

**CHRONIC ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC
EXTRACT OF LEAVES OF *CLERODENDRUM VISCOSUM* BY
CARRAGEENIN INDUCED PAW OEDEMA IN WISTAR ALBINO RATS**

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ABSTRACT

Aims: The aim of the study was to investigate the anti-inflammatory of ethanolic extract of the leaves of *Clerodendrum viscosum* (EELCV) by carrageenin induced paw oedema in wistar albino rats.

Study design: Prospective

Place and Duration of study: Dept of pharmacology, Yenepoya Medical College, Yenepoya University, Derlakatte, Mangalore 575018, India. June 2010-August 2010

Methodology: Dried powdered leaves of *Clerodendrum viscosum* were subjected to soxhlet extraction by using 90 % ethanol. Based on acute oral toxicity study according to Organization for Economic Cooperation and Development (OECD) guidelines no. 423, three doses of the test drug was selected (75, 150 & 300 mg/kg) for rats, and were subjected to screening of anti-inflammatory activity.

Results: Oral administration of EELCV at doses of 150 and 300mg/kg showed significant ($P = .01$) and moderate anti-inflammatory activity ($P = .05$) respectively by carrageenin induced paw oedema in wistar albino rats compared to control.

Conclusion: Administration of EELCV orally at the doses of 150 and 300mg/kg showed significant ($P = .01$) and moderate anti-inflammatory activity ($P = .05$) respectively by carrageenin induced rat paw oedema in wistar albino rats. The percentage inhibition of the oedema at 3rd hour was 66.44 % and 63.75 % at the dose of 150 mg/kg and 300 mg/kg respectively.

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KEYWORDS: Anti-Inflammatory Activity, *Clerodendrum Viscosum*, Carrageenin, Indomethacin.

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INTRODUCTION

Since time immemorial, indigenous plants have been a major source of medicine. In folk medicine, they are used, in single or in combined forms for treating different types of inflammatory and arthritic conditions. Prolonged administrations of steroidal and non-steroidal anti-inflammatory drugs are known to be associated for their adverse effects. Herbal drugs have lesser side effects and are largely replaced by synthetic drugs.¹ Plants have, at one time, supplied virtually all cultures with food, clothing, shelter, and medicines. It is estimated that approximately 10 to 15 percent of the roughly 300,000 species of higher plants have a history of use in traditional medicine.²

MATERIAL AND METHODS

Institutional Animal Ethical Committee (Licence No. CPCSEA / 347) approval was obtained from Yenepoya University, Derlakatte, Mangalore, India, before conducting the experiments dated 6th May 2010. All animals were handled and taken care according to guidelines of "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, Revised 1985) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

PLANT MATERIAL

The whole plant was collected from rural region of Manjanady, in Mangalore region in the month of June – August 2010. It was authenticated by (Prof) Dr. Krishna Kumar G., Chairman, Dept of Applied Botany, Mangalore University, Mangalore. The herbarium of the plant (voucher specimen no YU/CV/2010) has been deposited at Museum of Department of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore.

EXTRACTION

Leaves of *Clerodendrum viscosum* were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered. About 1000 gm of air dried powdered material was extracted with 90% ethanol in a Soxhlet extractor for 36 hours. It was concentrated to dryness under reduced pressure and controlled temperature (40-50°C) using rotary evaporator. The ethanolic extract yielded a dark green slightly sticky mass weighing 155g. The ethanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 15.5% w/w with respect to dried leaf. The collected leaf extract was stored in a desiccator.

SAMPLE SIZE, GROUPING AND DOSE OF THE DRUGS

Animals were divided into 5 groups (Control, Standard & 3 groups of Test drug) containing 10 animals, making a total number of 50 animals.

DRUGS AND CHEMICALS

The standard Indomethacin was obtained from our institutional pharmacy and 1% gum acacia from Department of Pharmacology, YMC, Mangalore.

ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN-INDUCED RAT PAW EDEMA

In this method, rats were divided in to five groups of 10 animals each (Equal number of Male & Female). All the animals were pretreated with drugs / vehicle, for 10 days and 60 minutes before injection of carrageenan (0.1 ml of 1%) on 10th day. Carrageenan was injected into the sub plantar tissue of left hind paw of each rat on 10th day. Swellings of carrageenan-injected foot were measured at zero, first, second, third, fourth and sixth hour using Digital Plethysmometer (Rajesh Chemicals, Mumbai).The right hind paw was injected with 0.1 ml of vehicle. The animals received the standard drug indomethacin (10 mg/kg, p.o.) which served as reference standard and the control group received 1% gum acacia 3ml/kg. Three doses of the test drug were selected (75, 150 & 300 mg/kg) for test group of rats, and all the groups were subjected to screening for anti-inflammatory activity by carageenin induced paw oedema. The peak effect of the carageenin induced paw oedema was observed at the 3rd hr after the injection. The increase in paw volume at 3rd hr

78 was calculated as percentage compared with the volume measured immediately after injection of carrageenin for each
 79 animal was compared between groups.³

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82 **Table 1 : Showing Anti-Inflammatory effect of Ethanolic Extract of leaves of *Clerodendrum viscosum* by**
 83 **Carragenin Induced Paw Oedema in rats (Chronic Study)**

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Drugs	0hr	1 st hr	2 nd hr	3 rd hr	4 th hr	6 th hr	% Inhibition at 3 rd hr
Control (1% Gum acacia) (3ml/kg)	1.27±0.08	1.59±0.16	1.49±0.13	1.49±0.12	1.78±0.13	1.73±0.20	---
Indomethacin 10mg/kg	1.11±0.16	0.58±0.10***	0.54±0.09***	0.50±0.09***	0.52±0.12***	0.42±0.06***	66.44
EECLV 75 mg/kg	1.18±0.14	1.44±0.14*	1.39±0.10*	1.20±0.23*	1.40±0.10*	1.51±0.09*	19.46
EECLV 150mg/kg	1.18±0.14	0.90±0.12***	0.76±0.08***	0.54±0.16***	0.74±0.16***	0.77±0.11***	63.75
EECLV 300mg/kg	1.18±0.14	1.12±0.13*	1.39±0.23*	0.80±0.18**	1.19±0.13**	1.38±0.09*	46.30

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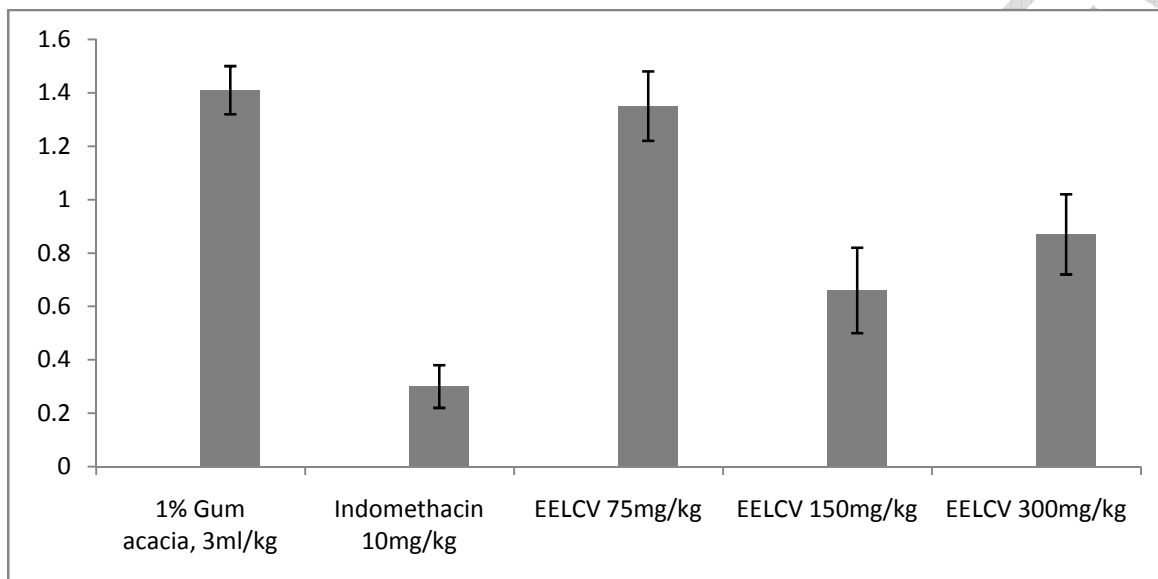
86 n=10. The observation are mean ± S.E.M. *P > .05, ** P =.05, *** P = .01 as compared to control (ANOVA followed
 87 by Dunnett's multiple comparison test)

88 **EELCV- Ethanolic Extract of the leaves of *Clerodendrum viscosum***

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94 **Graph 1 : Showing Anti-Inflammatory effect of Ethanolic Extract of leaves of *Clerodendrum viscosum* (EELCV)**
95 **by Carragenin Induced Paw Oedema in rats (Chronic Study) at 3rd hr**
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98 **The observations are mean \pm S.E.M. as compared to control**

99 **(ANOVA followed by Dunnett's multiple comparison test)**

100 **EELCV- Ethanolic Extract of the leaves of *Clerodendrum viscosum***
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102 **STATISTICAL ANALYSIS**

103 Results were expressed as mean \pm SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA)
104 followed by Dunnett's Multiple Comparison test. $P < .05$ was considered statistically significant.

