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ORIGINAL RESEARCH ARTICLE

Evaluation of Anti-Arthritis Activity of *Asystasia Dalzelliana* Leaves

Vishal Babushetty*, Chandrashekhar M Sultanpur

Department of Pharmacology, Government College of Pharmacy, Bangalore- 560027, India

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory, systemic autoimmune disease affecting people predominantly between the ages of 20-50 years with unpredictable course. About 1% of the world's population is afflicted by rheumatoid arthritis and it is two to three times more common in women than in men. The modern allopathic drugs available for treatment of rheumatoid arthritis offer only a temporary relief and are also associated with several side effects. Other alternative treatment for RA involves the use of herbal drugs because of their least/no side effects. The present research work was aimed at the scientific validation of the traditional use of *Asystasia dalzelliana* leaves in treatment of rheumatoid arthritis. Anti-arthritis activity of ethanolic extract of *Asystasia dalzelliana* leaves was evaluated by Freund's adjuvant induced arthritis model in rats. Paw edema, changes in organ weight, serum parameters such as SGOT, SGPT and ALP were estimated. Hind paw of experimental rats were also subjected for radiographic and histopathological examination for assessing the anti-arthritis potential of ethanolic extract of *Asystasia dalzelliana* leaves. The results of the current investigation concluded that extract of dose of 800mg/kg possess a significant anti-arthritis activity than the lower doses of 200mg/kg and 400mg/kg. The observed anti-arthritis activity of extract may be due to the presence of phytoconstituents such as alkaloid and flavonoids.

Keywords: Anti-arthritis, *Asystasia dalzelliana*, Freund's complete adjuvant, Aspirin.

INTRODUCTION

Rheumatoid arthritis is a chronic systemic inflammatory disease characterized by persistent symmetric inflammation of multiple peripheral joints. It is one of the most common inflammatory rheumatic diseases and is characterized by the development of a chronic inflammatory proliferation of the synovial linings of diarthrodial joints, which leads to aggressive cartilage destruction and progressive bony erosions. Untreated, rheumatoid arthritis often leads to progressive joint destruction, disability, and premature death [1].

Although the cause of rheumatoid arthritis is unknown, but it is supposed to be triggered by the combination of genetic susceptibility and exposure to environmental factors [2]. Advances have been made in elucidating subsequent steps in the pathogenesis of this disease. A critical role for T cells in the pathogenesis of RA is suggested by the strong association between RA and certain human leukocyte antigen (HLA) haplotypes. Recent data suggest that the destruction of rheumatoid joints is initiated by complex cell-cell interaction between antigen presenting cells and CD4+T cells. However it is thought that these cell-cell interaction result in the activation of macrophages and induction of the inflammatory process, culminating in degradation and resorption of cartilage and bone. Pro inflammatory cytokines particularly TNF and interleukin 1(IL-1) are critical components of this process [3].

Rheumatoid arthritis is predominantly a disease of the elderly, but childrens can also be affected by the disease. Nearly 46 million U.S. adults were reported to be arthritic, among this prevalence rate a quarter million are childrens and 60% are womens. While the prevalence of rheumatoid arthritis in Indian adults was reported to be about 0.75%. This prevalence rate is higher than that reported from China, Indonesia, Philippines and rural Africa [4].

The therapy for rheumatism includes medications with steroids, non-steroidal anti-inflammatory drugs (NSAIDS), disease modifying anti-rheumatic drugs (DMARDS) and immunosuppressant drugs. Unfortunately,

***Corresponding Author:** Vishal Babushetty**, Email:** vbabushetty@gmail.com, **Phone No:** +91- 9535488682

although these drugs have shown to improve signs and symptoms alter the natural history of the disease and improve quality of life but there is still no cure. In addition, these available therapies are associated with potential risks of death/ irreversible organ damage [5]. Other alternative treatment for RA is the use of herbal drugs. Even today 80% of the world population depends on plant derived medicines for the first line of primary health care because of least/no side effects [6,7].

