Design for Scientific RENAISSANCE

JABPAR

Effect of *Cyclamen Perciscum* Extract on the Inhibition of Amoebic Dysentery in Mice: Experimental Study

[1]Samia Khalil Mahmood, [1]Mohammad mossa,[1]Khalaf Y. Shamkhy,[1] Falah Hanash Nahar, [2]Rabab Qassim Saqir, [3]Al-Sultan I.I.

[1]Ministry of science and technology, Agriculture Research Directorate,Iraq

[2]Ministry of Health , Central Laboratory, Baghdad , Iraq

[3]Faculty of Veterinary Medicine, University Malaysia Kelantan, Malaysia

samkhm@yahoo.com

Article Info

Received: 10th June 2012
Accepted:20th August 2012
Published online: 1st September 2012

ISSN: 2231-8313

ABSTRACT

Hot and cold alcoholic extract of *Cyclamen Perciscum* plant were used in concentrated extracts (150, 300 and 450 mg/kg body weight) in an experimental trial to look for its effectiveness and inhibitory action for *Entamoeba histolytica* .This protozoon parasite is the cause of Amoebic Dysentery in humans and animals. The disease is categorised by the International Health Organization as one of the most ten dangerous communicable diseases. Following the experimental infection with this parasite to a Swiss mice type (Balb C), the results have shown in general a significant effectiveness of hot alcoholic extract in comparison with the cold alcoholic extract of *Cyclamen Perciscum* . Slight differences among the extracts used to treat infected mice with *Entamoeba histolytica* or to inhabit parasitism with the control group is demonstrated in all aspects of investigation carried throughout this experimental study.

Keywords: Amoebic Dysentery, Cyclamen Perciscum, Plant extraction, Entamoeba histolytica

1. Introduction

Entamoeba histolytica is one of the most dangerous and widely spread parasitic disease to the human as well as animals, which is considered to be the cause of Entamebiasis to human being. The main characteristic symptom observed is the severe diarrhoea called Dysentery associated with colic, fatigue, and bloody mucous excrements, plus other morphological changes in the liver, brain, lungs, and skin (Young, 2011). The common host for the parasites are the human, monkeys, cats, dogs, mice, and rats; but the intermediate carriers are birds and insects (Ryan and Ray, 2004). Infection of *E.histolytica* is worldwide known and the disease is one of the most important affections that end in death of patients .The disease is considered one of the most ten common diseases, as per the World Health Organization report (1998). Statistical report on the annual fatalities toll due to the infection of this parasite

has recorded to 500,000 cases (WHO, 1998). *E. histolytica* has two main phases, the Trophozoite phase Which is existing inside the lumen of large intestine and residing on mucosal tissue, 60% in cecum, 30% in rectum, and the rest are available in other parts of large intestine; causing sever ulcers due to the feeding on live intestine mucosal cells. Occasionally, Trophozoites move to circulation and reach the liver by hepatic portal vein. This form of parasite stay in the large intestine for certain period of time then turned to another phase of Encystations that will be eventually discharged with excrements, as a source of contagious to food and water in different means. The Encystation phase exist in the lumen of large intestine of the host which is called the resistance phase due to the encapsulation with a thick cell shield to protect the parasite against harsh ecological circumstances which make them survive in wet soil for 8 days and in cold places for 12 days at a temperatures of 4^0c .Cystic form can resist heat at 50^Oc and the sanitizer chlorine in water at a certain level but cannot survive at a high level of chlorine concentrations. It is a motionless phase on the contrary to the Trophozoite phase (DuPont, 1998). A well known herbal plant *Cyclamen Perciscum* is advocated by people for its wide use in various diseases. The *C. Perciscum* is composed of leaves that are oval with heart-shape base of serrated sides, its length is 3-12cm and the width is 2-7cm of dark green colour. It has flowers like crowns with several colours of red, white and dark or light pinks. It has been well known to public that the powder from this plant when used on the infected wounds, abscesses, infected eyes, feet fungal infection, dermatitis can cure or have a significant effect. It has a purgative and intestinal antihelmintic effect too. An extract from this plant is used to cure rheumatoid diseases, headache, goitre, and heart disease. The leaves after dryness convert to powder are used in curing the slight skin burns, skin cancer and sneaks bites. Flowers are also extracted for the usage of perfumes and other flavours of food and spices (Lvie, 2007). This study is aiming to look on the effect of the crude extract on the inhibition of *E.histolytica* infection using mice as an experimental model for human disease.

2. Methodology

Materials and Methods:

Experimental animal: Eighty male mice of Swiss (Balb C) type, 8 weeks of age has been used in the experiment. They were divided into 8 groups each group of 10 mice. All groups were exposed to the same circumstances like accommodation, room temperature, light, cage size, ventilation and feed.

