Vol. 6/2 (2006) 135 - 140

JOURNAL OF NATURAL REMEDIES

Anti-nociceptive activity of various extracts of *Dodonaea viscosa* (L). Jacq., leaves.

S. D. Joshi[1]*, V. D. Kulkarni[1], V. H. Kulkarni[1], H. M. Vagdevi[2], V. P. Vaidya[2], V. P. Veerapur[1], A. M. Badiger[3].

1. S. E. T's College of Pharmacy, Dharwad, Karnataka.
2. Kuvempu University, Shimoga, Karnataka.
3. K. L. E. S's College of Pharmacy, Hubli, Karnataka.

Abstract

Objective: The present study is aimed to evaluate the leaf extracts of *Dodonaea viscosa* for acclaimed antinociceptive activity on chemical and thermal induced pain. Materials and methods: Three experimental pain models viz. thermal stimulation by hot plate method, tail flick method using analgesiometer and writhing induced by glacial acetic acid were used for assessing antinociceptive activity of *D. viscosa*. Results: All the leaf extracts of *D. viscosa* showed antinociceptive activity in rats and mice. Conclusion: All tested extracts showed significant antinociceptive activity, among them ethyl acetate extract exhibited superior activity. Data suggests that the extracts of *D. viscosa* have an inhibitory activity on both peripheral and central pain mechanisms. The antinociceptive efficacy of the extracts may be attributed to the presence of flavonoids, sterols and saponins.

Key Words: *Dodonaea viscosa*, antinociceptive activity, ethyl acetate extract.

1. Introduction

In continuation of our interest in phytopharmacological investigation of the extracts of *Dodonaea viscosa* [1, 2], in the present study we report the antinociceptive activity.

D. viscosa (L). Jacq., (Fam: Sependaceae) popularly known as *aliar* and *vilayati mehandi* in India, is an evergreen shrub abundantly growing in Western Ghats of Karnataka, India. The species has been used in traditional medicine for snakebite and as febrifuge. It is also used to heal simple ulcer [3], fracture, sore and for immediate relief of pain of gum and teeth [4]. *D. viscosa* has been reported to posses local anaesthetic, smooth muscle relaxant [5], antimicrobial and antiinflammatory activity [6]. References reveal that the herbs rich in flavonoids show several biological activities such as antinociceptive, anti-inflammatory and antiulcer. A combination of these pharma-

* Corresponding author
E-mail: joshishrinivas@rediffmail.com

cological properties in a single medicinal substance is unique as most of the available antiinflammatory analgesics are ulcerogens.

Surveys conducted in tribal areas of Western Ghats of Karnataka revealed the use of leaves of this plant as a remedy in the treatment of snakebite, wounds, ulcers and for immediate relief of pain of gum and teeth. The objective of the present study was to investigate the possible analgesic potential of *D. viscosa* in peripherally and centrally mediated experimental models of pain.

2. Materials and methods

2.1 Plant material

The leaves of *D. viscosa* (L). Jacq., were collected in and around the city of Dharwad, Karnataka in the month of Sept, 2003 in its growth phase. The plant was authenticated by Prof. M. Jayaraj, Department of Botany, Karnataka University, Dharwad and voucher specimen of the plant was kept in the college herbarium.

2.2 Drugs and chemicals

Following drugs were chosen for the present study.

1. Naloxone (Triokaa Parenterals Pvt. Ltd., Ahmedabad)

2. Aspirin (AstraZeneca, Bangalore)

3. Pentazocine (Sigma Laboratories Pvt. Ltd.)

Petroleum ether, benzene, chloroform, acetone, ethyl acetate and glacial acetic acid used were of LR grade and procured from s.d. Fine Chemicals, Mumbai. Ethanol was of commercial grade. Double distilled water and ethanol were used throughout the experiment.

2.3 Preparation of the extract

The shade-dried powdered leaves (1 kg) were extracted exhaustively with 95% ethanol (3 cycles/h) in a Soxhlet apparatus by continuous heat extraction. The total ethanol extract was concentrated *in vacuo* to a syrupy consistency (yield 200 gm). The ethanol extract (EE) was used in different pain models.

