Exploration of Analgesic, Anti-Inflammatory, Neuropharmacological and Antidiabetic Activities of Clerodendrum viscosum Root Extract

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Abstract

In the traditional systems of medicine, Clerodendrum viscosum (CV) has important medicinal uses for the treatment of asthma, ulcer, inflammation, pyrexia, diabetes etc. In this study, analgesic, anti-inflammatory, neuro-pharmacological and antidiabetic effect of root extract of C. viscosum (CV) was investigated. Mice (Swiss albino) of either sex of 25-30 g were distributed into 4 groups as control (normal water), standard (STD), CV 250 mg/kg and CV 500 mg/kg (n=6). Analgesic effect was investigated by acetic acid initiated writhing test, formalin initiated paw licking test and hot plate test. Anti-inflammatory effect was investigated by xylene and croton oil initiated ear edema test and cotton pellet pleurisy test. Pentobarbital mediated sleeping time and hole cross tests were applied to assess neuro-pharmacological activity. Antidiabetic effect was evaluated by in-vitro alpha amylase inhibitory assay. In the acetic acid initiated writhing test, formalin test and hot plate test, the alcoholic extract of root showed significant antinociceptive activity (p<0.01) at 500 mg/kg dose. The test extract lowered inflammation in xylene, croton oil and cotton pellet test which was significant (p<0.001). The 500 mg/kg dose significantly (p<0.001) increased onset time of sleeping and significantly reduced sleeping duration (p<0.01) in sleeping time test. It also showed insignificant effect in hole-cross test. The extract exhibited a good alpha amylase inhibitory potential (IC50=1.24 mg/ml). C. viscosum root exhibited analgesic, anti-inflammatory, antidiabetic as well as CNS stimulant effect in model mice used for experimental purpose. More investigations are needed for the evaluation of such activities as well as the potential of this plant.

Key words: Clerodendrum viscosum, analgesic, anti-inflammatory, neuro-pharmacological, antidiabetic.

Introduction

Clerodendrum viscosum is a member of Lamiaceae family. In Bangladesh, it is commonly called Bhat. It is a perennial shrub or under-shrub having woody character with a height of 2-4 feet (Kirtikar and Basu, 2001; Das et al., 2010; Nayeem and Mehta, 2015). This plant is available as a weed which mostly found in the roadside and unused land. It has a great availability in Asia (tropical regions) including Bangladesh, India, Pakistan, Myanmar, Thailand and Sri Lanka. C. viscosum is rich of saponins, flavonoids, alkaloids and glycosides (Das et al., 2010; Shewale et al., 2012; Nadkarni and Nadkarni, 1976). From its flower, clerodin and hentriacontane were isolated. It is a natural health remedy used popularly as antiseptic and expectorant in Bangladeshi traditional practices. In the ethnomedicine, it also has uses in the alleviation of...
tumors, leprosy, scorpion sting and skin diseases (Shewale et al., 2012; Nadkarni and Nadkarni, 1976). The plant also has antipyretic, tonic and anthelmintic properties. The leaf and root of *C. viscosum* have wide use in the treatment of diabetes, asthma, tumors, convolution, dandruff, pyrexia, ascaricide, gravel, malaria, scorpion sting, snakebite, scabies, sore, spasm and tumor (Nandi and Lyndem, 2015). Previous workers reported analgesic, anti-inflammatory, hypoglycemic and cytotoxic effect of *C. viscosum* leaves extract (Tanny et al., 2021). Another study mentioned that ethanolic root extract of *C. viscosum* has antioxidant and analgesic effects (Sumi et al., 2015).

