



ANTHELMINTIC ACTIVITY OF PIPERINE FROM BLACK PEPPER

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ABSTRACT

Piperine is an alkaloid responsible for the pungency of black pepper & long pepper. The dried unripe fruits of *Piper nigrum* belong to the family Piperaceae, it is well known for its reducing inflammation, improving digestion, and relieving pain and asthma. Traditionally it is claimed to be used in the house hold for carminative and aromatic. In present study different concentration of piperine 2.5 mg/ml, 5 mg/ml, 10 mg/ml were investigated for its anthelmintic activity. The result indicates that the 10 mg/ml piperine posses significant anthelmintic activity. The paralysis time [P] and death time [D] is comparable with standard Albendazole 20 mg/ml. Dose dependent effects were observed with 2.5 mg/ml, 5 mg/ml and 10 mg/ml.

Key words: Piperine, Anthelmintic activity, Albendazole

INTRODUCTION

Helminthiasis is a disease in which a part of the body is infested with worms such as pinworm, roundworm or tape worm. Typically, the worms reside in the gastrointestinal tract but may also burrow into the liver and other organs. They produce harmful effect on host by depriving him of food, causing blood loss and by secreting toxins.¹ globally; over 3.5 billion people are infected with intestinal worms, of which children between 5–15 years account

for the highest infection rate of about 400 million cases of worm burden that are mainly attributed to poor sanitation and hygiene. In India, infections with these parasites are regarded as amongst the most common public health problems, particularly in rural areas and urban slums.² According to the WHO, only a few drugs are used in treatment of helminthes in humans. Anthelmintics from natural sources could play a key role in the treatment of this parasite infections.³ In the recent years; the importance of Herbal drugs in Medicine has

tremendously increased because of their fewer side effects. Consequently, the demand for the herbal formulation is increasing day by day.⁴Piperine is an alkaloid found naturally in plants belonging to the Piperaceae family such as *Piper nigrum* commonly known as black pepper, a household spice and *Piper longum* L, commonly known as long pepper. Piperine is the major pungent substance in these plants and is isolated from the fruit of the black pepper and long pepper plants. Piperine comprises 1 to 99% of these plants. There is also preliminary evidence that it may have some antimycobacterial, anticonvulsant and anti carcinogenic properties. Although this piperine has many useful claims, the mechanism of its medicinal effects is not understood.⁵ Gastro intestinal Helminthes are resistant to currently available drugs. So the discovery and development of new chemical substances for helminthes control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substance.⁶

MATERIALS & METHODS

Collection

Fruits of *Piper nigrum* were collected from an Ayurvedic shop located in Proddatur, kadapa, Andhra Pradesh in the month of January 2013. The foreign, earthy matter and residual materials were removed carefully from the fruits and then cleaned and dried in the shade. It was then mechanically reduced to coarse powder form.

Isolation of Piperine:

Placed 15g of ground *Piper nigrum* in a 250 ml Soxhelt apparatus, added 150 ml of 95% ethanol and 5 boiling chips, and heated at reflux for 2h. Filtered the mixture by suction filtration and then concentrated the filtrate to a volume of 10-15ml by simple distillation. To 10mL of a 10% solution of

KOH in 95% ethanol contained in a 125mL Erlenmeyer flask added the concentrated pepper extract. Heated the resulting solution and add water drop wise. A yellow precipitate formed. Added water until no more solid appears to form and then allowed the mixture to stand at least overnight collect the solid by suction filtration and recrystallized it with 10-20 ml of acetone..

Animals:

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. Earthworms 3-5 cm in length and 0.1-0.2 cm in width were used for all experimental protocol.

Drugs and Chemicals:

Albendazole, 95% Ethanol, Potassium hydroxide, Silica gel, Toluene, Ethyl acetate and are all analytical grade.

Anthelmintic Assay:

Anthelmintic activity was carried as per the method reported by *Rajesh. R et al.*, with minor modifications. All the extracts and the standard drug solution were freshly prepared before starting the experiments. *Pheretima posthuma* was placed in petridish containing three different concentrations (2.5, 5, 10 mg/ml) of piperine solutions. Albendazole is used as a standard reference. Each petridish was placed with 4 worms and observed for paralysis (or) death. Observations were made for the time taken to paralyze and / or death of individual worms. Paralysis was said to occur when the worms do not move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour.¹⁰

Dose Selection:

Albendazole 20 mg/ml and Piperine 2.5, 5, 10 mg/ml dissolved in normal saline.¹¹

EXPERIMENTAL DESIGN

The animals were divided into 5 groups each group contains 4 animals.

