



ANTHELMINTIC POTENTIAL OF CRUDE EXTRACT OF *CAMELLIA SINENSIS* (GREEN TEA)

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ABSTRACT

The purpose of the undertaken study was the evaluation of anthelmintic activity of *Camellia sinensis* (green tea) aqueous extract in experimental adult earthworm's *Pheretima posthuma*. The aqueous extract as well as control and standard drug for different concentration of 25 mg/ml, 50 mg/ml, 100 mg/ml were tested which included determination of paralysis time and death time in minutes. The extract showed the anthelmintic activity in dose dependent manner. Piperazine citrate was used as standard.

Keyword: *Camellia sinensis*, green tea, *Pheretima posthuma*, anthelmintic activity.

INTRODUCTION

Helminths (Greek word meaning worms)¹, it has plagued in humans prior to our recorded olden times. Helminths are considered most common agents of human's infections especially in developing countries and actively participate in global burden of diseases. Eggs of these intestinal worms found in the human mummified feces dating thousands of years ago^{2, 3, 4}. In 2002 it was estimated 1.5 billion with *Acaris*, 1.3 billion with hook worm and 1.1 billion with *trichoris* infection were diagnosed⁵. World Health Organization estimated 2 billion people infected with helminths and it was also estimated that 100% of all age group of school children are at risk of morbidity⁶. The major phyla of helminths are nematodes (round worms) which are soil-transmitted helminths that mostly cause the intestinal infection, filarial worms cause the onchocerciasis and lymphatic filariasis, while platyhelminths (flatworms) also known as trematodes like schistosomes and cestodes causes cyticercosis⁷⁻¹⁰. The plant of *Camiellia sinensis* is an inhabitant of Southeast Asian region commonly known as green tea. Whereas currently, it is cultivated in more than thirty countries of world. It is considered as most consumed beverage of world. Primarily, green tea is being widely consumed in China, Pakistan, India, North Africa and Middle East¹¹. *C. sinensis* plant contain more than 700 chemicals most of them closely related to human health like theobromine, gallotannic acid, vitamins (C, E and K), caffeine polysaccharides¹². *Camellia sinensis* has high content of polyphenols which is responsible for bitter taste¹³. Poly phenol is effective against many diseases like osteoporosis, liver disease. It has also been also considered that the extract of *C. sinensis* is effective against bacterial and viral infections¹⁴. It has been also revealed in previous study that green tea extract has beneficial anthelmintic effect¹². Additionally some studies suggested that intake of green has a beneficial impact on cognitive function, bone density, kidney stones and dental caries¹⁵⁻¹⁶. The present preliminary study was conducted on crude aqueous extract of *Camiellia sinensis* (green tea) for the evaluation of anthelmintic

activity against model worm adult earthworm (*Pheretima posthum*).

MATERIALS AND METHOD

Preparation of extract

The Green tea was purchased from the retail market in Karachi-Pakistan in reasonable price in large quantity for performing various biological activities. The extract was prepared in the concentration of 5% in distilled water¹⁷. The aqueous extract was prepared by boiling method of tea in water bath by constant agitation for 15 minutes. Later, all the coarse suspended particles of tea were first removed by using strainer and then by passing via 0.22µm filter¹⁸. The extract was stored in refrigerator in small vials as aliquots till use.

Selection of worms

The anthelmintic activity was done by following the method with slight modifications¹⁹. The assay was carried out on adult earthworm (*Pheretima posthuma*) owing to its resemblance in terms of anatomy and physiology with the intestinal roundworm parasite of human beings²⁰⁻²². One of the plausible reasons for selecting this worm as a model for study is its easy availability²³. Because of this reason; earthworms have been used extensively for in vitro initial investigation of anthelmintic compounds²⁴⁻²⁵.

Collection of worms

Earthworms of approximately with the size of 3 to 5 cm in length and 0.1 to 0.2 cm width, measured by ruler²⁵ were collected from moist garden soil of Federal Urdu University of Arts, science and Technology-Karachi Pakistan with the help of zoologists. All the worms were washed with normal saline in order to remove the soil particles, debris and fecal contents²⁷ and kept them in phosphate buffer saline (PBS)²⁸.

Anthelmintic assay

The earthworms were divided into three groups. Each group consisting of six earthworms (approximately equal size). Group first serve as control, receive only normal saline; Group second serve as standard, receive standard Piperazine

citrate and Group third serve as aqueous extract of different concentration²⁹. All the extracts and the standard (Piperzine citrate) solution in distilled water were freshly prepared before starting the experiments.

