

Attenuation of Sub Acute Inflammation by Ethanol Extract of *Clerodendrum viscosum* Linn. Leaves in Wistar Albino Rats

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Abstract

Inflammation is a pathological response of vascular tissue for harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes. In our study we have tried to evaluate attenuation effect of Ethanolic extract of *Clerodendrum viscosum* leaves in Wistar albino rats. Ten Wistar albino rats were randomly assigned to 5 groups. Group 1 served as Controls which received 1 % gum acacia, 3ml/kg, Group 2 received standard drug Indomethacin, 10mg/kg and Group 3, 4 & 5 received the test drug at the dose of 75, 150 & 300 mg/kg. All the drugs were given orally. Rats were anesthetized with pentobarbitone (30mg/kg, intraperitoneally) and sterilized cotton pellet was implanted subcutaneously. All animals received drugs once a day for 7 day and on 8th day rats were sacrificed by using high dose of Thiopentone sodium. The average weight of the cotton pellets (dry & wet) and transudative weight were recorded and compared between the groups. Ethanolic extract of *Clerodendrum viscosum* leaves showed significant sub acute anti-inflammatory activity at the dose of 150mg/kg and 300mg/kg by cotton pellet induced granuloma pouch in Wistar albino rats when compared to controls.

Keywords: *Clerodendrum viscosum* Linn. Ethanolic extract, Attenuation, Sub Acute Inflammation

Introduction

Inflammation is a physiological as well as pathological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism of the body to protect itself from stimuli but may lead to complication on chronicity. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases.⁽¹⁾

There are various inflammatory mediators to an inflammatory reaction that contributes to the associated symptoms and related tissue injury. The process of edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation.⁽²⁾ Various drugs including steroidal and non steroidal anti-inflammatory drugs are used to control the inflammatory reactions. Currently available NSAIDs in the market carries the risk of side / adverse effects either individually or in combination will have their own limitations. About 34-46% of the users of NSAIDs usually suffer from some gastrointestinal symptoms due to inhibition of the cytoprotective. Cyclooxygenase enzyme in gastric mucosa. Hence novel anti-inflammatory drugs are still under intense research especially from herbs.⁽³⁾

The genus of *Clerodendrum* is widely distributed in tropical and subtropical regions of the world. About five hundred species or more of the plants belonging to this genus are identified till now, which includes shrubs mainly. Ethno-pharmacological importance of various species of *Clerodendrum* genus has been recorded in various Indian systems of medicines and also mentioned as folk medicines. This genus is still being used as medicines especially by Indians, Chinese,

Japanese and other countries for the treatment of various life threatening disorders like syphilis, typhoid, cancer, jaundice and hypertension. The leaves are slightly bitter and is used to treat inflammation, skin diseases and is found beneficial in small pox.⁽⁴⁾ The plant parts are also used in Indigenous system of medicine in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy.⁽⁵⁾

Hence, keeping this in mind the present study was selected to evaluate the attenuation of sub-acute inflammation by Ethanolic extract of *Clerodendrum viscosum* Linn leaves in Wistar albino rats.

Materials and Methods

Institutional Animal Ethics Committee (IAEC), approval was obtained from Yenepoya Medical College, Yenepoya University, Derlakatte, Mangalore, Karnataka, India. Ref PhD 1/2010 dated 6th May 2010 before commencing the experiments. All the animals were handled and taken care according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSE), New Delhi, India.

Procedure

Wistar Albino rats randomly were divided into 5 groups of 10 rats each. Cotton wool as an adsorbant was weighed (20±1 mg) and made into pellet. Each pellet was sterilized initially in a hot air oven set at 120° for 2 hr. The rats were anaesthetized with pentobarbitone sodium (30 mg/kg, intraperitoneally). The nape of the back of the neck was thoroughly shaved, swabbed with 70% ethanol by using cotton and

sterilized cotton pellet was implanted subcutaneously. Group 1 served as Controls which received 1 % gum acacia, 3ml/kg, Group 2 received standard drug Indomethacin, 10mg/kg and Group 3, 4 & 5 received the test drug at the dose of 75, 150 & 300 mg/kg. All the drugs were given orally. Pellets from each animal was dissected out followed by drying at 60°C for 18 hr and finally its weight was noted. The average weight of each cotton pellet of all the groups was calculated. Dry weight (DW), wet weight (WW) and transudative weight (TW) of all the groups were calculated and compared to control values.^(6,7)