Bangladesh has a very broad biodiversity of medicinal plants with a long tradition of use having great phytotherapeutic properties (Shukla, 2009). These plants’ uses are based on historical and contemporary knowledge, clinical evidence and many have no supporting evidence at all. Their integration into conventional medicine is largely empirical and solely based on the patients’ positive experiences (Khanom et al., 2000). The findings of the above-mentioned studies on *C. viscosum* leaves extract formed the basis of our hypothesis that the root extract of the plant could have bioactivity beneficial for human health. Thus, we have been prompted to carry out *in vivo* evaluation of the root extract of the plant in animal models. Hence, the present study was aimed to assess the analgesic, anti-inflammatory, neuro-pharmacological and anti-hyperglycemic activities of ethanolic extract of *C. viscosum* root in different experimental models to justify the traditional and folkloric attributes.

**Methods and Materials**

*Plant extract preparation:* *C. viscosum* roots were collected from Dhamrai area of Dhaka district. It was then taxonomically identified from the Botany Department, Jahangirnagar University, Savar, Dhaka, Bangladesh. The collected roots were then washed in water, shed dried and pulverized to make extractable powder which was subsequently extracted using soxhlet extractor with ethanol. Extract was dried and a viscous semi solid extract was found which was preserved for tests.

*Experimental animals:* In the present study, (Swiss albino) both male and female mice were used. Age was about 6-7 weeks with 25-30 g of weight. Animals were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka and they were kept at standard room conditions (temperature: 27.0±1.0°C, relative humidity: 55-65% and 12 h light/12 h dark cycle). They had easy access to food and water *ad libitum*. The university animal ethical authority approved all the experimental protocols.

*Acetic acid initiated writhing test:* Twenty-four mice were divided into four groups and each contained six mice. Mice were pretreated with the normal water (10 ml/kg) in group-1, group-2 with diclofenac-Na (100 mg/kg) and group-3 and 4 with *C. viscosum* (250 mg/kg and 500 mg/kg), respectively. After 45 min, 0.7% acetic acid was injected to each mouse. The number of responses from writhing was recorded during a subsequent 5 min after 15 min of the acetic acid injection (Koster et al., 1959). The percentage inhibition was calculated using the following formula:

$$\text{% Inhibition} = \frac{\text{Mean number of wriths by control} - \text{Mean number of wriths by treated group}}{\text{Mean number of wriths by control group}} \times 100$$

*Formalin initiated paw licking test:* Twenty-four mice were divided into four groups and each contained six mice. Mice were pretreated with the normal water (10 ml/kg) in group-1, group-2 with diclofenac-Na (100 mg/kg) and group-3 & 4 with *C. viscosum* (250 mg/kg and 500 mg/kg), respectively. The left hind paw was injected with 2.7% formalin one hour after the administration of drug. The time of paw licking was recorded. Animals were observed for two phases as acute phase (5 min post formalin) and delayed phase (5 min from 20th min post formalin) (Hunskaar and Hole, 1987).
**Hot plate test:** Twenty-four mice were divided into four groups and each contained six mice. Mice were pretreated with the normal water (10 mL/kg) in group-1, group-2 with tramadol (10 mg/kg) and group-3 & 4 with *C. viscosum* (250 mg/kg and 500 mg/kg), respectively. The temperature was set at 55 ± 2°C on the heated plate. A cylinder was placed on the hot plate and animals were taken in the cylinder. The time from placement to the feeling of discomfort (such as licking of the paws or jumping off the surface) was recorded as latency time. Mice showing more than 10s as baseline latency were exempted from the study. The cut-off time was set at 15s for hot plate. The latency time of feeling discomfort was estimated at 0, 30, 60, 120, and 180 min post administration of the test drug (Eddy and Leimback, 1953).

**Xylene initiated ear edema test:** Twenty-four mice were divided into four groups and each contained six mice. Mice were pretreated with the normal water (10 mL/kg) in group-1, group-2 with diclofenac-Na (100 mg/kg) and group-3 and 4 with *C. viscosum* (250 mg/kg and 500 mg/kg), respectively. After one hour, 20 μL of xylene was administered on the anterior and posterior surfaces of the right ear lobe. The left ear was used as control. After one hour of xylene administration, both ears were cut, made a circular section and taken weight. % of weight of ear was calculated as the inflammation (Dai et al., 1995).

