Ethanol extract of Clerodendrum viscosum vent Roots: Investigation of analgesic and anti inflammatory effects in male adult Swiss albino mice.

Prasanth K.G1, Anandbabu A1, Johns Tom1, Dineshkumar B2, Krishnakumar K3 Geetha G4 Venkatanarayanan R5*

1Dept. of Pharmacology, PSG College of Pharmacy, Coimbatore – 641 004
2Dept. of Pharmaceutics, St James college of Pharmaceutical Sciences, Chalakudy
3Dept. of Pharmaceutical analysis, St James college of Pharmaceutical Sciences, Chalakudy
4Dept. of Pharmaceutical Analysis, PSG College of Pharmacy, Coimbatore – 641 004
5Dept. of Pharmacology, RVS College of Pharmacy, Coimbatore – 641 402

Email: venkatnarayananr@gmail.com

Received 17 September 2012; Accepted 05 October 2012

Abstract

Background: Clerodendrum viscosum (verbenaceae) leaves and roots has been used as an antiseptic, anti inflammatory and antipyretic in Indian medical traditional system. The present study was designed to investigate the analgesic and anti inflammatory activity of ethanol extract of Clerodendrum viscosum root (EECVR). Coarse powders of Clerodendrum viscosum root was subjected to successive maceration process with petroleum ether, ethyl acetate, chloroform and ethanol. These extracts were subjected to phytochemical analysis. Estimation of total alkaloid was performed for EECVR using UV-Vis spectroscopy. Analgesic activity of EECVR was determined by hot plate, tail immersion, acetic acid induced writhing and formalin test. Anti-inflammatory activity of EECVR was investigated by carrageenan induced paw edema in Swiss albino mice. Phytochemical test showed presence of alkaloids in EECVR. Total alkaloid content was found to be 1.4 %w/w. The EECVR (at a dose 200 and 400 mg/kg) showed potent analgesic activity (p<0.001) against acetic acid induced writhing and delayed phase of formalin test when compared to tail immersion and tail flick method. This extract also exhibited significant (p<0.001) anti-inflammatory effect in carrageenan induced paw edema. In this study, EECVR (at a dose 200 and 400 mg/kg) showing appropriate anti-inflammatory and analgesic effect which may act through the cyclooxygenase enzyme system. Moreover, presence of alkaloid in EECVR may be the responsible for the potential anti-inflammatory and analgesic activity.

© 2012 Universal Research Publications. All rights reserved

Key words: Analgesic; anti inflammatory; Clerodendrum viscosum roots.

Introduction

Inflammation and pain are formally defined as an unpleasant sensory and emotional incident coupled with tissue injury. They act as warning signs against disorders of the body function. Chronic inflammatory diseases remain as one of the major health problems in the world. Currently drugs such as steroids and NSAIDs are used in the treatment of such diseases.[1] Prolonged usage of NSAIDs lead to serious adverse reactions like gastrointestinal perforation, ulceration and hemorrhage. Steroids cannot be used for a long term therapy due to their toxicity. These results thus limit their therapeutic usefulness when long-term treatment is necessary.[2]

Alternative medicines mainly herbal medicines are available for the treatment of chronic inflammatory diseases. Herbal medicines have several advantages such as effectiveness, safety, affordability and acceptability.[3] Medicinal plants has been used in the Indian traditional system of medicine and have shown experimental or clinical anti-inflammatory activity.[4,5,6] Therefore, medicinal plants need to be investigated by scientific methods for their anti-inflammatory activity.

Clerodendrum viscosum (verbenaceae) leaves and roots has been used as an antiseptic, anti inflammatory and antipyretic in Indian medical traditional system. The present study was designed to investigate the analgesic and anti inflammatory activity of ethanol extract of Clerodendrum viscosum root (EECVR). Coarse powders of Clerodendrum viscosum root was subjected to successive maceration process with petroleum ether, ethyl acetate, chloroform and ethanol. These extracts were subjected to phytochemical analysis. Estimation of total alkaloid was performed for EECVR using UV-Vis spectroscopy. Analgesic activity of EECVR was determined by hot plate, tail immersion, acetic acid induced writhing and formalin test. Anti-inflammatory activity of EECVR was investigated by carrageenan induced paw edema in Swiss albino mice. Phytochemical test showed presence of alkaloids in EECVR. Total alkaloid content was found to be 1.4 %w/w. The EECVR (at a dose 200 and 400 mg/kg) showed potent analgesic activity (p<0.001) against acetic acid induced writhing and delayed phase of formalin test when compared to tail immersion and tail flick method. This extract also exhibited significant (p<0.001) anti-inflammatory effect in carrageenan induced paw edema. In this study, EECVR (at a dose 200 and 400 mg/kg) showing appropriate anti-inflammatory and analgesic effect which may act through the cyclooxygenase enzyme system. Moreover, presence of alkaloid in EECVR may be the responsible for the potential anti-inflammatory and analgesic activity.

