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EVALUATION OF ANTI-HELMINTHIC ACTIVITY OF POLY HERBAL SIDDHA FORMULATION PAANDU LEGIYAM IN EARTHWORM MODEL

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ABSTRACT

Anemia is a major public health problem. It affects people of all ages, especially pregnant women and children. According to statistics, globally, anemia affects 41.8% of pregnant women and 47.4% of preschool children. Moreover, it has negative effects on health and development, including neonatal and perinatal mortality, low birth weight, premature birth, and developmental delays of the children. Various factors such as iron and micronutrient deficiencies, gastrointestinal tract parasitic infestation (hookworm) and viral infections are known to cause anaemia. Helminthic infestations in children's contributes high prevalence of anaemia and hence research focuses on alleviating parasitic infestation may offers beneficial remedy. Commercially available drugs lie albendazole, mebendazole, levamisole and pyrantel pamoate found to offer symptomatic relief, but the major limitation of availing these drugs relies on resistance and reoccurrence of infestation. From the ancient time, herbal medicines have been used for the welfare of mankind as medicines to cure the series of diseases. New drugs of herbal origin are crucial because they are cheap, have little side effects and in accordance with WHO, about 80% of the world population are still depends mainly on plant based drugs. Paandu legiyam (PL) is a versatile siddha formulation indicated for treatment of anemia associated with parasitic infection in childrens, In consideration of this statement present study aimed at investigating the anthelmintic activity of PK using pheretima posthuma (earth worm) model. Results of this study reveals that maximum time take for the test drug PL at the dose of 1 gm to cause paralysis of worms is about 122.5 ± 9.4 mins, similarly the time taken of PL at the dose of 2 gm would be 97 \pm 11.6 mins for standard drug albendazole it was 39.5 \pm 6.4 mins. Similarly maximum time take for the test drug PL at the concentration of 1gm to cause death of worms is about 242.8 \pm 22.6 mins, similarly the time taken of PL at the dose of 2gm would be 182.5 \pm 7.72 mins for standard drug albendazole it was 115.3 ± 5.6 mins. In conclusion from the data's of the study it was clear that the test drug PL possess significant anti-helminthic property.

KEY WORDS: Anemia, Helminthic infestations, Siddha, Paandu legiyam, Anti-helminthic, Albendazole

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1. Introduction

Anaemia is one of the most widespread disorders of blood which affect the populations of all ages throughout the world. It is a public health problem that affects populations in both rich and poor countries [1]. However, the incidence of this disorder is higher in the developing countries than in the developed countries [2] due to poverty and lack of hygiene. The situation is aggravated by factors such as nutritional deficiencies and high prevalence of parasitic gastrointestinal infections which cause heavy loss of blood. Other conditions, such as malaria and haemoglobinopathies are also responsible [3].

In many parts of the developing world, parasitic worms and anemia are of considerable public health and economic importance. Soil-transmitted helminth (STH) infections are common parasitic infections in the tropics and subtropics [4]. More than 568 million school-age children live in areas where these parasites are intensively transmitted, and are in need of treatments. Although the global target is to eliminate morbidity due to STH infections in children by 2020 [5], these infections are still huge health problems in Ethiopia affecting millions of school-children. The prevalence may reach up to 83% among residents in some rural regions of the country [6].

The use of medicines from herbal origin for the management and treatment of human diseases is as old as human existence [7]. Interestingly, there has been increased attention in herbal medicine worldwide because of its significant contribution to health care delivery and as an alternative to clinical practice [8]. These herbal supplements have been demonstrated to be a good source of bioactive compounds (phytochemicals) which are useful for the treatment of myriad of ailments [9,10]. Some of the phytochemicals present in herbal preparations include phenols, phytates, flavonoids, alkaloids and steroids [11]. Most of the medicinal plants are still being used in rural areas. Hence, proper evaluation of the acclaimed activities of these herbal therapeutics ascertains their level of safety and efficacy.

