

Dose dependent potency and efficacy of ethanolic extract of leaves of *Clerodendrum viscosum* as an anti-arthritic agent in wistar albino rats

Chandrashekar R^{1*}, Sheeba Damodar KP², Mohandas Rai³

¹Assistant Professor, ³Professor & HOD, AJ Institute of Medical Sciences & Research Centre, Mangalore, ²Associate Professor, Dept. of Pharmacology, Academy of Medical Sciences, Periyaram, Kerala

*Corresponding Author:

Email: chandumoon47@gmail.com

Abstract

Introduction: Arthritis is a chronic inflammatory disorder involving abnormal activity of immune system which usually affects elderly people, even though seen in younger generation in few cases. Many drugs are available in the market but carry its own drawbacks and limitations. Hence, the search for the new drugs is still under research.

Objectives: Evaluation of anti-arthritic activity of Ethanolic Extract of Leaves of *Clerodendrum viscosum* in Wistar Albino rats.

Materials and Method: Wistar Albino rats were divided into five groups of 10 rats each. Complete Freund's adjuvant (CFA) was injected to sub plantar region of the left hind paw of each rat. Administration of 1% Gum acacia (3 ml/kg, orally), Ethanolic extract of Leaves of *Clerodendrum viscosum* (75, 150 & 300mg/kg) and Indomethacin (10 mg/kg, orally) was started on the same day and continued for 21 days. Paw volume and body weight were recorded on the same day. Digital plethysmometer was used to record paw volume. On 5th day, the volume of paw was recorded again to assess the primary followed by measuring the paw volume of the non-injected paw regularly. From day 13th to 21st, was drug free day and on 21st day the body weight and paw volume were recorded and the secondary lesions were graded.

Results: It was observed that *Clerodendrum viscosum* Ethanolic extract at doses of 150 mg/kg showed significant anti-arthritic activity on 5th day (p<0.01), 21st day (p<0.01) and Arthritic index (p<0.01). *Clerodendrum viscosum* Ethanolic extract at the dose of 300mg has also showed significant anti-arthritic activity on 5th day (p<0.05), on 21st day (p<0.01) and Arthritis index (p<0.01) by Complete Freund's adjuvant (CFA) induced Arthritis in Wistar albino rats

Conclusion: Ethanolic Extract of Leaves of *Clerodendrum viscosum* has shown dose dependant Potent and efficacious anti-arthritic activity in Wistar Albino rats

Keywords: Arthritis, *Clerodendrum viscosum*, Complete Freund's adjuvant, Ethanolic Extract, Wistar Albino rats

Introduction

Arthritis is a chronic inflammatory disorder that affects 0.5-1% of the world population with more women being affected than men.⁽¹⁾

Current treatments include non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and biologics, but none are curative and there is a significant 'non-responder' rate.^(2,3) Rheumatoid arthritis is systemic inflammatory disease in which the destruction of articular cartilage leads to bone deformity and loss of joint function and ultimately severe pain. It is the most common inflammatory arthritis affecting approximately 1-2% of the general population worldwide i.e. 20 million people worldwide. Incidence increases with age, with women being affected three times more than men (Arthritis Research Campaign). The risk of incidence appears to be greatest for women between 40 and 50 years of age, and for men somewhat later, which makes the rheumatoid arthritis to be an age-related immune disorder.^(4,5)

However, besides their high cost, prolonged use of many of these drugs is associated with severe adverse reactions such as gastric and duodenal ulcers, complications in the small intestine and colon can occur, which cause colitis, bleeding, perforation, stricture, and chronic problems such as iron deficiency anemia and protein loss and toxicity. A report also states that, NSAIDs treatment enhances joint destruction in arthritis and inhibits glycosaminoglycan

synthesis. Recently, there has been an increasing interest in natural food for scavenging the free radicals because of their wide acceptance.⁽⁶⁾

The plant *Clerodendron viscosum* Vent. is an indigenous medicinal plant widely distributed in various parts of India, Ceylon, Malaya and Bangladesh.⁽⁷⁾ The genus *Clerodendrum* (Family: Lamiaceae/ Verbenaceae) commonly known as "Ittae" (Madras malligae) is very extensively distributed in tropical and subtropical regions of the world and it is comprised of small trees, shrubs and herbs. First description of the genus was given by Linnaeus in 1753, with the identification of *C. infortunatum*. After a decade, in 1763 Adanson changed its Latin name "*Clerodendrum*" to its Greek form "*Clerodendron*". In Greek word Klero means chance and dendron means tree i.e. chance tree which means the tree which does not bring good luck like *Clerodendron infortunatum* or the tree which brings good luck like *C. fortunatum*. After about two centuries in 1942 Moldenke readopted the Latinized name '*Clerodendrum*', which is now presently used by taxonomists for the classification and of the genus and species. *Clerodendrum phlomidis* (L) Gamble (verbinaceae), a related species has shown anti-arthritic activity in one of the previous study.⁽⁸⁾