© 2012 Universal Research Publications. All rights reserved

Key words: Analgesic; anti inflammatory; Clerodendrum viscosum roots.
report for analgesic and anti-inflammatory effect of *Clerodendrum viscosum* Vent roots. Therefore, the present study was designed to investigate analgesic and anti-inflammatory effect effects of chloroform extract of *Clerodendrum viscosum* Vent roots in animal model.

**Materials and methods**

**Plant materials**

*Clerodendrum viscosum* Vent roots were collected from Pallakad district Kerala in the month of September and October 2010. The roots were inspected to be healthy and botanically identified and authenticated by Dr. G.V.S. Moorthy, Plant Biotechnologist, botanical survey of India, Coimbatore. The herbarium *Clerodendrum viscosum* Vent roots were deposited in botanical survey of India (BSI) against voucher no. BSI/SRC/5/23/10-11/Tech1152. The *Clerodendrum viscosum* Vent roots after collection were dried at room temperature (27-30°C) for 40-50 days. After complete drying, the dried root materials were grounded into coarse powder using domestic electric grinder and used for extraction.

**Preparation of plant extract**

Coarse powders of *Clerodendrum viscosum* root (30g) was subjected to successive maceration process with 300 ml of Pet. ether, chloroform, ethyl acetate, ethanol and aqueous) in a shaker system at room temperature. Then each extracts were filtered using and Whatman No.1 filter paper. The filtrate was subjected to evaporation under reduced pressure to obtain dried extracts.

**Phytochemical studies**

Our earlier studies (Prasanth et al., 2012) reported that ethanol extracts of *Clerodendrum viscosum* root showing presences of alkaloid.[8]  

**Total alkaloid estimation using Spectrophotometric method**

Based on our earlier studies (Prasanth et al., 2012), the ethanol extract of *Clerodendrum viscosum* root (EECVR) was subjected to total alkaloid estimation using spectrophotometric method. The absorbance was measured at 435 nm.[9]  

**Acetic acid - induced writhing**

Acetic acid induced writhing was performed and assessed by the abdominal contraction. The animals were divided into four groups. Each groups having six mice. Group 1 – Control (animals received with vehicle (0.3% w/v sodium carboxy methyl cellulose, p.o.,), Group 2 – Animals treated with 200 mg/kg of EECVR (p.o.,), Group 3 - Animals treated with 400 mg/kg of EECVR (p.o.,), Group 4 - Animals treated with 10mg/kg of pentoazocine (i.p,) as standard drug. After that animals were analyzed for noxeseptive action. Then the animals were placed individually in the hot plate and the jumping response was recorded.

**Formalin test**

Twenty micro liters of 1% formalin was administrated subcutaneously to the dorsal surface of the right paw to the pretreated animals. The animals were divided into four groups. Each groups having six mice. Group 1 – Control (animals received with vehicle (0.3% w/v sodium carboxy methyl cellulose (p.o.,), Group 2 – Animals treated with 200 mg/kg of EECVR (p.o.,), Group 3 - Animals treated with 400 mg/kg of EECVR (p.o.,), Group 4 - Animals treated with 10mg/kg of indomethacin (p.o.,) as standard drug. Writhing was induced to all groups after 30 minutes of treatment by administering 1% of acetic acid (at a dose 10ml/kg, (i.p).). The number of abdominal writhes (full extension of both hind paws) was cumulatively recorded every 5min over a period of 20 min.[13]
for 5 min after formalin injection (first phase) and 15-30 min after formalin injection (second phase).\textsuperscript{[14]}

