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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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Authors' contributions

This work was carried out in collaboration between all authors. Author RC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SNR managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aim of the study was to investigate chronic anti-inflammatory activity 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, Karnataka, 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 for anti-inflammatory activity.
Results: Oral administration of EELCV at doses of 150 mg/kg (P = .01) and 300mg/kg (P = .05) has shown significant anti-inflammatory activity by carrageenin induced paw oedema in Wistar albino rats compared to control. A significant inhibition of oedema

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formation was also observed at 4th hour.
Conclusion: Administration of EELCV orally at the doses of 150 mg/kg (*P* = .01) and 300mg/kg (*P* = .05) showed significant anti-inflammatory activity by carrageenin induced paw oedema in Wistar Albino rats. The percentage inhibition of the oedema at 3rd hour was 63.75 % for the dose of 150 mg/kg and 46.30 % for the dose of 300 mg/kg. A significant inhibition was also observed at 4th hour.

Keywords: Anti-inflammatory activity; clerodendrum viscosum; carrageenin; indomethacin.

1. 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 being 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 % of the roughly 300,000 species of higher plants have a history of use in traditional medicine [2].

2. MATERIALS 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 (Ref No Ph. D 1 / 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.

2.1 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, Karnataka, India.

2.2 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 (40-50ºC) 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.

2.3 Sample Size, Grouping and Dose of the Drugs

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

2.4 Drugs and Chemicals

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

2.5 Anti-Inflammatory Activity by Carrageenin-Induced Rat Paw Edema

In this method, rats were divided into five groups of 10 animals each (Equal number of Male and Female). All the animals were pretreated with drugs / vehicle, for 10 days. Carrageenin (0.1 ml of 1%) was injected into the sub plantar tissue of left hind paw of each rat on 10^{th} day. Swellings of carrageenin-injected foot were measured at zero, first, second, third, fourth and sixth hour using Digital Plethysmometer. The right hind paw was injected with 0.1 ml of vehicle. The animals which received indomethacin (10 mg/kg, p.o.) served as reference standard and the control group received 1% gum acacia 3 ml/kg. Three doses of the test drug were selected (75, 150 and 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 carrageenin induced paw oedema was observed at the 4^{th} hr after the injection. The increase in paw volume at 3^{rd} hr was calculated as percentage compared with the volume measured immediately after injection of carrageenin for each animal and was compared between groups [3].

2.6 Statistical Analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P* =.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Oral administration of EELCV at doses of 150 mg/kg (P = .01) and 300mg/kg (P = .05) showed significant anti-inflammatory activity by carrageenin induced paw oedema in Wistar albino rats. The percentage inhibition of the oedema at 3^{rd} hour was 63.75 % for the dose of 150 mg/kg and 46.30 % for the dose of 300 mg/kg compared to control and reference group (Table 1 and Graph 1).

Pain, inflammation and pyrexia are the most usual disturbing symptom in day to day life. Plenty of drugs are available in the market for relieving these symptoms and which are also sold over the counter. However, they have high tendency of causing adverse drug reaction from a trivial nausea and vomiting to gastric irritation leading to peptic ulcer, perforation and may also even lead to death [4]. The main undesirable side-effects of aspirin (Irreversible COX inhibitors) taken by mouth are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. In children and adolescents, aspirin is no longer indicated to control flu-like symptoms or the symptoms of chickenpox or other viral illnesses, because 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 India has a tradition of using herbal preparation from centuries especially in dakshina kannada (Mangalore) district, Karnataka, India. 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 mg/kg (P = .01) and 300mg/kg (P = .05) showed significant anti-inflammatory activity by carrageenin induced rat paw oedema in Wistar albino rats (Table 1 and Graph 1). The percentage inhibition of the oedema at 3rd hour was 63.75 % at the dose of 150 mg/kg and 46.30 % at the dose of 300 mg/kg respectively. A significant inhibition was also observed at 4th hour. (Table 1 and 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, improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities [11]. Probably, presence of phenolic compounds could be the reason for its anti-inflammatory activity.

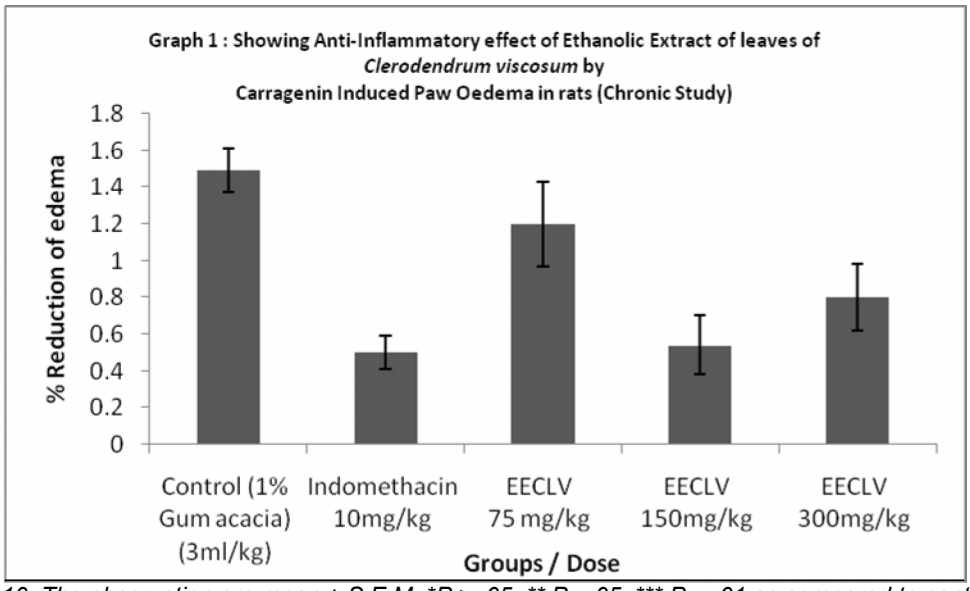
Table 1. Showing anti-inflammatory effect of ethanolic extract of leaves of *Clerodendrum viscosum* by carrageenin induced paw oedema in rats (chronic study)

Drugs	0 hr	1st hr	2nd hr	3rd hr	4th hr	6th hr	% Inhibition at 3rd hr
Control (1% Gum acacia) (3ml/kg), p.o	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, p.o	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, p.o	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, p.o	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, p.o	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

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

EELCV- Ethanolic Extract of the leaves of Clerodendrum viscosum.

p. o – Per orally.



*n=10. The observation are mean ± S.E.M. *P > .05, ** P =.05, *** P = .01 as compared to control (ANOVA followed by Dunnett's multiple comparison test).*
EELCV- Ethanolic Extract of the leaves of Clerodendrum viscosum

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.

4. CONCLUSION

Administration of EELCV orally at the doses of 150 mg/kg (P = .01) and 300mg/kg (P = .05) showed significant anti-inflammatory activity by carrageenin induced rat paw oedema in wistar albino rats. The percentage inhibition of the oedema at 3rd hour was 63.75 % at the dose of 150 mg/kg and 46.30 % at the dose of 300 mg/kg respectively. A significant inhibition was also observed at 4th hour.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. 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 (Ref No Ph. D 1 / 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.

and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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