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## Comparative studies on *in vitro* nematicidal (anthelmintic) activity of cow urine and cow urine distillate

K.Krupanidhi<sup>1</sup>, T.R.Prashith Kekuda\*<sup>2</sup>, B.K.M.Bhramaramba<sup>1</sup>,  
R.M.Shrungashree<sup>2</sup>, S.V.Suchitra<sup>2</sup>, R.Kavya<sup>2</sup>

<sup>1</sup>Dept. of Pharmaceutical Microbiology and Biotechnology, S.C.S College of Pharmacy,  
Harapanahalli - 583 131, Karnataka, (INDIA)

<sup>2</sup>Dept. of Microbiology, S.R.N.M. National College of Applied Sciences, Shivamogga-577201, Karnataka, (INDIA)  
Tel : 09844428934

E-mail: prashith\_kekuda@rediffmail.com

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### INTRODUCTION

The helminths are invertebrates characterized by elongated, flat or round bodies. There are three major groups of helminths containing members that have man as their main hosts, these being the Flukes (Trematodes), the Tapeworms (Cestodes), and the Roundworms (Nematodes). Some of the infections caused by helminthic parasites in man are *Ascariasis*, *Toxocariasis*, *Filariases*, *Trichurias*, *Enterobiasis*, *Hydatidosis*, *Onchocerciasis*, *Tapeworm infections*, *Hookworm infections etc.* Drugs such as Mebendazole, Albendazole, Piperazine citrate etc., have been prescribed to treat helminthic infections. Parasitic infections affect millions of people world-wide afflicting considerable human and other animal suffering. Many parasitic infections are increasing throughout the world. The impact of Human Immunodeficiency Virus (HIV) and AIDS has seen the emergence of "new" opportunistic parasites as well as the increased prevalence of other recognized types. Climatic changes induced through global warming have aided the spread of many parasite diseases, whilst starvation and the breakdown in sanitation that accompanies war have seen the re-emergence of others. The appearance of drug resistance has also dramatically in-

fluenced the ability to treat and control many parasite diseases.

There are several *in vitro* and *in vivo* models for finding anthelmintic activity of compounds. The objectives of the present investigation are to find out anthelmintic activity of Cow urine and its derivative namely Cow urine distillate, which is produced by subjecting Cow urine to distillation process, and to compare anthelmintic activities of Cow urine and Cow urine distillate using earthworm model.

### MATERIALS AND METHODS

#### Collection of Cow urine

Cow urine was collected in a clean container from a variety called *Amrit Mahal*, an indigenous variety of cow, which grazes in forest and not given any artificial feed. It is known that the cows which feed on natural ingredients will give urine with appreciable medicinal values.

#### Preparation of Cow urine distillate

Cow urine distillate was prepared by subjecting the filtered cow urine to distillation process. The distillation process was carried at low temperature (50°C). The

cow urine taken in a round bottom flask was kept on a heating mantle. The flask was connected to water cooled condenser in one end and the other end of the condenser was attached to a beak which is inserted into a container for collecting the distillate. The distillate obtained by the process was subjected to anthelmintic activity in different concentrations.

### Anthelmintic assay

#### Collection of Indian earthworms

In this study, earthworm model was selected as the earthworms are easily available and used widely for the initial evaluation of anthelmintic activity of compounds. The worms were procured from the Agricultural department, Harapanahalli. Equal sized ( $\pm 1$  cm) worms were selected for the study.

#### Standard drug

Albendazole was used as reference standard for anthelmintic study.

#### Methodology

There are several *in vitro* models for evaluation of Anthelmintic activity of compounds. The assay was carried according to the method which used earthworm as model organism<sup>[1]</sup>. The assay was performed on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Normal saline was used as the solvent. Albendazole was diluted with saline to obtain 0.1, 0.2 and 0.5% concentration which served as standard and poured into respective Petri dishes. 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0% concentration of Cow urine and Cow urine distillate were prepared in saline and poured into respective labeled Petri dishes. Six earthworms of nearly equal size were placed in each Petri dish at room temperature. The time taken to complete paralysis and death were recorded. The experiments were done in triplicate. The mean lethal time for each concentration was recorded. The time taken by worms to become motionless was noted as paralysis time, the earthworms were frequently applied with external stimuli, which stimulates and induce movement in the earthworm if alive. No movement even after applying stimuli was recorded as the death of the worm.

## RESULTS AND DISCUSSION

The TABLE 1 reveals anthelmintic activity of various concentrations of standard drug and Cow urine. Cow urine in 0.1%, 0.2% and 0.5% concentration was not found to exhibit any activity against test worms when compared to standard drug. 1% Cow urine was also found to be totally ineffective in causing paralysis or death of the worms. But 2% and 5% concentrations of cow urine were found to be active against test worms while the time taken for paralysis and death were far higher when compared to standard. The time required for paralysis and death decreased on increasing the concentration of the cow urine indicating dose dependent activity of the cow urine. In 5% concentration, the time for paralysis was nearly three hours and death was 190 minutes.