**Carrageenan induced paw edema**

The animals were divided into four groups. Each group having six mice. Group 1 – Control (animals received with vehicle (0.3% w/v sodium carboxy methyl cellulose (p.o.)), Group 2 – Animals treated with 200 mg/kg of EECVR (p.o.), Group 3 - Animals treated with 400 mg/kg of EECVR (p.o.), Group 4 - Animals treated with indomethacin at a dose of 10mg/kg (p.o.) as standard drug. Initially the normal paw volume of each animal was measured and after 30 minutes of the treatment, 0.05ml (1%) Carrageenan was administered into the right hind paw of each mouse. The thickness of the injected paw was measured before and at 15 and 30 min intervals and 1,2,3,4 and 6hr after Carrageenan treatment by using Plethysmometer. A significant reduction in the paw volume compared to the vehicle treated control animals was considered as anti-inflammatory response. The percentage of inhibition was also calculated.\textsuperscript{[15]}

\[
\% \text{ Inhibition} = \frac{\text{VT} - \text{VO}}{\text{VT}} \times 100
\]

\(\text{VT} = \text{paw volume of the rat before administration of carrageenan}\\
\text{VO} = \text{paw volume of the rat after administration of carrageenan at different time intervals}
\]

**Statistical analysis**

Results are expressed as mean ± SEM. Statistical analysis of the data was done using one way ANOVA, followed by Tukey’s multiple comparison test and the results were considered significant when \(P<0.05\).

**Results**

**Phytochemical study and total alkaloid content**

Ethanol extract of *Clerodendrum viscosum* root (EECVR) showed presence of alkaloid and total alkaloid content was found to be 1.4 %w/w using spectrophotometric method.

**Analgesic activity by hot plate method**

The EECVR (at a dose 200 mg/kg) showed no analgesic effect on tail flick induced analgesic effect (Fig. 1). But the EECVR (at a dose 400 mg/kg) exhibited significant (\(p>0.05\)) analgesic effect when compared with control group. The animal showed significant (\(p=0.001\)) analgesic effect with 10mg/kg of Pentazocine as standard drug.

**Figure 1:** Effect of aqueous root extract of *Clerodendrum viscosum* vent on hot plate induced analgesia in mice. All the values are expressed as mean ±SEM (n=6) and analyzed using one way Anova. ***\(p>0.001\) when compare with the control.

**Analgesic activity by tail flick method**

In the tail flick model, there was no analgesic effect observed in the 200mg/kg of the EECVR after 1hr of the treatment. But the 400mg/kg of the EECVR extract showed significant analgesic effect (\(p>0.05\)) when compared with control group. The reaction time increases in the standard drug treated group after 1 hr of the drug administration (\(p>0.001\)) when compared to the untreated group (Fig. 2).

**Acetic acid induced writhing**

Fig. 3 showed the analgesic effect of EECVR on acetic acid induced writhing. The EECVR at a dose of 200mg/kg and 400mg/kg exhibited significant (\(p>0.001\)) reduction of writhing in a dose dependant manner, when compared with the control group. The standard drug Indomethacin (10mg/kg) also showed significant effect when compared with control group. The higher dose of EECVR (400mg/kg) showed almost equal effect with the standard drug Indomethacin.

**Formalin test**

There was a significant, dose dependent reduction in the delayed phase of formalin induced pain response in mice (Fig. 4) (\(p>0.001\)). No effect was observed in the first phase for both the doses .The standard drug aspirin 100mg/kg also showed the effect in the delayed phase (\(p>0.001\)). The higher dose of EECVR 400mg/kg showed almost equal effect when compared with the standard drug.
The results showed that extract inhibited dose dependent inhibition in the hind paw edema of rats. The EECVR showed anti-inflammatory activity related to prostaglandin release. Acetic acid induced writhing is associated with the release of endogenous substances like prostaglandin, histamine, bradykinin etc. The alkaloid present in EECVR may be a potent inhibitor of prostaglandin in both doses (200mg/kg & 400mg/kg) which may be the reason for the analgesic activity of the extract. Alkaloids are commonly found with analgesic and anti-inflammatory property. The result obtained from the tail immersion and hot plate methods indicate that the extract has activity only through the periphery not through central. Hot plate method and tail immersion method are the most common tests of nociception which is based on a phasic stimulus of high intensity. Pain induced by thermal stimulus of the hot plate and tail immersion is specific for central mediated nociception through opioid receptors. This effect is also confirmed through formalin induced analgesic activity. The extract of EECVR showed significant effect in the delayed phase. Formalin test helps to understand the mechanism of pain and analgesia. In the formalin induced pain model, pain produced is in two phases. The first phase starts from 0-5 minutes which is mediated through central nociception which is based on a phasic stimulus of high intensity. Pain induced by thermal stimulus of the hot plate and tail immersion is specific for central mediated nociception through opioid receptors which reflects the rise in level of substance. Second phase starts from 15-45 minutes which is mediated through the release of inflammatory mediators like prostaglandin at the peripheral site causing pain. 