Anthelmintic observation

Observations were recorded for the time taken until the paralysis as well as death of an individual worm occurred³⁰⁻³¹. The paralysis was declared when the worms were not able to move even in normal saline³². Death was considered when the worms lost their motility followed with fading away of their body colors³³.

Table 1: Anthelmintic activity of control and standard

Concentration mg/ml	Control		Standard	
	Paralysis time (mins)	Death time (mins)	Paralysis time (mins)	Death time (mins)
25mg/ml	96.46 + 0.46	160.06+0.11	18.36 + 0.15	54.36 + 0.40
50mg/ml	78.23 + 0.25	142.2+ 0.34	12+ 0.00	46.46 +0.40
100mg/ml	62.43+ 0.11	111.7+ 0.26	08.84 + 0.03	13.6+ 0.17

Values are the mean ± S.E.M. of control and standard drug on three earthworms. Control is normal saline while standard drug is piperzine citrate.

Table 2: Anthelmintic activity of crude extract of *Camellia sinensis* (Green tea)

Concentration mg/ml	Crude extract of <i>Camellia sinensis</i> (Green tea)	
	Paralysis time (mins)	Death time (mins)
25mg/ml	44.86+ 0.41	120.46 +0.25
50mg/ml	22.16+ 1.06	92+0.51
100mg/ml	10.7 + 0.43	80.7+ 0.51

Values are the mean ± S.E.M. of aqueous extract of *Camellia sinensis* (Green tea) on three earthworms.

RESULTS AND DISCUSSION

Anthelmintics are the drugs that expel out parasitic worms (helminthes) from the body, by either causing paralysis or by directly killing them³⁴. Moreover, the conventionally employed agents for the treatment of helminthic infections are also acquiring resistance with chemotherapeutic agents because of the exposure of constant same chemical nature compound toxicity complications³⁵, un-economical and nonavailability of drugs in remote areas³⁶. In the current study, in earthworms, the aqueous extract of tea (*Camellia sinensis*) showed anthelmintic activity. Earthworms belonging to control group showed paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e. 96.46 + 0.46 mins, 78.23 + 0.25 mins and 62.43+ 0.11 mins while; death time 160.06 +0.11mins, 142.2+ 0.34 mins and 111.7+ 0.26 mins respectively as mentioned in Table 1. On the other hand, the standard reference compound piperzine citrate showed the time of paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e. showed the time of paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e 18.36 + 0.15 mins, 12+ 0.00 mins and 08.84 + 0.03mins respectively while; death time at 54.36 + 0.40 mins, 46.46 +0.40 mins, 13.6+ 0.17 mins respectively as indicated in Table 1. In case of aqueous extract of tea (*Camellia sinensis*)group, the paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e. 44.86+ 0.41 mins, 22.16+ 1.06 mins, 10.7 + 0.43 mins respectively death time at 120.46 +0.25 mins, 92+0.51 mins, 80.7+ 0.51 mins respectively as indicated in Table 2. The activity was observed in dose dependent way in all groups including control, standard drug ad extract. The results of similar study earlier conducted¹² that noted the aqueous extract of tea (50mg/ml) showed paralysis within 27.02 min & time of death 47.07 mins while, aqueous extract (100mg/ml) showed paralysis within 21.19 min & time of Death 37.02 mins. Piperzine citrate causes flaccid paralysis and expel out the worm by peristalsis. According to mechanism of action, piperzine citrate induces paralysis by increasing the chloride ion conductance of worm muscle membrane which is responsible of hyper polarization and reduced excitability that leads to muscle relaxation and results in expulsion of the

worm by the action of peristalsis³⁷. The literature reports reveal that caffeine, theobromine, polysaccharides, gallotannic acid, vitamins (C, E and K), amino acids, flavanoides and some other phytoconstituenits³⁸. Phytochemical analysis of the crude extracts revealed presence of flavonoids as one of the very important chemical constituents. Polyphenolic compounds show anthelmintic activity³⁹. Similarly, in the present investigation also the observed anthelmintic potential of test extract may be due to presence of similar phytoconstitutes¹².

CONCLUSION

The current study is the preliminary evaluation of crude extract of *Camellia sinensis* (green tea) having anthelmintic potential. Globally, today helminthic infections both in humans and cattles are a serious growing issue due to the availability of limited and effective anthelmintic drugs. The encouraging results of the undertaken study create further hope to explore in depth, design thorough experiments for better conclusion and ultimately develop a therapeutic molecule later. In the current scenario, there is a dire need to have an effective anthelmintic compound but also play a role in combating the developing resistance issues of current conventionally employed anthelmintic therapeutics as a good substitute.

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