



Assessment of Anthelmintic Activity of *Gymnema sylvestre* Leaves against *Pheretima posthuma* - An *In vitro* Design

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Abstract

Back ground: Herbs have always been the principal form of medicine in India. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants. Helminthic infections are among the global public health problems. Due to high cost of standard drugs and resistance, rural dwellers use herbal preparations. However, efficacy and mechanisms of action remains elusive. **Aim:** This laboratory study was carried out to explore the anthelmintic activity of *Gymnema sylvestre* leaf extract aligned with Indian earthworms *Pheretima posthuma*. **Method:** The adult Indian earthworm *Pheretima posthuma* was used for the proposed anthelmintic study. Ethanolic and aqueous extracts of *Gymnema sylvestre* leaves was investigated for their anthelmintic activity by dividing different animal groups. Different concentrations like 30, 60 and 100mg/ml of both extracts and 30mg/ml of albendazole as standard drug solution were used in the present research. **Results:** Ethanolic and aqueous extracts of *Gymnema sylvestre* leaves were found to show a potent anthelmintic activity when compared to standard drug albendazole. **Conclusion:** Further studies are required to isolate the active principles or phytomolecule which are responsible for the activity of their mechanisms and toxicity can pave the way toward new medicines.

Key words: Indian medicinal plants, *Gymnema sylvestre*, anthelmintic activity, *Pheretima posthuma*.

1. Introduction

Medicinal plants which act as therapeutic agents are also a good source of information for a wide variety of phytochemical constituents which can be developed as drugs with precise and good selectivity. These are the bank of potentially useful active constituents which could serve as novel leads and clues for newer drug design. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and saponin compounds. Correlation between the phytoconstituents and the therapeutic activity of plant is desirable to know for the synthesis of compounds with particular activities to treat different health ailments and chronic diseases as well. Owing to the significance in the above context, such preliminary phytochemical screening of plants is the need of the hour in order to identify newer therapeutic agents with improved efficacy. Number of studies has also reported around the world [1-3].

Infections with helminths, or parasitic worms, influence more than two billion people worldwide. They are the most common infectious agents of humans in developing countries and produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Parasites have been of concern to the medical



field for centuries and the helminths still cause considerable problems for human being and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance. Modern synthetic medicines are very effective in curing diseases but also cause a number of side effects. Crude drugs are less efficient with respect to cure of disease but are relatively free from side effects. A large number of medicinal plants are claimed to possess anthelmintic property in traditional system of medicine and are also utilized by ethnic groups worldwide [4-6].

Helminths are parasitic worms; they are most common infectious agents of humans in developing countries and produce a global burden of disease. Helminths have plagued humans since before the era of our earliest recorded history. There are two major phyla of helminths. The nematodes (roundworms) include the major intestinal worms, also known as soil transmitted helminths. Whereas platyhelminths (flatworms) include flukes also known as trematodes and tapeworms. It is estimated that approximately one-third of almost three billion people that live on less than two US dollars per day in developing regions of Sub-Saharan Africa, Asia, and America are affected with one or more helminth. The high medical, educational and economic burden of helminth infections provides an important rationale for launching a global assault on parasitic worms. However, the tools we currently have for controlling worm infections are limited, of the 1,556 new chemical entities marketed between 1975 and 2004, only four drugs- albendazole, oxamniquine, praziquantel and ivermectin were developed to treat helminthiasis [7,8].

Nowadays multiple drug resistance has been developed due to the indiscriminate use of the antimicrobial drugs commonly used for treatment of infectious diseases. It can also cause hypersensitivity, immune-suppression and allergic reactions. Recently the indiscriminate use of anthelmintic produced toxicity in humans. Hence the development and discovery of new anthelmintic are being derived through plants. Herbal medicine is still the main source of medicine and about 70-80% of the whole population, mainly in developing countries for primary healthcare because of better cultural acceptability, better compatibility, with the human body and fewer side effects [9].

Gymnema sylvestre (*G. sylvestre*) also known as madhunashini (Sanskrit) and cherukurinja (Tamil) belonging to the family of Asclepiadaceae is a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. It is a potent anti-diabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. In addition, it possesses wound healing, anti-inflammatory, anti-obesity, treatment of snake bite and anthelmintic properties [10,11].



Figure 1: *Gymnema sylvestre* plant



Figure 2: *Gymnema sylvestre* leaves



Figure 3: *Gymnema sylvestre* dried leaves and leaf powder

The aim of the present research is to screen the anthelmintic properties of *Gymnema sylvestre* leaf extract aligned with Indian earthworms *Pheretima posthuma*.

Materials and Methods

Drugs and chemicals

Albendazole, normal saline, distil water and ethanol were used during the experimental protocol.

Plant collection

The leaves of *Gymnema sylvestre* belonging to the family of Asclepiadaceae were collected from local area of Ghatkesar, Medchal, Telangana, India.

Plant extraction

Leaves were dried at room temperature (25 to 35 °C) and powdered with the help of an electrical grinder. The fine material was subjected to extraction by Soxhlet extractor using ethanol and distil water as solvents. The extracts were allowed to dry at 100 °C in water bath. The crude extract was stored at 4 °C in airtight bottles in refrigerator.



Worms' collection

Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworms of 3-5cm in length and 0.1-0.2cm in width were used for all experimental protocol. The earthworms resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.

