INTERNATIONAL RESEARCH JOURNAL OF PHARMACY **ISSN 2230 – 8407**
Available online http://www.irjponline.com
Research Article

ANTINOCICEPTIVE ACTIVITY OF COLD WATER EXTRACT OF *DESMODIUM TRIFLORUM* IN RATS

Ratnasooriya Wanigasekara Daya[1]*, Jayakody Anusha Chandra[1], Ratnasooriya Chatura Dayendra Tissa[1] and Weerasekera Kumudu Rupika[2]
[1]Department of Zoology, University of Colombo, Colombo 03, Sri Lanka
[2]Department of Nidhana Chikitsa, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka

Article Received on: 20/05/11 Revised on: 29/06/11 Approved for publication: 12/07/11

*Prof. W. D. Ratnasooriya, E-mail: wdr@zoology.cmb.ac.lk

ABSTRACT
The aim of this study was to examine antinociceptive potential of cold water extract (CWE) of *Desmodium triflorum* (Family: Fabaceae / Leguminosae) plant. One of four doses of CWE (dose 4000 mg/ml, 2000 mg/ml and 1000 mg/ml) or 1 ml tap water was orally administered to male wistar rats and antinociceptive activity was ascertained using tail flik and hot plate techniques. The results showed that CWE possessed marked antinociceptive activity when evaluated in the hot plate test but not in the tail flick test. Antinociceptive action was dose-dependent. Further, it had a rapid onset (1h) and a short duration (3h) of action. The CWE was safe and was not accompanied with side effects even after sub chronic administration. The antinociception action was mediated by supraspinal mechanisms, possibly via alkaloids and flavonoids. It is concluded that CWE of *D. triflorum* may be used as a cheap, orally active safe antinociceptive drug.
KEY WORDS: *Desmodium triflorum*, antinociception, pain, toxicity, Sri Lanka.

INTRODUCTION

Desmodium triflorum (L) DC (Family: Fabaceae/ Leguminosae), Hin- undupiyali in Sinhala, is a very small perennial weed with numerous, long, slender branches rooting at nodes. Its leaves are small, alternate, stipulate and trifoliate. Flowers are irregular, bisexual, very small and bright purple in colour. This plant is generally found in grassy places, lawns, roadsides and in open places in tropical countries including Philippine, Java, Taiwan, India and Sri Lanka. In Sri Lanka, it is very common in low country.[1]

Phytochemically, it is shown to contain urosolic acid, vitexin, genistin [2] a variety of flavonoids[3] and alkaloids (such as hypaphorine, betaine)[4]. In the folkloric and traditional medicine, it is claimed to be used in the treatment of liver congestion, chronic ulcers, dysentery, diarrhoea, urinary retention, snake bite poisoning (particularly against Russell's viper), dysmenorrhea and in moderate to strong stomach aches[3,4,5]. In Sri Lankan folkloric medicine and traditional medicine it is also considered as a strong aphrodisiac[5]. In the treatment of stomach aches usually fresh juice is recommended. Its use in stomach aches and dysmenorrhea suggest that the fresh juice of the plant may possess antinociception action. Infact, experimentally, methanol, hexanol,[6,7] ethyl acetate and petroleum ether fractions[6] of this plants have been shown to possess antinociception action. But, the potential implications of these finding are limited in traditional medicine since fresh water extracts (either cold or hot) is generally used. The aim of this study is therefore to investigate the antinociceptive potential of *D. triflorum* cold water extract. This would help to scientifically justify its use in aqueous form in traditional medicine of Sri Lanka as a painkilling drug.

MATERIAL AND METHODS

Collection and Authentication

Mature plants were uprooted from grassy areas at the premises of University of Colombo, Sri Lanka, in July 2010. The plant was identified and authenticated by Prof. (Mrs.) A. N. Senaviratne of the Department of Plant sciences, University of Colombo. Voucher specimen WDR/JRAC/5/2010) is deposited at the museum of Department of Zoology, University of Colombo.

Preparation of the cold water extract (CWE) of *D. triflorum*

Plants were washed in tap water and 20 g was macerated in 5 ml tap water using porcelain mortar and pestle for 5 min. A green CWE was obtained by squeezing the macerated material through a muslin cloth. Three doses were made from this CWE (4000, 2000 and 1000 mg/ml) to be administered orally to rats.

