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Antinociceptive, Sedative and Muscle Relaxants Activity of *Caralluma tuberculata* N E Brown

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Abstract

The current study was designed to assess the preliminary antinociceptive, sedative and muscle relaxants activates of crude extract and various solvent fractions of Caralluma tuberculata using established in-vivo protocols. The results illustrated significant (P<0.05) antinociceptive activity of extract/fractions of the plant in a dose dependent manner (50 and 100 mg/kg i.p.). When studied in open field test, the extracts and fractions of the plant demonstrated significant (P<0.05) sedative effect. Similarly, the extracts and fractions had (P<0.05) muscle relaxant effect on traction test. However, hexane was the only fraction which did not exhibit significant activity in either of the tests. The current investigation, suggest that *Caralluma tuberculata* contain potential molecules with antinociceptive, seadtive and muscle relaxant activities.

Keywords: Antinociceptive; Sedative and muscle relaxants activity

Introduction

Caralluma tuberculata belong to family Ascalpadaceae. It has an endangered plant used to cure diabetes and to control fat accumulation. *C. tuberculata* is herb growing in dry hilly area of K.P.K Dir, Pakistan. It is also known as Pamanky in Pashto and cooked and eaten as a vegetable [1]. *C. attenuata* is also used as a folk medicine for the treatment of diabetes and rheumatism. The literature revealed that *C. tuberculata* contains luteolin-4′-O-neohesperidoside with a significant anti-inflammatory and antinociceptive activity [2,3]. Some of biological activity of the plant such as phytochemical composition, phytotoxic potential, and antioxidant capacity has already been reported [4,5].

The purpose of the current study was to evaluate the antinociceptive, sedative and muscle relaxant activity of crude extracts and various fractions of *C. tuberculata* in various animal models.

Material and Methods

Plant Material: *C. tuberculata* was collected from the mountain area of Mago, Razagram, Toormang, Dir, Khyber Pakhtunkhwa Pakistan in the month of November 2011. The plant was identified by Ghulam Jelani Department of Botany University of Peshawar Pakistan.

Extraction and fractionation: Shade dried plant of *C. tuberculata* was filled in the flask and extracted successively with methanol solvent in soxhlet extractor for 4 h. The solvent extract was concentrated under reduced pressure at 45°C using a rotary evaporator, and black crude extract obtained was suspended in water and successively partitioned with n-hexane, chloroform and ethyl acetate fractions.

Acetic acid induced writhing test

The analgesic activity was carried out using NMRI mice (18-22 g) of either sex. Animal were divided into five groups (n=6). The group I and II were injected with normal saline (10 ml/kg, i.p.) and Ibuprofen (150 mg/kg, i.p.), while the remaining groups were treated with the extract/fractions of the plant (50 and 100 mg/kg, i.p.) after the above treatment animals were injected i.p. with acetic acid (1%). The abdominal constriction (writhing) was counted for 10 min after 5 min of acetic acid injection [6].

The apparatus used in the activity was consist of an area of white wood (150-cm diameter) enclosed by stainless steel walls and divided into four squares by black lines. The open field was placed in a light and sound-attenuated room. Animals were acclimatized under red light (40 Watt red bulb) 60 min before the start of the experiment in laboratory with food and water available *ad libitum*. Animals were administered with normal saline and the extract/fractions of the plant (50 and 100 mg/kg, i.p.). After 30 min each mouse was placed in the center of the box and the numbers of lines crossed were counted [7].

Traction test

In this procedure, a metal wire coated with rubber was used, both ends of which were rigidly supported with stands about 60 cm above the laboratory bench. Different groups (n=6) were treated with diazepam (1 mg/kg), distilled water (10 ml/kg) and the extract/fractions of the plant (50 and 100 mg/kg, i.p.). The animals were exposed to the traction test after 30, 60 and 90 min of treatment. Each animal was hung by their hind legs from the wire and the time of hanging was recorded for 5s. Failure to hang for less than 5s was considered as the presence of muscle relaxant activity and vice versa [7].

Statistical analysis

Results are expressed as mean ± S.E.M. One-way ANOVA was used for comparison test of significant differences among groups followed by Dunnet's multiple comparison post test. A level of significance (P<0.05 or 0.01) was considered for each test.

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Results and Discussion

Effect of extract/fractions in antinociceptive activity

The results of antinociceptive activity of extract/fractions of the plant are shown in Figure 1. Pain reduction was observed in a dose dependent manner. Crude extract antagonized noxious stimuli of acetic acid to 80% at 100 mg/kg i.p (Figure 1A). Upon fractionation, hexane was found insignificant in pain reversal (Figure 1B). However, chloroform and ethyl acetate fractions demonstrated marked activity 60.10 and 64.75% at 100 mg/kg i.p. (Figure 1C and 1D).

Effect of extract/fractions in sedative activity

As shown in Table 1, the extract/fractions of the plant showed significant sedative activity in open field test. Apart from hexane, extract and fractions illustrated significant (P<0.05) sedative activity in open field test.

Effect of extract/fractions in traction test

The effect of extract/fractions of the plant in traction is displayed in Table 2. The crude extract and fractions of the plant provoked significant (P<0.05) activity in traction test (muscle relaxant).

