

EVALUATION OF IN-VITRO ANTHELMINTIC ACTIVITY OF THE ROOTS OF *ZIZIPHUS OENOPLIA* LINN, MILL. (RHAMNACEAE)

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ABSTRACT

Background: Helminth infections are most common infections in man which affects a large proportion of world's population which interrupts their quality of life. The aim of this study is to prove the anthelmintic activity of the roots of *Ziziphus oenoplia* linn (Rhamnaceae).

Methods: Four groups each containing three earthworms of approximately same size were released into 10ml of desired formulation. Each group was treated with one of the following; Albendazole 40mg/ml in Carboxy Methyl Cellulose (CMC) as standard and ethanolic extracts 5mg/ml, 10mg/ml and 50mg/ml of the root powder *Ziziphus oenoplia* in CMC. The time taken for the paralysis and death of earthworm by ethanolic extract was compared with that of standard Albendazole.

Result: The result of this study shows that the anthelmintic activity of the drug increases with increase in the concentration of ethanolic extract of the root powder of *Ziziphus oenoplia*. In comparison with the standard drug Albendazole, the time taken for the paralysis and death of the earthworm by the ethanolic extract is found to be desirable.

Conclusion: The present investigation proves that the alcoholic extract of *Ziziphus oenoplia*, exerts significant activity against roundworm infections. We hope that in future the shrub *Ziziphus oenoplia* will be able to replace the anthelmintic drugs which have an increased risk of drug interactions.

Keywords: Anthelmintic, *Ziziphus oenoplia*, Ethanolic extract, Albendazole, Earthworms.

INTRODUCTION

Helminths are parasitic worms. Some helminthes are haemophagus i.e. blood eating while others are tissue grazers. Intestinal infections with worms can be more easily treated than those infections occurring in other locations in the body; because the worms need to be killed by the drug and the drug need not be absorbed when given by oral route. Helminths are the most common infectious agents of human in developing countries that produces a global burden of disease that exceeds better-known disease conditions like, malaria and Tuberculosis (TB).

The World Health Organization (WHO) estimates that a staggering two billion people harbor parasitic worm infections. India, in spite of better health education and diagnostic facilities available, intestinal helminthic infestations continues to be a threat to mankind especially due to the impact of high worm burden on malnourished and school going children. If left untreated, results in multi-year, chronic inflammatory disorders that cause both concurrent and delayed-onset pathology to the afflicted. Recent detection of some of these helminths in Human Immunodeficiency Virus (HIV) positive patients are some of the reported facts which indicate the magnitude of the problem and need for control of these soil transmitted helminthes particularly targeting the children and pregnant women¹. Anthelmintics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. Anthelmintics act either locally or systemically. They expel worms directly from the Gastro intestinal tract (GIT) or destroy helminths that invade organs and tissues.

To be an effective anthelmintic, a drug must be able to penetrate the cuticle of the worm or enter its alimentary tract. Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. Because of increasing anthelmintic resistance and impact of conventional anthelmintics on the environment, it is important to look for alternative strategies against gastrointestinal nematodes². Use of herbs could be one of the major options to control these pathologies. The plant *Ziziphus oenoplia* linn., mill., (Rhamnaceae) popularly known as "KARKANDHAUH" in Ayurvedic system of medicine, is traditionally used as anthelmintic, hepatoprotective, stomachalgia and in digestive disorders^{3,4,5,6}. The plant is reported to possess alkaloids ziziphine α , β , betulinic acid, carbohydrates and tannins^{7,8}. Literature

survey revealed that the root extract of *Ziziphus oenoplia* has not yet been screened for its anthelmintic activity. Therefore an attempt has been made to evaluate anthelmintic activity of roots of *Ziziphus oenoplia* on adult earthworm *Pheritima posthuma*.

MATERIALS AND METHODS

Plant collection and authentication

Ziziphus oenoplia roots were collected from the local areas of Avadi, Chennai dist., Tamilnadu, India. The collected roots were shade dried in the laboratory for 7 days. After complete drying the roots were coarsely powdered in a grinding mill and stored at room temperature in a closed air tight container for further use.

Plant authentication was obtained from the National Institute of herbal medicine (Plant anatomy research centre) Reg.no of the certificate (PARC/2010/498).

Worm collection

Indian earthworm *Pheritima posthuma* (annelida) were collected from Madras Medical College (MMC) garden and authenticated by the Zoological Survey of India, Santhome, Chennai dist, Tamilnadu, India.