Asystasia dalzelliana commonly known as violet Asystasia (Marathi: Neelkanth) belongs to family Acanthaceae. It is a perennial branched herb, about 60-100m found in kerala, Karnataka and western India. The juice of the leaves of plant *Asystasia dalzelliana* has been traditionally used in treating rheumatoid arthritis in tropical Africa [8]. But scientific investigations on anti-arthritis activity of ethanolic extract of *Asystasia dalzelliana* leaves have not yet been performed which may support its uses in traditional medicine. Hence the present study was undertaken to evaluate anti-arthritis activity of ethanolic extract of *Asystasia dalzelliana* leaves in rats.

MATERIALS AND METHODS

Plant collection and extraction

The fresh leaves of *Asystasia dalzelliana* were collected from Bidar (District of Karnataka state, India) in the month of July 2011 and authenticated (Batch No= VBS- 104) by Dr.P. Santhan, Plant Taxonomist, Natural Remedies Pvt. Ltd, Bangalore. Prior to use, it was ensured that the leaves were free from contamination, sand and no microbial growth. Dried powder leaves of *Asystasia dalzelliana* were successively extracted with ethanol by soxhlet apparatus for 72 hours. The residue obtained after extraction was then concentrated in a rotavapor under reduced pressure. Dried the residue and stored in a desiccator. This residue was used for further study. Phytochemical screening of extract had revealed the presence of alkaloids, flavonoids and tannins as its active constituents [9].

Animals

Swiss albino mice weighing 20-25g and Swiss albino rats weighing 150-180g of either sex were used for the study. Animals were housed at temperature of 25±2^{o}C and relative humidity of 30-60%. 12:12 hr light and dark cycles was followed and was fed with standard feeding pellets and water *ad libitium*. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Government College of Pharmacy, Bangalore, with Ref. No. GCP/IAEC/02/2010-11.

Acute toxicity study

Animals were fasted overnight; however water made available *ad libitium*, after which ethanolic extract was given at one of the four fixed-dose levels (5, 50, 300, and 2000 mg/kg) as per OECD-423 guidelines to five male and five female mice. The animals were observed for toxic symptoms such as behavioural changes, locomotion, convulsions and mortality for 72hr. Animals treated with highest dose were kept observation for 14 days for further toxicity study[10].

Standard drugs

Freund's complete adjuvant (Sigma-Aldrich Chemical Co. USA) and Marker enzymes AST, ALT and ALP estimation kits (Erba diagnostics, Mannheim) were used for experiments. All other experimental chemicals and solvents used were of analytical grade.

Anti-arthritis activity

Freund's adjuvant induced arthritis in rats:

Animals were divided into six groups containing six animals each. Arthritic syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw. Group I served as vehicle control and received 2% gum acacia, Group II served as arthritis control-untreated received 2% gum acacia, Group III received Aspirin (360mg/kg p.o) served as reference standard and extract of *Asystasia dalzelliana* leaves (EAD) of doses of 200mg/kg, 400mg/kg and 800mg/kg were administered to Group IV, Group V and Group VI respectively. The drug treatment was started from 14th day of adjuvant induction and terminated on 28th day.

The changes in paw volume was measured weekly by using Plethysmograph. On 29th day blood samples were collected by retro orbital punture, serum was separated by centrifugation and used for estimation of marker enzymes like Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) using markted kits. Animals were then sacrificed by cervical dislocation, change in organs weight such as liver, kidney and spleen was measured on a weighing balance. Hind paw of the animals were subjected to radiographic analysis and histopathological study[11, 12, 13]. Radiographic analysis was recorded on a digital system and seimen's X-rays machine to evaluate the bone damage at joints of hind paw.

Statistical Analysis

The observations are represented as mean ± SEM. The data were processed by one-way analysis of variance (ANOVA) followed by Dunnet's't'- test. *P≤0.05 was considered significant.

RESULTS

Acute toxicity study

The dizziness was observed at higher dose of ethanolic extract and no death was reported. The highest dose choosen for the acute oral toxicity was 2000mg/kg, hence 1/10th of this dose 200mg/kg was taken as initial effective dose and next higher doses 400mg/kg and 800mg/kg were selected for the present study.

Freund's adjuvant induced arthritis in rats

1. Paw edema: The challenge with CFA (1%, 0.1ml) showed significant increase in paw edema which has reached to peak and remained constant by the end of 2nd week in arthritis control as compared to vehicle control. Extract of dose of 200mg/kg has shown moderate effect on prevention of paw edema, but the treatment with standard aspirin and extract doses of 400mg/kg and 800mg/kg has shown significant prevention of paw edema as compared to arthritis control (**Table 1 & Fig 1**).

