Antinociceptive Potential of Viscosine Isolated form *Dodonaea viscosa* in Animal Models

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Summary: *Dodonaea viscosa* was selected in this study based on its ethno-medicinal applications for treating various painful conditions. In earlier studies crude extracts of *Dodonaea viscosa* showed significant actions as local anesthetic, smooth muscle relaxant, antibacterial, anti-inflammatory, antifungal, anti-ulcerogenic, anti-ascariasis, anthelmintic, cardiac depressant, hypotensive, uterine relaxation and vasoconstrictor activity in different experimental models. In an effort to identify the potential analgesic components of the plant, the principal flavonoid constituent, 5,7,4'-trihydroxy-3,6-dimethoxyflavone (viscosine) was isolated. When tested using the acetic acid-induced writhing and hot plate analgesic models, viscosine (30, 45 and 60 mg/kg), showed significant ($p < 0.05$) antinociceptive activity in a dose-dependent manner. It is concluded that viscosine has antinociceptive therapeutic potential through both central and peripheral mechanisms and can be used a template compound as a painkiller.

Keywords: *Dodonaea viscosa*; Acetic acid; Hot plate; Antinociceptive.

Introduction

Dodonaea, the genus consist of 60 species, is mostly distributed in the hot areas of various countries like Pakistan and India. *Dodonaea viscosa* (L.) Jacq. (syn-*Ptelea viscosa* Linn.) belongs to the family Sapindaceae which includes 150 genera and approximately 2000 species. It is a flowering ever green shrub and traditionally used as a folk medicinal preparations for combating various diseases and pathological conditions. It has been reported as antiviral, analgesic, antimicrobial, anti-inflammatory, rheumatism, gout, spasmolytic, laxative, hypotensive agent, hemorrhoids, fractures and snake bites [1, 2]. Furthermore, the crude plant has been investigated for various other pharmacological actions and exhibited local anesthetic, smooth muscle relaxant, antifungal, anti-ulcerogenic, anti-ascariasis, anthelmintic, cardiac depressant, uterine relaxation and vasoconstrictor activities [2]. Nociception is obnoxious sensory and emotional experience related to real or possible tissue damage. It involves a psychological component which can change its perception and undergo integration in higher centers especially in the brain. Pain may be acute or chronic, but in both conditions badly affect the quality of life of affected peoples. Different therapeutic options in the form of synthetic agents are available for the management of pain but they are suffering from some serious toxicities. In this connection, natural healing agents are gaining interest in the discovery of effective molecules with considerable safety. It is a common perception among the public that natural products are safe and effective with minimum side effects so in continuation of the research work of our team on plant extract and isolated compounds we performed this study.

The present study deals with the exploration of our isolated compound for its antinociceptive potential.

Results and Discussion

The peripheral analgesic activity was evaluated by using the acetic acid-induced writhing model of analgesia test [3, 4]. Intraperitoneal injection of acetic acid produced over 50 writhes in the vehicle-treated control group within the 20 min of observation period (Fig. 1). As shown in Fig. 2,

viscosine suppressed the writhing response in a dose-dependent manner. All doses of viscosine demonstrated significant (p>0.05) effect with aspirin. Various authors have shown that intraperitoneal injection of acetic acid triggers the release of predominantly prostaglandins e.g. PGE-2 [5] along with a variety of other mediators including bradykinin [6, 7] and pro-inflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α [8]. The demonstration of inhibition of the writhing response by aspirin in the present study together with previous reports showing the inhibitory effect of other non-steroidal anti-inflammatory drugs like indomethacin and diclofenac [9] further highlights the prominent role of the cyclooxygenase pathway in acetic acid-induced writhing response. The exact mechanism of viscosin in suppressing the acetic acid-induced writhing response, however, remains to be established.

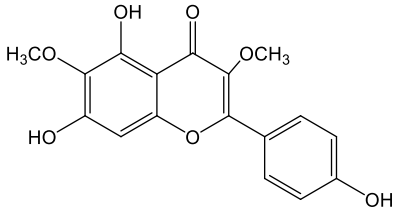

Fig. 1. Viscosine isolated *D. viscosa*.

The hot plate or thermal induced hyperalgesia test was used to investigate the central thermal anti-nociceptive effect. This model is mostly used to estimate supra-spinal analgesia in compounds and so far considered selective model for evaluation centrally acting analgesic drugs, like morphine and its analogues, however peripheral anti-nociceptive agents are found to be inactive in this model [9]. Viscosine increased the reaction time of treated mice in a dose dependent manner (Fig. 3). Significant activity (P<0.05) were recorded for all tested doses of viscosine while the activity at 60 mg/kg, which was over 4-fold enhancement over untreated control group, was comparable with that of the positive control, Tramadol (p>0.05). It is thus likely that viscosine exert its effect by modulating the spinal and supra-spinal receptors of the central opioid pathway. The therapeutic effect of plants is directly attributed to presence of active constituents therefore we subjected the *Dodonaea viscosa* for isolation. The present research work strongly supports the ethno-medicinal use of this plant for the treatment of various painful conditions.