**Croton oil initiated ear edema test:** Mice were distributed into 4 groups containing 6 animals in each group. Group-1 control group received normal water (10 mL/kg), group-2 (STD) was given diclofenac-Na (100 mg/kg). Groups 3 and 4 were provided with CV extract (250 mg/kg and 500 mg/kg, respectively). After a time of one hour, 15 μL croton oil was applied on the right ear lobe and on left ear lobe 15 μL acetone. The posterior surfaces of both ears were preferred. One hour later of application of croton oil, both ears were cut, made circular sections and weighed (Zitterl-Egleser et al., 1997).

**Cotton pellet mediated granuloma formation test:** Cotton pellets of 10 ± 1 mg were weighed, sterilized and subcutaneously impregnated one pellet on left and another on the right side of the abdomen of the mice, using mild chloroform anesthesia. Group specific drugs were administered to different groups of cotton pellet impregnated mice once-daily for 7 days. Group-1 (control group) received normal water (10 mL/kg) and group-2 (STD) received diclofenac-Na (100 mg/kg). Test extracts were administered to groups 3 and 4. At the 8th day mice were sacrificed. Cotton pellets were eliminated, dried at 60°C for 24 hours and weighed dry cotton (Swingle and Shideman, 1972). The reduction of the cotton weight indicates the attenuation of inflammation.

**Pentobarbital mediated sleeping time test:** Mice were distributed into 4 different groups (n=6). Group-1 (control group) received normal water (10 mL/kg), group-2 (STD) received diazepam (5 mg/kg). Groups 3 and 4 were provided with CV extract 250 mg/kg and 500 mg/kg respectively. After 30 min. pentobarbital (45 mg/Kg, IP) was applied for inducing sleep. If mice were found staying immobile and lost righting reflex after positioning back, they were considered asleep. The sleep latency time was recorded by calculating the time interval between pentobarbital injection and immediate onset of sleep. Then total sleeping time was also recorded (Rakhshandah and Hosseini, 2006).

**Hole cross test:** The method applied followed the test as described by Takagi et al. (1971). Mice were distributed into 4 different groups (n=6). Group-1 (control group) received normal water (10 mL/kg), group-2 (STD) received diazepam (5 mg/kg). Groups 3 and 4 were provided with CV extract (250 mg/kg and 500 mg/kg, respectively). A box with 30x20x14 cm, a hole of 3 cm in diameter that is at a height of 4.5 cm from the floor was prepared with a dividing wall. The number of free movement of the animals through the hole from one to another chamber was recorded for 2 minutes. The readings were taken at 0, 30, 60, 120, 180 and 240 min post administration of the test drug.

**Evaluation of in-vitro antidiabetic properties (α-amylase method):** Starch-iodine method as described by Chakrabarti, 2014 was used for α-amylase inhibitory assay. In the pre-labeled test tubes, 1 ml
sample (extract or standard) of different concentrations (2, 1, 0.5 mg/ml) was taken. To each of the test tube, 20 μL of α-amylase was added. The solution was incubated for 10 minutes at 37°C. Then 1% starch solution (200 μl) was added to the test tube. The mixture was re-incubated at 37°C for 1 hour. After that 1% iodine solution (200 μl) was added to each test tube following the addition of 10 ml distilled water. Finally, absorbance of the mixture was read at 565 nm. At the same procedure, test sample, substrate and α-amylase blank were estimated. Each of the experiment was repeated and % α-amylase inhibition was measured by \(\left[1-\frac{(SA-SBB-SMB)}{AAB}\right]\times 100\), where, SA= Absorbance of sample, SBB = Substrate blank, SMB = Sample blank, AAB = α- amylase blank.

**Statistical analysis:** For the inhibition assay, Microsoft Office Excel (2007) tool was used. Animal experiment data was analyzed by one-way ANOVA using SPSS 16.0. Dunnet’s post hoc test was followed and data presented indicated mean ± SEM. The experimental results of test groups were taken into comparison against control group. p<0.05 was considered statistically significant, p<0.01 highly significant and p<0.001 very highly significant respectively.