Phytochemical screening

The CWE was tested for the presence of (qualitative) alkaloids, flavonoids, phenols, cumarins, tannins, steroids and peptides as described by Fransworth.[8]

Experimental Animals

Healthy adult Wistar rats (males 200-250g and females 200-225g) purchased from the Medical Research Institute, Borella, Sri Lanka, were used. They were maintained singly in plastic cages in the Department of Zoology, University of Colombo under standardized animal house condition (temperature: 28-30^{0}C: photoperiod: approximately a 12-h light: dark cycle: and relative humidity 50-55%) with free access to tap water and pelleted food (Master Feeds Limited., Colombo, Sri Lanka) containing 19.5% proteins, 7.5% oil, 4.5 % fiber, 7.9 % ash, 0.48 % methylamine, 0.9% calcium and 0.7% phosphorus.

All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guide lines (guiding principles in the use of animals in toxicology, adopted by the Society of Toxicology in 1999) and rules of the Department of Zoology, Faculty of Science, University of Colombo for animal experimentations.

Evaluation of Antinociceptive activity using Hot plate and Tail flick test

Thirty rats were selected, and divided into five groups. Different concentrations of water extract or vehicle (control) was administered orally in the following manner: Group 1(n = 6) with 1 ml of water; Group 2 (n = 6) with 1 ml of 1000 mg/ml of extract; Group 3 (n = 6) with 1 ml of 2000 mg/ml extract; Group 4 (n = 6) with 1 ml of 4000 mg/ml extract; Group 5 (n = 6) with 1 ml of 133.5 mg/kg of Aspirin (State Pharmaceutical Cooperation, Sri Lanka) as a positive control.

Three to four hours before treatment (pre treatment) and then at hourly intervals for 4 h post – treatment, the rats were subjected to hot plate and tail flick test[9]. A cut off time of 20s was used to avoid tissue damage in hot plate test and 5s in tail flick test. In the hot plate test, the time taken to lick either hind paw or to jump up (reaction time) when placed on an enclosed hot plate (model MK 35 A Muroma Co. Ltd., Tokyo, Japan) maintained at 50^{0}C was recorded. In the tail flick test, the time taken to flick the tail (the reaction time) when the tail was immersed (5-6 cm from the tip) in a water bath at 55^{0}C was noted[9].

Evaluation of Sedative activity

Twelve rats were randomly divided in to two groups (n = 6 / group). One group was orally treated with 4000 mg/ml dose of CWE of *D. triflorum*. The other group was orally treated with 1 ml of tap water. One hour post treatment, each of these rats was individually placed in the center of a standard rat hole-board apparatus and observed for 7.5 min. During this period, the number of rears, number of head dips, locomotory activity and number of faecal boluses expelled were monitored. The time spent on a head dip was then computed[10].

Evaluation of Toxicity

Twelve rats were randomly assigned in to two equal groups (n = 6/group). One group was orally treated with the high dose of CWE and other with 1 ml of tap water (control) daily for 30 consecutive days. The food and water intake of each rat were noted. Further, these rats were observed daily for overt signs of toxicity (salivation, lacrimation, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of fur, diarrhoea or urination) behavioral abnormalities (impairment of spontaneous movements, climbing, cleaning of face, ataxia and other postural changes) aversive behaviors (biting and scratching behavior licking tail, paw and penis, intense autogrooming or vocalization) and stress (exopthalmia and fur erection). One day post treatment, blood was drain from the tail of these rats under ether anesthesia with aseptic precautions. Blood was allowed to clot at room temperature (28^0 C) and serum separated. Serum GOT and GPT, Creatinine and Urea were determined using Randox (Randox Laboratories Ltd., Antrim, UK) according to manufactures instructions. These rats were then sacrificed, stomachs excised and cut opened along the graterer curvature. The stomachs were rinsed with tap water and observed microscopically for erythromatous regions and mucosal haemorrhagic lesions in the glanduler portion.

Statistical Analysis

The data were expressed as mean ± SEM. Dose dependency was determined using Sperman's linear regression method. Statistical comparision were made using one way ANOVA followed by Turkey's post hoc test and Mann-Whitney U-test as appropriate. Significance was set at $P \leq 0.05$.

RESULTS

Phytochemical screening

CWE of *D. triflorum* contained alkaloids, phenolics, flavonoids and amino acids.

Antinociceptive activity

None of the doses of CWE of *D. triflorum* induced in significant change ($P > 0.05$) in the reaction time of the tail flick test. (Data not shown). On the other hand, the mid and high dose of CWE induced a significant prolongation of the reaction time ($P < 0.05$) at 1st (49.54 % to 56.45%), 2nd (33.28% to 40.33 %) and 3rd (42.75 % to 50.11%) hour of treatment. The effect was dose

dependent. (r = 1) The reaction time returned to normalcy by the 4th hour. Aspirin induced significant ($p < 0.05$) prolongation of reaction time up to 4 h. (see Table 1). Further, none of the doses of CWE induced Starub's tail reaction.