The main objective of anthelmintic treatment is to control morbidity and eradicate infection burden, interrupt transmission and complete eradication of the parasite reservoir [12]. WHO has recommended only four drugs: albendazole, mebendazole, levamisole, and pyrantel pamoate for soil-transmitted helminthiasis and these have been in use for several decades [13]. The main challenges in animal and human health are a wide range of parasitic diseases and the emergence of resistance to anthelmintic drugs. Resistance to anthelmintic drugs is a persistent problem worldwide and needs urgent solution [14]. Hence exploration of drug from alternate therapeutic origin which overcomes the resistance grabs higher attention in recent times.

Siddha medicines use versatile ingredient from herbal, minerals, metals, animal products and even from marine origin. Guidelines of siddha regulates the procedure of purification, detoxification, processing and quality mean of formulating the siddha drugs. Each formulation is made of hundreds of potential phytotherapeutics which can reveal beneficial pharmacological activity in the biological system. Paandu legiyam (PL) is a versatile siddha formulation indicated for treatment of anemia associated with parasitic infection in childrens, In consideration of this statement present study aimed at investigating the anthelmintic activity of PK using Pheretima posthuma model.

2. Materials and Methods

2.1. Anthelmintic activity using Pheretima posthuma Model

2.1.1. Earthworm

Indian adult earthworms (Pheretima posthuma) were collected from local vendor and washed with normal saline were used for the anthelmintic study. The earthworms of 4-6 cm in length and 0.1-0.2 cm in width were used.

2.1.2. Methodology [15]

The worms were acclimatized to the laboratory condition one week prior to the experimentation. The earthworms were divided into three groups of four earthworms in each group of two per petri dish. Albendazole at the concentration of 200mg/10ml was served as standard. Clean and sterile petri plates were used for the study. Group I served as low dose treated group of which the worms were exposed to 1 gm of Paandu legiyam and Group II served as high dose treated group of which the worms were exposed to 2 gms of the Paandu legiyam. Group III served as standard drug treated group of which the worms were exposed to 2 gms of the Paandu legiyam. Group III served as standard drug treated group of which the worms were exposed to Albendazole 200mg.

2.2. Grouping

Group I – Worms exposed to PL 1 gm per dish Group II – Worms exposed to PL 2 gms per dish Group III – Worms exposed to Albendazole 200mg/ml Earthworms of nearly equal size in length and width are taken for each concentration and placed in Petri dishes at room temperature. The time taken for complete paralysis and death are recorded. The mean paralysis time and mean death time for each dose was calculated .The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli, which stimulates and induce movement in the earthworms.

3.Results

3.1. Effect of Paandu legiyam on duration of paralysis in Earth worms

The result obtained from the present clearly indicates that the test drug PL possess significant antihelminthic property. The result obtained from the present clearly indicates that the test drug PL possess significant anti-helminthic property. Maximum time take for the test drug PL at the dose of 1 gm to cause paralysis of worms is about 122.5 ± 9.4 mins, similarly the time taken of PL at the dose of 2 gm would be 97 \pm 11.6 mins for standard drug albendazole it was 39.5 \pm 6.4 mins at the concentration of 200mg/10ml. Data's represented in table 1.

 Table 1: Effect of Paandu legiyam on Paralysis duration

 in Earth worms

Group	Treatment	Time taken for paralysis (min)
Ι	PL 1 gm	122.5 ± 9.469
П	PL 2 gms	97 ± 11.63
III	Albendazole 200mg/ml	39.5 ± 6.455

Each value represents the mean \pm SD. N=3

3.1. Effect of Paandu legiyam on death induction time in Earth worms

Maximum time take for the test drug PL at the concentration of 1gm to cause death of worms is about 242.8 \pm 22.6 mins, similarly the time taken of PL at the dose of 2gm would be 182.5 \pm 7.72 mins for standard drug albendazole it was115.3 \pm 5.6 mins at the concentration of 200mg/10 ml. As shown in figure 1 and table 2.



