



Research Article

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

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ABSTRACT

Inflammation is an important feature of many diseases. It is the response of a tissue to an injury, infection, irritation of foreign substance. In fact, it is a part of host defense, but when it is severe, it may be far worse than the diseases itself and in extreme condition, it may be too fatal also. There is an increasing demand for the medicinal plants in developing countries like India. Attention has to be given to assess the medicinal value of such plants to explore the potential drugs out of it. The aim of the study was to investigate acute anti-inflammatory activity of ethanolic extract of the leaves of *Clerodendrum viscosum* (EELCV) by carrageenin induced paw oedema in Wistar Albino rats. 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 were selected (75, 150 and 300 mg/kg p.o). Oral administration of EELCV at doses of 150 and 300mg/kg showed significant ($p < 0.01$) and moderately significant acute anti-inflammatory activity ($p < 0.05$) respectively by carrageenin induced paw oedema in Wistar Albino rats compared to control.

Keywords: Anti-inflammatory activity, *Clerodendrum viscosum*, Carrageenin, Indomethacin.

INTRODUCTION

Pain and inflammation are the most common symptoms of many diseases afflicting millions of people worldwide.^{1,2} Even though there are effective medicines used to reduce these manifestations,³ traditional medicine practitioners, mainly in developing countries have been using herbal medicines to treat various ailments including pain and inflammation.⁴ Nature has provided a complete store-house of natural remedies to cure many ailments.⁵ This is where, nature⁶ provides us a good source in the form of herbs, plants, algae's etc to cure many diseases without much toxic effects. Research on medicinal plants is an important field of biochemical research in India.⁷ Inflammation is a manifestation involved in localized increase in the number of leukocytes and a variety of complex inflammatory mediators. It also involves synthesis and release of prostaglandins at injured site of inflammation. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research on plants with age old folkloric used as pain relievers, anti-inflammatory agents, and therefore be considered as a fruitful and logical research strategy in the search for novel analgesic and anti-inflammatory drugs.⁸

Currently Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) and steroidal anti-inflammatory are used in the treatment of inflammation. Existing synthetic molecule likes NSAIDs and selective COX-2 inhibitors have potential to increase the incidence of adverse cardiovascular thrombotic effects.⁹ On the other hand steroids have immense potential in the treatment of inflammation, but due to their adverse effects it can be used only for short periods.¹⁰ So, in order to overcome

these drawbacks, there is need to focus on the scientific exploration of herbal drugs having fewer side effects.

The genus *Clerodendrum* (Family: Verbenaceae) is very widely distributed in tropical and subtropical regions of the world. More than five hundred species of this genus have been identified till now, which includes small trees, shrubs and herbs. Ethno-medical importance of various species of *Clerodendrum* genus has been reported in various indigenous systems of medicines and also as folk medicines. The genus is being used as medicines especially in countries like India, China, Thailand, Korea and Japan as an indigenous system of medicine for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension. Few species of the genus like *Clerodendrum inerme*, *C. thomasonae*, *C. indicum*, and *C. speciosum* are ornamental and being cultivated for aesthetic purposes. The powder/paste form and the various extracts of root, stem and leaves are reported to be used as medicine for the treatment of asthma, pyreticosis, cataract, malaria, and diseases of skin and lungs. In order to prove these ethno-pharmacological claims, some of these plant species are being extensively studied for their pharmacological activities using various animal models. Along with biological studies, its isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied.¹¹ Hence the rationale of the study was to carry out acute anti-inflammatory activity of ethanolic extract of the leaves of *Clerodendrum viscosum* (EELCV) by carrageenin induced paw oedema in wistar albino rats.

MATERIALS AND METHODS

Institutional Animal Ethical Committee approval was obtained on 6th May, Ref. No. Ph.D 1/2010 from Yenepoya University, Derlakatte, Mangalore, India, before conducting the experiments.

Plant Material

In the month of June – August 2010, the whole plant was collected from rural area of Manjanady located in Mangalore district. It was authenticated by (Prof) Dr. Krishna Kumar G, Chairman, Dept of Applied Botany, Mangalore University, Mangalore, Karnataka, India. 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.

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 viscous 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 and 3 groups of Test drug) containing 10 animals, making a total number of 50 animals.

