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NOTE

In vitro Anthelmintic Activity of Aerial Parts of *Vetiveria zizanioides* Linn. Nash

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Keeping in view the traditional claim of use of leaf juice of khus grass as anthelmintic, the aqueous extract of leaves of *Vetiveria zizanioides* (Linn.) Nash was tested for anthelmintic activity against Indian earthworm (*Pheretimaposthuma*), roundworm (*Ascaridia galli*) and tapeworms (*Raillietina spiralis*). Various concentrations of extract were tested and results were expressed in terms of time for paralysis and time taken for death of worms and helminths. Dose dependent activity was observed in the aqueous extract thus may be useful as an anthelmintic.

Key Words: Anthelmintic, *Vetiveria zizanioides* (Linn.) Nash, Piperazine citrate.

Vetiveria zizanioides (Linn.) Nash (Poaceae), popularly known as khus grass, has been known in India since ancient times. It is the major source of the well-known oil of vetiver, which is used in medicine and in perfumery[1]. In India, the roots have been used for making screens, mats, hand fans and baskets. Different morphological parts of the grass are used for various ailments, such as boils, burns, epilepsy, fever, scorpion sting, snakebite and sores in the mouth. The root extract is used for headache and toothache, the leaf paste is used for lumbago, sprain and rheumatism, the stem decoction for urinary tract infection, the leaf juice as an anthelmintic, the vapours for malarial fever and the root ash is given for acidity relief[2]. The plant have been reported to have antimycobacte-rial[3], antibacterial[4], antipyretic[5], antioxidant[6], larvicidal, repellent and insecticidal properties[7].

Traditionally, leaves of *V. zizanioides* are used as anthelmintic but scientifically it is not revealed yet. Thus, the present study was designed to evaluate the *in vitro* anthelmintic activity of aqueous extract of *V. zizanioides*.

Piperazine citrate (CDH Ltd.) used as standard. The leaves of *V. zizanioides* were collected from the local market of Muzaffarnagar and authenticated in the Department of BPharmacognosy, S. D. College of Pharmacy & Vocational Studies, Muzaffarnagar, India. A voucher specimen (PK-113) has been deposited in the herbarium.

Preparation of extract: Aqueous extract of dried and powdered leaves was prepared by cold maceration method for 24 to 48 h. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and dried (aqueous extract of *V. zizanioides*: AVZ, 9.7 % w/w). The crude extract was subjected to chemical characterization and evaluation of anthelmintic activity.

Animals: Indian adult earthworms (*Pheretima posthuma*), roundworm (*Ascaridia galli*) and tapeworms (*Raillietina spiralis*) were used to evaluate anthelmintic activity *in vitro*. Earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. Roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the fecal matter. These intestines were then dissected and worms were collected and kept in normal saline solution. Earth worms were used for anthelmintic activity[8] due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings[9]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*[10]. The average size of earthworm, round worm and tapeworm was 6-8, 5-7 and 6-8 cm respectively. Earthworm and helminths were identified in Department of Pharmacognosy, S. D. College of Pharmacy & Vocational Studies, Muzaffarnagar, India.

Anthelmintic activity: The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*[11]. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation of anthelmintic activity[12].

TABLE-1
ANTHELMINTIC ACTIVITY OF AQUEOUS EXTRACT OF *Vetiveria zizanioides* LEAVES (AVZ)

Groups	Conc. Used (mg/mL)	Time taken for paralysis (P) and death (D) of worms (mins)					
		Pheretima posthuma (Earthworm)		*Ascaridia galli* (Roundworm)		*Raillietina spiralis* (Tapeworm)	
		P	D	P	D	P	D
Control	-	A	A	A	A	A	A
AVZ	25	21.18 ± 0.56	28.91 ± 0.73	22.78 ± 0.77	49.08 ± 0.58	27.53 ± 1.00	52.18 ± 0.95
	50	12.16 ± 0.75	16.35 ± 0.57	15.58 ± 0.67	40.20 ± 0.65	17.75 ± 0.89	42.87 ± 0.60
	75	07.79 ± 0.98	10.37 ± 0.55	09.55 ± 0.76	32.08 ± 0.58	8.48 ± 0.71	26.59 ± 0.78
	100	02.71 ± 0.47	06.40 ± 0.56	04.72 ± 0.71	28.08 ± 0.58	5.23 ± 0.60	20.50 ± 0.76
Piperazine citrate	10	15.16 ± 0.58	29.39 ± 0.58	10.17 ± 0.59	11.38 ± 0.66	23.07 ± 0.58	53.33 ± 0.88

Results are expressed as Mean ± SEM (n=6). 'A' indicates absence of activity in 24 h of administration

Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier[13]. Test samples of aqueous extract of *V. zizanioides* were prepared at the concentrations, 25, 50, 75 and 100 mg/mL in distilled water and six worms *i.e. Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each Petri dish containing 25 mL of above test solution of extracts. Piperazine citrate (10 mg/mL) was used as reference and distilled water as control[14]. This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis when no movement of any sort could be observed except when the worms were shaken vigorously and time for death of worms after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 ºC). All the results are shown in Table-1.

Statistical analysis: All the results are expressed as mean ± SEM, where n = 6.

Preliminary phytochemical studies on *V. zizanioides* revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins and triterpenoids. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. From the result aqueous extract of *V. zizanioides* show an anthelmintic activity when compared to the standard drug. Each crude extract at the concentration of 25, 50, 75 and 100 mg/mL produced anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/mL concentration. The same effects were observed with reference drug piperazine citrate. Phytochemical analysis of the crude extracts revealed the presence of tannins as one of the chemical constituents. Tannins were shown to produce anthelmintic activity[15]. Chemically tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics *e.g.*, niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[16]. It is possible that tannins contained in the extracts of *V. zizanioides* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal[17] or glycoprotein on the cuticle of the parasite[18] and cause death.

Conclusion

The traditional claim of *V. zizanioides* leaves as an anthelmintic has been confirmed as the aqueous extract showed activity against *Pheretimaposthuma*, *Ascaridia galli* and *Raillietina spiralis*. Further studies are necessary to isolate and reveal the active compound contained in the crude extracts of leaves of *V. zizanioides* responsible for activity and to establish the mechanism of action.

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