Fig 1: Anti-arthritis activity of *Asystasia dalzelliana* leaves against Freund's adjuvant induced arthritis in rats

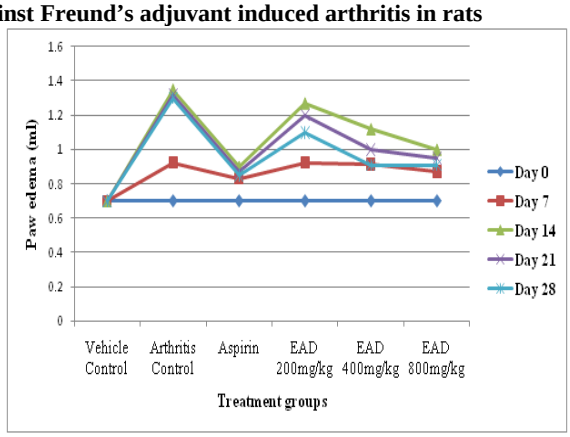


Table 1: Anti-arthritis activity of *Asystasia dalzelliana* leaves against Freund's adjuvant induced arthritis in rats

Treatment Group	Day 7	Day 14	Day 21	Day 28
Arthritis Control	0.92±0.028	1.35±0.016	1.32±0.019	1.30±0.019
Standard Aspirin	0.83±0.006*	0.90±0.011***	0.87±0.011***	0.85±0.015***
EAD 200mg/kg	0.92±0.012	1.27±0.009**	1.20±0.014***	1.10±0.026***
EAD 400mg/kg	0.91±0.016	1.12±0.009***	1.0±0.085**	0.91±0.070***
EAD 800mg/kg	0.87±0.015	1.0±0.072***	0.95±0.050***	0.91±0.050***

Values expressed as mean ± SEM (n=6) $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared to arthritis Control.

2. Biochemical estimation: The CFA (1%, 0.1ml) in arthritis control showed significant elevated levels of SGOT, SGPT and ALP as compare to vehicle control. The extract of dose of 200mg/kg significantly decreased the levels of SGOT and ALP but has insignificant effect on SGPT level. However Standard aspirin and extract of doses of 400mg/kg and 800mg/kg has shown significant effect of preventing the elevated levels of SGOT, SGPT and ALP as compared to arthritis control (**Table 2 & Fig 2**).

Fig 2: Anti-arthritis activity of *Asystasia dalzelliana* leaves against Freund's adjuvant induced arthritis in rats

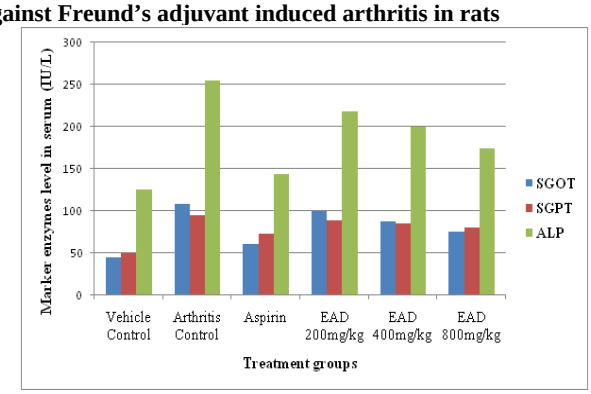


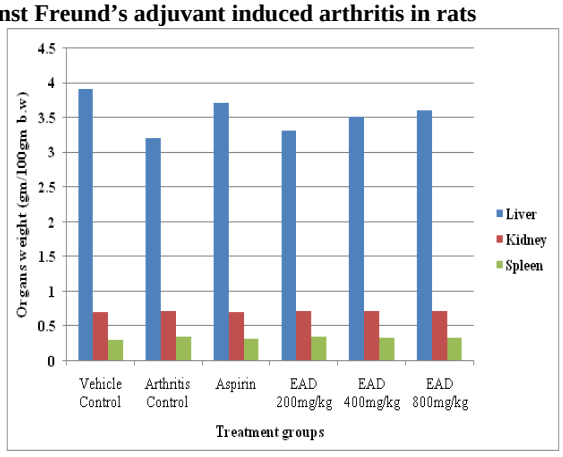
Table 2: Anti-arthritis activity of *Asystasia dalzelliana* leaves against Freund' adjuvant induced arthritis in rats