Results

It was observed that Ethanolic extract *Clerodendrum viscosum* Linn. leaves has shown very significant sub acute anti-inflammatory activity with respect to wet weight ($p < 0.05$), dry weight ($p < 0.01$) and transudative weight ($p < 0.01$) at the dose of 150mg/kg and with respect to wet weight ($p < 0.01$), dry weight ($p < 0.01$) and transudative weight ($p < 0.01$) at the dose of 300mg/kg by cotton pellet induced granuloma pouch in Wistar albino rats compared to control (Table 1 & Fig. 1) with significant mean percentage of inhibition of sub acute inflammation (Table 2).

Table 1: Sub-acute anti-inflammatory activity of EELCV by cotton pellet induced granuloma pouch in Wistar albino rats

Groups / Drugs / Dose	WW (mg)	DW (mg)	TW (mg)
Group 1. Control (1% Gum acacia) 3ml/kg, p.o	204.5±3.31	57.03±0.88	151.2±2.24
Group 2. Standard (Indomethacin) 10mg/kg, p.o	114.7±2.89***	44.82±1.10***	76.49±2.18***
Group 3. EECVL 75mg/kg, p.o	194.9±2.68*	53.99±1.82*	149.7±2.62*
Group 4. EECVL 150mg/kg, p.o	186.9±7.29**	50.02±2.55***	83.79±2.37***
Group 5. EECVL 300mg/kg, p.o	144.5±6.31***	45.55±1.13***	77.43±1.87***

All observations are mean ± S.E.M compared to control. Statistical Method: ANOVA, Post hoc: Dunnett's multiple comparison test. * $p > 0.05$ - Not Significant, ** $p < 0.05$ -Significant, *** $p < 0.01$ - Highly Significant, WW-Wet weight, DW- Dry weight, TW-Transudative weight EECVL- Ethanolic Extract of *Clerodendrum viscosum* Linn. Leaves, p.o- per oral.

Table 2: Mean Percentage inhibition of sub-acute inflammation by EECVL in Wistar albino rats

Groups / Drugs / Dose	Wet Weight (%) (Mean Percentage of Inhibition)	Dry Weight (%) (Mean Percentage of Inhibition)	Transudative Weight (%) (Mean Percentage of Inhibition)
Control (1% Gum acacia) 3ml/kg, p.o	0	0	0
Standard (Indomethacin) 10mg/kg, p.o	43.91	21.40	49.41
EECVL 75mg/kg, p.o	4.69	5.33	0.99
EECVL 150mg/kg, p.o	8.60	12.29	44.58
EECVL 300mg/kg, p.o	29.33	20.03	48.78

Discussion

Our previous study has shown acute as well as chronic analgesic activity of Ethanolic extract of *Clerodendrum viscosum* Linn leaves in Wistar albino rats.⁽⁸⁻¹⁰⁾ The granuloma due to inflammation is a characteristic feature of sub-acute inflammatory reaction.⁽¹¹⁾ Cotton pellet granuloma method is a valid and widely used method to assess the transudative, exudative and a proliferative phases of inflammation especially of subacute type. The transudative fluid adsorbed by the cotton pellet greatly contributes for the wet weight of the granuloma, whereas the dry weight contributes for the amount of granuloma tissue formed.

In our study we found that, Ethanolic extract of *Clerodendrum viscosum* Linn. leaves has shown anti-inflammatory activity on sub-acute inflammation by cotton pellet induced granuloma pouch in wistar albino rats at the dose of 150 and 300 mg/kg. There was significant reduction with respect to wet weight, dry weight and transudative weight at the dose of 150 and 300mg/kg.

Conclusion

This study has given substantial evidence that Ethanolic extract of *Clerodendrum viscosum* Linn. leaves could play a vital role even in sub-acute inflammatory condition at the dose of 150 and 300mg/kg. However further studies involving the assay of inflammatory mediators would be suggestive to know its exact role in extenuating the sub-acute inflammation.

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