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Original Research Article

Evaluation of Antihistaminic Activity in Moringa Oleifera Seeds: A Phytochemical and Pharmacological Investigation

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Abstract: In recent years, the herbal medicines have been extensively used in various diseases because of their safety profile. Helminthic infections are the most common infection in human beings affecting a large Proportion of the world's population. Anthelmintic drugs are the drugs. Which are used to kill or reduce the number of helminthic parasites in the intestinal tract or Tissues of the body. Moringa oleifera belonging to the moringaceae family is a highly valued Plant, distributed in many countries of the tropical and subtropical. The standard drugs such as piperazine citrate, albendazole mebendazole and thiabendazole possess some side effects Such as nausea, vomiting, stomach and abdominal pain, headache, dizziness, and temporary Hair loss etc. But the herbal drug shows fewer side effects. The plant which shows muscle Relaxant property may also shows antihelminthic activity. So we have selected Moringa Oleifera which is an herbal drug and shows muscle relaxant property. Hence the present study was undertaken for phytochemical evaluation and to test the antihelminthic activity of Methanolic extract of Moringa oleifera seed. Preliminary phytochemical investigation indicates the presence of alkaloids, glycosides, flavonoids, steroids, carbohydrates and tannins. Indian Adult earthworms (Pheretima posthuma) were used to study antihelminthic activity. Various concentrations of extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendezol(20mg/ml) was used as a reference standard and distilled water as a Control group [1].

Keywords: Medicinal Plants, Anthelmintic Activity, Helminthiasis Infection, Anthelmintic Drug, Phytochemical Screening, Evaluation Test.

INTRODUCTION

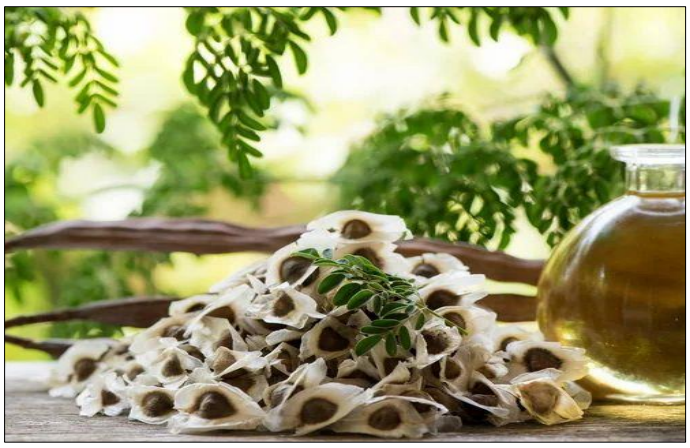
Worldwide, the use of traditional medicines (TMs) has a long history and encompasses an easily accessible and affordable source of treatment. In India, one of the earliest uses of TM is cited in Rig Veda, a compilation of Hindu holy verses (1600-3500 BC). Traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs1. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting ingredients are sometimes marketed for uses contemplated in the traditional healing systems from which they emerged [2], that were never Medicinal plants constitute a very important part of biological heritage of the earth. Traditional societies place a high value on this inheritance, which they express through their ultimate relationship with nature.

Helminth infections are among the commonest infections in man, affecting a large proportion of the world's population. In developing countries they pose a major threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia, and Pneumonia [3]. The world health organization reveals that over two billion people suffering from parasitic

worm infection [7]. It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced by parasitic worm infection [4]. The prevalence of parasitic helminths typically displays a negative binomial distribution with in an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individual are likely to become ill and to perpetuate infection within their [5]. Antihelminthic are drugs that may act locally to expel worters from the OTTOR systemically to eradicate adult helminths or Development form that effect organs and tissue of the body. This helminths infection also causes acute as wel chronic ill health.

