimulating factor, interferon's, and PGDF. These mediators are responsible for the pain, destruction of bone, and cartilage that can lead to severe disability [14]. However, standard drug and HARE extract can significantly suppress the swelling of the paws and also decreases the paw volume in both acute and chronic phase which may be due to the suppression of inflammatory mediators released due to induction of Freund's adjuvant. Although the actual mechanism of suppressing inflammation is not known, it can be correlated with the presence of alkaloids and flavonoids in suppressing the inflammation and antioxidant activity. As the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. Earlier findings suggest that absorption of 14°C-glucose and 14°C-leucine in rat's intestine was reduced in the case of inflamed rats [24]; but on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it shows that the anti-inflammatory drugs correct the decreased/deranged absorption capacity of intestine during inflammation. The increased body weight during the treatment of standard drug, HARE extract, may be due to the restoration of the absorption capacity of intestine. The extract also shows a significant effect on various blood and serum parameters. Formaldehyde-induced arthritis is one of the most commonly used acute model for assessing anti-arthritic potential of plant extracts. The development of edema in the paw of the rat after injection of 0.1 mL of 2% v/v formaldehyde is due to the release of histamine, serotonin, and the prostaglandin like substances at the site of injection [23]. Inhibition of paw edema in formaldehyde-induced arthritis may be due to the significant anti-inflammatory potential of HARE.

CONCLUSION

On the basis of the results obtained in this study, we conclude and propose that possibly, the potent anti-arthritic effect of aerial parts of hydroalcoholic HARE may be through maintenance of synovial membrane, thereby inhibiting cytokines and leukotriene infiltration inhibition as evidenced in paw edema volume. In turn, protecting synovial membrane and improving health status through anti-inflammatory properties of HARE. Improvement in health parameters considers in this study, including HB, ESR, and body weight indicating its beneficial effects while recovery from arthritis. From the results observed from the current investigation, it is concluded that the HARE possesses potent anti-arthritic activity since it give a positive result in controlling inflammation in acute and chronic arthritic models in Wistar strain albino rats.

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AUTHORS' CONTRIBUTIONS

Ms. Radhika Chikatipalli Research scholar; conducted research work and consolidated results with discussion and conclusions.

Dr. K. Saravanakumar: Research supervisor; supervised the research work and guided to draft this research article as per the journal's instructions.

Dr. Chandra Sekhar Kothapalli Bonnoth: Research co-supervisor; supervised the research work and guided to draft this research article as per the journal's instructions.

CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

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