

ORIGINAL RESEARCH ARTICLE

Investigation of Anthelmintic Activity of *Withania somnifera*Laxmi Patidar¹, Vikas Patidar¹, Kratika Daniel^{2*}, Vivek Daniel², Swapnil Goyal³¹Research scholar, Mandsaur Institute of pharmacy, Mandsaur, M.P, India²Department of Pharmaceutical Chemistry, Mandsaur Institute of pharmacy, Mandsaur, M.P, India.³Department of Pharmacology, Mandsaur Institute of pharmacy, Mandsaur, M.P, India

Received 25 Aug 2012; Revised 03 Dec 2012; Accepted 14 Dec 2012

ABSTRACT

The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Helminth infections are the most common health problems in India and in developing countries. Present study evaluates the anthelmintic activity of various extracts of leaves of *Withania somnifera* using Indian earthworms i.e. *Pheritima posthuma* (5-7cm size). Different extracts (Petroleum ether, Chloroform, Methanolic and hydroalcoholic 50% extracts) of leaves of *Withania somnifera* at the concentration of 10, 20 and 50 µg/ml were tested. The results obtained indicate that *Withania somnifera* has anthelmintic activity that supports the folk medicinal use of the plant. The effects of standard drug as albendazole (10 µg/ml) also evaluated. The results of present study indicate that the hydroalcoholic and chloroform extract significantly demonstrated paralysis and also caused death of worms in dose dependent manner.

Key words: Helminth, *Withania somnifera*, albendazole, anthelmintic.**INTRODUCTION**

Natural products are important sources for biologically active drugs [1]. There has been a growing interest in the study of medicinal plants as natural products in different parts of the world [2]. Natural products including plants, animals and minerals have been the basis of treatment of human diseases [3] problems with drug-resistant microorganisms, side effects of current and emerging diseases where no medicines are available have stimulated renewed interest in plant as a important source of new medicines. Current estimates indicates that about 80 % of people in developing countries still rely on traditional medicine based largely on various species of plant and animals for their primary healthcare [4].

The development of anthelmintic drug-resistance in helminthes against synthetic drugs have been reported in number of countries [5-8] which gives a clear indication that control programs based exclusively on their use are not sustainable. Even though the majority of infections due to worms are limited to tropical regions, these can develop in individuals who had visited such infected areas and then, can transmit the disease in temperate regions [5]. Some anthelmintic drugs such as praziquantel and albendazole, are contraindicated

for certain groups of patients like pregnant and lactating woman [6]. This has led to the increase in interest of ethno medical practices across the world for the use of medicinal plants in treatment of helminthic diseases [7]. Plants are known to provide a rich source of herbal anthelmintic, antibacterial and insecticides remedies [8, 9]. A number of medicinal plants have been used in the treatment of parasitic infections in man as well as in animals [10-12].

Withania somnifera also known as Indian ginseng or Ashwagandha is a plant belonging to Solanaceae family. Traditionally, it has been used in treatment of various disorders such as inflammation, fevers, and to protect against infection or illness [12]. The chemistry of *Withania* species has been extensively studied and several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids, tannin etc. have been identified, extracted, and isolated. At present, more than 12 alkaloids, 40 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated and reported from aerial parts, roots and berries of *Withania* species. [13 - 19]

Scientifically various pharmacological activities have been reported anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems.^[13] The mechanisms of action for these properties are not fully understood. Toxicity studies reveal that ashwagandha appears to be a safe drug.

In search anthelmintic activity, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in-vitro* because they resemble intestinal worms in their interaction with anthelmintic and their easy availability. It has been demonstrated that all anthelmintic are toxic to earthworms also and thus support their use in the investigation of anthelmintic activity^[17]. The present research is thus motivated by the need to find new substances of natural origin which possess anthelmintic activity with a low degree of toxicity for humans^[18].