Our previous studies related to ethanolic extract of leaves of *Clerodendrum viscosum* has shown acute and chronic anti-inflammatory activity in different animal models of inflammation.^(9,10) In view of this, the present study was undertaken to evaluate anti-arthritic activity

of ethanolic extract of leaves of *Clerodendrum viscosum* in Wistar Albino rats as this species is most commonly seen in this part of region.

Materials and Method

Freund's adjuvant induced Arthritis model was used to assess the anti-arthritic activity in Wistar rats.⁽¹¹⁾ Wistar Albino rats were divided into five groups of 10 rats each. Complete Freund's adjuvant (CFA) consists of *Mycobacterium butyricum* (Difo) 0.05% suspended in heavy mineral/ paraffin oil was prepared by thoroughly grinding with a pestle and mortar to give a final concentration of 6 mg/ ml. On the first day, they were injected with CFA (0.1ml) in to sub plantar region of the left hind paw by a 26 gauge needle. Administration of 1% Gum acacia (3 ml/kg, orally), Ethanolic extract of Leaves of *Clerodendrum viscosum* respectively and Indomethacin (10 mg/kg, orally) which served as reference standard was started on the same day and continued for 21 days. Paw volume and body weight were recorded on the same day. Digital plethysmometer (Rajesh Chemicals, Mumbai, India) was used to record raw volume. On 5th day, the volume of paw was recorded again to access the primary lesion and influence of Test / STD drug on this phase.

Thereafter the severity of the adjuvant induced arthritis was followed by measuring the paw volume of the non-injected paw regularly. From day 13th to 21st, the animals did not receive any treatment and on the 21st day the body weight and paw volume were recorded and the secondary lesions were graded^(12,13) (Table 1).

Results

Table 2: Anti-arthritic activity of EELCV by Complete Freund's Adjuvant induced arthritis in Wistar albino rats

Groups / Drugs / Dose		Chronic inflammation		
		Mean decrease in paw		Arthritis Index
Groups / Drugs	Dose	5 day (ml / %)	21 day (ml / %)	21 day (%)
Control (1% gum acacia), p.o	3 ml/kg	1.41±0.08	1.39±0.08	7±0.59
Standard (Indomethacin), p.o	10 mg/ kg	0.55±0.08 (60.99) (p<0.01)	0.02±0.003 (98.56) (p<0.01)	2.3±0.36 (p<0.01)
EELCV, p.o	75 mg/ kg	1.02±0.10 (27.65) (p>0.05)	1.25±0.02 (9.92) (p>0.05)	6.5±0.61 (p>0.05)
EELCV, p.o	150 mg/ kg	0.59±0.10 (58.15) (p<0.01)	0.14±0.09 (89.92) (p<0.01)	3.3±0.42 (p<0.01)
EELCV, p.o	300 mg/ kg	0.76±0.14 (46.09) (p<0.05)	0.89±0.16 (35.97) (p<0.01)	3.1±0.31 (p<0.01)

Table 1: Parameters considered

Parts observed	Observation	Grading's
Ear	Absence of swellings	0
	Presence of swelling	1
Nose	No swelling of connective tissue	0
	Intense swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Fore paws	Absence of inflammation	0
	Presence of inflammation	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Severe inflammation	3

Grading of primary, secondary and arthritic lesion is as follows

- Assessment of primary lesion:** Percent inhibition of paw volume of adjuvant injected left paw over the control measured on 5th day
- Assessment of secondary lesion:** Percent inhibition of paw volume of non-injected right paw over the controls measured on 21st day
- Assessment of arthritic index:** It is calculated as sum of the scores of primary and secondary lesion. The average of treated animals is compared with control groups.

The total percentage change is calculated as:
Percentage inhibition of injected left paw on 5th day + Percentage inhibition of non-injected right paw on 21st day + Percentage inhibition of Arthritis index.