**Results and Discussion**

In the traditional system of treatment, several plants are used to provide recovery from pain and inflammation. This claimed clinical efficacy has to be justified in a scientific pathway. *C. viscosum* has been used for various remedies as different parts of this plant are good source of phytoconstituents. Therefore, we have selected *C. viscosum* for the present experimental study.

The analgesic effects were justified by three different animal models, which explained analgesic properties to two separate phases (thermal and chemical) of noxious stimuli (Victor *et al.*, 2004). The writhing model by acetic acid is known as the visceral pain model (Vyklicky, 1979), and the result (Fig. 1) showed that both the doses of *C. viscosum* root extract resulted significant decrease in the writhing number compared to the control. The extract at 500 mg/kg showed significant (p<0.01) analgesic power which indicates that the extract possesses slightly lesser antinociceptive effect as compared to the reference drug.

Formalin test is a more valid analgesic test model and it is better correlated to the clinical pain (Tjolsen *et al.*, 1992; Ghannadi *et al.*, 2005). The study result (Fig. 2) indicated that the plant extract at 500 mg/kg caused a significant decrease in licking time at first 5 min (p < 0.01) and also in second 5 min. (p < 0.05) by the mice injected with formalin. The capacity of CV extract to hindrance the late phase of the formalin test strongly reflects its association in peripheral analgesic mechanism.

Data of the hot plate model was presented in fig. 3 which showed the slight insignificant increase in latency time of the CV extract at 250 mg/kg. At 500 mg/kg, CV significantly (p<0.01) improved the pain tolerance after 60 min. of treatment which was dose dependent and the extract showed lesser activity than the reference drug (tramadol, 10 mg/kg).

![Figure 1](image1.png) **Figure 1.** Effect of CV 250 mg/kg and CV 500 mg/kg in The acetic acid initiated writhing test.

![Figure 2](image2.png) **Figure 2.** Effect of CV 250 mg/kg and CV 500 mg/kg in Formalin initiated paw licking test.
In anti-inflammatory activity evaluation test, *C. viscosum* ethanolic extract at 250 mg/kg suppressed ear swelling in mice induced by xylene which was significant (p < 0.05). The reduction of inflammation was very highly significant (p < 0.001) at 500 mg/kg when compared with control. Prominent anti-
inflammatory activity was exhibited by diclofenac-Na (100 mg/kg) which claimed that it might inhibit the substance P release or inhibit its action (Fig. 4).

The anti-inflammatory effect of *C. viscosum* against ear edema mediated by croton oil was presented in fig. 5. The CV extract at both the doses (250 mg/kg and 500 mg/kg) reduced the croton oil mediated swelling of ear which was very significant (p < 0.001). The result was comparable with the anti-inflammatory effect (p < 0.001) of diclofenac-Na (reference drug).

The CV extract increased the onset of pentobarbital mediated sleeping time which was significant (p < 0.001) at both 250 mg/kg and 500 mg/kg doses. The total sleeping time was reduced by the extract which was also significant at 250 mg/kg (p < 0.05) and 500 mg/kg dose (p < 0.001) in mice (Fig. 7) when compared with control.

In hole cross test, the locomotor activity was increased by CV in mice at both the doses when compared with control at 60, 120, 180 and 240 minutes (Figure 8), although the stimulatory effect was more prominent at higher dose (500 mg/kg).

The CV ethanolic extract was evaluated for its inhibitory effect on α-amylase enzyme by *in-vitro* method. The alpha amylase inhibitory potential of the extract have been shown in the figure 10. The CV extract showed good alpha amylase inhibitory ability with the IC₅₀ value of 1.24 mg/ml. The standard reference drug acarbose showed IC₅₀ value of 0.33 mg/ml.