Acetic acid induced writhing test has been primarily used by various research groups for the assessment of antinociceptive of natural compounds worldwide [8,9]. Acetic acid caused the release of different endogenous noxious mediators such as bradykinin, serotonin, histamine, substance P [10-12]. The resulting pain is symbolized by contraction of the abdominal muscle accompanied by an extension of the forelimbs and body elongation. The peripheral nociceptive fibers are sensitive to both narcotics analgesic and non-steroid anti-inflammatory drugs [13]. The extract/fractions of the plant (50 and 100 mg/kg, i.p.) showed marked reduction in the abdominal constriction provoked by the acetic acid in a dose dependent manner. Consequently, one possible mechanism of antinociceptive activity of the extract/fractions of the plant could be due to the blockade of the effect or the release of endogenous substances (arachidonic acid metabolites) that excite pain nerve endings.

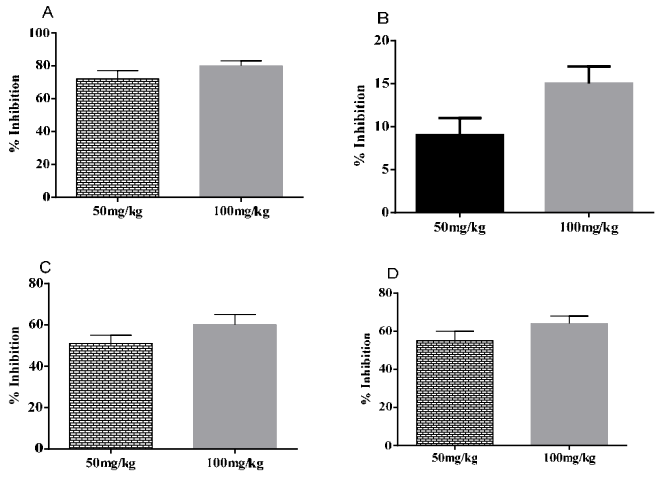

Figure 1. Percent antinociceptive effect of extract/fractions (A) crude extract, (B) hexane, (C) chloroform and (D) ethyl acetate fraction of *Caralluma tuberculata* in acetic acid induced writhing test. Data presented as mean ± S.E.M, (n=6) compared with control. Ibuprofen (150 mg/kg, i.p.) produced 81% activity.

Sample	Dose	No. of lines crossed
Control	10 ml/kg	130 ± 3.95
n- Hexane	50 mg/kg	126 ± 4.55
	100 mg/kg	131 ± 4.25
CHCl3	50 mg/kg	119 ± 3.90
	100 mg/kg	112 ± 3.65*
ETOAC	50 mg/kg	100 ± 3.85*
	100 mg/kg	95 ± 4.10*
MeOH	50 mg/kg	105 ± 4.55
	100 mg/kg	99.0 ± 2.98*
Bromazepam	5 mg/kg	9 ± 0.55***

Data represent the number of lines crossed by animal in box, 30 min after treatment with normal saline (10 ml/kg, control), SLO (50, 100 and 200 mg/kg) or bromazepam (5 mg/kg). Data presented as mean ± S.E.M, (n=6). *P<0.05, ***P<0.001, all compared with control.

Table 1. Effect of crude extract and fractions of *Caralluma tuberculata* in locomotive test (sedative activity)

Group	Dose	Traction test (%)		
		30 min	60 min	90 min
Control	10 ml/kg	0 ± 0.00	0 ± 0.00	0 ± 0.00
Diazepam	0.25 mg/kg	100** ± 0.00	100** ± 0.00	100** ± 0.00
n- Hexane	50	3 ± 0.23	5 ± 0.59	12 ± 0.95
	100	4 ± 0.45	9 ± 0.90	16 ± 0.95
CHCl3	50	5 ± 0.06	8 ± 0.07	17.10
	100	11 ± 0.95	20 ± 1.19	27.45 ± 1.10
ETOAC	50	0 ± 0.00	10 ± 0.75	15 ± 1.25
	100	12 ± 0.89	23 ± 1.05	32.30 ± 2.15*
MeOH	50	5 ± 0.3	11 ± 0.58	15 ± 1.75
	100	13 ± 0.90	21 ± 1.54	29.22 ± 1.45*

Values represent the percentages of mice (n=6) showing negative effects in the traction test 30,60 and 90 min after treatment with distilled water (10 ml/kg), extract/fractions 50 and 100 mg/kg) or diazepam (0.25 mg/kg). Data presented as mean ± SEM (n=6) *P\0.05 and** P\0.01, both compared with controls.

Table 2. Percent effect of extract/fractions of *Caralluma tuberculata* in traction test.

The open field assay is frequently employed as a prognostic test for the assessment of the sedative properties of natural agents [7]. Pretreatment of mice with extract/fractions of the plant showed dose dependent reduction in locomotive activity in open field test as compared to control. The reduction in the frequency and amplitude of motion could be attributed to the sedative effect of *C. tuberculata*. Bromazepam was more prominent in its effect [14]. The resulting sedative effects of extract/fractions of the plant were similar to standard drug, bromazepam.

The muscle relaxant activity of extract/fractions of the plant was assessed in traction test which is generally used for the said purpose [7]. The results showed significant muscle relaxant activity of the extract/fractions of the plant after 90 min of treatment.

Conclusion

It is concluded that the extract/fractions of *C. tuberculata* possessed strong antinociceptive, sedative and muscle relaxant components. Further studies on the isolation could be helpful in the identification of individual constituent responsible for current show.

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