Sedative activity

The high dose of CWE did not significantly change ($P > 0.05$) any of the parameters of rat hole-board test: (control vs treatment) rearings 15.66 ± 1.41 vs 15.83 ± 2.35: head dips 6.00 ± 0.85 vs 8.83 ± 1.30: time/dip 0.95 ± 0.07 vs 0.81 ± 0.17: locomotory activity 14.16 ± 1.25 vs. 14.83 ± 2.24: faecal boluses 1.66 ± 0.84 vs 1.16 ± 0.79.

Evaluation of toxicity

None of the rats were morbid. There was no apparent suppression in food and water intake. Further, deaths were not encountered. The high dose of CWE did not induce overt signs of toxicity, behavioural abnormalities, aversive behaviours or stress. None of the serum parameters determined was significantly altered ($P > 0.05$). (control vs treatment): SGOT 56.75 ± 11.34 vs 53.50 ± 9.88 U/L : SGPT 25.83 ± 2.28 vs 27.33 ± 2.64 U/L : serum Urea 38.03 ± 3.38 vs 38.42 ± 3.88 mg/dL: serum Creatinine 0.88 ± 0.25 vs 1.05 ± 0.26 mg/dL. In addition, no erythromatous regions or gastric haemorrhagic lesions were evident in CWE treated rats as in the control.

DISCUSSION

This study examined the antinociceptive potential of cold water extract of *D. triflorum* plant. The results show, for the first time, that CWE has marked oral antinociceptive action, when evaluated in hot plate test but not in tail flick test. This suggests that the antinociceptive action of CWE is mediated centrally at supraspinal level: hot plate test predominately measures supraspinally organized responses[11] whilst the tail flick test predominately measures antinociception mediated via spinal mechanisms [11]. Positive results in the hot plate test also suggests that CWE is effective against neurogenic pain [12]. However, we cannot exclude the possibility that the CWE is also effective against continuous inflammatory pain[14] or visceral pain , since methanolic extract of *D. triflorum* has been shown to display positive results in formalin test and writhing tests [4, 7].

The antinociceptive action of CWE was dose - dependent. This indicates that the action was treatment related, genuine and possibly receptor mediated. Further, the antinocoception action had a quick onset (within 1 h) and short duration of action (about 3 h). The former observation a desirable feature for a painkilling drug but not the later. The quick onset of the action also indicates quick absorption and also suggests that the CWE induced antinociception is mediated by constituent/s that is/are directly available and not through its/their metabolite/s. Another interesting feature of the CWE, is that it is well tolerated: neither produced overt signs of toxicity nor hepatotoxicity (in terms of SGOT and SGPT levels) nor renotoxicity (in terms of serum creatinine and urea levels) nor stress (as judged by exopthalmia and fur erection) nor aggressive beheviours.

In rats, food deprivation induces antinociception[13]. But such a mode of action is unlikely in this study as food was available *ad libidum* and there was no apparent suppression of food and water intake. Stress is known to induce antinociception[14] but CWE was not stressogenic. Thus, CWE was unlikely to alleviate pain by this mechanism. Sedation impairs pain [14] and several sedative drugs have pain relieving actions[14]. However, this mode of action is also unlikely to be operative here since none of the parameters of the rat hole-board test was impaired by CWE; hole-board test is a validated, sensitive and widely used to evaluate potential sedative agents[10]. Non impairments of parameters in the rat hole board test also suggests that the CWE lacks CNS depressant activity. Opiodergic mechanisms are also unlikely to be operative in this study since CWE did not elicit Struab's tail reaction [9]. In contrast, inhibition of both phases of formalin test [7] with methanolic extract of *D. triflorum* suggests the operation of opiodergic mechanisms in inducing antonociception. Further, alkaloides and flavonoids were present in the CWE and others have shown their presence in extracts of *D. triflorum* made in polar solvent[3, 4]. Several plant alkaloids and flavonoids possess potent antinociceptive action[15,16]. Thus, antinociceptive action of CWE could be attributed alkaloids and / or flavonoids. Free radicals are now implicated in pain[17]. *D. triflorum* is shown to possess potent antioxidant[3] and free radical scavenging[3] activity. Thus, it is possible that this mode of action, at least partly, could be playing a role in evoking antinociceptive activity by the CWE. Obviously, further studies are needed to elucidate the precise mode of antinociceptive action of CWE.

In conclusion, this study, shows for the first time, that CWE of *D.triflorum* is an orally active, potent and safe painkilling drug. Further, a scientific justification for the use of CWE of *D,triflorum* by Sri Lankan traditional practitioners as an analgesic is provided.