Certain plants have been used in folk medicine for the relief of pain and inflammation. The root of *C. viscosum* was claimed to exert analgesic and anti-inflammatory action and suggested by folk medical practitioners for the same purpose (Nandi and Lyndem, 2015). The analgesic, anti-inflammatory, neuro-pharmacological and antidiabetic effects of the CV extract were explored in this study.

The analgesic activity was explored by three different animal models, which could provide reactions to two separate phases of noxious stimuli (thermal stimulus and chemically induced tissue damage) (Victor *et al.*, 2004). The peritoneal fluids of PGE₂ and PGF₂α, serotonin and histamine are increased by acetic acid and this theory is used for the exploration of peripheral analgesics (Deraedt *et al.*, 1980; Collier *et al.*, 1968). Central analgesic activity was explored by hot plate test due to having several benefits, especially the sensitive action to strong analgesics with less tissue damage.

*C. viscosum* root extract significantly decreased the writhes as compared to the control. The extract at
500 mg/kg exhibited significant (p<0.01) antinociceptive power which indicates its antinociceptive capacity that is slightly lower than the reference drug utilized in the present study. Generally, acetic acid helps to liberate endogenous substances like histamine, serotonin, prostaglandins substance P and bradykinins (Konaté et al., 2012). These substances excite nerve endings to cause pain. Locally situated peritoneal receptors are involved to cause the response of abdominal constrictions (Bentley et al., 1983). This method has an association with prostanoids that increases the PGE_2 and PGF_2 alpha level in peritoneal fluids (Derardt, 1980) and products of lipooxygenase (Roberts and Morrow, 2001). The acetic acid mediated writhes were reduced by plant extract which indicates the peripherally mediated analgesic effect through inhibition of PGs and other endogenous substances synthesis and release. (Tadiwos et al., 2017).

Formalin test, a more valid analgesic test, has a better correlation with clinical pain (Tjolsen et al., 1992; Ghannadi et al., 2005). It is a biphasic test model that measures both neurogenic (first phase) and inflammatory (second phase) pain. Neurogenic pain (0-5 min) is due to the direct stimulation of nociceptors and measures pain from central effects. The second phase (15-30 min) is qualitatively different from the first phase. It depends on the peripheral inflammation where release of chemical mediators from damaged cells are responsible and stimulate nociception following induction of pain (Hunskaar and Hole, 1987). This test in general estimates a long lasting nociceptive stimulus that resembles clinical pain (Tjolsen et al., 1992). This test is thus used as a basic pain research tool for studying the mechanisms of analgesic agents as it has connection to tissue injury. Agents acting mainly on the CNS inhibit both phases equally whereas drugs acting on periphery inhibit the late phase. Result of this study presented in the figure 2 indicates that alcoholic extract of CV at 500 mg/kg significantly decreased licking time at first 5 min. (p<0.01) as well as at second 5 min. (p<0.05) in the mice injected with formalin. The inhibitory ability of CV extract in the late phase more prominently indicates that this has an involvement in action that is mediated from periphery which is probably by the inhibition of PG synthesis.

To elucidate centrally mediated antinociceptive responses hot plate and tail clip tests are useful and mainly focuses the changes above the spinal cord level (Vongtau et al., 2004). This probably acts by inhibiting descending pain pathway (Richardson et al., 1998). A number of complex processes are responsible for modulating pain which include opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti, 1983; Headley, 1985; Wigdor, 1987; Pasero, 1999). Data in the current model exhibited that the CV extract showed dose-dependent increase in the pain tolerance level.

There are different irritant agents (e.g., xylene, croton oil, phenol, capsaicin, histamine etc) which causes ear edema in mice and these models are widely used for the identification of topical anti-inflammatory effect with its possible mechanism of action (Gábor, 2000). Neurogenous swelling induced by xylene is a common inflammatory model which was selected for vascular permeability test through the association of substance P (Luber-Narod, 1997). Xylene induces instant irritation, leading to fluid accumulation and edema in the mouse ear which characterizes acute inflammatory response (Atta and Alkofahi, 1998). In this experiment, the C. viscosum ethanolic extract suppressed xylene initiated ear swelling in mice.