106 **RESULTS AND DISCUSSION**

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108 Oral administration of EELCV at doses of 150 and 300mg/kg (Table 1 & Figure 1) to Wistar Albino rats showed significant
109 ($P = .01$) and moderate anti-inflammatory activity ($P = .05$) respectively by carrageenin induced paw oedema in Wistar
110 Albino rats. The percentage inhibition of the oedema at 3rd hour was 53.19 % and 38.29% at the dose of 150 mg/kg and
111 300 mg/kg respectively compared to control and reference group (Table 1 & Graph 1).
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113 Pain, inflammation and pyrexia are the most usual disturbing symptom in day to day life. Plenty of drugs are available in
114 the market for relieving these symptoms and which are also sold over the counter. However, they have high tendency of
115 causing adverse drug reaction from a trivial nausea and vomiting to gastric irritation leading to peptic ulcer, perforation
116 and may also even lead to death.⁴ The main undesirable side-effects of aspirin (Irreversible COX inhibitors) taken by
117 mouth are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. In children and adolescents,
118 aspirin is no longer indicated to control flu-like symptoms or the symptoms of chickenpox or other viral illnesses, because
119 of the risk of Reye's syndrome.⁵ Reversible COX inhibitors have not shown any superiority over aspirin in therapeutic

effects of safety in over dose. Nimesulide has been banned in most western country due to its hepatotoxic effects. Even though paracetamol is commonly prescribed analgesic and antipyretic drug, but it lacks anti inflammatory effects and also not safe in over dose. Selective COX 2 inhibitors have been least prescribed in last few years due to its adverse cardiac events and many have been withdrawn from the market.⁶ Corticosteroids have anti inflammatory effects, but lacks analgesic and antipyretic effects. Moreover the long term administration of corticosteroids has its own adverse effects and also delays wound healing process.^{7, 8} In recent years; active principles of different chemical structures have been isolated from plants possessing anti-inflammatory activity. Southern part of our country has a tradition of using herbal preparation from centuries especially in dakshina kannada (Mangalore) district. However no scientific data are available for many herbal drugs which are locally used. Hence evaluating these drugs, it would be worthwhile to have scientific approach of using them. Hence in this study, an indigenous plant called *Clerodendrum viscosum* mentioned in Charaka and Sushruta treaties as an analgesic, anti-inflammatory and antipyretic drug and also in folk medicine was taken to evaluate acute anti-inflammatory activity of EELCV by carrageenin induced paw oedema in Wistar Albino rats.⁹

Administration of EELCV at doses of 150 and 300mg/kg (Table 1 & Figure 1) showed significant ($P = .01$) and moderate anti-inflammatory activity ($P = .05$) respectively by carrageenin induced rat paw oedema in Wistar Albino rats. The percentage inhibition of the oedema at 3rd hour was 66.44 % and 63.75 % at the dose of 150 mg/kg and 300 mg/kg respectively (Table 1 & Graph 1). Preliminary phytochemical screening of EELCV shows the presence of phytosterols, triterpenoids, flavinoids, lactones, fats and fatty acid, glycoside, phenolic compound and tannins.¹⁰ Phenolic compounds possess biological properties such as antiapoptosis, antiaging, anticancer, anti-inflammatory, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.¹¹ Probably, presence of phenolic compounds could be the reason for its anti-inflammatory activity.

However further studies are required to evaluate its comprehensive analysis including quantitative / semi quantitative analysis, characterize its chemical structure and assess its pharmacotherapeutical activities with exact mechanism of action as an anti-inflammatory agent.

CONCLUSION

Administration of EELCV orally at the doses of 150 and 300mg/kg showed significant ($P = .01$) and moderate anti-inflammatory activity ($P = .05$) respectively by carrageenin induced rat paw oedema in wistar albino rats. The percentage inhibition of the oedema at 3rd hour was 53.19 % and 38.29% at the dose of 150 mg/kg and 300 mg/kg respectively.

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