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**Anthelmintic activity of aqueous and methanolic extract of
Krumina tablet - A polyherbal formulation**

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Abstract

The present study was done with the aim to evaluate anthelmintic activity of aqueous and methanolic extract of krumina tablet - a polyherbal formulation containing traditionally used herbs via., *Embelia Ribes*, *Acorus calamus*, *Caesalpinia crista*, *Butea monosperma*, *Operculina turpethum*, using adult earthworm *Pheritima posthuma*. The aqueous and methanolic extracts of krumina tablet were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole was used as a reference standard and gum acacia in saline as a control group and it was found that the aqueous extract showed higher activity than methanolic extract.

Key-Words: Anthelmintic activity, Krumina tablet, *Pheritima posthuma*

Introduction

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics^{1,2, 3}. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Treatment with an antihelminthic drug kills worms whose genotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. Intestinal worm infections in general are more easily treated than those in other locations in the body⁴. Development of resistance to most of the commercially available anthelmintics became a severe problem worldwide. So the use of synthetic anthelmintics can lead to resistance of parasites⁵.

Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human and could be of value in preventing the development of resistance⁶.

Krumina tablet is one of the traditional polyherbal preparations, containing traditionally used herbs via., *Embelia Ribes*, *Acorus calamus*, *Caesalpinia crista*, *Butea monosperma*, *Operculina turpethum*. They are also used as important ingredients in folklore medicine in many Asian countries. The present study was perform to study the anthelmintic activity of aqueous and methanolic extract of Krumina tablet on Indian earth worms (*Pheretima posthuma*).

Material and Methods

Procurement of Product

Krumina tablet, a product of Sharangdhar pharmaceutical Pvt Ltd., is an Ayurvedic preparation was purchased from Local medical shop from Akulj city, Solapur district, Maharashtra.

Preparation of the aqueous extract

100 gm of Krumina Tablet was suspended in 1.0 liters of distilled water then stirred magnetically for 24 hours and kept for 7 day at room temperature. The extract was double filtered by using musline cloth and whatmann No. 1 filter paper. The extract was concentrated to dryness in a water bath at controlled temperature (50 -60°C). The dried aqueous extract of Krumina Tablet was stored in desiccators under controlled conditions till it used for experimental purpose.

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Preparation of methanolic extract

100 gm of Krumina Tablet was suspended in 1.0 liters of methanol then stirred magnetically for 24 hours and kept for 7 day at room temperature. The extract was double filtered by using musline cloth and whatmann No. 1 filter paper. The extract was concentrated to dryness in a water bath at controlled temperature (50 - 60°C). The dried methanolic extracts of Krumina Tablet was stored in desiccators under controlled conditions till it used for experimental purpose.

Experimental animals

Anthelmintic activity was evaluated on adult Indian Earthworms (*Pheretima posthuma*) due its anatomical and physiological resemblance with the intestinal round worm of human beings^{7,8}. The Indian adult earthworms *Pheretima posthuma* (Annelida) were collected from moist soil of the field and washed with normal water and saline solution to remove soil and fecal matter. The Earth worms of 4-8 cm in length and 0.2- 0.3 cm in width were used for all experimental parameters.

Drugs and Chemicals used

Albendazole (Glasko Smith Kline) was used as reference standard purchased from local medical shop, Akluj. Chemicals methanol (S.D fine chemicals, Mumbai), Gum Acacia (S.D fine chemicals, Mumbai).

Preparation of test sample

Samples for experiments were prepared by dissolving extract to obtain a stock solution of 100 mg/ml, from the stock solution, different working dilutions were prepared to get concentration range of 10, 20 and 40 mg/ml of aqueous extracts (AKC) and of 10, 20 and 40 mg/ml of methanolic extracts (MKC). For present study Albendazole taken as standard drug. The concentration of standard drug was prepared in 1% gum acacia to give 20 mg/ml concentration.

Storage of Drug Solution

Fresh drug solutions were prepared. The solutions were kept in air- tight amber colored bottles and stored at room temperature till use.

Experimental Animals Groups Dividing

The Indian adult Earth worms can be divided into eight groups. Each group consist six earth worms. Group-I is contain Vehicle (1% gum acacia in normal saline), Group-II containing Albendazole as a reference standard (20 mg/ml) , Group-III containing aqueous extract (10 mg/ml), Group-IV containing aqueous extract (20 mg/ml), Group-V containing aqueous extract (40 mg/ml), Group-VI containing methanolic extract (10 mg/ml), Group-VII containing methanolic extract (20 mg/ml) and Group-VIII containing methanolic extract (40 mg/ml).