### Discussion

Carrageenan has been widely used as an inflammatory agent capable to induce experimental inflammation to evaluate the anti-inflammatory property of the drugs. This experimental model showed a high degree of reproducibility. Inflammation produced by carrageenan is in two different phases. Initial phase is attributed by the release of histamine, bradykinin and serotonin which causes increase in vascular permeability. The later phase is related to prostaglandin release. The EECVR showed dose dependent inhibition in the hind paw edema of rats. The results showed that extract inhibited the second phase of carrageenan induced paw edema, which mainly involves arachidonic acid metabolites. EECVR showed anti-inflammatory activity similar to the positive control drug indomethacin, a NSAID which is known to nonselectively inhibit the action of the cyclooxygenase enzyme. This confirms the anti-inflammatory property of the EECVR through inhibition of cyclooxygenase enzyme system. However, further work need to be carried out to evaluate the effect of EECVR via the enzymatic activity of cyclooxygenase enzyme system.

Phytochemical evaluation showed that Clerodendrum viscosum roots having alkaloid content. The spectrophotometric alkaloidal estimation indicated that EECVR contains good quantity of alkaloids. EECVR (at a dose of 200mg/kg) showed appropriate anti-inflammatory and analgesic effects in animal model, which may act through the cyclooxygenase enzyme system. Moreover, presence of alkaloid in EECVR may be the responsible for the potential anti-inflammatory and analgesic activity. Therefore, Clerodendrum viscosum roots could be used to treat inflammatory conditions. In our laboratory, isolation and characterization of alkaloid in EECVR are in progress to understand the anti-inflammatory property of the drugs. This experimental model showed a high degree of reproducibility. Inflammation produced by carrageenan is in two different phases. Initial phase is attributed by the release of histamine, bradykinin and serotonin which causes increase in vascular permeability. The later phase is related to prostaglandin release. The EECVR showed dose dependent inhibition in the hind paw edema of rats. The results showed that extract inhibited the second phase of carrageenan induced paw edema, which mainly involves arachidonic acid metabolites. EECVR showed anti-inflammatory activity similar to the positive control drug indomethacin, a NSAID which is known to nonselectively inhibit the action of the cyclooxygenase enzyme. This confirms the anti-inflammatory property of the EECVR through inhibition of cyclooxygenase enzyme system. However, further work need to be carried out to evaluate the effect of EECVR via the enzymatic activity of cyclooxygenase enzyme system. Acetic acid induced writhing is associated with the release of endogenous substances like prostaglandin, histamine, bradykinin etc. The alkaloid present in EECVR may be a potent inhibitor of prostaglandin in both doses (200mg/kg & 400mg/kg) which may be the reason for the analgesic activity of the extract. Alkaloids are commonly found with analgesic and anti-inflammatory property. The result obtained from the tail immersion and hot plate methods indicate that the extract has activity only through the periphery not through central. Hot plate method and tail immersion method are the most common tests of nociception which is based on a phasic stimulus of high intensity. Pain induced by thermal stimulus of the hot plate and tail immersion is specific for central mediated nociception through opioid receptors. This effect is also confirmed through formalin induced analgesic activity. The extract of EECVR showed significant effect in the delayed phase. Formalin test helps to understand the mechanism of pain and analgesia. In the formalin induced pain model, pain produced is in two phases. The first phase starts from 0-5 minutes which is mediated through central nervous system by the activation of peripheral nociceptive receptors which reflects the rise in level of substance. Second phase starts from 15-45 minutes which is mediated through the release of inflammatory mediators like prostaglandin at the peripheral site causing pain.

### Conclusion

In the present study, phytochemical evaluation showed that EECVR having alkaloid content. The spectrophotometric alkaloidal estimation indicated that EECVR contains good quantity of alkaloids. EECVR (at a dose of 200 and 400 mg/kg) showed appropriate anti-inflammatory and analgesic effects in animal model, which may act through the cyclooxygenase enzyme system. Moreover, presence of alkaloid in EECVR may be the responsible for the potential anti-inflammatory and analgesic activity. Therefore, Clerodendrum viscosum roots could be used to treat inflammatory conditions. In our laboratory, isolation and characterization of alkaloid in EECVR are in progress to evaluate their analgesic and anti-inflammatory activity.


Source of support: Nil; Conflict of interest: None declared