2.4 Phytopharmacological studies

The coarse powder of the leaves of *D. viscosa* (900 g) was used for sequential extraction with different solvents in their increasing order of polarity. The extracts obtained were petroleum ether (60-80°) extract (PEE), benzene extract (BE), chloroform extract (CE), acetone extract (AE), ethyl acetate extract (EAE) and chloroform: water extract (ACE).

The dried extracts were subjected to various chemical tests to detect different phytoconstituents [7, 8]. Selected extracts based on phytochemical screening were used for antinociceptive studies. The three experimental pain models; thermal stimulations by hot plate method and tail flick response by analgesiometer and writhing induced by glacial acetic acid were used for assessing analgesic effects.

2.5 Animals

Adult albino mice (18-22 g, 6 animals per group per treatment) were used for the abdominal constriction test. While healthy albino rats (150-200 g, 6 animals per group per treatment) were used for the tail flick and hot plate tests. They were housed individually in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30-70%. A 12:12 h light and dark cycle was followed.

The rats were fed with animal feed pellets manufactured by Hindustan Lever (India) Ltd., Mumbai and water was provided *ad libitum*. The study protocol was approved from Animal Ethical Committee of the Institution.

2.6 Acute toxicity study

Healthy albino mice of either sex weighing 20-25 g and of 90 days age were used to determine the safer dose by up and down staircase method [9]. The animals were starved overnight before the experimental procedure. Extracts were suspended in 1% sodium CMC in water and were administered orally to animals in ascending order and widely spread doses, say 10, 30, 100, 300, 1000 and 3000 mg/kg. The animals were observed continuously for two hour, frequently for the next four hours and there after for overnight mortality. After administration of the extract, Irwin test was conducted, where animals were observed for behavioral changes.

LD_{50} was extrapolated by making use of graphical methods to rats; $1/8^{th}$ of Lethal dose was taken as the screening dose.

2.7 Evaluation of analgesic activity

2.7.1 Tail Flick method [10]

The heat intensity of thermal stimulation (Techno Analgesiometer) was adjusted such that rats had control tail flick latency of 3-4 sec. A cut-off latency of 10 sec was used to prevent thermal injury. The initial reaction time was recorded in thirty animals and then they were divided into five groups of 6 rats each. First group received ethyl acetate extract, second group received naloxone, and third group received naloxone and ethyl acetate extract, while the fourth and fifth received ethanol and petroleum ether extract respectively at a dose of 375 mg/kg body weight. Tail flick latency in seconds was recorded every 30 min for a duration of 3 h after drug administration.

2.7.2 Glacial acetic acid (GAA) induced writhing in mice [11]

Male albino mice were divided in to five groups of 6 animals each. Acetic acid (0.75%) was administered *i.p.* into the mice of all groups (10 ml/kg) to induce pain. First group of the animals received only acetic acid and served as control, second group received aspirin (100 mg/kg) and served as positive control, while the third, fourth and fifth groups received ethanol, ethyl acetate and petroleum ether extracts respectively at a dose of 375 mg/kg body weight.

All the extracts were administered orally by using intragastric tube 15 min prior to the administration of acetic acid injection. A significant reduction in the number of constrictions when compared with vehicle treated animals was considered as antinociceptive response.

The contraction of abdominal muscle together with stretching of the hind limbs was cumulatively counted over a period of 20 min, beginning 5 min after GAA injection. Antinociceptive activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pretreated with the extracts using the ratio- (Control mean - treated mean) x 100/ control mean.

2.7.3 Hot plate method [12]

Healthy albino rats (125-150g) were divided into five groups each consists of six animals. First group was administered 5% gum acacia at the dose of 5 ml/kg and served as control, second group received pentazocine (5 mg/kg) and served as positive control while the third, fourth and fifth groups received ethanol extract, ethyl acetate extract and petroleum ether extract respectively at a dose of 375 mg/kg orally. The time of reaction to pain stimulus (interval between placing the rat in the hot plate and the lick or jump response) of the rat placed on the plate heated at 55°C ± 0.5°C was recorded every hour for duration of 3 h after drug administration.

2.8 Statistical analysis

All the results are reported as Mean ± SEM. Statistical significance was analyzed employing one-way ANOVA followed by Post-hoc Dunnett's test using GraphPad Prism version 4.0. Values of P less than 0.05 were considered significant.