MATERIALS AND METHODS

Plant Material

Leaves of *W. somnifera* (Solanaceae) were purchased from local market of Mandsaur, Madhya Pradesh, India in September 2011. The plant material was identified and authenticated by Dr. Gyanendra Tiwari (Scientist from K.N.K. College of horticulture, Mandsaur) and herbarium was submitted in Department of Pharmacognosy at Mandsaur Institute of Pharmacy, Mandsaur, India.

Preparation of Extract

The plant materials were cleaned, dried under shade and pulverized by using grinder. 500g of the powder of plant was successively extracted with Petroleum ether, chloroform, methanol and hydroalcoholic (50%) using Soxhlet apparatus. The percentage yield of different extracts Petroleum ether, chloroform, methanols, hydroalcoholic (50%) were found to be 1.61 %, 3.46 %, 13.62 % and 15.66 % respectively. The Preliminary Phytochemical were performed for the presence of different phytoconstituents like alkaloids, flavonoids, tannin, steroids and saponin

Animals

The earthworm *Pheretima posthuma* (Annelida, Megascolecidae) was used for evaluating the anthelmintic activity the earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworm *Pheretima posthuma* as it has anatomical and

physiological resemblance with the intestinal roundworm parasites of human beings, hence can be used to study anthelmintic activity.

Anthelmintic activity

The anthelmintic activity was performed according to the method of Kratika *et al* 2010^[19-22]. The animals were divided into six groups containing six earthworms in each group. All the extracts of *W. somnifera* were dissolved in minimum quantity of DMF and then final volume was adjusted to 10mL with Normal saline. The reference standards and extract solutions were prepared freshly before starting the experiment. Albendazole was used as standard, where normal saline solution used as control. All the earthworms were released into 10mL of respective formulation as follows: vehicle control (5% DMF in normal saline), Albendazole (10µg/mL), Petroleum ether extract (10, 20 and 50 µg/mL), Chloroform extract (10, 20 and 50 µg/mL), methanolic extract (10, 20 and 50 µg/mL), and hydro alcoholic extract (10, 20 and 50 µg/mL).

Observations were made for the time taken to paralyze or death of individual worms. Paralysis was said to occur when the worms do not receive any sense even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color^[23], when dipped in warm water (50°C). All the result was expressed as a mean ± SEM of six animals in each group.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis showed the presence of flavonoids, steroids alkaloids and tannins like phytoconstituents in extracts of *W. somnifera*. *In vitro* anthelmintic activity results shown in Table 1, the predominant effect of albendazole on the worm is to cause flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis^[23].

From the results (**Figure 1**) it is observed that Chloroform extract and Hydroalcoholic (50%) extract of *W. somnifera* showed significant anthelmintic activity. The graph revealed dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. The petroleum ether extract at dose 5, 10 and 50 µg/ml concentrations paralysis was observed respectively at 20.74±0.23, 16.45±0.19 and

7.15±0.05 min and death at 25.66±0.27, 20.42±0.47 and 11.48±0.17 min post - exposure. In case of chloroform extract at 5, 10 and 50 µg/ml produced paralysis within 22.43±0.19, 13.18±0.16 and 7.52±0.04 min, respectively, while death was observed within 30.58±0.20, 18.47±0.19 and 12.43±0.19 min respectively. In case of Methanolic extract at 5, 10 and 50 µg/ml produced paralysis within 22.01±0.21, 14.54±0.16 and 8.20±0.06 min, respectively, while death was observed within 28.23±0.27, 18.50±0.22 and 10.55±0.10 min respectively. In case of Hydro alcoholic (50%) extract at 5, 10 and 50 µg/ml produced paralysis within 12.84±0.15, 8.17±0.016 and 5.00±0.10 min, respectively, while death was observed within 20.17±0.21, 12.24±0.08 and 8.16±0.06 min respectively. The standard drug albendazole (10 µg/ml) showed paralysis at 4.01±0.11 min and death occurred after 10.27±2.18 min. The results were compared with the standard drug, albendazole and it was found that all extract posses anthelmintic activity in dose dependent manner.

