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Anthelmintic activity of aqueous extracts of some Saponin containing medicinal plants

Lipi Purwal[1], Vivek Shrivastava[1], Krishna K Makode[1], Umesh K Jain [1]*

[1]Bhopal Institute of Technology and Science -Pharmacy,
Bhojpur Road, Bangrasia, Bhopal (M.P).

ABSTRACT

Helmintic infections are now being recognized as cause of chronic ill health and sluggishness amongst the tropical people. The aim of present study was to evaluate anthelmintic prospective of crude aqueous extracts of some saponins containing medicinal plants. The dried roots and leaves of W. somnifera and dried flowers and leaves of C. officinalis were used for the preparation of the extracts. Four concentrations (2, 4, 6 and 8 mg/ml) of aqueous extracts were investigated for in vitro anthelmintic activity employing Indian adult earthworms (Pheretima posthuma), which involved determination of time paralysis (P) and the time of death (D) of the worms. Albendazole (10 mg/ml) was included as standard reference and distilled water as control. All the four aqueous extracts of roots and leaves of W. somnifera (L.) and dried flowers and leaves of C. officinalis (L.) showed significant anthelmintic activity. All the investigated extracts showed significant difference ($p<0.001$) against negative control. The study confirmed the anthelmintic potential of these extracts and further studies are suggested to isolate the saponins and other active principles responsible for the activity.

Key words: *Withania somnifera, Calendula officinalis, Pheretima posthuma*; Anthelmintic activity.

INTRODUCTION

Helminthosis play a critical role in small ruminant production leading to huge financial losses particularly in areas where extensive grazing is accomplished [1]. Development of resistance to most of the commercially available anthelmintic has become a severe problem globally [2] and evaluation of the activities of medicinal plants claimed for anthelmintic property is getting attention these days [3-7]. *W. somnifera* (Hindi–Ashwagandha, English–Indian Ginseng/winter cherry) is an official drug mentioned in the Indian Herbal Pharmacopoeia and Ayurvedic

Pharmacopoeia [8,9]. The plant has been reported to have anticancer, anabolic, antisertogenic [10] anti-inflammatory, antistress, antioxidant, immunomodulatory, heamopoietic and rejuvenating properties [11]. The chemistry has been extensively studied and over 35 chemical constituents had been extracted, isolated and identified in *W. somnifera*. The biologically active chemical constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitoindoside IX and X) [11]. *Calendula officinalis* has been reported to have wound healing, antioxidant immunostimulent, hepatoprotective, antibacterial and antifungal activity [12].The various chemical constituents present in *C. officinalis* are terpenoids, flavonoids, coumarins, volatile oil and quinones [12] .The flowers of *C. officinalis* contains flavonol glycosides, triterpene oligoglycosides, saponins and sesquiterpene glucosides [13].The present study was under taken to evaluate the *in vitro* anthelmintic activity of crude extracts of dried leaves and roots of *W. somnifera* and dried leaves and flowers of *C. officinalis* employing different concentrations against Indian adult earthworms (*Pheretima posthuma).*

METRIALS AND METHODS

Plant Material

The dried roots and leaves of *W. somnifera* and dried flowers and leaves of *C. officinalis* were used for the preparation of the extracts. The dried roots and leaves of *W. somnifera* and dried flowers and leaves of *C. officinalis* were collected in the month of Jan 2010 from medicinal garden of Bhopal Institute of Technology & Science –Pharmacy, Bhopal, Madhya Pradesh, India. The samples were authenticated by Dr. Pradeep Tiwari, Deparment of Botany, Dr. Hari Singh Gour University, Sagar, M.P., India, where the voucher specimens (Bot/Her's/A-551) and (Bot/Her's/3201) respectively were deposited.

Preparation of aqueous extracts:

The plant materials (dried roots and leaves of *W. somnifera* and dried flowers and leaves of *C. officinalis*) was collected and shade dried for several days .The parts were powdered with the help of electric grinder. The aqueous extract of dried roots and leaves of *W. somnifera* and dried flowers and leaves of *C. officinalis* were prepared by decoction method [14]. The aqueous extractive value for roots and leaves of *W. somnifera* were 18.5% and 16.7% respectively on dried weight basis and that for aqueous extract of *C. officinalis* flowers and leaves were 17.7% and 15.7% w/w respectively.

Preparation of test sample:

Samples for *in-vitro* study were prepared by dissolving the all extracts in distilled water so as to get final concentration of 2, 4, 6 and 8 mg/ml strength.

Standard drug:

Albendazole was purchased from P.K. Scientific, Bhopal.

Used organisms:

Indian adult earthworms (*Pheretima posthuma* (Annelida)) 10-12 cms in length and 0.2-0.3cms in width were taken for all the experimental protocol. Indian adult earthworms were obtained from water logged area of moist soil and washed with normal saline to remove all faecal matter.

Doses:
Aqueous extracts of dried roots and leaves of *W. somnifera* and dried flowers and leaves of *C. officinalis* were investigated at 2, 4, 6 and 8 mg/ml strength for anthelmintic activity.

