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INVESTIGATION OF *IN-VITRO* ANTHELMINTIC ACTIVITY OF *CISSAMPELOS PAREIRA* LINN AGAINST *PHERETIMA POSTHUMA*

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ABSTRACT

The world health organization estimates that a staggering 2 billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants has an alternative source of anthelmintics. In view of this, an attempt has been made to study the anthelmintic activity of whole plant of *Cissampelos pareira*. The activity was checked by in-vitro anthelmintic model by using earthworm. In this current study, alcoholic and aqueous extract at various concentrations (5, 10, 25, 50, 100 mg/ml) were used and studied for paralysis and death of earthworm. All the extract was found not only to paralyze (Vermifuge) but also to kill the earthworm (vermicide). All the extract have significant activity but aqueous extract (100mg/ml) was found to be more effective to execute the earthworm. From this study it is concluded that *Cissampelos pareira* have potent anthelmintic activity and can be used in the treatment of helminthiasis.

INTRODUCTION: Helminth infections are among the widest spread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia.

Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas [2]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural antihelmintics [1].

Cissampelos pareira (L.) Hirsuta (family: Menispermaceae), popularly known as patha in Ayurveda (Indian Traditional System of Medicine) a wound healer and antidote, paste of roots are used in fistula, pruritis, skin disorders and snake poison externally [2]. Internally it is useful in anorexia, indigestion, abdominal pain, diarrhoea and dysentery. Plant is a blood purifier and has anti-inflammatory properties [2].

It is also used in cough and as it purifies breast milk, it is used in various disorders of breast milk secretion. It is a potent diuretic. The root possesses astringent, mild tonic, diuretic, stomachic, analgesic, antipyretic and emmenagogue properties [3]. They are frequently prescribed for cough, dyspepsia, dropsy, urino-genital troubles such as prolapsus uteri, cystitis, haemorrhage and menorrhagia, and calcular nephritis [4]. The leaf juice possesses antiseptic, anthelmintic, insecticidal

and parasiticidal properties, used to check hemorrhage from cuts burns and wounds [5]. The leaves of *Cissampelos pareira* have been reported to be a rich source of alkaloids [6, 7].

However, scientific study of this plant in relation with the potentiality as effective antihelmintic agent is still fragmentary. The present study was therefore carried out to evaluate the claimed antihelmintic effect of *Cissampelos pareira* leaves using in-vitro model of *Pheretima posthuma*.

MATERIAL AND METHODS:

Plant Material: The plants of *Cissampelos pareira* Linn were collected from the National botanical research institute, Lucknow, Uttar Pradesh, India and were authenticated by Prof J. P. Shukla, Department of Botany, D.B.S College, Kanpur, where a voucher specimen (PH/CP/20) is deposited for further reference.

Preparation of Extracts: For the preparation of aqueous extract, aerial part was collected, shade dried at room temperature, pulverized and extracted with water by maceration process for 24 hrs. The extract was concentrated in a rotary flash evaporator and dried in desiccator.

Animals: Indian adult earthworms (*Pheretima posthuma*) were used to study antihelmintic activity [8]. The earthworms were collected from moist soil and washed with normal saline to remove all fecal matter. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the antihelmintic activity [9].

Antihelmintic activity- For the antihelmintic activity of plant extract of *Cissampelos pareira,* Indian adult earthworms (*Pheretima posthuma*) of 3-4 cm in length and 0.1-0.2 cm in width were used. The animals were divided into seven groups containing six earthworms in each group. Different dilutions of drug extract (5, 10, 25, 50 and 100 mg/ml) has been made and then the volume was adjusted to 10 ml with normal saline water. All the dilutions of drug extract and standard drug solution were freshly prepared before starting the experiments. Different extract dilutions and standard drug solution were poured in different petri dishes. All the earthworms were washed in normal saline before they were released into 10 ml of respective formulation. Observation were made for the time taken to paralyze (Paralysis was said to occur when the worm did not revive even in normal saline) and death (Death was concluded when the worms lost their motility followed with their body colors fading away) [10]. All the results were expressed as a mean ± SEM of six animals in each group.

RESULT AND DISCUSSION: Preliminary phytochemical screening showed the presence of phenolic compounds, alkaloids, saponins etc like phytoconstituents in the extract of *Cissampelos pareira.* Some of these phytoconstituents may be responsible to show a potent antihelmintic activity. From the observations made all the aqueous extract dilutions of *Cissampelos pareira* was found to show a potent antihelmintic activity when compared to the standard drug. The aqueous extract of *Cissampelos pareira* at 10mg/ml concentration shows paralysis at 29.33 min and death at 49.67 min, whereas 25 mg/ml shows paralysis at 14.33min and death at 27.50 min.

At 50mg/ml concentration time has been taken for paralysis is 7.50 min and death at 14.67 min, while at the maximum concentration of drug i.e. 100 mg/ml requires time for paralysis is 3.67 min and for death of earthworms is 5.67 min. The standard drug Piperazine citrate shows paralysis at 18.50 min and death at 60.29 min at 15 mg/ml concentration. The two concentrations (50, 100 mg/ml) of this plant show good antihelmintic activity as compared to standard drug. All the values are expressed as mean ± SEM (n=6).

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [11, 12, 13]. The extract of *Cissampelos pareira* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml in shorter time as compared to reference drug Piperazine citrate.

From the above results, it is concluded that the aqueous extract of entire plant of *Cissampelos pareira* have potent antihelmintic activity when compared with the conventionally used drug. Further studies using in vivo models are required to carry out and establish the effectiveness and pharmacological rationale for the use of *Cissampelos pareira* as an antihelmintic drug. The drug may be further explored for its phytochemical profile to identify the active constituent responsible for antihelmintic activity.

TABLE 1: *IN-VITRO* ANTIHELMINTIC ACTIVITY OF *CISSAMPELOS PAREIRA* EXTRACT

Group	Treatment	Concentration (mg/ml)	Time(min) Paralysis	Time(min) Death
I	Vehicle	-	-	-
II	Piperazine Citrate	15	18.50±0.31**	60.29±0.26**
III	Aqueous extract	5.0	50.38±0.28	-
IV	Aqueous extract	10	29.33±0.27	49.6±0.22
V	Aqueous extract	25	14.33±0.27*	27.50±0.21*
VI	Aqueous extract	50	7.50±0.26**	14.67±0.20**
VII	Aqueous extract	100	3.68±0.24**	5.67±0.20**

All value represents Mean ± SEM from six observations, where n=6 in each group. (*p<0.01, **p<0.001 shows significant)

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