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Research Article

IN VITRO ANTHELMINTIC EVALUATION OF *KUDARKIRUMI NAASINI CHOORANAM* BY EARTHWORM MOTILITY ASSAY (EUDRILUS EUGENIAE)

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ABSTRACT

Kudarkirumi Naasini Chooranam is a classical Siddha polyherbal formulation traditionally used for the management of intestinal helminthic infestations. The present study was undertaken to evaluate the in vitro anthelmintic activity of this formulation using the earthworm motility assay with *Eudrilus eugeniae* as the experimental model. The formulation was prepared according to standard Siddha procedures and tested at different concentrations to assess its biological activity. The anthelmintic activity was evaluated by recording the time taken for paralysis and death of the earthworms after exposure to the test solutions. The results demonstrated a concentration-dependent effect, with higher concentrations producing a faster onset of paralysis followed by death of the worms. The observed activity is likely due to the interference of active constituents with the neuromuscular coordination and physiological functions of the parasites. The findings of this study indicate that *Kudarkirumi Naasini Chooranam* possesses significant anthelmintic potential and supports its traditional use in the management of intestinal worms.

INTRODUCTION

A commonly used in vitro technique for assessing the biological activity and possible toxicity of test substances is the earthworm motility assay employing *Eudrilus eugeniae.* Because of its well-developed neuromuscular system, appropriate body size, and ease of handling, *Eudrilus eugeniae*, sometimes known as the African nightcrawler, is extensively employed in laboratory experiments.

Earthworms are a useful model organism for initial screening of anthelmintic and toxic effects because their physiological and neuromuscular responses are thought to be similar to those of intestinal helminths.

This experiment is predicated on the idea that physiologically active substances disrupt the worm's regular neuromuscular function, resulting in decreased motility, paralysis, and ultimately death. The biological potency of the sample can be evaluated by subjecting earthworms to varying doses of the prepared test solution and timing how long it takes to cause paralysis and death. Dose-dependent action is seen by the decrease in paralysis and death time with increasing concentration. Therefore, the earthworm motility assay offers a quick and easy way to assess the test sample's anthelmintic or poisonous potential.

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Collection and Authentication of Raw Material

The raw drugs required for the preparation of "*Kudarkirumi Naasini Chooranam*" were brought from a reputable raw drug shop in Nagercoil. The herbal raw drugs were verified and authenticated by the Head of the Department of Gunapadam of Government Siddha Medical College, Palayamkottai.

Purification

- *Palasu*- Soak the seeds in water, remove the outer skin, dry the seeds and grind them into a powder.
- *Omam*- Soak the seeds in a lime water and dry it.
- *Vaividangam*- dry them in the sun and store them away.
- *Perungayam*- Use this either by roasting it over a charcoal fire, or by soaking it in lotus leaf juice for one *Nazhigai* (approximately 24 minutes) and then drying it.

Procedure

Grind all the ingredients into coarse fine powder. Keep it in storage container.

Preparation of Test Solution

The volume of working solution needed for the experiment determines how much distilled water is needed. Typically, 5–10ml of solution were made for each concentration in the earthworm motility test. To create a homogenous solution, the test sample was precisely weighed and dissolved in a tiny amount of distilled water while being gently stirred. Distilled water was progressively added to the final volume after complete dissolution in order to get the required concentration, such as 25, 50, 100, and 200mg/ml. Different concentrations were prepared using the serial dilution approach. To guarantee homogeneity, the produced solutions were properly mixed before being used. For the motility test, freshly made test solutions were utilized right away to prevent the active ingredients from deteriorating. All solutions were stored in clean labeled containers until use in the experiment.

Preparation of Test Solutions

Prepare different concentrations of the sample using distilled water.

