

Received: 08th Nov-2012Revised: 14th Nov-2012Accepted: 14th Nov-2012

Research article

**CHRONIC CENTRAL AND PERIPHERAL ANALGESIC ACTIVITY OF ETHANOLIC
EXTRACT OF THE LEAVES OF *CLERODENDRUM VISCOSUM* IN RODENT MODELS**Chandrashekar. R*¹ and S.N. Rao²*¹ & ²Dept of Pharmacology, Yenepoya Medical College, Yenepoya University, Derlakatte, Mangalore
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ABSTRACT : The aim of the study was to investigate the chronic peripheral analgesic activity of Ethanolic Extract of the leaves of *Clerodendrum viscosum* (EECV) by acetic acid induced writhing reflex test in mice and chronic central analgesic activity of EECV by tail immersion method in rats. Dried powdered leaves of *Clerodendrum viscosum* were subjected to solvent 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 and were subjected to chronic analgesic activity. EECV showed significant chronic peripheral analgesic activity ($p < 0.01$) in mice in the dose of 200 mg / kg and moderate analgesic activity at the dose of 400 mg/kg ($p < 0.05$) as compared to control and the standard drug Indomethacin. But failed to show any chronic central analgesic activity by tail immersion method at any of the three doses selected compared to control and standard drug Pentazocin in rats.

Keyword: Analgesic, Ethanolic extract, *Clerodendrum viscosum*, Writhing reflex test, Tail immersion test

INTRODUCTION

Pain, inflammation and fever are very common symptoms in human beings. Several plants and their products are claimed and proved to possess analgesic and antipyretic property (B.K. Nanda et al., 2009). The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments (Balunas MJ et al., 2005). Plants are indispensable sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs (Hedge et al., 1990). The genus *Clerodendrum* L. (Family: Lamiaceae) is very widely distributed in tropical and subtropical regions of the world. More than five hundred species of the genus are 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 as folk medicines. The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various lifethreatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension. Few species of the genus like *Clerodendrum inerme*, *C. thomsonae*, *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 blood, skin and lung. To prove these ethno-medical claims, some of these species are being extensively studied for their biological activities using various animal models. Along with biological studies, isolation and identification studies of chemical constituents and its correlation with the biological activities of the genus has also been studied. The major chemical components reported from the genus are phenolics, steroids, di- and triterpenes, flavonoids, volatile oils, etc (Neeta Shrivastva et al., 2007). Preliminary phytochemical screening has shown that leaves of *Clerodendrum viscosum* possess phytosterols, triterpenoids, flavinoids, alkaloids, saponins, carbohydrate, phenolic compound and tannins (Chandrashekar et al 2012).

MATERIALS AND METHODS

Institutional Animal ethical committee approval was obtained from Yenepoya University before conducting the experiments.

Animals

Equal number of Swiss albino mice weighing 25-30 g were divided into 5 groups having 10 animals in each group (5 males & 5 females) for acetic acid induced writhing reflex test in mice and equal number of male and female Swiss albino rats weighing 150 - 200 g were taken and divided into 5 groups having 10 animals in each group (5 males & 5 females) for tail immersion test in rats. Animals were acclimatized under standard laboratory condition and were kept in 12hr day and night cycle before the start of the experiment for seven days. Animals were handled carefully according to Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Plant material

The leaves of the plant were collected from rural region of manjanady, in Mangalore region in the month of Sept – Nov 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 Yenepoya University, Mangalore.

Extraction

Leaves of *Clerodendrum viscosum* were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered (1kg). The coarse powder was subjected to solvent extraction in Soxhlet extractor using 90 % ethanol. The ethanolic extract was concentrated by vacuum distillation to dryness; the yield obtained was 12.5% w/w with respect to dried leaf. The collected leaf extract was stored in a desiccator. A suspension of the extract prepared in 1% gum acacia was used in experimental studies.

Drugs and Chemicals

The drugs and chemicals were Indomethacin, Pentazocin (Yenepoya pharmacy, Mangalore), Gum acacia, Ethanol (Rajesh chemicals, Mumbai).