3. Results

Preliminary phytochemical screening of *D. viscosa* revealed the presence of sterols, flavonoids, tannins, saponins, carbohydrate and alkaloids. The dose of the extracts determined by up and down staircase method was found to be 1/8th of Lethal dose (3000 mg) that is 375 mg/kg body weight.

Preliminary investigations with ethanol extract of the leaves exhibited a significant antinociceptive activity in all the models and further bioactivity guided fraction studies showed the antinociceptive activity in the order of EAE>EE>PEE.

The tail flick reaction time increased significantly ($p<0.01$) in rats after oral administration of *D. viscosa* leaf extracts (Table 1). Peak analgesic effects of all the extracts were observed after 60 min of oral administration. Naloxone pretreatment significantly reduced the antinociceptive effect of the ethyl acetate extract. Ethyl acetate extract showed a highest analgesic activity (6.5 sec at 60 min) followed by ethanol extract (5.5 sec at 60 min) and petroleum ether extract (4.5 sec at 60 min).

Similar results were obtained from acetic acid induced writhing test, showing significant activity for all extracts (Table 2). The activity was highest for ethyl acetate extract and lowest for petroleum ether extract and the activities were comparable to that produced by aspirin.

The hot plate method also showed higher analgesic activity in ethyl acetate extract and followed by ethanol and petroleum ether extract (Table 3). Reaction time of treated animals one hour after treatment was significantly higher in comparison to the control and became even more so after two and three hours.

4. Discussion

The results of the present study revealed the potency of leaf extracts of *D. viscosa* as good antinociceptive agents. Thermal stimulation models (tail flick test and hot plate method) and writhing induced by acetic acid involve central [9] and peripheral [10] mechanisms respectively.

Table1.
Antinociceptive effect of *Dodonaea viscosa* leaf extracts on reaction time to tail flick test in rats.

Group	Drug	Control	Reaction intervals (seconds) at time (min)					
			30	60	90	120	150	180
I	EAE	3.6 ± 0.10	5.8 ± 0.08*	6.5 ± 0.16*	5.7 ± 0.08*	4.6 ± 0.08*	4.1 ± 0.03*	3.7 ± 0.07
II	NLX	3.2 ± 0.08	2.3 ± 0.05*	2.3 ± 0.12*	2.4 ± 0.07*	2.5 ± 0.07*	2.5 ± 0.07*	2.6 ± 0.08*
III	NLX+EAE	4.3 ± 0.27	4.2 ± 0.24	4.6 ± 0.07	4.5 ± 0.07	4.2 ± 0.06	4.0 ± 0.16	3.6 ± 0.08*
IV	EE	3.4 ± 0.07	4.9 ± 0.07*	5.5 ± 0.08*	5.1 ± 0.08*	4.1 ± 0.08*	3.9 ± 0.07*	3.6 ± 0.05*
V	PEE	3.5 ± 0.07	4.2 ± 0.07*	5.0 ± 0.08*	4.6 ± 0.05*	4.1 ± 0.05*	3.7 ± 0.08**	3.4 ± 0.05

Naloxone (NLX) (1mg/kg *i.p.*) was given 10 min prior to EAE. Dose of EAE, EE & PEE-375mg/kg, *p.o*; Values are mean ± SEM, (n = 6); *p < 0.01, **p < 0.05 compared to control group.

Table 2.
Effect of leaf extracts of *Dodonaea viscosa* on glacial acetic acid (GAA) induced writhing in mice.

Group	Drug	Dose	Writhing count	% inhibition
I	Control	0.75% GAA	44 ± 1.94	——
II	Standard	100 mg/kg Aspirin	25 ± 1.04*	43.18
III	EE	375 mg/kg	32 ± 1.00*	27.27
IV	EAE	375 mg/kg	30 ± 1.00*	31.81
V	PEE	375 mg/kg	35 ± 0.73*	20.45

Values are mean ± SEM, (n = 6); *p < 0.01 compared to control group.

Table 3.
Antinociceptive effect of *Dodonaea viscosa* leaf extracts on thermal stimulation (Hot plate method) in rats.