Treatment Group	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)
Vehicle Control	44.19±2.188	49.50±1.443	124.37±0.019
Arthritis Control	107.60±2.136	93.70±2.887	254.28±3.423
Standard Aspirin	60.10±2.140***	72.48±2.092***	143.72±3.909***
EAD 200mg/kg	99.00±2.582*	88.39±1.882	218.35±4.997***
EAD 400mg/kg	86.62±2.041***	84.86±2.092*	199.00±3.270***
EAD 800mg/kg	74.25±2.327***	79.55±2.777**	174.12±3.639***

Values expressed as mean ± SEM (n=6) $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ as compared to arthritis Control.

3. Organ weight changes: The challenge with CFA (1%, 0.1ml) showed significant increase in kidney and spleen weight and a decrease in liver weight in arthritis control as compared to vehicle control. Standard aspirin has shown significant reduction in weight of kidney and spleen and has increased the weight of liver as compared to arthritis control. Extract of dose of 200mg/kg has not shown significant changes in the organ weight of arthritic rats. However doses of 400mg/kg and 800mg/kg significantly prevented the changes in liver and spleen weight, whereas the effect on kidney was insignificant as compared to arthritis control (**Fig 3**).

Fig 3: Anti-arthritic activity of *Asystasia dalzelliana* leaves against Freund's adjuvant induced arthritis in rats



4. Radiographic evaluation: Radiographic examination of CFA treated hind paw in arthritis control revealed several soft tissue swelling and

narrowing of joint spaces as compared to vehicle control. Extract of doses of 200mg/kg and 400mg/kg shown moderate effect on change in joint architecture. Treatment with standard aspirin and extract dose of 800mg/kg has shown considerable reduction in soft tissue swelling and narrowing of the joint space as compared to arthritis control (**Fig 4**).

Fig 4: Showing the X-ray of hind paws of vehicle, arthritis control, standard and test group animals

Fig A: Vehicle control **Fig B: Arthritis control** **Fig C: Aspirin**

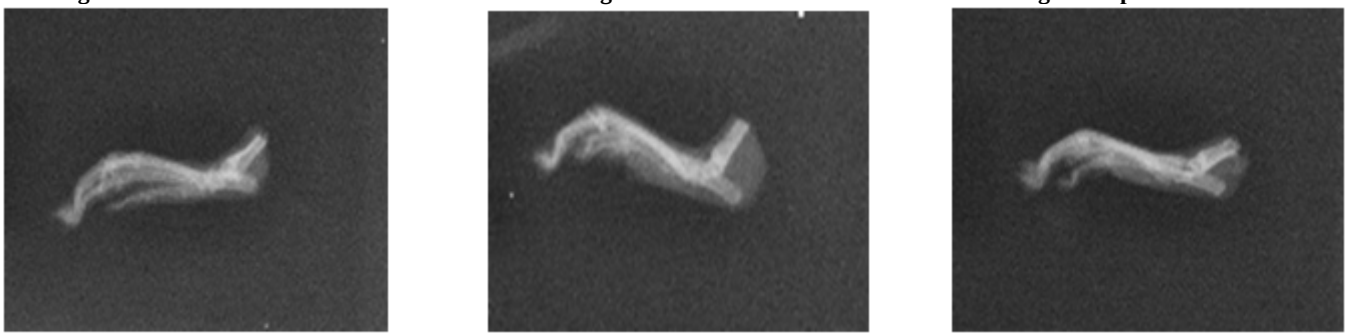
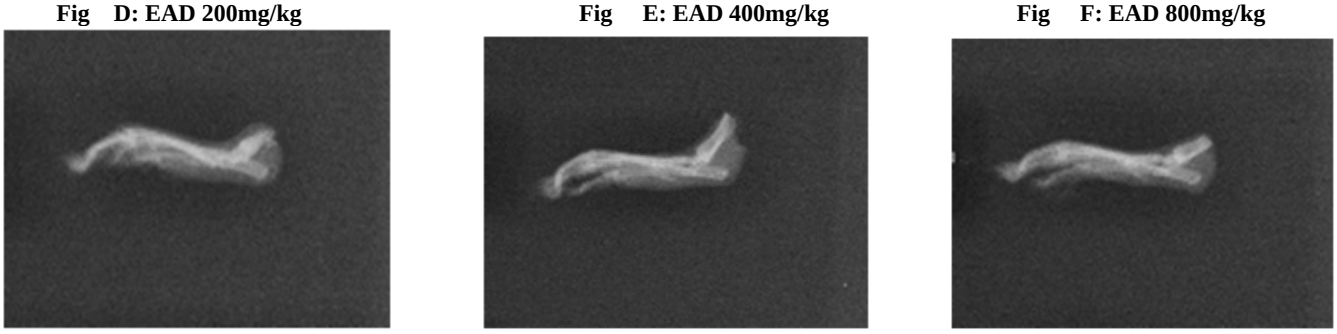


