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EVALUATION OF ANTHELMINTIC ACTIVITY OF THE METHANOLIC EXTRACT OF AMORPHOPHALLUS PAEONIIFOLIUS TUBER

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ABSTRACT

Methanolic extracts of the tuber of *Amorphophallus paeoniifolius* were investigated for its antihelmintic activity against *Pheretima posthuma* and *Tubifex tubifex*. The extract with the concentrations of 25, 50 and 100 mg/ml were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. The extract exhibited significant antihelmintic activity at highest concentration of 100 mg/ml. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control. The extracts were found not only to paralyze (Vermifuge) but also to kill the earthworms (Vermicidal).

INTRODUCTION: People living in poverty in developing countries often suffer from helminth infections, which more often physically impair their hosts than kill them. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates [1]. As an important component of complementary and alternative medicine, traditional Ayurvedic medicinal plants may be useful model for the discovery and development of new chemical substances for helminth control which are generally considered to be very important sources of bioactive substances [2]. Helminthiasis, or infection with parasitic worms, affects over two billion people worldwide. Human beings can spread these pathogens to previously uninvolved population through travel, migration, and military operations.

Worms pathogenic for human beings are *Metazoa*, classified into roundworms (*nematodes*) and two types of flatworms, flukes (*trematode*s) and tapeworms (*cestodes*). These biologically diverse eukaryotes vary with respect to life cycle, bodily structure, development, physiology, localization within the host, and susceptibility to chemotherapy. Immature forms invade human beings via the skin or gastrointestinal tract and evolve into well-differentiated adult worms that have characteristic tissue distributions. With few exceptions, such as Strongiloides and Echinococcus, these organisms cannot complete their life cycles, i. e., replicate themselves, within the human host. Therefore, the extent of exposure to these parasites dictates the severity of infection, and reduction in the number of adult organisms by chemotherapy is sustained unless reinfection occurs. The prevalence of parasitic helminths typically displays a negative binomial distribution within an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individuals are most likely to become ill and to perpetuate infection within their community [3].

Antihelmintics are drugs that either kill (vermicide) or expel (vermifuse) infesting helminths. Helminthiasis is prevalent globally (1/3 of world's population harbors them), but is more common in developing countries with poorer personal and environmental hygiene. Multiple infestations in the same individual are not infrequent. In the human body, G. I. T. is the abode of many helminths, but some also live in tissues or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of ill health [4]. The antihelmintic assay was carried as per the method of Ajaiyeoba *et al.,* [5] with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* and *Tubifex tubifex* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [6, 7, 8, 9]. Because of easy availability, earthworms have been used widely for the initial evaluation of antihelmintic compounds *in vitro* [10, 11, 12, 13, 14].

The tubers of wild plants are highly acrid and cause irritation in throat and mouth due to excessive amount of calcium oxalate present in the tubers. The tubers are anodyne, anti-inflammatory, antihaemorrhoidal, haemostatic, expectorant, carminative, digestive, appetizer, stomachic, antihelmintic, liver tonic, aphrodisiac, emmenogogue, rejuvenating and tonic. They are traditionally used in arthralgia, elephantiasis, tumors, inflammations, hemorrhoids, hemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic, constipation, helminthiasis hepatopathy,

splenopathy, amenorrhea, dysmenorrhoea, seminal weakness, fatigue, anemia and general debility [15]. The tuber is reported to have antiprotease activity [16], analgesic activity [17], cytotoxic activity [18] and CNS depressants activity [19]. Antihelmintics from the natural sources may play a key role in the treatment of these parasite infections. In view of this, an attempt has been made to study the antihelmintic activity of the tuber of *Amorphophallus paeoniifolius*. In this study, methanolic extracts were used and studied for paralysis and death *of Pheretima posthuma* and *Tubifex tubifex.* The objective of the present research is to prove the traditional anthelmintic use of the plant *A. paeoniifolius.*

MATERIALS AND METHODS:

Plant: The tuber of *Amorphophallus paeoniifolius* (Dennst.) Nicolson var.*campanulatus* (Decne) (Areaceae), was collected from Asansol, West Bengal, India. The tuber of *Amorphophallus paeoniifolius* (Dennst.) Nicolson var. *campanulatus* (Decne) Sivadasan (Areaceae), was identified with the Herbarium of Botanical Survey of India, Botanic Garden, Howrah with ref no. CNH/I-I/ (272)/ 2008/ Tech. II/ 314.