Group	Drug	Reaction time in seconds		
		1 h	2 h	3 h
I	Control (5% gum acacia)	18.0 ± 1.2	18.1 ± 1.0	18.4 ± 0.8
II	Pentazocine 5 mg/kg	20.1 ± 0.2	25.1 ± 1.2*	29.0 ± 0.8*
III	EE	19.3 ± 0.4	21.2 ± 0.7*	24.2 ± 0.3*
IV	EAE	20.1 ± 1.4	24.4 ± 0.9*	27.3 ± 0.3*
V	PEE	19.2 ± 0.8	20.8 ± 1.4*	23.0 ± 1.4*

Values are mean + SEM, (n = 6); *p < 0.01 compared to control group.

Antinociceptive effect observed in both these experiments with *D. viscosa* extracts indicates the involvement of both peripheral and central mechanisms.

All the extracts showed significant antinociceptive activity, whereas ethyl acetate extract exhibited superior activity than other extracts. Pretreatment with the opioid antagonist naloxone, partially reduced the antinociceptive effect of ethyl acetate extract.

This indicates the involvement of endogenous opioid peptides in mediation of antinociceptive response of *D. viscosa* leaf extracts. As the analgesic effect is reduced partially after naloxone, some other nonopioid mechanisms may also be involved. It is possible that extracts of *D. viscosa* may modulate some other neuro-transmitters/neuromodulators involved in the regulation of pain sensitivity.

Acetic acid is believed to trigger the production of irritant substances within the peritoneum, which cause the writhing response [13]. The effect of the extracts against the noxious stimulus indicates that they depressed the production of the irritants and thereby reduced the number of writhes in the mice.

The extract of *D. viscosa* is reported to contain tannins, saponins, traces of alkaloids and flavonoids like quercitin [14]. It has been observed that the flavonoid substances produced significant opioid and ATP sensitive (K^+_{ATP}) channel-mediated antinociceptive response like morphine.

Thus it can be concluded that the antinociceptive activity of the plant may be due to the presence of flavonoids, sterols and saponins. Further studies are necessary to elucidate the molecular mechanism of antinociceptive action and to determine the bioactive molecule (s) responsible for aforesaid activity.

5. Acknowledgements

Authors convey their sincere thanks to Shri. H. V. Dambal, President, Soniya Education Trust for providing all the research facilities. Authors are also grateful to Dr. M. Jayaraj, Professor, Dept. of Botany, Karnataka University for authentication of the title plant.

References

1. Joshi SD, Aravind MB, Veerapur VP, Shastry CS. (2003) *Indian Drugs*, 40(9): 549.

2. Veerapur VP, Badiger AM, Joshi SD, Nayak VP, Shastry CS. (2004) *Indian J. Pharm. Sci.*, 66(4): 407.

3. Perry LM, Metzger J. (1980) *Medicinal plants of East Southeast Asia*, MIT Press, Cambridge Mass and London: England; 375.

4. Kirtikar KR, Basu BD. (1993) *Indian Medicinal Plants*, II Edn., Vol. I, International Book Publisher: Dehradun; 641.

5. Rajas A. (1996) *Planta Med.*, 62: 154.

6. Getie M, Rietz R, Huschka C. (2003) *Fitoterapia*, 74: 139.

7. Harborne JB, (1998) *Phytochemical methods*, Chapman and Hall: London; 60.

8. Kokate CK. (1991) *Practical Pharmacognosy*, Vallabh Prakashan: London; 107.

9. Ghosh MN. (1984) *Fundamentals of Experimental Pharmacology*, II Edn., Scientific Book Agency: Calcutta; 153.

10. Davies OL, Raventos J, Walpole AL. (1946) *Br. J. Pharmcol.*, 1: 255.

11. Witkin LB, Heubner CF, O'Keete E, Spitalitta P, Plummer A J. (1961) *J. Pharmacol. Exp. Ther.*, 133: 400.

12. Turner RA. (1965) *Screening Methods in Pharmacology*, Academic Press: New York; 100.

13. Bartolinin AM, Galli A, Ghelardini C, Gioti A, Malcangio M, Malmberg-Aiella P, Zucchi PL. (1987) *Br. J. Pharmcol.*, 92: 711.

14. Yoganarasimhan SN. (1996) *Medicinal Plants of India*, Vol. I, Interline Publishing Private Limited: Bangalore; 178.