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Comparative studies on anthelmintic activity of natural sweeteners

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ABSTRACT

The aqueous extract of natural sweeteners was investigated for anthelmintic activity using earthworms (*Pheretima posthuma*), tapeworms (*Raillietina spiralis*) and roundworms (*Ascaridia galli*). Various concentrations (100-300 mg/ml) of Sweeteners extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded. Extract of honey exhibited high significant anthelmintic activity at highest concentration of 300mg/ml. The result shows that aqueous extract possesses vermifugal activity and found to be effective as an anthelmintic.

Keywords: Anthelmintic, Natural sweeteners, Piperazine citrate

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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 helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia [1]. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas [2]. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases [3]. Hence there is an increasing demand towards natural anthelmintics.

Natural sweeteners are a healthier choice and they are gentler than sugar, they don't affect our health like refined sugar artificial and sweeteners. Honey is the natural sweet substance produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. It is useful in oxidative stress [4], effective treatment for conjunctivitis in rats, inflammation and has antibacterial activity allergenic condition etc [5-8]. There are two types of jaggery available in India. One is from sugarcane (*Saccharum officinarum*) and the second type is from Palmyra palm tree (*Borassus flabellifer*). Jaggery or cane jaggery (from *Saccharum officinarum*) is a traditional unrefined non-centrifugal sugar consumed in Asia, Africa and South America. It is made for direct consumption. This type of sugar is a concentrated product of cane juice without separation of the molasses and crystals, and can vary from golden brown to dark brown in color. It contains up to 50% sucrose, up to 20% invert sugars, moisture content of up to 20%, and the remainder made up of other insoluble matter such as ash, proteins and bagasse fibers. It useful in anemia, rickets, helps heal ulcers and wounds, constipation. Palm Jaggery (from *Borassus flabellifer* lin) contains carbohydrates, appreciable amounts of vit. B complex and minerals (iron is present in an easily assimilatory form). The concentration of amino acids in palm jaggery is much higher than in cane-jaggery. It is used as an energy food for convalescents and is govern in anaemia. It exhibits a mild laxative effect. The jaggery solution is reported to be an excellent food for typhoid patients. The low sodium and high potassium value indicates the possibility of its use in hypertension and oedema due to heart and liver diseases. It can be proscribed for diseases characterized by a marked loss of potassium and also as a diuretic.

MATERIALS AND METHODS

Preparation of extract

Aqueous extract (Maceration method) Powdered material of Natural sweeteners (500gm) was kept for maceration with 1000 ml of distilled water for 12 hrs. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and Concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be 30 percent.

Animals

Adult earthworms (*Pheretima posthuma*), Roundworm (*Ascaridia galli*) and Tapeworms (*Raillietina spiralis*) were used to evaluate anthelmintic activity *in vitro*. Earthworms were collected near the swampy water along Vishnu road Vishnupur, roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughterhouse and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected and worms were collected and kept in normal saline solution. The average size of earthworm was 6-8 cm, average size of roundworm was 5-7 cm and average size of tapeworm was 6-8 cm. Earthworm and helminthes were identified in Dept. of Zoology, D.N.R.College, Bhimavaram and services of veterinary practioners were utilized to confirm the identity of worms.

Drugs and chemicals

Piperazine citrate (Glaxo Smithkline) was used during the experimental protocol.

Anthelmintic activity

The anthelmintic assay was carried out as per Ajaiyeoba method [9]. The assay was performed *in vitro* using adult earthworm (*Pheretima posthuma*) owing to its Anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity [10-12]. Use of *Ascaridia galli* and *Raillietina spiralis* species as a suitable model for screening of anthelmintic drug was advocated earlier [13,14]. Test samples of the extract was prepared at the concentrations, 100, 200 and 300 mg/ml in distilled water and six worms i.e. *Pheretima posthuma*, *Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg/ml) was used as reference standard and distilled water as control [15-18]. This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). All the results were shown in Table.1 and expressed as a mean \pm SEM of six worms in each group.

RESULTS AND DISCUSSION

From the observations made, higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for all worms. Aqueous extract showed Anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 300mg/ ml concentration, for all three types of worms. Extract exhibited more potent activity at lower concentration (100 mg/ml) against (roundworm) *Ascaridia galli*. Evaluation of anthelmintic activity was compared with reference standard Piperazine citrate (Table.1). Honey

showed less paralytic time and death time when compared to Cane jaggery and Palm jaggery. The orders of anthelmintic activity of natural sweetening agents are as follows:

Honey > Palm Jaggery > Cane Jaggery

Acidic pH level of natural sweeteners prevents the growth of many helminthes and Natural Sweeteners has a saturated mixture of monosaccharides. This mixture has a low water activity; most of the water molecules are associated with the sugars and few remain available for helminthes, so it is a poor environment for their growth.

Groups	Concentration	<i>Pheretima posthuma</i> (Earth worm)		<i>Ascaridia galli</i> (Roundworm)		<i>Raillietina spiralis</i> (Tapeworm)	
		Paralytic time (mean& SEM)	Death time (mean& SEM)	Paralytic time (mean& SEM)	Death Tim (Mean&SEM)	Paralytic time (mean&SEM)	Death time (Mean&SEM)
Control (Water only)	---	---	---	---	---	---	---
Honey	100mg	116 ± 1.4	180 ± 1.7	90± 2.4	150±1.9	123 ±1.4	190±1.7
	200mg	21 ± 0.8	31 ± 0.7	18± 0.8	25± 0.7	27 ± 0.8	41± 0.1
	300mg	19 ± 0.3	30 ± 0.9	14± 0.3	22± 0.4	25± 0.3	45± 1.9
Cane jaggery	100mg	178 ± 1.9	230 ± 2.3	138 ± 1.4	230±2.3	178 ±1.4	230±1.3
	200mg	51 ± 1.2	78 ± 1.2	31 ± 0.8	78±1.2	51 ± 0.2	78±1.2
	300mg	23 ± 0.6	46 ± 0.3	19 ± 0.6	46±0.3	23 ± 0.6	46±1
Palm Jaggery	100mg	157 ± 1.3	215 ± 3.4	137 ± 1.3	215±1.4	157 ±1.3	215±1.4
	200mg	39 ± 0.5	58 ± 1.6	39 ± 1.5	58 ± 0.6	39 ± 0.5	58 ± 0.6
	300mg	25 ± 0.1	55 ± 0.9	19 ± 0.1	55±12.1	25 ± 2.1	55±0.1
Piperazine citrate (Standard)	10 mg/ml	23±1.15	61±0.88	11±0.43	39±0.6	23±0.4	55±0.68

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