Among the various human being and cattle's. More than half of the population is suffering from the worm infection and mostly cattle's Suffers from this worm infections. In most of the developing countries the major health concern is helminths infection because they Predispose humans to other infection such as fungal and bacterial infections. Intestinal infections with worm can easily treated Because it can easily be killed by the drug and this drug needs not to be absorbed when given by oral route [6]. This antihelminthic drug sometimes produces some side effects. This side effect includes abdominal pain, loss of appetite. Nausea, vomiting, headache, diarrhoea [9]. Antihelminthic produces from the natural sources play key role in the treatment of this Parasite infection. In ethno medicine (study of the traditional medicine practice by various ethnic groups) at least 80% of the world Population in developing countries uses plant material as there source of primary health care [8]. Due to this it increases problems of Development of resistance in helminths against antihelminthic. So the medicinal plants are used for antihelminthic activity. There is an increase in the antihelminthic resistance and the impact of conventional antihelminthic on the environment. So it is important to Have alternative method against gastrointestinal nematode [10]. Moringa oleifera is the most widely cultivated spices belonging to the family moringaceae, It is the only genus in the family moringaceae. Moringa oleifera is the fast-growing, drough-resistant tree and it is widely cultivated in tropical and subtropical area. It is natinf in north-western India [11]. The leaves and young seeds pods of Moringa oleifera are used as vegetables. It is used in water purification and in herbal medicine. The leaf powder is used for washing hands. The plant which shows muscle relaxant property may also shows antihelminthic activity. So we have selected Moringa oleifera which is an herbal drug and shows muscle relaxant property [12].

Kingdom-Plantae
Class-Angiosperms
Order-Brassicales
Family- Moringaceae
Genus-Moringa
Species-Oleifera
Bionominal Name- Moringa Oleifera



1.1 Helminthiasis Infection

Helminthiasis, also known as worm infection, is any macroparasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminths.

There are numerous species of these Parasites which are broadly classified into

1) Tapeworms
2) Flukes
3) Roundworms

They often live in the gastrointestinal tract of their hosts, but they may also hurrow into other organs, where they induce physiological damage. Helminthiasis is prevent globally about 1/3 rd of worlds population harbours them, but is more common in developing countries with personal and environmental hygiene [15]. Causative organism harms the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins Anthelmintic are drugs that either kill or expel infesting helminthes [14].

1.2 Sign and Symptoms

Nausea
Vomiting
Abdominal Pain

Diarrhoea
Loss of appetite
Visible worms in the stools
Weight loss [14]

1.3 Location
Found in human intestinal tract, urinary tract or blood stream [16]

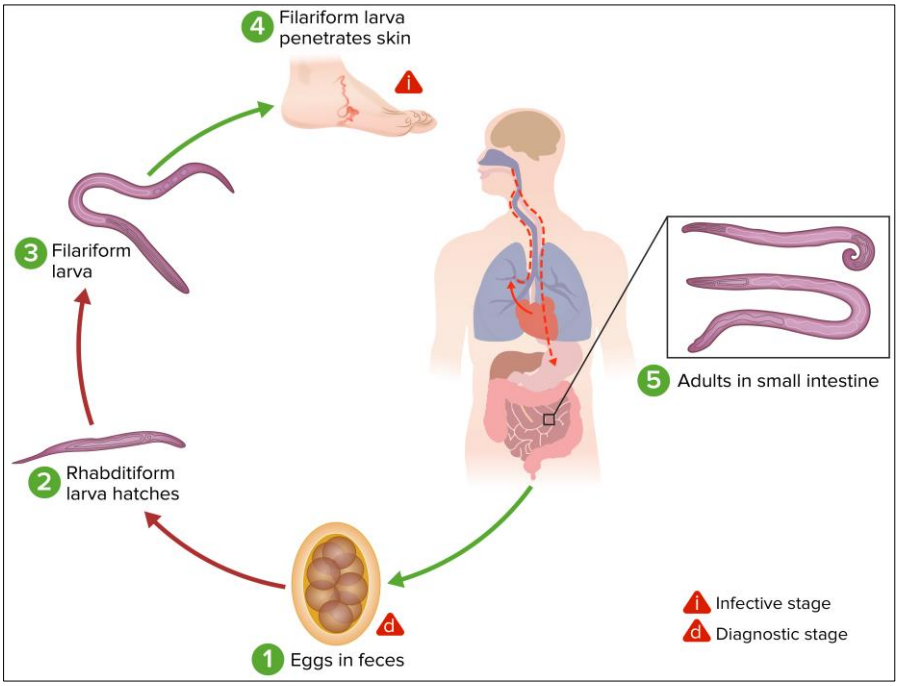
1.4 Transmission
Direct contact with contaminated soil often when walking with barefoo Helminths can spread through contaminated water, food, waste soil and blood [17].

1.5 Anthelmintic Medication Work
Binding to nerves and muscle cells and causing paralysis and eventually death of the parasite [18].