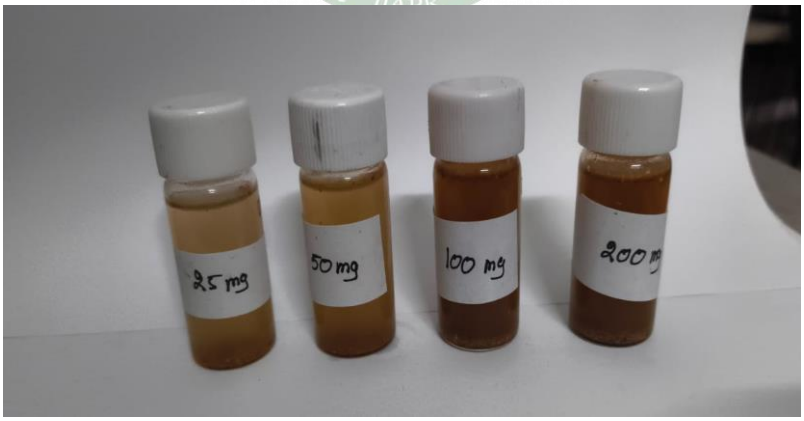
MATERIALS AND METHODS

Ingredients of *Kudar Kirumi Naasini Chooranam*

S.No	Name	Botanical Name	Parts Used	Quantity
1.	*Palasu vidhai*	*Butea monosperma*	Seed	20gm
2.	*Perungayam*	*Ferula asafoetida*	Gum resin	10gm
3.	*Omam*	*Trachyspermum ammi*	Seed	10gm
4.	*Vaividangam*	*Embelia ribes*	Seed	5gm
5.	White sugar	---	---	45gm

Concentration	Preparation Method
25 mg/mL	Dissolve 25mg sample in 5ml distilled water
50 mg/mL	Dissolve 50mg sample in 5ml distilled water
100 mg/mL	Dissolve 100mg sample in 5ml distilled water
200 mg/mL	Dissolve 200mg sample in 5ml distilled water

Preparation of Test Samples for Anthelmintic Assay



Materials Required

The materials used for the earthworm motility assay included healthy adult earthworms measuring approximately 8–10cm in length and weighing about 1–1.5 g, the prepared test sample, distilled water which served as the control, and normal saline (0.9% NaCl) for cleaning the earthworms. Petri dishes or clean beakers were used as experimental chambers, and filter paper was placed inside the dishes to maintain moisture and facilitate exposure of worms to the test solution. Forceps were used for careful handling of earthworms to avoid physical injury during the experiment. A stopwatch was used to record paralysis and death time accurately. A sterile needle was used to confirm the death of earthworms by checking the absence of response to stimulation. Laboratory gloves were worn during the experiment to maintain hygiene and prevent contamination. All materials were cleaned and prepared before starting the assay to ensure accurate experimental results.

Earthworm Motility Assay

Healthy adult earthworms were collected from moist soil and carefully transported to the laboratory

under suitable conditions. The collected earthworms were washed gently with tap water followed by normal saline (0.9% NaCl) solution to remove adhering soil particles and contaminants. After washing, the earthworms were placed on moist filter paper for 5–10 minutes to remove excess surface moisture and to maintain physiological conditions.

Clean Petri dishes were prepared by lining them with filter paper, and approximately 5–10ml of each prepared test solution (25, 50, 100, and 200mg/mL) was added to the respective dishes. One earthworm was placed in each Petri dish containing the test solution, while a separate dish containing distilled water was used as the control. The experiment was conducted at room temperature.

The time was recorded immediately after placing the earthworm into the solution. Continuous observation was carried out to monitor worm movement. Paralysis time was noted when the earthworm stopped spontaneous movement and responded only to vigorous shaking. Death time was recorded when the worm showed no movement even after needle stimulation and exhibited fading of body colour. Each concentration was tested in triplicate to ensure accuracy, and mean values were calculated for data analysis. All experiments were conducted carefully to avoid mechanical injury and environmental stress to the earthworms.

Determination of End Points

The determination of end points was carried out by monitoring the paralysis and death of earthworms after exposure to the test solutions. Paralysis time was recorded when the earthworm completely stopped spontaneous movement but showed slight movement only when shaken vigorously with forceps. The worm was considered to be in the paralysis stage when normal wriggling and locomotor activity were absent.