Anthelmintic activity of different concentrations of  
**TABLE 1: *In vitro* anthelmintic activity of different concentrations of standard drug and Cow urine**

Sl no	Compound	Concentration (in %)	Time in minutes	
			For paralysis	For death
01	Control (normal saline)	0.9	NP	ND
		0.1	60	86
02	Standard (Albendazole)	0.2	58	83
		0.5	46	72
		0.1	NP	ND
03	Test (CU)	0.2	NP	ND
		0.5	NP	ND
		1.0	NP	ND
04	Test (CU)	2.0	255	310
		5.0	175	190

CU-Cow urine, Readings are average of 3 trials; NP- No paralysis observed even after 8 hours; ND- No death

**TABLE 2: *In vitro* anthelmintic activity of different concentrations of standard drug and Cow urine distillate**

Sl no	Compound	Concentration (in %)	Time in minutes	
			For paralysis	For death
01	Control (normal saline)	0.9	NP	ND
		0.1	60	86
02	Standard (Albendazole)	0.2	58	83
		0.5	46	72
		0.1	NP	ND
03	Test (CUD)	0.2	NP	ND
		0.5	93	360
		1.0	40	78
04	Test (CUD)	2.0	20	50
		5.0	9	35

CU- Cow urine, Readings are average of 3 trials; NP- No paralysis observed even after 8 hours; ND- No death

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standard drug and Cow urine distillate was depicted in TABLE 2. The result shows that cow urine distillate is more potent in its nematicidal activity against worms tested. 0.1% and 0.2% concentrations of Cow urine distillate were ineffective in causing paralysis and death of worms. But time taken for paralysis and death at 0.5% concentration were 93 and 360 minutes respectively. Significant anthelmintic activity was recorded in Cow urine distillate at concentrations 1%, 2% and 5%. All concentrations were found to be superior in acting against worms when compared to standard. The time for paralysis and death reduced significantly as the concentration increased suggesting dose dependent activity of Cow urine distillate. The time required for paralysis and death of worms in 5% concentration were less than ten minutes and over half an hour. The readings obtained are comparable with the standard drug, Albendazole.

Nowadays there is risk of development of resistance in helminthic parasites against the drugs given to treat infections. The ayurvedic approach of using plants in treating diseases have shown to be promising in acting against helminthic worms as evidenced by several literatures. There are several reports revealing *in vitro* as well as *in vivo* anthelmintic activities of plant extracts and synthetic chemicals. The crude latex of *Calotropis procera* was investigated for anthelmintic activity using adult earthworms. The fresh and aqueous extracts of dried latex exhibited dose dependent activity<sup>[2]</sup>. The anthelmintic effect of the crude methanol extract of *Xylopiya aethiopica* was evaluated in rats experimentally infected with the rat hookworm *Nippostrongylus brasiliensis*. The extract at the dose of 0.8 g/kg, 1.0 g/kg, 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0 g/kg produced deparasitization rates of 21%, 47%, 51%, 50%, 63% and 76% and were significant ( $P > 0.05$ ) when compared to untreated control rats<sup>[3]</sup>. Chloroform extracts of stem and root of *Punica granatum* and *Artrmisia siversiana* were investigated for anthelmintic activity against *Syphacia obvelata*, *Nippostrongylus brasiliense* and *Hymenolepis nana* *in vivo*. Both extracts were able to eliminate *H. nana* from mice. Their potency was less as compared to Quinacrine<sup>[4]</sup>. In an *in vitro* anthelmintic activity, against free living soil nematode, employing extracts of *Berlina grandiflora* and purified betulinic acid, the major

triterpenoid found in *Caenorhabditis elegans*, it was found that the extracts possess anthelmintic activity<sup>[5]</sup>. Anthelmintic activity of essential oils of *Cymbopogon martini* was evaluated on adult Indian earthworms *Pheretima posthuma* and results showed that the volatile oil of *C. martini* flower required less time to cause paralysis and death of the earthworms<sup>[6]</sup>. Seeds of *Butea monosperma* administered as crude powder (CP) at doses of 1, 2 and 3 g/kg to sheep naturally infected with mixed species of gastrointestinal nematodes exhibited a dose and a time-dependent anthelmintic effect<sup>[7]</sup>. An enterically coated, tableted formulation of 0, 0-dimethyl-0-1, 2-dibromo-2, 2-dichloroethyl phosphate given to 18 dogs was found to clear 86.5 per cent of the dogs of hookworm infections when given orally at a level of 15 mg/kg<sup>[8]</sup>. *In vitro* and *in vivo* data on the benzimidazole compound indicate anthelmintic potential when introduced directly into the abomasums of sheep<sup>[9]</sup>.

## CONCLUSION

Anthelmintic activities of Cow urine and Cow urine distillate have been investigated *in vitro* using earthworm model. The cow urine and its derivative are found to possess active principles responsible for anthelmintic activity. From the results, it is evident that cow urine distillate is more efficient in its activity, against worm model, when compared to cow urine. Cow urine was found to possess activity only in high concentration. The results of the study clearly reveal dose dependent activity i.e., more the concentration higher the activity. The experimental findings justify the objectives of the study and further studies involving *in vivo* models of anthelmintic activity could permit evaluation of the potential of cow urine and its derivate (Cow urine distillate) as cheaper and potential nematicide. Thus, the consumption of cow urine and Cow urine distillate singly or with certain herbal compounds could be a cheaper and alternate way to prevent parasitic infections.

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