The observation are mean \pm S.E.M. $p > 0.05$ - Not Significant, $p < 0.05$ -Significant, $p < 0.01$ - Highly Significant as compared to control

(ANOVA followed by Dunnett's multiple comparison test)

EELCV- Ethanolic Extract of the Leaves of *Clerodendrum viscosum*, p.o- per oral

Discussion

Adjuvant induced arthritis is believed to be due to an immunological process associated with the development of delayed hypersensitivity to a component of the injected mycobacterial adjuvant.⁽¹⁵⁾

Rat models are useful for studies of the pathogenesis of rheumatoid arthritis (RA) since rats are extraordinarily sensitive to induction of arthritis with adjuvants. Injection of not only the classical complete Freund's adjuvant but also mineral oil without mycobacteria and pure adjuvants such as pristane and squalene, induce severe arthritis in many rat strains.⁽¹⁵⁾

Our study has shown that *Clerodendrum viscosum* Ethanolic extract at doses of 150 mg/kg showed significant anti-arthritic activity on 5th day ($p < 0.01$), 21st day ($p < 0.01$) and Arthritic index ($p < 0.01$). *Clerodendrum viscosum* Ethanolic extract at the dose of 300mg has also showed significant anti-arthritic activity on 5th day ($p < 0.05$), on 21st day ($p < 0.01$) and Arthritis index ($p < 0.01$) by Complete Freund's adjuvant (CFA) induced Arthritis in Wistar albino rats (Table 2 & Fig. 1-3).

Conclusion

Ethanolic Extract of Leaves of *Clerodendrum viscosum* has shown dose dependant Potent and efficacious anti-arthritic activity in Wistar Albino rats. However, further studies are required to evaluate its exact mechanism at molecular level as a potent and efficacious anti-arthritic agent.

References

1. Firestein G.S. Evolving concepts of rheumatoid arthritis. Nature (London). 2003;423:356-61.
2. Strand V, Kimberly R, Isaacs JD. Biologic therapies in rheumatology: lessons learned, future directions. Nat Rev Drug Discov.2007;6:75-92.
3. Julià A, Erra A, Palacio C, Tomas C, Sans X, Barceló P, Marsal S. An eight-gene blood expression profile predicts the response to infliximab in rheumatoid arthritis. PLoS One.2009;4(10):e7556.
4. Hoff M, Haugeberg G. Using hand bone mass measurements to assess progression of rheumatoid arthritis. Ther Adv Musculoskelet Dis.2010;2:79-87.
5. Charles G, Helmick et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part I.2008;58:15-25.
6. Syed Uzair AS, Huma Jawed, Shahid IA, Anjum S, and Shabana US (2013) The Anti-Arthritic and Immune-Modulatory Effects of NHAG: A Novel Glucosamine Analogue in Adjuvant-Induced Arthritis. Bio Med Research International Volume 1-3.
7. Al-Amin, M.; Akter, S; M. Rahman A. Diversity of Forest Undergrowth of North Eastern Region of Bangladesh, Research Journal of Agriculture and Biological Sciences.2007;3(3):143148.
8. D. Kilimozhi, V. Parthasarathy, N. Amuthavalli. Effect of *Clerodendrum phlomidis* on adjuvant induced arthritis in rats - a radiographic densitometric analysis. International Journal of Pharm Tech Research.2009;1(4):1434-144.
9. Chandrashekar R, S. N. Rao. Acute Anti-Inflammatory Activity of Ethanolic Extract of Leaves of *Clerodendrum viscosum* by Carrageenin Induced Paw Oedema in Wistar Albino Rats. Int. J. Res. Ayurveda Pharm.2013;4(2):224-227.
10. Chandrashekar R, S. N. Rao. Chronic Anti-Inflammatory Activity of Ethanolic Extract of Leaves of *Clerodendrum viscosum* by Carrageenin Induced Paw Oedema in Wistar Albino Rats. British Journal of Pharmaceutical Research.2013;3(4):579-586.
11. Newbould, B.B., Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. Br J PharmacolChemother.1963;21:127-36.
12. Chandrashekar R, Rao S.N. Chronic Anti-Inflammatory Activity of Ethanolic Extract of Leaves of *Leucas indica* By Carrageenin Induced Paw Oedema in Wistar albino rats. International Journal of Universal Pharmacy and Bio Sciences. 2013;2(2):79-84.
13. D'Arcy P.F., Howard E.M., Muggleton P.W. and Jowsend S.B. The anti-inflammatory action of griseofulvin in experimental animals, J. Pharm. Pharmacol. 1960;12:659-665.
14. John T. Sharp, Byron H. Waksman, Carl M, Pearson. Sarabelle Madoff. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. Iv. Examination of tissues and fluids for infectious agents. Arthritis and Rheumatism. 2005;4(2):169-175.
15. Holmdahl R1, Lorentzen JC, Lu S, Olofsson P, Wester L, Holmberg J, Pettersson U. Arthritis induced in rats with non-immunogenic adjuvants as models for rheumatoid arthritis. Immunol Rev. 2001 Dec;184:184-202.