Preparation of the extract

The powdered root was repeatedly extracted using continuous percolation in a Soxhlet apparatus with 500ml of each solvent of increasing polarity starting with hexane, ethyl acetate, ethanol, distilled water. The extracts were evaporated at room temperature and preserved properly before subjecting to anthelmintic activity.

Preparation of the test sample

The test sample was prepared by dissolving 5mg, 10mg, 50mg of ethanolic extract residue of the root of *Ziziphus oenoplia* in CMC and the standard extract (Albendazole) was prepared by dissolving 40mg of drug in CMC^{9,10}.

Evaluation of anthelmintic activity

The ethanolic extract root of *Ziziphus oenoplia* was screened for anthelmintic activity by using adult Indian earthworms, *Pheritima posthuma* by using the method of Mali. *Pheritima posthuma* was selected in view of its anatomical and physiological resemblance with

the intestinal round worm parasite of human beings. Four groups each containing six earthworms of approximately equal size (12-14cm) was released into 10ml of desired formulation. Each group was treated with one of the following vehicle: Albendazole 40mg/ml in CMC as standard and the ethanolic extract 5mg/ml, 10mg/ml, 50mg/ml of root powder of *Ziziphus oenoplia* in CMC as test^{11,12,13}. Observations were made for the time taken for paralysis and death of individual earthworm. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed by fading of body color^{14, 15,16}.

RESULTS AND DISCUSSION

After subjecting the earthworms to the crude ethanolic extract of the root of *Ziziphus oenoplia* it is observed that the earthworms lost

their motility and produced dose dependant paralysis which eventually progressed to death. Concentrations of the ethanolic extract 5mg, 10mg, 50 mg/ml have produced paralytic effect at 38.58 minutes, 29.14 minutes and 20.15 minutes and mortality was confirmed at 60.01 minutes, 48.54 minutes and 31.24 minutes respectively on the observation of haemorrhagic and necrotic spots on the worms after their death with higher concentration (Table 1.0)

The time taken for paralysis and death eventually decreases, as the concentration of ethanolic extracts of the root powder of *Ziziphus oenoplia* progressively increases.

The effect of ethanolic extract was compared with the standard drug Albendazole (40mg/ml) which produced death within 20.62 minutes.

Table 1: Shows the Anthelmintic activity as revealed from the extract of root of *Ziziphus Oenoplia*.

Type of vehicle	Concentration mg/ml	Time taken for paralysis (in minutes)	Time taken for death (in minutes)
Albendazole (standard)	40	10.33	20.62
Ethanolic extract (test sample) of the root powder of <i>Ziziphus oenoplia</i>	5	38.58	60.01
	10	29.14	48.54
	50	20.15	31.24

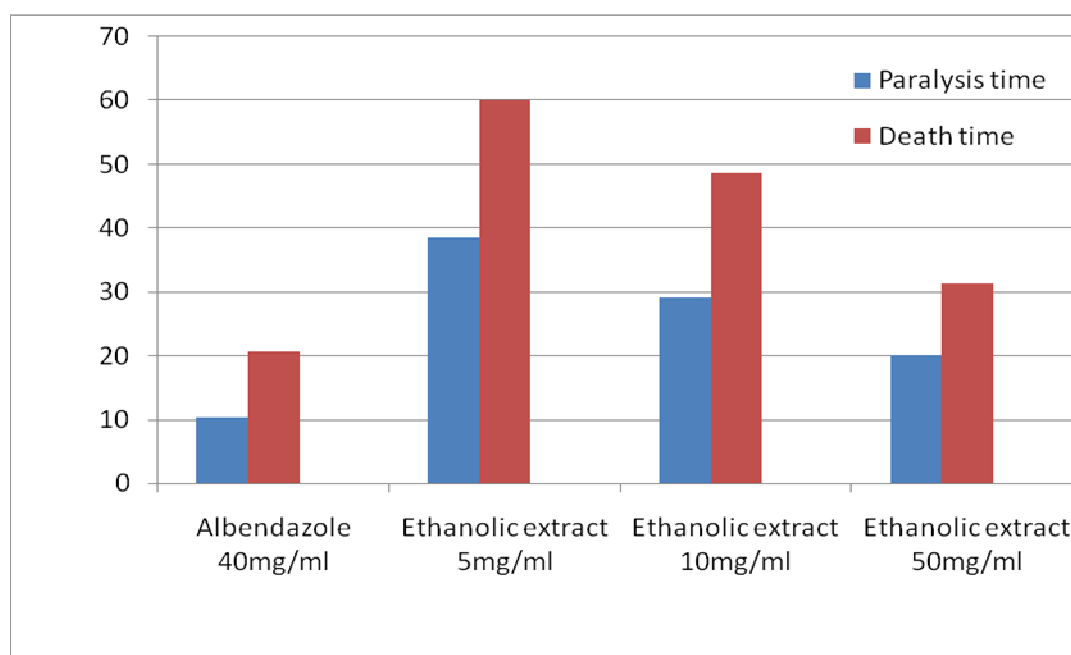


Fig. 1.1: is the diagrammatic representation of Anthelmintic activity as revealed from the extract of root of *Ziziphus Oenoplia*.