Fig D: EAD 200mg/kg **Fig E: EAD 400mg/kg** **Fig F: EAD 800mg/kg**



5. Histopathological changes in joints: Histopathological studies of hind paw joints in vehicle control rats shown intact articular cartilage and normal synovial lining. Distorted articular cartilage and inflamed cells like lymphocytes and esinophiles were abundant at synovial lining with arthritis control rats. The extract of doses of 200mg/kg and 400mg/kg has shown mild inflammatory condition at synovial lining. However the standard aspirin and extract of 800mg/kg has maintained intact articular cartilage and has reduced the number of inflamed cells like lymphocytes and esinophiles as compared to arthritis control (**Fig 5**).

Fig 5: Showing histopathological changes of hind paw joints of vehicle, arthritis control, standard and test group animals

Vehicle Control **Arthritis Control** **Standard Aspirin**

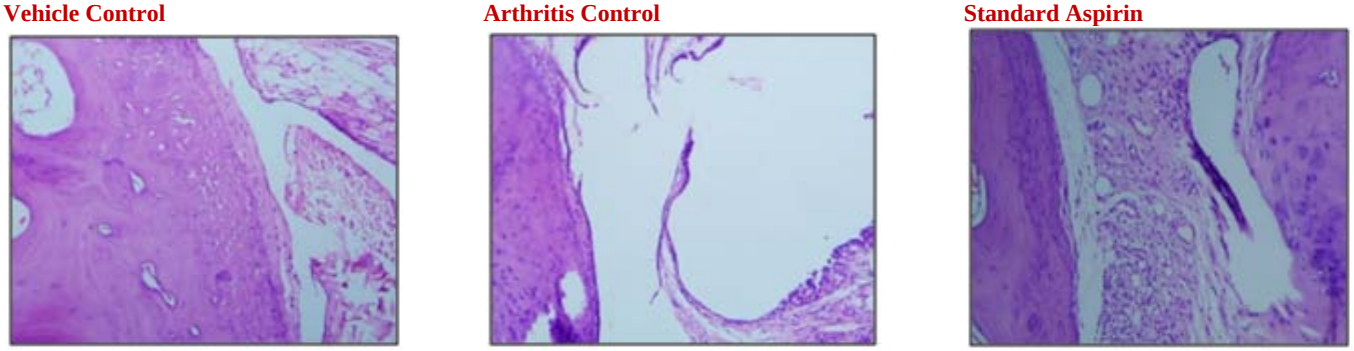


Fig A, H&E, 100x **Fig B, H&E, 100x** **Fig.C, H&E, 100x**

EAD 200mg/kg **EAD 400mg/kg** **EAD 800mg/kg**

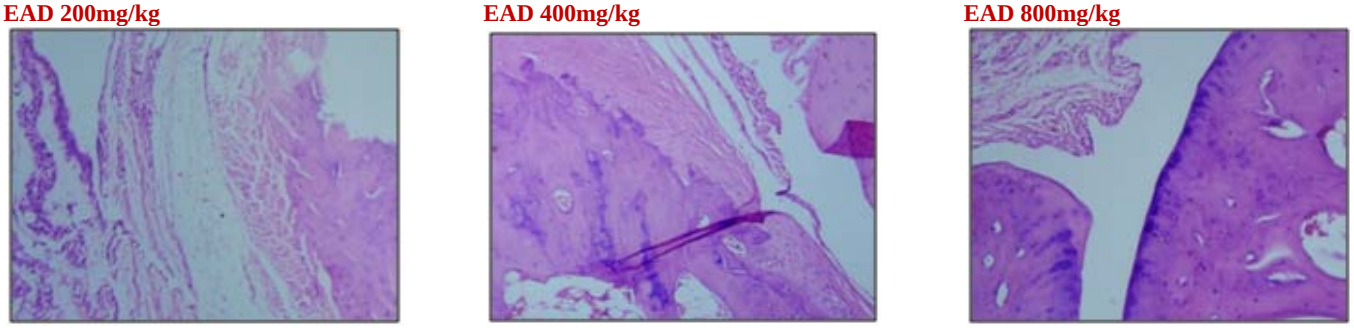


Fig D, H&E, 100x **Fig E, H&E, 100x** **Fig.F, H&E, 100x**

DISCUSSION

Adjuvant arthritis is characterized by chronic proliferative and inflammatory reactions in synovial membranes, producing pain, disability and eventually destruction of joints. Experimental evidence suggests that an autoimmune process

involving T lymphocytes is responsible for the generation of adjuvant arthritis[14, 15]. In the present study, when the animals were challenged with CFA (1%, 0.1ml) produces mono-articular arthritis, which was evident from the significant increase in paw edema in arthritis control. However the doses of 400mg/kg and 800mg/kg of extract were shown significant prevention in paw edema on 21st and 28th day of adjuvant injection.

Assessment of the serum levels of AST, ALT and ALP provides an excellent and simple tool to measure anti-arthritic activity of the target drug. Serum AST and ALT has been reported to play a vital role in the formation of biologically active chemical mediators such as bradykinins in inflammatory process. Elevated levels of serum ALP in adjuvant induced arthritic rats can be due to increase in the liver and bone fraction. This in turn implicates a localized bone loss in the form of bone erosion and peri articular osteopenia, as the enzyme is released into circulation in the course of bone formation and resorption[16]. In the present study, when the animals were challenged with CFA (1%, 0.1 ml) it produced significant elevated serum AST and ALT levels (estimated as serum SGOT and SGPT) and also elevated serum ALP levels in arthritis