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Short Communication

In Vitro Evaluation of Anthelmintic Activity of Glycerine on Indian Earthworms

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ABSTRACT
One of the well-known pharmaceutical ingredients- Glycerine showed significant anthelmintic results after lot many experimental trials. Thus Glycerine is acting as a potent anthelmintic agent and it does so by exerting its osmotic dehydration property. Experimental trials were carried out using Indian earthworms (Pheretima Posthuma). The various concentrations were tested for Anthelmintic activity & Albendazole is used as a standard for comparison.
This is the first attempt to reveal the anthelmintic activity of glycerine which is not yet reported anywhere. The effect of Glycerine against parasite was observed in very shorter time. It shows paralysis time 101 seconds and death time 104 seconds which is much superior to that of Albendazole (14.53 minutes and 23.46 minutes). By *in vitro* evaluation it is proved that Glycerine potentiates paralysis in earthworm & ultimately it results in death of worms.
Keywords: Anthelmintic, Albendazole, Glycerine, Tween 80.

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INTRODUCTION
Development of anthelmintic resistance and high cost of conventional anthelmintic drugs requires alternative sources of anthelmintics. Infection with helminths or parasitic worms, affect more than two billion people worldwide. [1] It is often found in the developing countries. Helminth infection produces a global burden of disease which often leads to malnutrition, anaemia, eosinophilia and pneumonia. [2] Helminthosis plays an important role in economic losses. [3] Major threat to the control of parasites is their survival against drugs which are generally effective at the recommended dose. Anthelmintics are the drugs used to treat helminth infections by expelling them from body either stunning or by killing them without damaging host cells. They are either vermifuges (those that stun) or vermicides (those that kill).

Glycerine is a clear, oily viscous liquid with somewhat sweet taste. It is manufactured from petroleum, or glycerides in fats. Glycerine shows hydrophilic properties- water loving, therefore easily mixes with water.

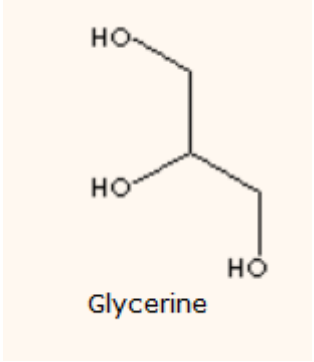

Figure 1: Structural formula of Glycerine

SYNONYMS:

Glycerol
Glycerine
1,2,3Trihydroxypropane
1, 2, 3 propanetriol

MATERIALS AND METHODS

Materials:

Glycerine was collected from Vikhe Patil Memorial Hospital. Tween 80 and Albendazole were obtained from the institute, Vikhe Patil Pharmacy College, Ahmednagar. While performing the experiment, we have confirmed that Tween 80 is not showing any anthelmintic activity at the given concentration.

Preparation of solutions:

Preparation of Albendazole (1.5%) solution- Albendazole has Minimum Inhibitory Concentration of 1.5%. 1.5 mg Albendazole was accurately weighed. It is mixed with 0.1% Tween 80 and volume is made up to 100 ml with distilled water. It will give 1.5 % Albendazole solution.

Preparation of glycerine solution-

Solutions of glycerine with concentration ranges 0.5%, 1%, 1.5%, 2% were prepared using distilled water.

Selection of worms:

The assay was performed using adult earthworm, Pheretima Posthuma. [4] These earthworms show anatomical and physiological resemblance with the intestinal round worm parasites of human beings. As they are easily available, widely used for the initial *in vitro* anthelmintic evaluations. [5, 6] Adult earthworms were collected from moist soil. They are washed properly to remove all faecal matters and then used for the study. The earthworms of 5-6 cm length and 0.1-0.2 cm widths were used for all experimental protocol.

Grouping of worms:

Six groups, having six earthworms in each group were prepared. Group I serves as a 'control' and receive only distilled water. Group II serve as 'standard' and receives 1.5% Albendazole solution. Group III, IV, V, and VI receive glycerine preparations with concentration 0.5%, 1%, 1.5%, 2% respectively.

Observations were made for the paralysis time and death time of individual earthworm. Six groups, six earthworms in each group were prepared.

Sr. No	Group	Condition	Concentration (%)
1.	I	Control (Distilled Water)	---
2.	II	Standard (Albendazole)	1.5%
3.	III	Glycerine	0.5%
	IV		1 %
	V		1.5 %
	VI		2 %

Table 1: Scheme Used for Evaluation of Anthelmintic activity of Glycerine.
All the results were expressed as mean± SEM.

Probable mechanism of action:

Glycerine is an osmotic dehydrating agent. Probably the death of earthworms occurs due to severe dehydration, i.e. drainage of water content from earthworm's body. The additional purgative feature may be useful in expelling dead worms out of the body, which is helpful against reducing the requirement of multiple drug therapy.

Statistical analysis:

Data was analysed by applying t-test on each group. P values were calculated in each case and consequences were noted. Statistical analysis for both paralysis time and death time of earthworm were carried out and results are shown in Table No 2.

RESULT & DISCUSSION:

Results obtained from the experiment confirm the anthelmintic activity of glycerine with shortest time of paralysis and deaths. Paralysis was confirmed when earthworms lost their motility and death was confirmed with fading of their colour. No activity was observed even after dipping them in warm water (50° C).

Group	Treatment		Concentration	Time taken for paralysis (min)	Time taken for death (time)
1	Control	I	-	-	-
2	Standard (Albendazole)	II	15 mg/ml	14:53±0.342***	23:46±0.03011***
3	Glycerin	II I	0.5 ml	101:52±0.3421***	104:09±0.3336***
		I V	1.0 ml	01:41±0.003651***	01:44±0.003651***
		V	1.5 ml	01:17±0.007923***	01:20±0.005774***
		V I	2.0 ml	00:21±0.004773***	0:25±0.004773***

Table 2: Anthelmintic activity of glycerine on Pheretima Posthuma
Values are expressed as Mean± SEM, ***p<0.0001 when compared with standard drug

Hence the above results prove that the activity of glycerine is much superior as compared to Albendazole. All the concentrations were found to possess anthelmintic activity.

CONCLUSION:

In vitro evaluation of glycerine on earthworms showed that it has profound activity against worms and it is much better than standard (Albendazole) at very low concentrations. It achieves the better therapeutic effect in lower dose with no side effects. The probable mechanism of action is also supportive to the study. In future we may use glycerine directly as an anthelmintic or as an adjunct to reduce the dose of drug.

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