1.6 Treatment (Standard Drug)
1. Mebendazole:
Adverse Effect:
1) Diarrhoea, nausea and abdominal Pain
2) Allergic reaction
3) Loss of hair
4) Granulocy topenia have been reported with high [19]

2. Albendezol
Adverse Effect:
1) Gastrointestinal side effects, dizziness, prolonged use has caused headache, fever, jaundice

3. Thiabendazol
Adverse Effect:
1) Itching
2) Abdominal Pain
3) Nausea, vomiting [21]



METHODOLOGY

1. Plant Collection
The fresh seeds of the plant were collected in the month of Mary and June from the local areas of Swami chincholi Puse, India. The plant material was washed with distil water and shade dried, pulverized in mechanical.

2. Drugs and Chemicals
Albendazole suspension and methanol were used during the experimental protocol. All the chemicals used are Laboratory and Analytical grade.

Preparation of Plant Extract
Seeds were collected and washed thoroughly in water, chopped, air dried for a week and pulverized in electric grinder. Weighed 50 gm of seed powder, add 200 ml of methanol store this combination for 7 days and Filter it by whatmann filter paper. The extract was stored at 40C until used. The prepared extract is used for the antihelminthic activity.

4. Selection of Experimental Model
Indian adult earthworms (Pheretima posthuma) were used to carry out the experiment. The earthworms were collected from the Junior College Khutbav Tal:Daund Dist:Pune. Worms were washed with normal saline to remove all faecal matter Ready availability, anatomical and physiological resemblance of Pheretima posthuma made it to be used initially for in-vitro evaluation of Anthelmintic activity.

2.1 Phytochemical Evaluation

It is performed on plant extract or separated phytoconstituents to explore the possible primary and secondary active constituents present in the extract or phytochemicals.

It Includes:

Screening for primary metabolites
Screening for secondary metabolite

7.2 Qualitative Photochemical Screening for Primary Metabolites

It is performed on plant extract/ phytoconstituents to explore carbohydrates proteins, amino acids, fats and fixed oils present in it.

Test for Carbohydrates

About 50mg of the extract is dissolved in Send distilled water and filtered. The filtrate is tested for the presence of carbohydrates: 2ml of filtrate, 2drops of alcoholic solution of anapthol is added. Mixture is shaken well and Iml concentrated H2SO4 is added slowly along the sides of test tube and is observed for colour. The formation of violet ring at the junction of two liquids indicates the presence of carbohydrate.

a) Fehling's Test

2ml of filtrate is neutralized with alkali. The mixture is heated with Iml of Fehling's solution A and B each and observed for precipitate formation. Red precipitate formed indicates the presence of reducing sugars.

b) Benedict's Test

To 0.5ml filtrate, 0.5ml benedicts reagent is added. The mixture is heated on a boiling water bath for 2 minutes and observed for precipitate formation. Formation of orange red precipitate indicates the presence of reducing sugars.

2. Test for Proteins and Amino Acids

100mg of extract is dissolved in 10ml of distilled water, filtered through wattmann number filtrate paper and filtrate subjected to test for proteins and amino acids.

a) Biuret Test

To Iml of filtrate. Iml 10% NaOH solution is added heated to boil. To this a drop of CuSO4 is added and observed for colour. Formation of purple violet colour indicates the presence of proteins.

3. Test of Fixed Oils and Fats

a) Saponification Test

Treat the extract with few drops of 0.5N alcoholic KOH solution and a drop of phenopthalein solution. The resultant is heated on a water bath for about 1-2 hours. Formation of soap due to neutralization of alkali indicates the presence of fatty material.

73 Qualitative phytochemical screening for secondary metabolites-It is performed on plant extract/phytoconstituents to explore alkaloids, glycosides. Steroids, terpenoids, phenolic compounds, Tannins, flavonoids and saponins present in it.

1. Test for Alkaloids

About 50mg of solvent free extract is dissolved in the same solvent used for extraction and filtered. The filtrate is tested for the presence of alkaloids.

a) Mayer's Test

To 0.5ml of filtrate, drops of mayer's reagent solution of potassium mercurie iodide) is added along the sides of the test she and observed for precipitate. The formation of creamy precipitate indicates the presence of alkaloids.

b) Wagner's Test

To 0.5ml of filtrate, 2 drops of wagner's reagent (solution of lodine in potassium iodide) is added along the sides of the test Tube and observed for the precipitate. The formation of reddish brown precipitate indicates the presence of alkaloids.

c) Dragendroff's Test

To 0.5ml of filtrate, 2drops of dragendroff's reagent is added and observed for precipitate. The formation of prominent Reddish brown colour precipitate indicates the presence of alkaloids.