ACUTE TOXICITY STUDIES

As there was no references for the dose of *Clerodendrum viscosum*, EECV was studied for acute oral toxicity study as per revised OECD guidelines. EECV was devoid of any toxicity in mice when given in doses up to 2000 mg/kg by oral route. Its 1/5th (i.e. 400 mg/kg) and 1/10th (i.e. 200 mg/kg) and 1/20th (i.e. 100 mg/kg) of 2000 mg/kg were used to test for analgesic activity in mice and 75 mg/kg, 150 mg/kg & 300 mg/kg were used to test for analgesic activity in rats (Dose conversion from mice to rat) (OECD/OCDE guidelines., 2001).

Acetic acid induced writhing reflex of EECV in mice

In this method, mice in groups of 10 each were treated with vehicle (1% gum acacia, orally) (10ml/kg, orally), EECV (75 mg/kg, 150 mg/kg & 300 mg/kg orally), and Indomethacin (10 mg/kg orally) was used as positive control. Analgesic activity of EECV was assessed by counting the number of writhes induced by 0.6% (10ml / kg Intraperitonally) of acetic acid. Numbers of writhes per animal were counted following 10min. For chronic study the animals received the drug every day for ten days and 1 hour before conducting the experiment on tenth day. A writhe was considered when animal showed contraction of abdomen with simultaneous stretching of at least one hind limb. Protection against writhing was taken as an index of analgesia (Table 1 & Figure 1).

It is calculated as

Average number of writhing in control group - Average number of writhing in treated group / Average number of writhing in control group × 100.

Table-1: Showing analgesic effects of Ethanolic Extract of the leaves of *Clerodendrum viscosum* (EECV) by acetic acid induced writhing reflex in mice (Chronic Study)

Treatment	Number of writhing \pm SEM	% Inhibition
Control (Gum acacia 1%) (3ml/kg)	28.9 \pm 1.12	
Indomethacin (10 mg/kg)	14.9 \pm 0.83***	48.44
EECV (100 mg/kg)	27.5 \pm 0.96*	4.84
EECV (200 mg/kg)	18 \pm 1.77***	37.71
EECV (400 mg/kg)	24.2 \pm 0.91**	16.26

n=10. The observation are mean \pm S.E.M. *p> 0.05, **p<0.05, *** p< 0.01 as compared to control (ANOVA followed by Dunnett's test)

EECV- Ethanolic Extract of the leaves of *Clerodendrum viscosum*

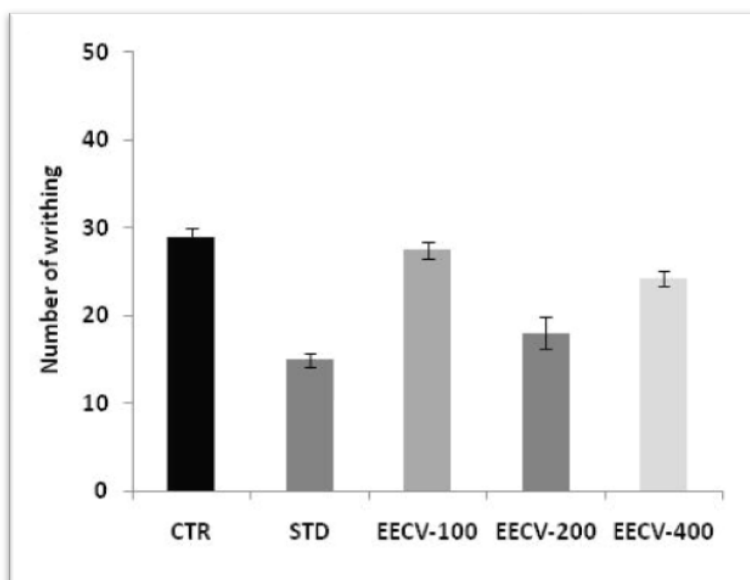


Figure 1: Analgesic effects of EECV by acetic acid induced writhing reflex test in mice (Chronic Study)
n=10. The observation are mean \pm S.E.M. *p> 0.05, **p<0.05, *** p< 0.01 as compared to control (ANOVA followed by Dunnett's test)