From the above result, it is concluded that the extracts of the plant have potent anthelmintic activity when compared with the conventionally used drugs and is equipotent to standard drug. Further results, using *in vivo* models are required to carry out and establish the effectiveness and pharmacological rationale for the use of the plant as anthelmintic drug.

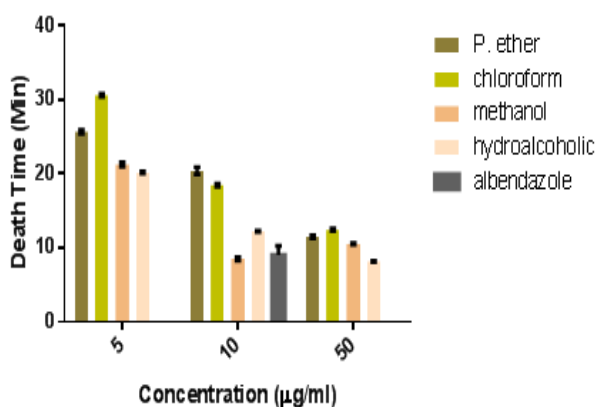
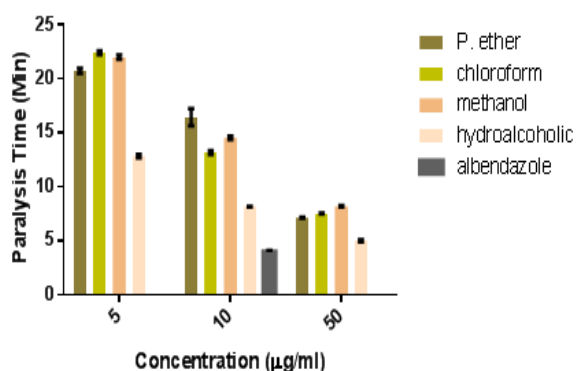


Table 1: Anthelmintic activity of extracts of *W. somnifera*

Groups	Concentration (µg/ml)	<i>Pheretima posthuma</i>	
		Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)
1	Control saline solution	---	---
2	Pet Ether extract	5	20.74±0.23
	10	16.45±0.19	20.42±0.47
	50	7.15±0.05	11.48±0.17
3	Chloroform extract	5	22.43±0.19
	10	13.18±0.16	18.47±0.19
	50	7.52±0.04	12.43±0.19
4	Methanolic extract	5	22.01±0.21
	10	14.54±0.16	18.50±0.22
	50	8.20±0.06	10.55±0.10
5	Hydro alcoholic extract (50%)	5	12.84±0.15
	10	8.17±0.016	12.24±0.08
	50	5.00±0.10	8.16±0.06
6	Standard drug (Albendazole)	10	4.01±0.11
			10.27±2.18

Each value represents mean ± SEM (N=6).

Data were analyzed using ANOVA and expressed as Mean ± SEM (N =6) followed by Dunnett's test.

REFERENCES

- Saleh Alqasoumi, I., Mohammad Al-Dosari, S. Abdul Malik, Alsheikh, M. and Maged Abdel-kader, S.: Res. J. Med. Plants, 2009.3(1): 9-15 .
- Gazzaneo, Ir., Paiva de lucena, R.F. and Paulino de Albu querque, U.: J. Ethnobiol. Ethnomed. 2005. 1: 9 .
- Patwardhan B. et al; Ayurveda and natural product drugs discovery. Current science. 2004. 86.25.789-799.
- Dahanukar, S.A., Kulkarni, R.A., Rege, N.N. Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology. 2000.32.,81-118.
- Dhar D.N., Sharma R.L. and Bansal G.C., Gastrointestinal nematodes in sheep in Kashmir, *Vet. Parasitol*, 11, 271-7 (1982)
- Jackson F., Anthelmintic resistance, the state of play, *British Vet. J.*, 149, 123-38 (1993).
- Van Wyk J.A., Malan F.S. and Randles J.L., How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics, *Vet. Parasitol.*, 70, 111-22 (1997).
- Waller P.J., The global perspective of anthelmintic resistance in nematode parasites of sheep excluding Australasia, *Proc. 4th Intl. Cong. Sheep Vet.*, Armidale, Australia, 59-63 (1997).
- Bundy D.A., Immunoepidemiology of intestinal helminthic infection I: The global burden of intestinal nematode disease, *Trans Royal Soc Trop Med Hyg*; 8, 259- 61 (1994).