2. Test for Glycosides

For detection of glycosides about 50mg of extract is hydrolysed with concentrated HCl for 2hrs on a water bath and filtered. The hydrolyzed is subjected to following test.

a) Borntragers Test

To 2ml of hydrolysate, 3 ml of chloroform is added and shaken well. To the separated chloroform layer, Iml of 10% ammonia Solution is added and observed for colour. Formation of pink colour indicates the presence of anthraquinone glycosides.

b) Keller-Killiani Test

About 50mg of the extract is dissolved in 2 ml of glacial acetic acid and 2drops of 5% ferric chloride solution is added and Mixed to this 1 ml of H2SO4 is added, reddish brown colour appears at the junction of two liquid layers and the upper layer appears Bluish green colour indicating the presence of steroidal glycosides.

3. Test for Phenolic Compounds and Tannins

a) FeC13 Test

About 50mg of extract is dissolved in 2 ml of distilled water and then 2 drops of neutral 5% FeCl3 solution is added and Observed for colour. Formation of blur, green or black colour indicates the presence of phenolic compounds and tannins.

b) Lead Acetate Test

About 50mg of extract is dissolved in 2 ml of distilled water and to this 3ml of 10% lead acetate solution is added and observed för the precipitate. The formation of white precipitate indicates the presence of phenolic compounds and tannins.

c. Test for Flavonoids

a) Alkaline reagent test: 10mg of extract is dissolved in 2 ml of water and treated with 1 ml of 10% ammounium hydroxide solution and observed for the colouration 2 drops of dilute HCl is added and again observed for the discolouration. The formation of an intense yellow colour which turns to colourless on addition of dilute HCl indicates the presence of flavonoids [22].

EXPERIMENTAL DESIGN

Methanol extract from the seed of Moringa Oleifera were investigated fo thelmintic activity against Pheretima posthuma. Various concentrations (25,50 and 100 mg/ml) of extract was tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and saline water as control. The assay was performed on adult Indian earthworms, Pheretima posthuma due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro, The anthelmintic activity was carried out as described by using standard protocols, with minor modifications The Indian earthworm (Pheretima posthuma) of nearly equal size, five in each group was taken for the experiment.

8.1 Methods

1) Five groups of approximately equal sized Indian earthworms consisting of one earthworms in each group were released into 10 ml of desired formulation.
2) Group first serve as control, receive only water (negative). Group second serve as standard receive standard drug albendazole (positive) of 20 mg concentration. Group third serve as low dose of extract. Group forth serve as medium dose and Group fifth serve as high dose.
3) Observations were made for the time taken to paralysis and death of individual worms.
4) Maximum time is 77 minutes. Time
5) For paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in waru water (50 0C).
6) Death was included when the worms lost their motility followed by white secretions and Fading away.

RESULTS AND DISCUSSION

RESULT

Preliminary phytochemical screening has shown the presence of carbohydrates, alkaloids, glycosides, steroids, terpenoids, tannins and phenolic compounds m ethanolic extracts of plants was illustrated in Table.

Table No. 1: Phytochemical screening for methanolic extract of Moringa Oleifera

Test	Methanolic Extract
Test For Carbohydrates	
Molish's test	+Ve
Fehling's test	+Ve
Benedict's test	+Ve
Test for proteins and amino acids	
Biuret test	-Ve
Ninhydrin test	-Ve
Test for alkaloids	
Mayers test	+Ve
Wagners test	+Ve
Dragendroff's test	+Ve
Test for Glycosides	
Kellar killani test	+Ve
Borntragers test	-Ve
Test for tannins and phenolic compounds	
Ferric chloride test	+Ve
Lead Acetate test	+Ve
Test for flavonoids	
Alkaline reagent test	-Ve
Test for fixed oils and fats	
Saponification test	-Ve

Fig. 1: Photographs of antihelminthic activity Methanolic extract of Moringa oleifera seed after experiment