Anthelmintic assay [7,12-14]

Ethanollic and aqueous extracts of *Gymnema sylvestre* leaf extract was investigated for their anthelmintic activity against *Pheretima posthuma*. The earthworms were divided in to eight groups containing six earthworms in each group (Table 1). Both the extracts were dissolved in the normal saline at different concentrations and then the volume was adjusted to 20ml with normal saline.

The standard drug solution was prepared with distil water and volume was adjusted 20ml with normal saline. Both of the extracts and standard drug solution were freshly prepared before starting the experiment. Different concentrations 30, 60 and 100mg/ml of both extracts and 30mg/ml of standard drug solution at the volume of 20ml were poured in different petridishes. All the earthworms before released in petridishes were washed in normal saline solution.

Each group of six earthworms was released in to 20ml of prepared formulations as following manner observations were made for the time taken to paralyze and death of individual earthworm. Time for paralysis was noted until there was no movement could be observed in earthworms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their mortality followed with fading away of their body colour.

Table 1: Animal groups

1 st Group	Normal saline as control
2 nd Group	Albendazole (30mg/ml) solution as standard
3, 4, 5 groups	Ethanollic extract solution at different concentrations
6, 7, 8 groups	Aqueous extract solution at different concentrations



Figure 4: Earthworms used for anthelmintic activity

Result and Discussion

The main advantages of using *in vitro* assays to screen the anti-parasitic properties of the plants and plant extracts include low costs and rapid turnover which allow the screening of plants at large scale. In addition, these tests



measured the effect of anthelmintic activity directly on the processes of hatching, development and motility of parasites without interfering the internal physiological functions of the host.

The predominant effect of albendazole on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. From the observations, the ethanol and aqueous extracts of *Gymnema sylvestre* showed not only paralysis but also showed death of earthworms.

Both the extracts were found to show a potent anthelmintic activity when compared to standard drug. Aqueous extract at 30, 60, 100mg/ml concentrations show paralysis at 4.99 min, 4.37 min, 3.38 min and death at 16.00 min, 14.87 min, 13.82 min, whereas ethanolic extract at the similar concentrations shows paralysis at 3.63 min, 3.00 min, 2.12 min and death at 10.00 min, 8.30 min, and 7.45 min. Ethanolic extract at the concentration of 100mg/ml show shortest time of paralysis as compared to standard drug. The standard drug albendazole at 30mg/ml concentration shows paralysis at 2.26 min and death at 6.42 min of earthworms respectively (Table 2).

Table 2: Anthelmintic activity against *Pheretima posthuma*

Groups	Extract/Drug	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
1	Normal saline (Control)	-	-	-
2	Albendazole (Standard)	30	2.26±0.46	6.42±1.65
3	Ethanolic extract	30	3.63±1.61	10.00±0.35
4	Ethanolic extract	60	3.00±1.87	8.30±1.35
5	Ethanolic extract	100	2.12±1.78	7.45±0.33
6	Aqueous extract	30	4.99±0.24	16.00±1.55
7	Aqueous extract	60	4.37±1.66	14.87±0.25
8	Aqueous extract	100	3.38±1.88	13.82±0.32

The biological activities of the medicinal plants are due to the presence of secondary metabolites. The production of novel therapeutic drugs from plants are based on the reconnoitred of the phytoconstituents. The secondary metabolites demonstrated various health benefits like anti-diabetic, anti-cancer, anthelmintic, anti-bacterial, CNS depressant, etc. The flavonoids and polyphenol have strong anti-oxidants, which scavenged the free radicals and inhibitors of lipid peroxidation. Hence flavonoids and polyphenol have chief role for the therapeutic efficacy of medicinal plants, and researchers are more focused to explore their biological activities.

The researchers are giving more attention towards the medicinal plant for the development of anthelmintic drug because it gives new bioactive compounds with no or little side effects and easily accessible to the peoples of developing countries. Moreover, the medicinal plants have the best compatibility with human physiology than allopathic medicines.

Conclusion

Herbalism is very important, especially in the healthcare system of developing countries. In ancient Indian literature, medicinal plants are defined by a very broad logic as a possible source of therapeutic ingredients. The number of patients looking for alternative or herbal remedies is growing rapidly. For past era herbal medicine has integrated generations of practitioner treatment experience into the indigenous health system. Herbal medicines are not only cheap, but also culturally acceptable, very elastic to the human body, have few side effects in case of injury, and vary, so the demand for major medicines in poor countries is high.

Helminth infection is a parasitic disease common in human beings from several decades caused by parasites (roundworms, pinworms, hookworms etc). These helminths can be very dangerous for human health. They must be expelled via excreta using drugs. Several synthetic drugs are available to cure these diseases but these drugs are associated with serious side effects. To overcome this disadvantage, the researcher has surveyed flora literature.

From the results, it was concluded that both ethanolic and aqueous extracts of *Gymnema sylvestre* leaf have significant anthelmintic activity. From the results, *Gymnema sylvestre* has an anthelmintic activity have been confirmed as it displayed activity against the worm used in the present study. Future details research work on this plant will open a



new avenue of drug industries. However, further detailed study is needed to isolate and purification of constituents from the plant for anthelmintic activity. Further more research is required to found the mechanism of action of anthelmintic activity for these plants, and their toxicity.

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Author contributions

All authors contributed to data collection, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Conflict of interest

All authors declare that there is no conflict of interests regarding publication of this paper.

Ethical approval

Not required.

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