control. The leaves extract of dose 200mg/kg has produced significant decrease in the levels of SGOT and ALP but has no effect on SGPT levels. However the doses of 400mg/kg and 800mg/kg have produced significant decrease in elevation of SGOT, SGPT and ALP levels as compared to arthritis control. This decreased enzyme levels in adjuvant induced arthritis by the extract treatment emphasises that there might be decrease in bone loss and organ protective mechanism, which may be due to reduction in the release of chemical mediators of the inflammatory process.

The effect of extract on the organ weights like liver, spleen and kidney was also studied. When the animals were challenged with CFA (1%, 0.1ml) it produced significant increase in the kidney and spleen weight and decrease in the liver weight in arthritis control. Extract at all the three dose levels shown no effect on change in kidney weight. The 200mg/kg extract has shown insignificant effect on change in liver and spleen weight. However extract of doses of 400mg/kg and 800mg/kg has produced significant increase in liver weight and decrease in spleen weight as compared to arthritis control. The increase in spleen weight in the adjuvant induced arthritic rats was reported to be associated with splenomegaly, generalized lymphadenopathy and altered hepatic function[17]. Treatment with higher doses of extract has significantly decreased the spleen weight. Thus these findings indicate that there might be inhibition of lymphocytes and decreased immunological responses, which may be responsible for anti-arthritic potential of leaves extract.

The anti-arthritic effect of extract of *Asystasia dalzelliana* on localized bone loss in the form of bone erosion and periarticular osteopenia was further confirmed by radiographic examination. Soft tissue swelling around ankle joint of arthritic rat was considered to be due to edema of periarticular tissues such as ligament and capsule. Diminished joint space is the hallmark of arthritis, which is due to articular cartilage destruction brought by cytokines such as TNF- α and IL-1, which stimulate the release of proteolytic enzymes such as collagenases, glycohydrolases and neutral proteases. As a result, the pannus invades the joint and sub-chondral bones and eventually the joint is destroyed and undergoes fibrous fusion or ankylosis[18]. The damage of hind paw joints was further confirmed by histopathological changes in arthritis control. In present study the radiographical and histopathological examination revealed that the extract of dose of 800mg/kg has markedly inhibited the change in joint architecture as compared to arthritis control.

