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Research Article

IN VITRO ANTHELMINTIC ACTIVITY OF *FICUS BENGHALENSIS* LINN. LEAVES EXTRACTS

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ABSTRACT

The present laboratory study was carried out to investigate the anthelmintic properties of *Ficus benghalensis* Linn leaves extracts against Indian earthworms *Pheretima posthuma*. Alcoholic extract and aqueous extract of leaves were used as test solutions. Albendazole was included as standard drug and normal saline as control. Observations were made for the time taken to paralyze and death of the earthworm. Three concentrations 25mg/ml, 50mg/ml, 100mg/ml of each extract and standard drug at the concentration of 25mg/ml were studied. The results of study indicated that *Ficus benghalensis* Linn leaves extracts exhibited anthelmintic activity significantly in a dose dependent manner when compared with standard group. For the aqueous extract group the time of paralysis and death time was 3.92 minute and 13.72 minute, while alcoholic extract group showed shortest time of paralysis (P) at 2.12 minute and death (D) at 7.34 minute of earthworms at higher concentration 100mg/ml as compared to standard Albendazole (25mg/ml) paralysis 2.36 minute and death at 6.42 minute respectively. Alcoholic extract showed more pronounced effect than aqueous extract. Whereas the control group worms were observed for full day and night and there was no paralysis or death was found during that period.

Keywords: Anthelmintic activity, Albendazole, *Ficus benghalensis* Linn, *Pheretima posthuma*

INTRODUCTION

Infections with helminths, or parasitic worms, affect 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.[1] Parasites have been of concern to the medical field for centuries and the helminths still cause considerable problems for human being and animas. 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.[2] 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.[3]

Ficus benghalensis Linn. Syn.-*Ficus Indica* Linn, family-Moraceae. The plant is a large deciduous tree found throughout the forest tract of India, in sub-Himalayan region, Rohilkhand, in deciduous forests of Deccan and in all districts from sea level to 1200m, in deciduous and semi evergreen forests of south India. (The Wealth of India 1988)[4]. This plant is commonly known as Banyan tree or vata or vada tree in ayurveda. *Ficus benghalensis* is a remarkable tree from India that sends down its branches and great number of shoots, which take root and become new trunks. Traditionally all parts of the plant are astringent, acrid, sweet, refrigerant, anodyne, vulnerary, depurative, anti-inflammatory, ophthalmic, styptic, antiarthritic, diaphoretic, antidiarrhoeal, antiemetic and tonic.[5] It is used in Ayurveda for the treatment of diarrhea, dysentery and piles, teeth disorders, rheumatism, skin disorders like sores and to boost immune system, as a hypoglycemic.[6] Bark contains tannins, wax, esters and glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitostirol-alpha-D-glucose and meso-inositol. Two flavonoid compounds, viz. 5,7-dimethylether of leucopelargonidin 3-0-alpha-L-rhamnoside and 5,3-dimethyl ether of leucocyanidin 3-0-alpha-D galactosyl cellobioside were present in the bark of *Ficus benghalensis* [6,7]. Pharmacologically various extracts of *Ficus benghalensis* has shown analgesic and anti-inflammatory (Thakare V N *et al*., 2010)[7], anti-arthritic (Bhardwaj L K *et al*., 2010)[8] antioxidants (Shukla R *et al*., 2004)[9], antidiabetic (Sharma S *et al*., 2007)[10], immunomodulatory (Gabhe S Y *et al*., 2006)[11] and antimicrobial (Gayathri M *et al*., 2009)[12] activity in experimental animals.

The literature survey revels that, the leaves of *Ficus benghalensis* for anthelmintic activity, has not been systematically investigated so far. Therefore the present study was designed to investigate the anthelmintic properties of alcoholic and aqueous leaves extract of *Ficus benghalensis* against Indian earthworms *Pheretima posthuma.*

MATERIALS AND METHODS

Plant material

The fresh leaves of *Ficus benghalensis* were collected from the Jawaharlal Nehru Medical college campus (JNMC), Belgaum in the month of June-July 2010. The plant material was authenticated by Dr. Harsha Hegde, Scientist B, Regional Medical Research Center, Indian Council of medical Research (ICMR) Belgaum Karnataka. The voucher specimen (RMRC-508) has been deposited in ICMR herbaria and Department of Pharmacognosy, KLES College of Pharmacy, Belgaum, India.

Preparation of Extracts

The collected fresh leaves about 2kg were dried under shade for two weeks than powdered and stored in air tight plastic jar for further use. The dried leaves powder 450g was exhaustively extracted by hot continuous extraction using soxhlet apparatus with 95% ethanol at a temp. 70^0 C up to 42 siphons. The total alcoholic extract was filtered and concentrated by distillation process. The concentrated mass was dried under vacuum till constant weight. For aqueous extract the dried leaves powder 200g was macerated with 1000 ml chloroform water (1:9) for seven days. Chloroform water was used to prevent the growth of microorganism in the extract. The extractive was filtered and concentrated over a water bath and further dried in vacuum oven till constant weight.