CONCLUSION

Traditional claim of the roots of *Ziziphus oenoplia*, as an anthelmintic has been confirmed as the ethanolic extract shows good anthelmintic activity against *Pheritima posthuma*.

Future study of the experiment involving activity guided fractionation is under way and the study is also aimed at extensive investigation, isolation and purification of active phytoconstituents which is responsible for the activity.

The establishment of mechanism of action is required in the near future.

Finally the effectiveness and pharmacological rationale for the use of *Ziziphus oenoplia* needs to be put forth.

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REFERENCES

1. Crompton D W T, Montresor A, Nehshein, L Savioli, Controlling diseases due to helminth infections WHO; 2003.
2. Acharya De. Bundy D A, Immunoepidemiology of intestinal helminth infection I: The global burden of intestinal nematode disease. Trans Royal Soc Trop Med Hyg; 1994 .p.259-261.
3. The Ayurvedic Pharmacopoeia of India, New Delhi. The Controller of Publications; 2001.p.143.
4. Fabricant D S, Farnsworth N R. The value of plants used in traditional medicine for drug discovery; 2001. p.69-75.
5. Deepak and Shrivastava Anshu.. Indigenous Herbal Medicines: Tribal formulations and Traditional Herbal Practices; 2008. p.440.
6. Samwatsar S and Diwanji V B. Plants used for skin disease, cuts, wounds and bruises by the tribals of western MP, J Econ Tan Bot, Addl Ser; 1996 (12).P.192-195.
7. Sunit Suksamrarn, Narisara Suwannapoch, Natthachai Aunchai, Mayuso Kuno, Piniti Ratananukul, Rachada Haritakun, Chawewan Jansakul, Somsak Ruchirawat. Ziziphine N, O, P and Q, new anti plasmodial cyclopeptide alkaloids from *Ziziphus oenoplia* Linn., Mill var. brunoniana, Tetrahedron; 2005 (61).P.1175-1180.
8. Cassels B K, Eckhardt G, Kaussmann E U., Tschesche R; Cyclopeptide alkaloids of *Ziziphus oenoplia* Linn., Mill, Tetrahedron; 1974 (30).P. 2461-2466.
9. Veereshand kambboja S. Anthelmintic activity of *Ziziphus jujube* mill and lamk, Pharmacognosy journal; 2010 (2).p.63-65.
10. Chandrashekar D. Khadse Rajendra. Kakade B. In vitro Anthelmintic activity of Fenugreek seed extract against *Pherithuma posthuma*. International journal of research in pharmaceutical sciences; 2010 (3).p.54-57.
11. Mali R G, Shailaja Mahajan, Patil K S. Anthelmintic activity of root bark of *Capparis spinosa*. Indian J Nat Prod; 2005 (21).p.50-51.
12. Mali R G, Wadekar R R. In Vitro anthelmintic activity of *Baliospermum montanum* Muell. Arg roots. Indian J Pharm Sci; 2008.p.131-133.
13. Tagbota S, Townson S. Antiparasitic properties of medicinal and other naturally occurring products, Adv Parasitol; 2001(50).P.199-205.
14. Shivkar Y M, Kumar V L. Anthelmintic activity of latex of *Calotropis procera*. Pharma boil; 2003(41):263.p.518.
15. Bhabhani S Nayak, Prabhat K Jena, Nigam P Sahu, Udaya K Nayak, Balakrishna K Patro Comparative study of Anthelmintic Activity between aqueous and ethanolic extract of *Solanum surattense* Linn. International Journal of pharmacy and pharmaceutical sciences; 2009.p.103-107.
16. Chetan B Chavan, Maheshwar G Hogade, Somnath D Bhing, Mahesh Kumbhar, Ashpak Tamboli. In vitro anthelmintic activity of fruit extract of *Barleeia prionittis* Linn against *Pheretima posthuma*. International Journal of pharmacy and pharmaceutical sciences; 2010.p.49-50.