Extract may possibly act by decreasing synthesis/ release of T cell mediators such as IL, TNF- α as evident from decreased in spleen weight. These effects may probably attributed to presence of phytochemicals such as alkaloid and flavonoids in *Asystasia dalzelliana* leaves because a lot of these secondary plant metabolites identified so far exhibit anti-arthritic properties[13]. Hence one or more of these plant metabolites could be responsible for its anti-arthritis activity.

CONCLUSION

The present preclinical study had revealed the traditional use of *Asystasia dalzelliana* leaves in treatment of rheumatism. However the study also concludes that ethanolic extract of *Asystasia dalzelliana* leaves of dose of 800mg/kg exhibited significant anti-arthritis activity than the lower doses of 200mg/kg and 400mg/kg. Further studies like isolation and characterizations of active principals responsible for such activity are necessary to be executed.

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REFERENCES

1. Stephen JM, William FG. Pathophysiology of Disease: An Introduction to Clinical Medicine. 5th edition. McGraw-Hill publication; 2006.
2. Ollier WE, Harrison B, Symmons D. What is the natural history of rheumatoid arthritis?. Baillieres Best Pract Res Clin Rheumatol 2001; 15: 27-48.
3. Robbins, Cotron, Vinay k, Abdul KA, Nelson F. Pathologic Basis of Disease. 7th edition. Elsevier publication; 2008. p. 1306-1308.
4. Irfan AK, Atiya K . Herbal therapy for arthritis. Ukaaz publications; 2008. p. 15-72.
5. Susan JL, Arthur K. Pharmacological treatment of established rheumatoid arthritis. Best Pract Res Clin Rheumatol 2003; 17: 811-829.
6. Soeken KL, Miller SA, Ernst E. Herbal Medicines for the treatment of rheumatoid arthritis: a systematic review. Rheumatology 2003; 42: 652-659.
7. Sheetal V, Singh SP. Current and future status of herbal medicines. Veterinary World 2008; 1: 347-350.
8. Grabben GJH, Denton OA. Porta: Plant resources of tropical Africa 2. Backhuys publications 2004; 2: 100.
9. Kokate Ck, Purohit AP, Gokhale SP. Text book of pharmacology. 43rd edition. Nirali prakashan publication; 1996.
10. Ecobichon DJ. The Basic of Toxicology Testing. 3rd edition. CRC press; 1997. p. 43-86.
11. Mythilypriya R, Shanthi P, Sachdanandam P. Therapeutic effect of Kalpamruthaa, a herbal preparation on adjuvant induced arthritis in wister rats. J Inflammopharmacology 2008; 16: 21-35.
12. Jaijesh P, Srinivasan KK, Bhagath KP, Sreejith G, Raju SK, Sareesh NN *et al*. Anti-arthritic potential of the plants *Justicia gendarussa Burm F*. J Clinics 2009; 64: 357- 362.
13. Jaijesh P, Srinivasan KK, Bhagath KP, Sreejith G, Raju SK, Sareesh NN *et al*. Compering the anti-arthritis activities of the plants *Justicia gendarussa Burm F* and *Withania Somnifera Linn*. Int J Green Pharm 2009; 3: 281- 284.
14. Holoshitz J, Naparstek Y, Ben-Nun A, Cohen IR. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. J Science 1983; 219: 56-58.
15. Francischi JN., Yokoro CM, Poole S, Tafuri WL, Cunha FQ, Teixeira MM *et al*. Anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis. Eur. J. Pharmacol 2000; 399: 243-249.
16. Borashan FA., Ilkhanipoor M, Hashemi M, Farrokhi F. Investigation the effects of *Curcumin* on serum hepatic enzymes activity in a rheumatoid arthritis model. Electr. J. Biol 2009; 4: 129-133.
17. Ismail MF, EL-Maraghy SA, Sadik NAH. Diethylnitrosamine-inducehepatocarcinogenesis in rats: possible chemoprevention by blueberries. African J. Biochem. Res 2008; 2: 74-80.
18. Sudaroli M, Chatterjee TK. Evaluation of red and white seed extracts of *Abrus precatorius Linn.* against freund's complete adjuvant induced arthritis in rats. J. Med. Plants. Res 2007; 1: 86-94.