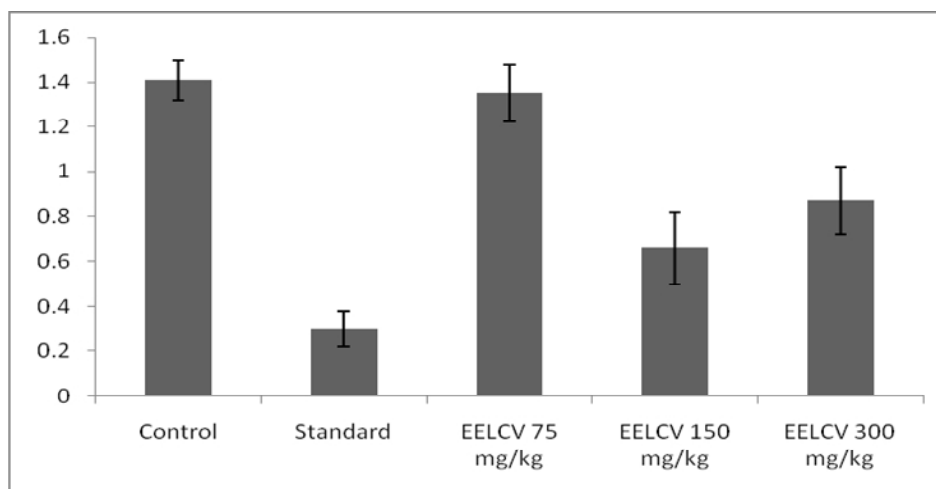
Drugs and Chemicals

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

Table 1: Acute Anti-Inflammatory effect of Ethanolic Extract of leaves of *Clerodendrum viscosum* (EECLV) by Carragenin Induced Paw Oedema in Wistar Albino rats

Drugs	0hr	1 st hr	2 nd hr	3 rd hr	4 th hr	6 th hr	% Inhibition at 3 hr
Control (1% Gum acacia) (3ml/kg) p.o	0.49±0.09	1.61±0.06	1.30±0.13	1.41±0.09	1.74±0.07	1.31±0.10	-
Indomethacin (10mg/kg) p.o	0.46±0.07	0.48±0.05***	0.72±0.03***	0.30±0.08***	0.40±0.10***	0.55±0.07***	78.72
EECLV (75 mg/kg) p.o	0.47±0.04	1.44±0.29*	1.29±0.07*	1.35±0.13*	1.49±0.09*	1.59±0.09*	4.2
EECLV (150mg/kg) p.o	0.31±0.06	0.48±0.07***	0.79±0.02***	0.66±0.16***	0.50±0.08***	0.59±0.08***	53.19
EECLV (300mg/kg) p.o	0.40±0.08	0.50±0.08***	0.82±0.02***	0.87±0.15**	1.28±0.14**	0.93±0.14**	38.29

n=10. The observation are mean ± S.E.M. *p> 0.05, **<0.05, *** p< 0.01 as compared to control (ANOVA followed by Dunnett's multiple comparison test), EECLV- Ethanolic Extract of Leaves of *Clerodendrum viscosum*, p.o – Per Orally



Graph 1: Anti-Inflammatory effect of Ethanolic Extract of leaves of *Clerodendrum viscosum* (EECLV) by Carragenin Induced Paw Oedema in Wistar Albino rats at 3rd hr

The observations are mean ± S.E.M. as compared to control (ANOVA followed by Dunnett's multiple comparison test) EECLV- Ethanolic Extract of Leaves of *Clerodendrum viscosum*

Anti-inflammatory activity by Carrageenin induced rat paw oedema

In this method, rats were divided in to five groups of 10 animals each (Equal number of Male and Female). All the animals were pretreated with drugs / vehicle, 60 minutes before injection of carrageenin (0.1 ml of 1%). Carrageenin was injected into the sub plantar tissue of left hind paw of each rat. Swellings of carrageenin injected foot were measured at zero, first, second, third, fourth and sixth hour using Digital Plethysmometer.^{12, 13} The right hind paw was injected with 0.1 ml of Normal Saline (vehicle). The animals which received indomethacin (10 mg/kg, p.o.) served as reference standard. Control group received 1% gum acacia 3ml/kg p.o. 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 evaluating acute anti-inflammatory activity. The peak effect of the carrageenin induced paw oedema was observed at the 3rd hour after the injection. The increase in paw volume at 3rd hour was calculated as percentage compared with the volume measured immediately after injection of carrageenin for each animal and collected data were compared between groups.

Statistical Analysis

Results are expressed as mean \pm SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison test. $p < 0.05$ was considered statistically significant.

RESULTS

Oral administration of EELCV at doses of 150 and 300mg/kg showed significant ($p < 0.01$) and moderately significant anti-inflammatory activity ($p < 0.05$) respectively by carrageenin induced paw oedema in Wistar Albino rats (Table 1 and Figure 1). 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 compared to control and reference group (Table 1 and Figure 1).

DISCUSSION

Pain, inflammation and pyrexia are the most usual disturbing symptoms 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 (OTC). 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.¹⁴ The main undesirable side-effects of aspirin (Irreversible COX inhibitors) taken by oral route 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.¹⁵ 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.¹⁶ Corticosteroids even though 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.^{17, 18} 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 by 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 treatises 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.¹⁹ Preliminary phytochemical screening of EELCV showed the presence of phytosterols, tri-terpenoids, flavonoids, lactones, fats and fatty acid, glycoside, phenolic compound and tannins.²⁰ Phenolic compounds possess biological properties such as anti-apoptosis, anti-aging, anti-cancer, anti-inflammatory, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.²¹ 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.

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