Evaluation of Anthelmintic Activity

The evaluation of anthelmintic activity was followed by earlier reported method⁷. Three different concentrations of aqueous and methanolic extract (as given earlier) were prepared and the group of six earthworms which having equal size were released into 10 ml of sample with desired concentration in petridish. Observations were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis in any sort could be observed, except when the worm was shaken vigorously. The worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Paralysis is assumed to occur when they do not revive even in saline solution. Potency is inversely proportional to time taken for paralysis and / or death of parasite. Observations were shown in table 1, regarding the anthelmintic activity of aqueous and methanolic extract of Krumina tablet –a polyherbal formulation on Indian Earthworms (*Pheretima posthuma*).

Statistical analysis

The results were expressed as mean \pm SEM and statistically analyzed by ANOVA followed by Dunnett's test, with level of significance set at $p < 0.05$.

Results and Discussion

In the present work, aqueous and methanolic extracts Krumina Tablet –a polyherbal formulation were used to evaluate anthelmintic activity against Indian earthworms (*Pheretima posthuma*), Each extract containing 10, 20, and 40 mg/ml produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. As shown in table no.1

Anthelmintic activity and some synthetic phenolic anthelmintics are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation⁹. Chemotherapeutic drugs against helminthes infection act mainly through three different mechanisms, such as, disruption of the neuromuscular physiology, blocking the energy metabolism, disrupting the highly efficient reproductive system of the parasites¹⁰. Several important anthelmintics cause paralysis by disrupting one or the other aspect of neuromuscular system¹¹. The possible mechanism of the anthelmintic activity of Krumina tablet cannot be explained on the basis of our present results. However, it may be due to its effect on inhibition of glucose uptake in the parasites and depletion of its glycogen synthesis. Krumina tablet may also have activated nicotinic cholinergic receptor in the worms resulting in either persistent depolarization or hyperpolarisation¹².

The alcoholic and methanolic extract of Krumina tablet exhibited marked anthelmintic activity in terms of causing paralysis and death of worms at concentration of 40mg/ml. The paralysis time was found to be about 28.3 minutes for aqueous extract and 35.0 minutes for methanolic extract as compare to the standard drug Albendazole it was found to be 25 minutes. Alcoholic extract caused death of worms at 41.5 minutes while methanolic extract took 42.0 minutes for death of worms as compare to standard drug Albendazole it was found to be 37.2 minutes. Gum acacia in saline did not revealed wormicidal activity.

The results of the above studies demonstrated that, the alcoholic and methanolic extract of Krumina tablet possess potent anthelmintic activity with varying magnitudes. But the aqueous extract of Krumina tablet showed highest activity, which is almost equal in effectiveness to the standard Albendazole. The difference in the time taken for induction of paralysis in both Albendazole and Krumina Tablet was almost same. Therefore potency of drug was found to be inversely proportional to the time taken for paralysis / death of worms. The higher concentrations of aqueous extract produced paralytic effect much earlier and necrotic spots were observed externally on the worms, as compare to methanolic extract. The effect of each extract was compared with Albendazole as standard drug (20 mg/ml).

Using the *Pheretima posthuma* as the animal models, we have shown that aqueous and methanolic extract of Krumina Tablet has potential to act against helminthiasis. Moreover, the extent of anthelmintic effect of the Krumina Tablet is comparable to that of standard drug, Albendazole being used against helminthiasis. This observation suggests that Krumina Tablet must contain lead compounds that may shown promising wormicidal activity.

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Table 1: Anthelmintic activity of aqueous and methanolic extracts Krumina Tablet –a polyherbal formulation on Indian Earthworms (*Pheretima posthuma*)

Name of Group	Name of extract & Concentration	Time taken for paralysis in minute (Mean \pm SEM)	Time taken for death in minute (Mean \pm SEM)
Group-I	Gum acacia in saline (1%)	---	---
Group-II	Albendazole (20mg/ml)	25.0 \pm 1.59	37.2 \pm 1.62
Group-III	AKC (10 mg/ml)	61.3 \pm 4.75**	86.5 \pm 1.73**
Group-IV	AKC (20 mg/ml)	40.5 \pm 1.18**	57.2 \pm 1.87**
Group-V	AKC (40 mg/ml)	28.3 \pm 0.95	41.5 \pm 1.98
Group-VI	MKC (10 mg/ml)	72.0 \pm 2.16**	79.8 \pm 1.25**
Group-VII	MKC (20 mg/ml)	52.2 \pm 1.62**	59.0 \pm 2.08**
Group-VIII	MKC (40 mg/ml)	35.0 \pm 1.57	42.0 \pm 1.51

Values are expressed as mean \pm SEM. (n=6), ANOVA followed by Dunnett's test, *p<0.05, **p<0.01 when group III to VIII were compared with Standard (Group-II).

AKC - Aqueous extracts of Krumina Tablet.

MKC- Methanolic extract of Krumina Tablet.

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