Experimental Methods
Preliminary Phytochemical Investigation:
The crude extracts were further subjected to phytochemical analysis as per the standard procedures. [15-16]

Anthelmintic Assay:
The anthelmintic assay was carried as per the method of Ajaiyeoba *et al*., [17] with necessary modifications. The assay was performed on Indian adult earth worm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being [18-19]. Because of easy availability earth worms have been widely used for the initial evaluation of anthelmintic compounds [20-21].
Eighteen groups of approximately equal size Indian earthworms consisting of ten worms in each group were released into 50 ml of desired formulation. Each group was treated with the following:

1. **Group 1** received distilled water which served as control.
2. **Group2** received Albendazole at the dose of 10 mg/ml as the standard.
3. **Group3, 4, 5, 6** received aqueous extracts of ***Withania somnifera*** **roots (WSR)** at the dose of 2, 4, 6, 8 mg/ml respectively.
4. **Group 7, 8, 9, 10** received aqueous extracts of ***Withania somnifera*** **leaves (WSL)** at the dose of 2, 4, 6, 8 mg/ml respectively.
5. **Group 11, 12, 13, 14** received aqueous extracts of ***Calendula officinalis*** **flowers (COF)** at the dose of 2, 4, 6, 8 mg/ml respectively.
6. **Group 15, 16, 17, 18** received aqueous extracts of ***Calendula officinalis*** **leaves (COL)** at the dose of 2, 4, 6, 8 mg/ml respectively.

Observations were made from the time taken for paralysis and / or death of individual earthworms. The time of paralysis was noted when worms do not revive even in the normal saline and when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility.

Statistical Analysis
The data were subjected to statistical analysis one-way ANOVA followed by Dunnett post-test was employed to identify pairs of results with significantly different means. Both ANOVA and post-test were performed by Graph- Pad InStat computer package

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening of all extract revealed the presence of saponins glycosides.
The aqueous extracts of both the plants **showed significant** anthelmintic activity as compared to negative control. Data (table 1) revealed that all the tested extracts of ***Withania somnifera*** and *Calendula officinalis* possess anthelmintic activity in dose dependent manner giving shortest

time of paralysis (P) and death (D) with 8 mg/ml concentration of aqueous leaf extract of ***W. somnifera.*** From the results shown in table 1, the predominant effect of Albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. The aqueous leaf extract of ***W. somnifera*** demonstrated paralysis as well as death of worms comparable to Albendazole especially at higher concentration of 8 mg/ml. The root extract of ***W. somnifera*** at concentration of 8 mg/ml also showed significant activity.

Table I: Screening of *In vitro* Anthelmintic Activity of *W. somnifera* and *C. officinalis.*

The aqueous leaf extract of ***W. somnifera*** *at the concentration of* 8 mg/ml caused paralysis in 20.63 min and time of death was 43.74 min while aqueous root extract of ***W. somnifera*** revealed paralysis at 29.0 min and death in 76.7min.

Part used	**Groups**	**Aqueous extract (mg/ml)**	**No. of worms used**	**Paralysis Time (min)** (Mean ±SE)	**Death Time (min)** (Mean ± SE)
Albendazole	AL10	10	10	26±1.52 [a]	48.3±1.97 [a]
Distill Water	DDW	-	10	0	0
***Withania somnifera* Roots (WSR)**	WSR2	2	10	177.9±2.37[a]	324.5±15.66 [a]
	WSR4	4	10	164.6±3.38 [a]	256.3±6.55 [a]
	WSR6	6	10	143.8±3.83 [a]	190.9±7.76 [a]
	WSR8	8	10	29.0±1.90 [a]	76.7±4.51 [a]
***Withania somnifera* Leaves (WSL)**	WSL2	2	10	165.1±3.83 [a]	294.3±8.11 [a]
	WSL4	4	10	122.4±3.39 [a]	189.8±5.34 [a]
	WSL6	6	10	30.63±2.45 [a]	76.4±4.71 [a]
	WSL8	8	10	20.63±1.33 [a]	43.74±2.88 [a]
***Calendula officinalis* Flowers (COF)**	COF2	2	10	167.6±2.517 [a]	642.8±2.32 [a]
	COF4	4	10	146.3±1.73 [a]	464.7±3.49 [a]
	COF6	6	10	134.8±1.67 [a]	272.3±3.24 [a]
	COF8	8	10	56.5±1.93 [a]	111.2±1.58 [a]
***Calendula officinalis* Leaves (COL)**	COL2	2	10	211.7±2.33 [a]	366.3±3.040 [a]
	COL4	4	10	150.7±2.09 [a]	291.8±2.064 [a]
	COL6	6	10	111.9±1.94 [a]	182.9±2.57 [a]
	COL8	8	10	87±2.611 [a]	168.6±1.950 [a]

Calendula officinalis flowers and leaf extracts were also shown to have anthelmintic activity but the anthelmintic effect with respect to paralysis time and death time was less as compared to both the extracts of ***W. somnifera.*** The crude extracts of ***C. officinalis*** flowers and leaf extracts demonstrated paralysis at 56.5 min and death of worms at 111.2 minutes.

Both the plants contain saponins and have also shown anthelmintic potential which are in accordance with previous reports which reveals that saponins are known to have anthelmintic activity [22].

CONCLUSION

The aqueous extracts of both the plants **showed significant** anthelmintic activity as compared to negative control. This result may lend support for the use of these plants as anthelmintic. From this study, both the plants investigated were found to be active as anthelmintic with regard to both paralysis and death times. This validates their uses in treating management of worm infestations. Further studies are required to establish the mechanisms of action.

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