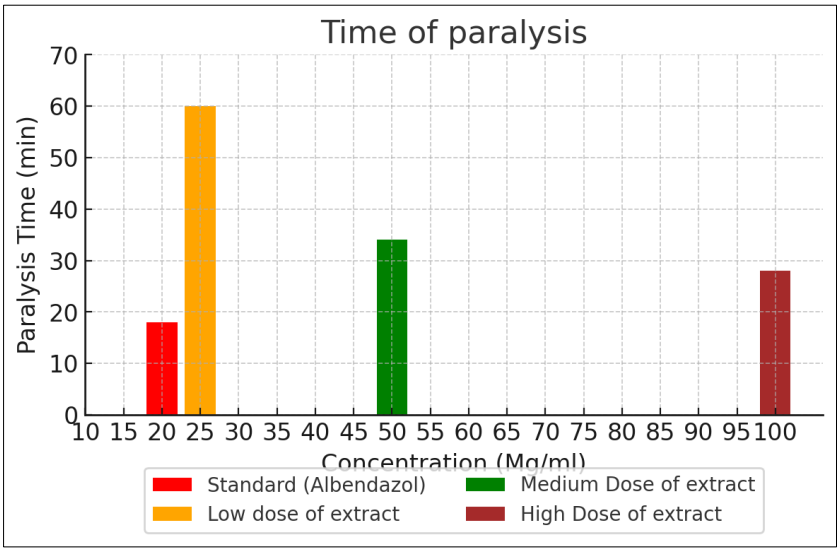
Figure no.2: Photographs of antihelminthic activity Methanolic extract of Moringa oleifera seed after experiment

Table No. 2: Time of Paralysis

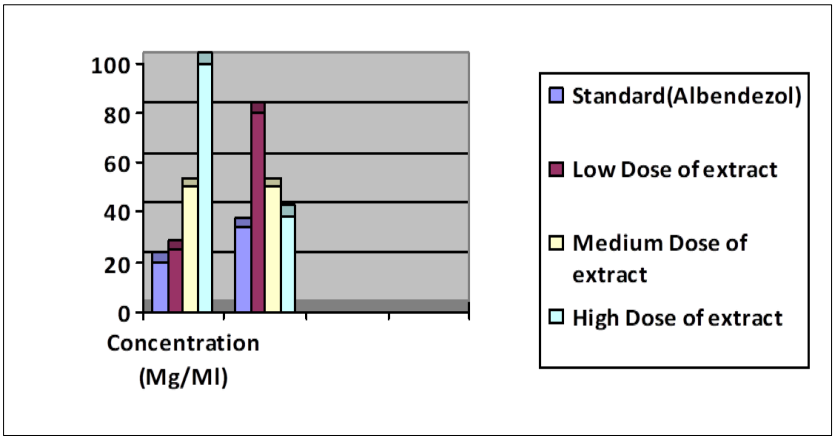
Group	Treatment	Concentrations (Mg/ml)	Paralysis time (min)
1	Standard (Albendezol)	20	18
2	Low dose of extract	25	60
3	Medium dose of extract	50	34
4	High dose of extract	100	28

Table No. 3: Time of Death

Group	Treatment	Concentration (Mg / Ml)	Death Time (Min)
1	Standard (Albendezol)	20	34
2	Low dose of extract	25	80
3	Medium dose of extract	50	50
4	High dose of extract	100	39



Graph of Time of paralysis



Graph of Time of Death

DISCUSSION

Moringa oleifera was collected and dried for a week. The Methanolic extract was prepare by dissolving Seed powder in methanol and store for 7 days. The percentage yield of Methanolic extract was found to be 3.33% w/w. Phytochemical screening was performed on Methanolic extract of Moringa oleifera. It shows the presences of carbohydrates, alkaloids, glycosides, steroids and terpenoids, tannins and phenolic compounds (Table). The presence of some of these phytochemical constituents may produce antihelminthic activity. The evaluation of antihelminthic activity was done. Five groups were taken consists of 1 earthworms in each group. Two parameters were observed that is time of paralysis and time of death was illustrated in Table and ... respectively. When the three concentrations of extract were compared with standard drug it shows activity in a dose-dependent manner showing maximum efficacy at high dose than to the medium dose followed by low dose.

The statistical values for time of paralysis is 71.25±1.10 > 80.5±2.02 > 84.25±1.70 > 89.25±0.47 it represents standard drug, High dose, medium dose, low dose respectively. The statistical values for time of death is 80 50 > 39> 34 it represents Low Dose, medium dose, high dose. Standard respectively. Further studies should be done to identify the active constituents responsible for the antihelminthic activity.

CONCLUSION

From our observations, higher concentration of extract produced paralytic effects earlier and the time taken for death was shorter when compared with other two concentrations, Methanolic extract of Moringa oleifera shows antihelminthic activity in Dose-dependent manner showing maximum efficacy at high dose (100mg/ml concentration). Antihelminthic activity of the extract was compared with the standard drug albendazole. From the above results, we can conclude that Moringa oleifera seed extract exhibited significant antihelminthic activity: therefore further study must be carried to know the active chemical constituents responsible for Antihelminthic activity.

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