Death time was determined by confirming the absence of any movement even after gentle stimulation using a sterile needle. The earthworm was considered dead when it failed to respond to mechanical stimulation, exhibited complete loss of motility, and showed noticeable fading or pale discoloration of the body. The paralysis and death times were recorded carefully using a stopwatch. Observations were made continuously for each concentration, and the experiment was repeated in triplicate to obtain mean values for statistical analysis. Care was taken to avoid physical damage to the earthworms during the determination of end points.

Table 1: Paralysis Time

S.No.	Concentration (mg/mL)	Earth Worm *Eudrilus eugeniae* (min)			
		Test - 1	Test - 2	Test - 3	Mean Paralysis Time
1	Control	0	0	0	0
2	25	45	48	46	46.3
3	50	30	32	31	31.0
4	100	18	20	19	19.0
5	200	8	9	7	8.0

$$Mean\ paralysis\ time \frac{T1 + T2 + T3}{3}$$

Calculations

25 mg/mL: (45 + 48 + 46) / 3 = 139 / 3 = 46.3 min
50 mg/mL: (30 + 32 + 31) / 3 = 93 / 3 = 31.0 min
100 mg/mL:(18 + 20 + 19) / 3 = 57 / 3 = 19.0 min
200 mg/mL: (8 + 9 + 7) / 3 = 24 / 3 = 8.0 min

Final Mean Paralysis Times

S.No.	Concentration (mg/mL)	Mean Paralysis Time (min)
1	25	46.3
2	50	31.0
3	100	19.0
4	200	8.0

Paralysis of the Earthworm was Observed After Exposure to 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml Concentration









Table 2: Death Time

S.No	Concentration (mg/mL)	Earth Worm *Eudrilus eugeniae* (min)			
		Test 1	Test 2	Test 3	Mean Death Time
1	Control	0	0	0	0
2	25	70	72	69	70.3
3	50	55	57	54	55.3
4	100	32	33	31	32.0
5	200	15	16	14	15.0