CTR- Control

STD- Standard

EECV- Ethanolic Extract of the leaves of *Clerodendrum viscosum*

Analgesic effects of EECV by tail immersion method in rats:

In this method, the rats in each group treated with vehicle (1% gum acacia, p.o) 10 ml/kg, EELI (75 mg/kg, 150 mg/kg, & 300 mg/kg orally), and pentazocin (30 mg/kg, Intraperitoneally) was used as positive control. For chronic study the animals received the drug every day for ten days and 1 hour before conducting the experiment on tenth day. They were placed in individual restraining cages leaving the tail hanging out freely. The distal 1-2cm of tail was immersed in water and maintained at 55 \pm 0.1°C.

The time taken by the rat to withdraw the tail from hot water was noted as reaction time. The reaction time noted before the administration of the drug was noted as zero minute. The cut off time to withdraw the tail was kept as 10 seconds. The mean reaction time was recorded for each group and compared between the groups (Table 2).

Table 2: Analgesic effects of EECV by tail immersion test in rats (Chronic Study)

Drugs	Time (0min)	Time (30min)	Time (60min)	Time (120min)	Time (180min)	Time (240min)	Time (360min)
Control (1% Gum acacia) (3ml/kg)	1.43±0.09	1.36±0.08	1.25±0.07	1.20±0.06	1.18±0.03	1.18±0.02	1.15±0.02
Standard (Pentazocine) (30mg/kg)	6.32±0.16***	5.70±0.04***	5.54±0.11***	5.53±0.02***	5.37±0.03***	5.23±0.03***	4.86±0.10***
EECV 75 mg/kg	1.43±0.06*	1.16±0.04*	1.46±0.08*	1.18±0.04*	1.16±0.03*	1.41±0.08*	1.29±0.08*
EECV 150mg/kg	1.32±0.09*	1.43±0.09*	1.48±0.09*	1.28±0.04*	1.33±0.08*	1.32±0.07*	1.27±0.08*
EECV 300mg/kg	1.54±0.10*	1.56±0.20*	1.40±0.06*	1.30±0.08*	1.28±0.05*	1.35±0.10*	1.39±0.05*

n=10. The observation are mean ± S.E.M. *p>0.05, *** p< 0.001 as compared to control
EECV- Ethanolic Extract of the leaves of *Clerodendrum viscosum*

STATISTICAL ANALYSIS

The observations are mean ± S.E.M. and analyzed by one way ANOVA followed by Dunnett's test. p < 0.05 was considered statistically significant.

DISCUSSION

Pain, inflammation and pyrexia are the most common disturbing symptom in day to day life. Numerous drugs are available in the market for relieving these symptoms and which are sold over the counter. However they have high tendency of having adverse drug reaction from a trivial nausea and vomiting to gastric irritation leading to peptic ulcer, perforation and even death.

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 (Macdonald's et al., 2002). 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 (Kearney PM et al., 2006). 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 (Mehlish DR., 2002). Opioids even though analgesic but lacks anti-inflammatory and antipyretic effects and not used for simple pain due to its abuse and dependence liability (Janssen P et al., 1963). 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 it would be worthwhile to evaluate these drugs and have a scientific approach of using them. Hence in this study, we have taken 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 (*The Ayurvedic pharmacopoeia of India.*, 2004).

The present study has shown that the EECV at dose 200mg/kg exhibited significant chronic peripheral analgesic activity ($p < 0.01$) and at the dose of 400mg exhibited moderate peripheral analgesic activity ($p < 0.05$) compared to control and standard drug Indomethacin in mice by acetic acid induced writhing reflex. But, it did not show any significant chronic central analgesic activity by tail immersion method when compared to control and standard drug Pentazocin at any of the three doses selected in rats.

However further studies are needed to evaluate its exact mechanism of action for its peripheral analgesic activity.

ACKNOWLEDGEMENT

The author is grateful to Dr.S.N.Rao Rao, Senior Professor & Head, Dept of Pharmacology, and Yenepoya University for providing the support to conduct this experimental research.

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