Animals

Indian adult earthworms (*Pheretima posthuma*) were selected for the in vitro anthelmintic assay. The earthworms were collected from department of zoology, R. L. Science College, Belgaum, Karnataka and washed with normal saline to remove all fecal matter. The earthworms of 3-5 c.m.in length and 0.1-0.2 c.m.in width were used for the present experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study the anthelmintic activity.[13]

In Vitro Anthelmintic Assay

Alcoholic and aqueous leaves extracts of *Ficus benghalensis* were investigated for their anthelmintic activity against *Pheretima posthuma*. The earthworms were divided in to eight groups containing six earthworms in each group. Both the extracts were dissolved in the normal saline at different concentrations and then the volume was adjusted to 20 ml with normal saline. The standard drug solution was prepared with distilled water and volume was adjusted 20 ml with normal saline. Both of the extracts and standard drug solution were freshly prepared before starting the experiment. Different concentrations 25mg/ml, 50mg/ml and 100mg/ml of both extracts and 25mg/ml of standard drug solution at the volume of 20 ml were poured in different petridishes. All the earthworms before released in petridishes were washed in normal saline solution.

The each group of six earthworms was released in to 20 ml of prepared formulations as following manner respectively.

1st group – Normal saline as control

2nd group - Albendazole (25mg/ml) solution as standard

3rd, 4th and 5th group – Alcoholic extract solution at different concentrations

6th, 7th and 8th group – Aqueous extract solution at different concentrations.

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. [14]

RESULT AND DISCUSSION

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.[15] From the observations (Table-1) the leaves extracts of *Ficus benghalensis* were 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 25mg/ml, 50mg/ml, 100mg/ml concentrations shows paralysis at 4.78 min, 4.26 min, 3.92 min and death at 15.76 min, 14.62 min, 13.72 min. whereas alcoholic extract at the similar concentrations shows paralysis at 3.82 min, 2.92 min, 2.12 min and death at 9.76 min, 8.04 min, and 7.34 min. Alcoholic extract at the concentration of 100mg/ml shoe shortest time of paralysis as compared to standard drug. The standard drug albendazole at 25mg/ml concentration shows paralysis at 2.36 min and death at 6.42 min of earthworms respectively.

Table 1: Anthelmintic activity of alcoholic and aqueous leaves extracts of *Ficus benghalensis* Linn against Indian earthworms - *Pheretima posthuma.*

Groups	Treatment of Drugs /extracts	Concentration mg/ml	Time taken for paralysis (P) in minute	Time taken for death (D) in minute
01	Control(Normal saline)	-	-	-
02	Albendazole(Standard)	25	2.36±0.64	6.42±1.92
03	Alcohalic extract	25	3.82±1.62	9.76±0.84
		50	2.92±1.16	8.04±1.46
		100	2.12±1.34	7.34±0.32
04	Aqueous extract	25	4.78±0.18	15.76±1.42
		50	4.26±1.04	14.62±0.96
		100	3.92±1.24	13.72±0.28

Results are expressed as Mean ±SEM (n = 6). Values are in minutes.

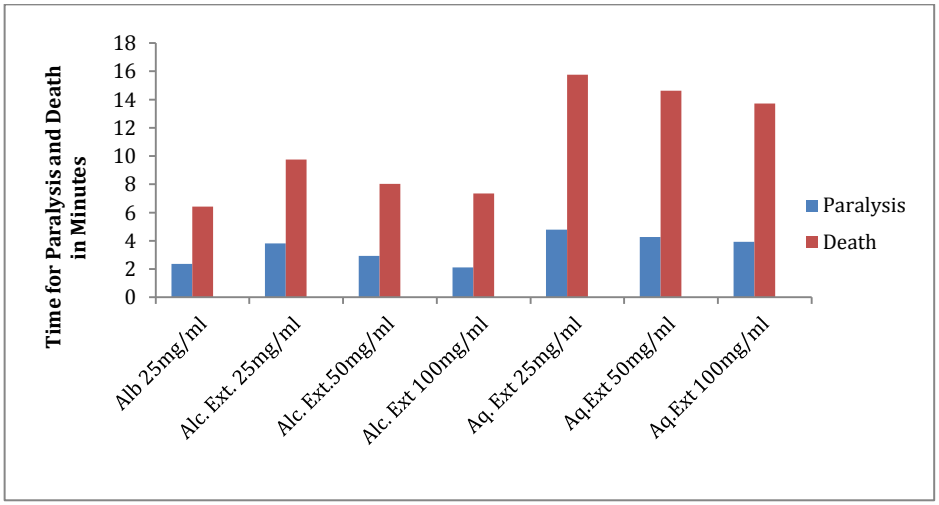

Fig-1; Comparative data of paralysis and death time for *Pheretima posthuma.*

CONCLUSION

From the above laboratory results, It is concluded that alcoholic extract and aqueous extract of *Ficus benghalensis* Linn leaves have a potent anthelmintic activity when compared with standard drug. In this present study anthelmintic assay was performed on the adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings.[13] Further studies are needed to establish the mechanism of action and isolation of phyto – constituents responsible for the anthelmintic activity.

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