2-o-tetranoilphorbol-13-acetate (TPA) and other phorbol esters are the main irritant agents of croton oil. TPA can activate protein kinase C (PKC), which activates mitogen activated protein kinase (MAPK) and phospholipase A2 (PLA2) which in turn, leads to the release of platelet activation factor (PAF). This cascade of events is responsible for permeability and dilution of vessels, migration of polymorphonuclear leukocytes and causes histamine
and serotonin release as well as cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) enzymes synthesis (Ferrandiz et al., 1996; Wang et al., 2001). COX, LOX and leukotriene B4 (LTB4) antagonists as well as corticosteroids exhibit topical anti-inflammatory activity in animal inflammatory models induced by croton oil or TPA (Murakawa et al., 2006). In this experiment, C. viscosum significantly inhibited the ear swelling.

In the inflammatory process, leukocytes, eosinophils, basophils, neutrophils and monocytes are activated during the inflammation and interact with inflammatory and immunological mediator cells. Metabolism of these cells causes reactive oxygen species (ROS) generation (Barnes et al., 2004). ROS by activating transcription nuclear factors and activator protein-1 (AP-1) triggers inflammation (Sugiura and Ichinose, 2008).

Anti-inflammatory activity of C. viscosum was also evaluated by cotton pellet granuloma for subacute inflammation. When cotton pellets are subcutaneously implanted in the rats, three phases of inflammatory response are observed. The first one is transudative phase which happens during the first 3 h. The second one is exudative phase (between 3 and 72 h) and last one is proliferative phase (between 3 and 6 days) after cotton implantation (Swingle and Shideman, 1972). Decrease in granuloma weight happens due to the suppression of proliferative phase (Kavimani et al., 1996). This study showed the reduction of dry weight of cotton pellet granuloma by C. viscosum extract as compared to the control group.

Central behavioral analysis can be evaluated by pentobarbital initiated sleeping test and hole cross test which sensitively evaluate CNS stimulating effect of drugs as well as plant extracts (Gupta et al., 1997; Takagi et al., 1971). Pentobarbital is a barbiturate of short duration of action and induces sedation in animals at appropriate dose by stimulation and post synaptic allosteric inhibitory modification of gamma-aminobutyric acid (GABA-an inhibitory neurotransmitter) receptors (Ffrench-Mullen et al., 1993). In this study, CV extended the onset time of sleep and lowered the sleeping duration when compared to control. This rationalizes that CV might have GABA inhibitory activity. In the hole cross test, the locomotor activity was also increased by CV which also justifies the CNS stimulating activity of the extract (Guaraldo et al., 2000).

Hyperglycemia is a state of rapid rise in blood glucose level due to continuous breakdown of starch by α-amylase enzyme from pancreas (Deshpande et al., 2009). Decreasing the post-prandial hyperglycemia by lowering the absorption of glucose from GI tract by α-amylase and α-glucosidase inhibition can be an approach for treating diabetes (Rhabasa-Lhoret and Chiasson, 2004). Therefore, this experimental approach investigated an in-vitro evidence for the potential inhibition of α-amylase enzyme by CV extract. Inhibitors of α-amylase (also called as starch blockers) slow down the metabolism of starch and sucrose, which in turn delays glucose and fructose absorption from GI tract and finally regulate blood glucose level (He et al., 2014; Dineshkumar et al., 2010). The outcome of this study revealed that the extract of CV inhibited alpha-amylase enzyme activity.

Conclusions

For thousand years, various types of health problems are alleviated by using medicinal plants. A huge number of medicinal plants with potential analgesic, anti-inflammatory and other properties are available in the nature. Test extract of C. viscosum reduced pain and inflammation, showed CNS stimulant effect as well as inhibited the alpha-amylase enzyme. Therefore, it showed significant analgesic, anti-inflammatory, CNS stimulant and antidiabetic effects and further investigations to establish the mode of action for these activities are required.

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References