In conclusion, viscosine displayed promising antinociceptive potential through peripheral and central mechanisms. Further detailed mechanisms of action studies including effects on nociceptive mediators are well merited.

Experimental

Collection and Identification of Plant Material

The collection of aerial parts of *Dodonaea viscosa* was undertaken from the hills of Kurram Agency, Khyber-Pakhtoonkhwa, Pakistan and under the close supervision of Dr. Ijaz Khan, a plant taxonomist at the Department of Botany, Post Graduate College, Kohat, Pakistan for the purpose of identification. The collected voucher specimen (DVPGCK-098) has been consigned in the herbarium of Department of Botany, Post Graduate College, Kohat.

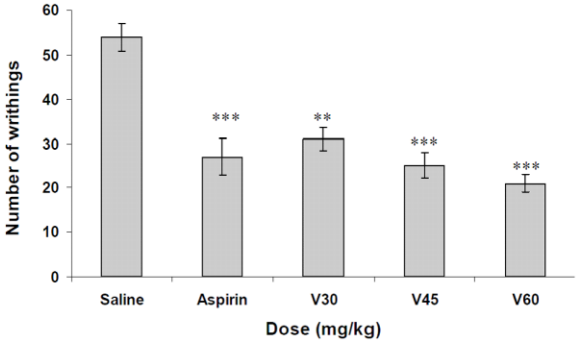

Fig. 2: Effect of Viscosine on acetic acid-induced writhing model. * p < 0.05, ** = p < 0.01, ** = p < 0.001.

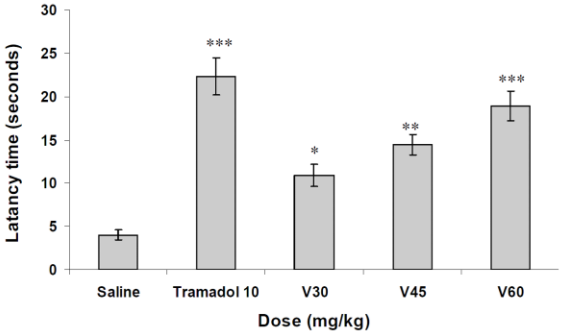

Fig. 3: Effect of viscosine in hot-plate latency test. * p < 0.05, ** = p < 0.01, ** = p < 0.001 (n = 5).

Extraction and Isolation

After drying plant material under shadow, the 20 kg was grinded into powder and subjected to extraction at room temperature with MeOH (35

L×3×15 days). Thereafter the solvent was removed, the remaining extract was suspended in H_2O and extracted with hexanes, $CHCl_3$, EtOAc, and *n*-BuOH and resultants fractions yield was hexanes (116 g), $CHCl_3$ (890 g), EtOAc (173 g), and *n*-BuOH (337 g) extracts respectively. The EtOAc extract was further sub-fractioned by using MPLC (medium pressure liquid chromatography) on silica gel with the solvents system of hexanes/$CHCl_3$, $CHCl_3$/EtOAc, EtOAc/MeOH, and MeOH and resultant elutes were 38 fractions (Fr. 1-Fr. 38). The viscosine, a pure compound (Fig. 2) isolated from Fr. 23 (3.8g) (obtained with hexanes/$CHCl_3$, 2:8, 5L) by column chromatography (CC) [silica gel, hexanes/EtOAc (6:4, 500 mL)]. Its structure was confirmed by comparison with analytical data available in literature [10].

Antinociceptive Activity

Acetic Acid-Induced Writhing Test

The peripheral anti-nociceptive action of viscosine was evaluated by using the acetic acid–induced writhing reflex model in mice (BALB/c, 18 – 22 g) [11]. Animals were divided into four groups (n=6) and receiving either viscosine (30, 45 or 60 mg/kg, i.p.), the positive control, aspirin (150 mg/kg p.o.) or the vehicle control (10 ml/kg, i.p.). After 30 min, writhing response was induced by administration of acetic acid (1.0%, v/v, 0.1 ml/10 g body weight). The number of writhes was counted for 20 minutes after acetic acid administration and percent analgesic activity calculated [11].

Thermal Nociception (Hot Plate Test)

The hot plate model was used for the evaluation of the central anti-nociceptive potential of viscosine. The mice were selected for experiment after initial screening by placing them on hot plate maintained at 50 ± 0.05 ^oC [12] and only those animals which responded within 15 s were selected for the test. Various doses of viscosine (i.p.), vehicle (i.p.) or Tramadol (10 mg/kg, p.o.) as a positive reference drug were administered 30 min prior to the placement on the hot plate. The parameters observed in these experiments include Jumping, withdrawal of the paws or licking of the paws (withdrawal response latency). The response latencies were recorded at interval of 0, 60, 120 and 240 min with consideration of a cut off period of 30 s to avoid unnecessary damage to the paw in the absence of response.

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