Figure 1: Anthelmintic activity of Paandu legiyam in Earth worms **Table 2: Effect of Paandu legiyam on death induction time in Earth worms**

Group	Treatment	Time taken for death (min)	
Ι	PL 1 gm	242.8 ± 22.65	
II	PL 2 gms	182.5 ± 7.724	
III	Albendazole 200mg/ml	115.3 ± 5.62	
Each value represents the mean \pm SD. N=3			

4.Discussion

Anaemia, is another global public health issue, affects around 40% school-age children in developing world [16]. Previous reports revealed that anaemia in schoolaged children is strongly associated with moderate and heavy hookworm infections [17]. Worm infection, particularly helminthiasis is one of the most common chronic infections of humans. Worldwide more than 200 million people harbor these infections [18]. It is major global health problem mainly in tropical countries. These infections are common risk factor to community health in the developing countries and responsible for several conditions such as pneumonia, anemia, eosinophilia, and malnutrition. Expelling or killing of helminths is the two primary mechanisms by which drugs produces anthelmintic effects [19].

Helminthic infestations are now being recognized as a cause of chronic ill health and sluggishness amongst the children. More than half of the population in the world suffers from worm infestations of one or the other. Helminthes also affect domestic animals and livestock causing considerable economic loss. Traditional system of medicine reports the efficacy of several natural products eliminating helminthes [20].

Drugs of natural origin have gained attention as a potential source of new therapeutic agents. Most of the clinically active drugs are either natural products or pharmacophore of the natural substance. It indicates the importance of drugs having natural sources in drug discovery process [21]. The use of plant as a source of medicine is as old as mankind and majority of the formulation in traditional Indian medicine system is of herbal origin [22]. The result obtained from the present clearly indicates that the test drug PL possess significant anti-helminthic property. Maximum time take for the test drug PL at the dose of 1 gm to cause paralysis of worms is about 122.5 ± 9.4 mins, similarly the time taken of PL at the dose of 2 gm would be 97 \pm 11.6 mins for standard drug albendazole it was 39.5 \pm 6.4 mins at the concentration of 200mg/10ml.

Helminths mainly reside in the gastrointestinal tract, but some are known to invade tissues. The harmful effects of infection manifest as food deprivation, blood loss, local organ injury, intestinal or lymphatic obstruction. Sometimes, the same effects are also produced by worm secreted toxins. Although helminthiasis is not fatal, it is a principal etiological factor responsible for morbidity that in turn influences personal and social health and productivity Some of these phytocomponents like alkaloids, tannins, phenols etc. may be accountable to have a significant anthelmintic activity [23]. It was reported that, tannins may to interfere with energy generation of worms by uncoupling oxidative phosphorylation or they binds to the free protein of the gastrointestinal tract of the worms and lead to death [24]. In another study, alkaloids were reported to cause paralysis of the worms by acting on its central nervous system [25]. The prime effect of albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Albendazole acts to increase chloride ion conductance of worm muscle membrane which produces hyperpolarization and excitability reduction that leads to muscle relaxation and flaccid paralysis of worms [26]. It was observed from the datas obtained from the present investigation that the maximum time take for the test drug PL at the concentration of 1gm to cause death of worms is about 242.8 ± 22.6 mins, similarly the time taken of PL at the dose of 2gm would be 182.5 ± 7.72 mins for standard drug albendazole it was 115.3 ± 5.6 mins at the concentration of 200mg/10 ml.

5. Conclusion

Infection of helminths is a major problem in human as well as in animals that in turn adversely affects the health and also causes drug resistance to other diseases. To overcome these problems, there is a need for studies focusing on natural sources such as plants which give new biologically active agents having no or fewer side effects and more compatible with human physiology. From the result of the study it was concluded that the test drug Paandu legiyam possess promising anthelmintic property.

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