- I. Earth worms treated with normal saline.
- II. Earth worms treated with Albendazole 20 mg/ml.
- III. Earth worms treated with Piperine 2.5 mg/ml.
- IV. Earth worms treated with Piperine 5 mg/ml.
- V. Earth worms treated with Piperine 10 mg/ml

RESULTS & DISCUSSIONS

The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*. Albendazole citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis so that they are expelled in the feces. As shown in Table. Piperine

exhibited anthelmintic activity in dose dependent manner taking shortest time for paralysis (P) and death (D) with 10mg/ml concentration. Hence piperine in its different concentration exhibited anthelmintic activity. It show shortest time of paralysis (P=24 min) and death (D=53min) in 10 mg/ml concentration, while the time of paralysis and death will increase in 5 mg/ml concentration (P=32min & D=62min) and in 2.5 mg/ml concentration (P=36min&D=71min) respectively as compare to Albendazole (20mg/ml) used as standard reference (P= 22 min& D= 48) and normal saline as control. The predominant effect of Albendazole on worm is to cause a flaccid paralysis those results in expulsion of the worm by peristalsis. Thus piperine showed significant Anthelmintic activity as compare to standard reference and control.

CONCLUSION

From the result, it is conclude that the isolated piperine 10 mg/ml showed significant anthelmintic activity when compared with the standard anthelmintic drug. However further *in vivo* studies needed to find out the potential pharmacological profile of Piperine as an anthelmintic drug.

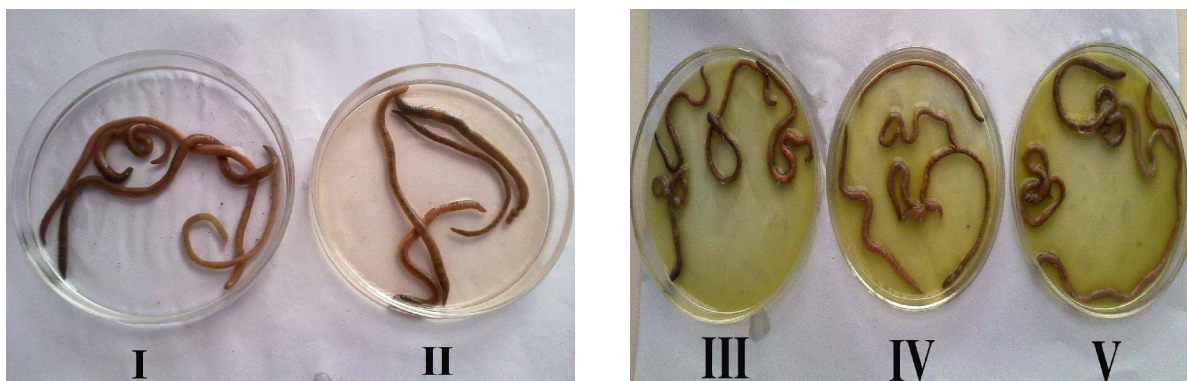
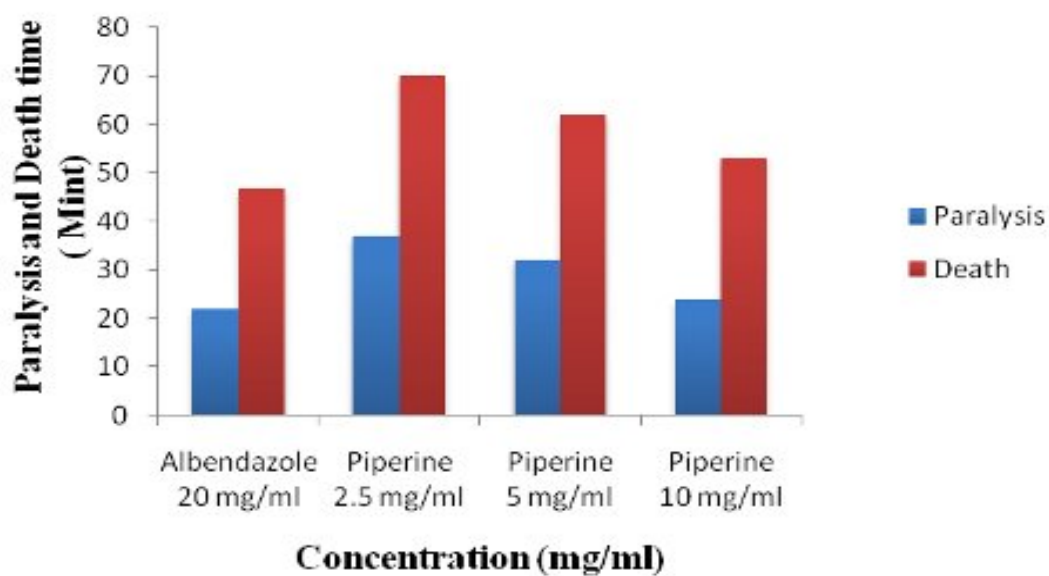


Fig 1: Anthelmintic activity of Piperine

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S. No.	Group	Concentration (mg/ml)	Paralysis time (Mints)	Death time (Mints)
1	Group I	Normal Saline	-----	-----
2	Group II	Albendazole	22 ± 3.51	48 ± 3.41
3	Group III	Piperine 2.5 mg/ml	36 ± 3.69	71 ± 6.83
4	Group IV	Piperine 5 mg/ml	32 ± 3.41	62 ± 6.13
5	Group V	Piperine 10 mg/ml	24 ± 2.75	53 ± 5.12

All values represent Mean +SD; n= 4 in each group.



Graph 1: Anthelmintic activity of Piperine

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