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NOTE

In vitro Anthelmintic Activity of Seeds of *Cicer arietinum* Linn.

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Methanolic extract of seeds of *Cicer arietinum* Linn. 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 methanolic extract of *Cicer arietinum* Linn. may be useful as an anthelmintic.

Key Words: Anthelmintic, *Cicer arietinum Linn*., Piperazine citrate.

The plant *Cicer arietinum* Linn. belonging to family Fabaceae is largely cultivated in most parts of India. Seed is aphrodisiac, anthelmintic, tonic, enriches the blood, cures skin diseases, inflammation and more especially of ear. It is also used as diuretic[1], halitosis, hepatosis, otitis, pharyngosis, pulmonosis and splenosis[2]. It is ingredient of a Unani anti-hypertensive drug Ajmaloon[3] and Nakhud[4]. Leaves are sour, astringent, improve taste and appetite, cure bronchitis, causes flatulence. Tart leaves are orexigenic, enterosis[1]. Isoflavonoids isolated from *C. arietinum* shows antifeedant activity[5]. Biochanin-A and formononetin isolated from *C. arietinum* were evaluated for management of diabetes mellitus[6]. Pangamic acid isolated from aqueous extract of *C. arietinum* has been evaluated for stamina building, antistress, antihyperlipidemic activity[7]. The aqueous seed coat extract exhibited diuretic activity[8].

Traditionally, *C. arietinum* is used as anthelmintic[2] but scientifically it is not revealed yet. Thus the present study was designed to evaluate this traditional claim. For this purpose the methanolic extract of seeds of *C. arietinum* was tested for anthelmintic activity against indian earthworm (*Pheretima-posthuma*), roundworm (*Ascaridia galli*) and tapeworms (*Raillietina spiralis*).

The seeds of *C. arietinum* were collected from the local market of Muzaffarnagar and authenticated in the Department of Pharmacognosy, S.D. College of Pharmacy & Vocational Studies, Muzaffarnagar, India (Voucher specimen no. PK-121).

Preparation of extract: The collected materials was dried under shade and then made into a coarse powder using grinder. The dried and powdered seeds of *C. arietinum* were defatted with petroleum ether (60-80 ºC). Then the dried marc was soaked in methanol overnight and extracted in a Soxhlet's apparatus for 24 h (methanolic extract of seeds of *C. arietinum*: MCA, 8.4 % 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[9] due to its anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoids*, of human beings[10]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*[11]. 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.*[12]. The assay was performed *in vitro* using adult earthworm (*Pheretima*

TABLE-1
ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACT OF *C. arietinum seeds*

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
MCA	25	14.67 ± 0.88	62.67 ± 0.71	20.17 ± 0.60	51.5 ± 0.76	25.5 ± 0.76	52.02 ± 0.86
	50	08.50 ± 0.99	52.17 ± 0.60	14.65 ± 0.56	42.53 ± 0.74	15.0 ± 0.58	42.67 ± 0.49
	100	06.50 ± 0.85	45.00 ± 0.58	08.17 ± 0.70	38.67 ± 0.88	05.07 ± 0.58	23.33 ± 0.88
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

posthuma) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation of anthelmintic activity[13]. Use of *Ascaridia galli* and *Raillietina species* as a suitable model for screening of anthelmintic drug was supported earlier[14]. Test samples of the extract were prepared at the concentrations, 25, 50 and 100 mg/mL in 1 % gum acacia in normal saline and six worms *i.e. Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) in each group 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 normal saline as control[15]. This procedure was adopted for all three different types of worms. All the test and reference drug solutions 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 were shown in Table-1.

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

Preliminary phytochemical studies on *C. arietinum* revealed the presence of alkaloids, carbohydrates, proteins, steroids, glycosides, flavonoids, tannins and phenolic compounds. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity. From the result of the methanolic extract of seeds of *C. arietinum* shows an anthelmintic activity when compared to the reference drug. Methanolic extract of seeds of *C. arietinum* at the concentration of 25, 50 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[16]. 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[17]. It is possible that tannins contained in the extracts of *C. arietinum* produced similar effects.

Conclusion

The traditional claim of *C. arietinum* seeds as an anthelmintic has been confirmed as the methanolic extract shows 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 *C. arietinum* responsible for activity and to establish the mechanism of action.

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