10. Savioli L., Crompton D.W.T. and Neira M., Use of anthelmintic drugs during pregnancy, *American Journal of Obstetrics and Gynecology*, 188, 5–6 (2003).
11. Alawa C.B.I., Adamu A.M., Gefu J.O., Ajanusi O.J., Abdu P.A., Chiezey N.P., Alawa J.N. and Bowman D.D., In vitro screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity, *Veterinary Parasitology*, 113, 73–81 (2003).
12. Satyavati G.V., Raina M.K. and Sharma M., *Medicinal Plants of India*, Vol. I, pp: 201–06. Indian Council of Medical Research, New Delhi (1976)
13. Lewis W.H. and Elvin-Lewis M.P.H., *Medicinal Botany Plants Affecting Man's Health*, John Wiley and Sons, New York (1977).
14. Nadkarni A.K., *Indian Materia Medica*, 3rd Ed. Popular Prakashan, Bombay, India (1954).
15. Chopra R.N., Chopra I.C., Handa K.L. and Kapur L.D., *Chopra's Indigenous Drugs of India*, U.N. Dhur and Sons (P) Ltd., Calcutta, India, 303 (1958).
16. Akhtar M.S., Iqbal Z., Khan M.N. and Lateef M. Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo–Pakistan subcontinent, *Small Rumin. Res.*, 38, 99–107 (2000).
17. Mishra L.C., Singh B.B., Dagenais S., Scientific Basis for the Therapeutic Use of *Withania somnifera* (Ashwagandha): A review. *Alternative Medicine Review*; 5(4), 334-346 (2000).
18. Sollmann T., Anthelmintics: Their efficiency as tested on earthworms, *J Pharm Exp Ther*; 12,129-70 (1918).
19. Shrivastava Kratika, Sureka Shruti, Goyal Swapnil, Panwar Mangal Singh, Dave Kapilesh, Singh Narendra. In-Vitro Anthelmintic Activity of Hydro Alcoholic Extract of Leaves of *Nyctanthes Arborescens* Linn. *Journal of Global Pharma Technology*, 2009, 1 (1): 101-104.
20. Kratika Shrivastava, Vivek Daniel, Pramod S. Kunwar, Swapnil Goyal, V.D. Gupta. Synthesis And Biological Activities Of Some Novel 1,2,4-Triazole [3,4-B][1,3,4] Thiadiazole Derivatives. *Journal of Global Pharma Technology*, 2010; 2(1): 72-80.
21. Kratika Shrivastava, Ritesh K. Sharma, Vivek Daniel, Mangal Singh, Swapnil Goyal. Synthesis and Antihelminthic Activity of Some Azole Derivative of Hippuric Acid. *International Journal of Pharmaceutical Sciences*, 2010; 2 (2): 502 – 507.
22. Vivek Daniel, Kratika Daniel, Swapnil Goyal, Mahendra S.Parihar, Mangal Singh. Synthesis and In-Vitro Anthelmintic Activity Of Some 4-Aminophenol Derivatives. *International Journal of Pharmaceutical Research*, 2010, 2(2): 21 – 24.
23. Chatterjee K.D., *Parasitology, Protozoology and helminthology*. 6th Ed. Calcutta, In Guha. Ray Sree Saraswaty Press Ltd. 1967. 168-9.