$$Mean\ death\ time \frac{D1 + D2 + D3}{3}$$

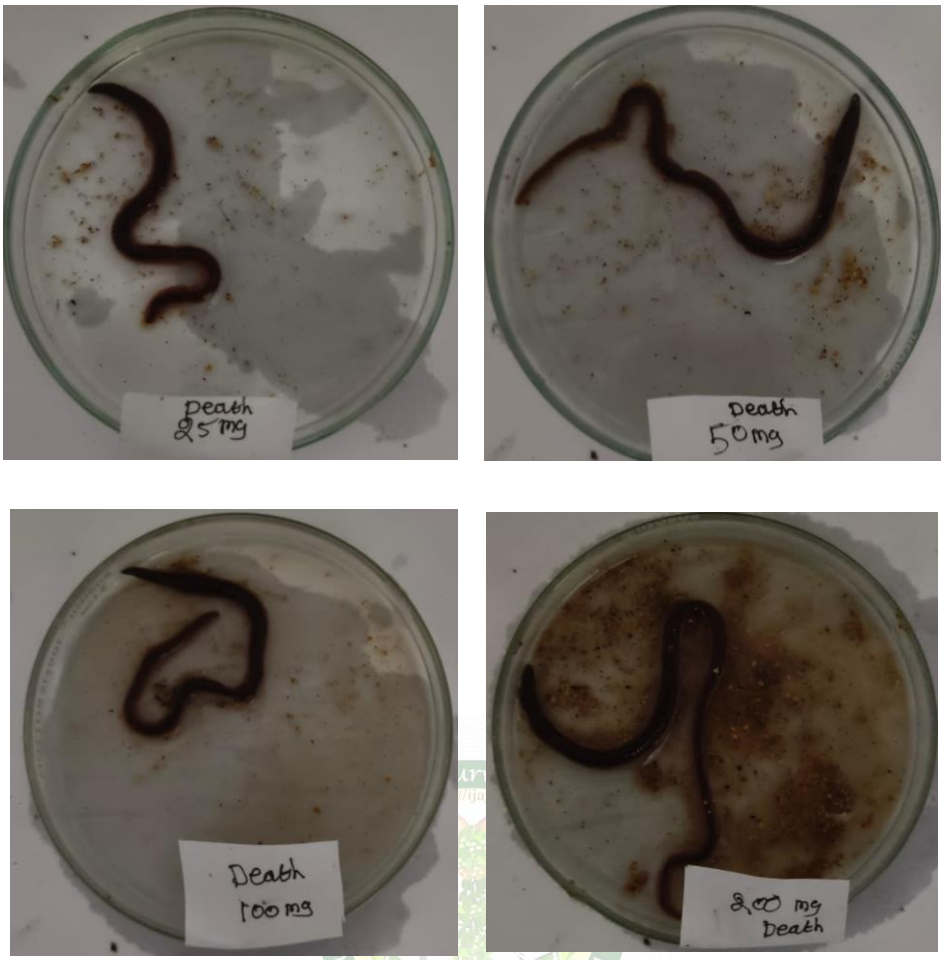
Calculations

25 mg/mL: (70 + 72 + 69) / 3 = 211 / 3 = 70.3 min
50 mg/mL: (55 + 57 + 54) / 3 = 166 / 3 = 55.3 min
100 mg/mL: (32 + 33 + 31) / 3 = 96 / 3 = 32.0 min
200 mg/mL: (15 + 16 + 14) / 3 = 45 / 3 = 15.0 min

Final Mean Death Times

Concentration (mg/mL)	Mean Death Time (min)
Control	—
25	70.3
50	55.3
100	32.0
200	15.0

Death of the Earthworm was Observed After Exposure to 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml Concentration



DISCUSSION

The present study evaluated the in vitro anthelmintic activity of *Kudarkirumi Naasini Chooranam* using the earthworm motility assay with *Eudrilus eugeniae* as the experimental model. The results clearly demonstrated a concentration-dependent anthelmintic effect, as evidenced by the progressive decrease in both paralysis time and death time with increasing concentrations of the formulation. At lower concentration (25mg/ml), the mean paralysis and death times were recorded as 46.3 minutes and 70.3 minutes respectively, indicating a slower onset of action. As the concentration increased to 50mg/ml and 100mg/ml, a marked reduction in paralysis (31.0 and 19.0 minutes) and death times (55.3 and 32.0 minutes) was observed. At the highest concentration tested (200mg/ml), the formulation exhibited rapid activity, producing paralysis within 8.0 minutes and death within 15.0 minutes. This significant reduction in time indicates enhanced efficacy at higher concentrations and confirms a clear dose-dependent response.

The anthelmintic activity observed may be attributed to the presence of active phytoconstituents in the formulation, which are known to interfere with the neuromuscular system of helminths. These compounds may cause paralysis by disrupting ion channels or neurotransmission, ultimately leading to death of the organism. The progressive loss of motility and eventual death observed in the earthworms further supports this mechanism of action.

The earthworm motility assay proved to be a simple, reliable, and reproducible method for evaluating anthelmintic activity. The results of this study validate the traditional use of *Kudarkirumi Naasini Chooranam* in the management of intestinal helminthiasis and suggest its potential as an effective natural anthelmintic agent.

CONCLUSION

Kudarkirumi Naasini Chooranam exhibited promising anthelmintic activity in the present in vitro study. The formulation effectively induced paralysis and death in the test organism, indicating its potential to act against intestinal helminths. These findings provide supportive evidence for its traditional use in Siddha medicine for the management of worm infestations.

The study highlights the relevance of exploring classical herbal formulations as potential sources of safe and effective anthelmintic agents. *Kudarkirumi Naasini Chooranam* may offer a natural therapeutic

option, particularly in regions where helminthic infections are prevalent.

Nevertheless, comprehensive studies including in vivo investigations, toxicity assessments, and controlled clinical trials are essential to further substantiate its efficacy and ensure its safe application in human therapeutics.

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