Preparation of extract: The tuber of the plant was dried in shade and made to fine powder using a laboratory mill. The dry powder is extracted with methanol using soxhlet extractor.

Phytochemical tests: Phytochemical screening of methanolioc extract indicated the presence of steroids, flavonoids, alkaloids and carbohydrates.

Worms: Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil. The average size of earthworm was 6-8 cm. They were washed with tap water for the removal of the adhering dirt. Aquarium worms *Tubifex tubifex* (Annelida) were collected from the local market. The average sizes of the worms were 1-1.5 cm. Both worm types were identified at the P. G. Department of Zoology, P.K.Roy College, Dhanbad, Jharkhand.

Chemicals:

- Piperazine Citrate (Glaxo)
- Methanolic extract of *Amorphophallus paeoniifolius*
- Double distilled water

Procedure: The antihelmintic assay was carried as per the method of Ajayieoba E.O. et al. with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. *Pheretima posthuma* worms are easily available and used as a suitable model for screening of antihelmintic drug was advocated earlier. The assay was also performed on the aquarium worm, *Tubifex tubifex*, because they belong to same group of Annelida (Mueller, 1774). 20 ml formulations containing three different concentrations, methanolic extract of tuber (25, 50 and 100 mg/ml in double distilled water) were prepared and taken in different petridishes and six earthworms (same type) were placed in the solutions respectively.

Similarly lump of *Tubifex* worms were placed in the test solutions. All the test solution and standard drug solution were prepared freshly before starting the experiments. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C. Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control [20, 21, 22]. Three sets of experiments were done for statistical significance.

RESULTS:

TABLE 1: THE ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACT OF AMORPHOPHALLUS PAEONIIFOLIUS

Groups	Concentration (mg/ml)	*Pheretima posthuma*		*Tubifex tubifex*	
		Paralyzing Time	Death Time	Paralyzing Time	Death Time
Distilled Water	–	–	–	–	–
Methanolic Extract	25	45.66±2.333	81.32±4.666	94.66±1.453	113.99±2.334
	50	37.33±1.202	66.66±2.655	52.00±2.309	65.00±3.837
	100	22.33±1.453	38.66±2.906	12.33±1.453	17.66±2.335
Piperazine Citrate	10	25±1.155	64±0.881	22.66±1.764	45.33±1.202

DISCUSSION: From the above study it was seen that the methanolic extract showed dose dependent antihelmintic activity as compared to a standard drug piperazine citrate (**Table 1**). The mean paralyzing time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 45.66, 37.33 and 22.33 minutes respectively. In the meantime piperazine citrate at a dose of 10 mg/ml causes paralysis in the above helminth in 25 minutes. The mean death time of *Pheretima posthuma* with the dose of 25, 50 and 100 mg/ml were found to be 81.32, 66.66 and 38.66 minutes respectively. In the meantime piperazine citrate at a dose of 10 mg/ml causes paralysis in the above helminth in 64 minutes.

The mean paralyzing time of *Tubifex tubifex* with the dose of 25, 50 and 100 mg/ml were found to be 94.66, 52.00 and 12.33 minutes respectively. In the meantime, piperazine citrate at a dose of 10 mg/ml cause paralysis in the above helminth in 22.66 minutes. The mean death time of *Tubifex tubifex* with the dose of 25, 50 and 100 mg/ml were found to be 113.99, 65.00 and 17.66 minutes respectively. In the meantime piperazine citrate at a dose of 10 mg/ml causes death in the above helminth in 45.33 minutes.

CONCLUSION: In conclusion, the traditional claim of tubers of *Amorphophallus paeoniifolius* as an antihelmintic have been confirmed as the methanolic extract of the tuber displayed significant activity against the worms used in the study. Further studies are going on to isolate and reveal the active compound (S) contained in the crude extracts of *A. paeoniifolius* and to establish the mechanism (S) of action for its anthelmintic activity.

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