REFERENCES

1. Dassanayake MD, Clayton WD. A revised Hand book to the Flora of Ceylon. Amreind; New Delhi; 1996.
2. Chio LC, Huang KF. Studies on the constituents of *Desmodium trflorum* (L.) DC. Providence University Publications, Taiwan. 1995; pp1-14.
3. Lai SC, Ho YL, Huang SC, Huang TH, Lai ZR,Wu CR Lian KY, Chang YS. Antioxident and antiproliferative activities of

Desmodium triflorum (L) DC. American J Chinese Med 2010; 38:329-342.
4. Gosal S, Srivastava RS, Benerjee PK, Dutta SK. Alkalodes of *Desmodium triflorum*. Phytochemistry 1971; 10: 3312-3313.
5. Jayaweera DMA. Medicinal Plants.(indigenous and exotic) used in Ceylon. 5th Edn., National Science Foundation, Sri Lanka; 2006.
6. Chowdhury KK, Saha A, Bachar SC, Kundu JK. Analgesic and anti-inflammatory activities of *Desmodium triflorum* DC. J Biol Sci 2005; 5; 581-583.
7. Lai SC, Peng WH, Huang SC, Ho, YL, Huang TH, Lai ZR, Chang YS. Analgesic and anti-inflammatory activities of methanol extact from *Desmodium triflorum* DC in mice. American J Chinese Med 2009; 37: 573-588.
8. Fransworth NR. Phytochemical screening. Chicago College of Pharmacy, University of Illinois 1998.
9. Langerman L, Zakowski MI, Piskown B, Grant GJ. Hot plate versus tail flick: evaluation of acte tolerance to continuous morphine infusion in the rat model. J Pharmacol Toxicol Methods 1995; 34: 23-28.
10. File SE, Wardrill A. Validity of head dipping as a measure of exploration in modified hole-board. Psychopharmacologia 1975; 44: 53-57.
11. Wong CH, Dey P, Yarmush J, Wu WH, Zbuzek VK. Nifidefine-induced analgesia after epidural injection in rats. Anesth Analg 1994; 79: 303-306.
12. Farsam H, Amanlou M,Dehpour R, JahanianiF. Anti-inflammatory and analgesic activity of *Biebersteinia multifida* DC. Root extract. J Ethnopharmacol 2000; 71: 443-447.
13. Givorn RM, Berka C, Bernston GG, Walker JM, Sandman CA. Effect of naloxone on analgesia induced by food deprivation. Life Sci 25: 885-888.
14. Rang HP, Dale MM, Ritter JM, Moore PK., Pharmacology, 5th Edn., Churchill Livingtone, London, New York; 2003.
15. Deraniyagala SA, Ratnasooriya WD, Goonasekera CL. Antinociceptive effect and toxicological study of the aqueous bark extract of *Barringtonia racemosa* on rats. J Ethnopharmacol 2003; 46; 1-6.
16. Govindarajan R, Vijayakumar M, Rao CV, Shirwaikar A, Kumar S, Rawat AKS, Pushpangadan P. Antiinflammatory and antioxidant activities of *Desmodium gangeticum* fractions in carrageenan-induced inflamed rats. Phytotherapy Res 2007; 21: 975-979.
17. Halliwell B. Free radical antioxidents in human disease: Curiosity, cause or consequence. Lancet 1994; 344: 721-744.

Table 1. Effect of orally administered of cold water extract (CWE) of *Desmodium triflorum* on the reaction time in hot plate test in rats.(mean ± SEM n = 6).

Groups	Pre treatment	Post treatment		
	1h	2h	3h	4h
Control (Tap water)	7.55 ± 0.26	8.98 ± 0.31	7.66 ± 0.16	7.11 ± 0.15	6.93 ± 0.26
CWE 1000 mg /ml	8.31 ± 0.15	10.93 ± 1.11*	9.41 ± 0.21*	9.13 ± 0.25*	8.10 ± 0 .31
CWE 2000 mg / ml	7.48 ± 0.22	13.42 ± 0.40*	10.21 ± 0.30*	10.15 ± 0.36*	7.85 ± 0.21
CWE 4000 mg / ml	8.11 ± 0.28	14.05 ± 0.16*	10.75 ± 0.32*	10.73 ± 0.43*	7.90 ± 0.30
Aspirin 133.5 mg/kg	9.09 ± 1.15	17.50 ± 2.30*	16.00 ± 2.10*	13.20 ± 1.10*	11.70 ± 1.10*

P > 0.05, as compared with control (ANOVA with Turkey's family error post hoc test)

Source of support: Nil, Conflict of interest: None Declared