Parasite Media and harvest inoculums: A special media for *E. histolytica* (liver infusion agar) prepared in a sterile test tubes. The Media was used after the addition of ampicilline 0.07 micro liter to eliminate all bacteria in the culture media. Samples were taken from infected excrements of confirmed patients suffering the disease and in the cystic phase. These samples were cultured in the prepared media which has enriched by 0.5ml of special sterilized dyes and starch. Culture media were incubated at 37^oc.The parasite culture was maintained every successive day in a new culture media till the parasite appears after 4-6 days (fig.1). The parasite moved to a new culture media twice a week for 3 successive weeks.

Only the cystic form is moved harvested and preserved in sterile test tubes with 10ml physiological solution at 4^{o}c until used in the oral drenching to mice (Cheesbrough, 2000).

Plant extract: The crude powder of *C. Perciscum* leaves, manufactured by Alzahoor Company, is used to prepare the hot and cold alcoholic extracts at concentrations of 150, 300 and 450 mg/kg of body weight.

Experimental design: Sixty mice were infected orally with a suspension of cystic form of the parasite using tephlon tube in a dose of 10000 cyst/cm^2. After 5 days of induced infection, the *E. histolytica* appeared in mice and confirmed by laboratory tests of mice excreta (fig.2). After conformation of mice infection with the parasite each group of 10 mice were treated orally (drenching methods) by one concentration of cold or hot alcoholic extracts. Two groups of ten mice each were given saline solution orally in the same drenching method and kept as control group for hot or cold infected –treated groups The treatment continues for 5 consecutive days. Mice were monitored closely in this post infected -treated time. Mice were allocated for close observation to record any change in activity and appearance of signs and symptoms as well as testing the excrements for detection to *E. histolytica.*

Statistical Analysis:

A statistical analysis was used according to the US statistical programme (Spss II), to assign differences between experimental groups and of the control.

3. Results and Discussion

A significant effect of hot alcoholic extract of *C. Perciscum* on the inhibition of *E. histolytica* activities was observed post treatment (Tables 1and 2), whereas there are significant differences among group activities after treatment at the level ($p< 0.05$). With regard to the effect of cold alcoholic extract the results were less significant in comparison with the effect of hot alcoholic extract (Tables 3 and 4).The interpretation for the results concerned with the effect of hot alcoholic extract treatment that is more significant and reliable for parasite inhibition in comparison to the cold alcoholic extract could be attributed to the use of high temperature of water bath (75^{o}c) that aid in complete extraction of the active substance from the plant leaves by concentrated ethyl alcohol(95%). This explanation does not apply to any other herbal extract may has the same inhibition process against certain bacteria which depend on quality, type and concentration of the active materials existed in the herbal. A recommend future studies regarding chemical analysis to this herbal plant to know the effectiveness, concentrations of trace elements of the metallic and non-metallic kinds and to exclude the harmful elements. Further investigation on the toxic effect of the extract is recommended.

Table 1: The effect of hot alcoholic extract on the clinical symptoms of *E. histolytica* infection as well as the percentages of infection in the treated mice groups.

Activities (signs and symptoms)	Infected- treated Groups			
	Control Group	150 mg/kg	300 mg/kg	450 mg/kg
Lack of Activity	+	+	+	+
Weakness	+	-	-	-
Lack of Appetite	+	+	+	-
Diarrhoea	+	+	-	-
% Infection	100%	50%	40%	10%

Table 2: The infection and mortality rates due to *E. histolytica* infection in the infected-treated groups with hot alcoholic extract.

Aspects	Infected- treated Groups			
	Control Group	150 mg/kg	300 mg/kg	450 mg/kg
No. Examined	20	20	20	20
% Infection	100	50	40	10
% Death	70	20	-	-

Table 3: The effect of cold alcoholic extract on the clinical symptoms of *E. histolytica* infection as well as the percentages of infection in the treated mice groups.

Activities (signs and symptoms)	Infected- treated Groups			
	Control Group	150 mg/kg	300 mg/kg	450 mg/kg
Lack of Activity	+	+	+	+
Weakness	+	+	-	-
Lack of Appetite	+	+	+	-
Diarrhoea	+	+	-	-
% Infection	100%	100%	80%	80%

Table 4: The infection and mortality rates due to *E. histolytica* infection in the infected-treated groups with cold alcoholic extract.

Aspects	Infected- treated Groups			
	Control Group	150 mg/kg	300 mg/kg	450 mg/kg
No .Examined	20	20	20	20
% Infection	100	100	80	80
% Death	80	80	80	90

There are no significant differences between groups ($p < 0.05$).

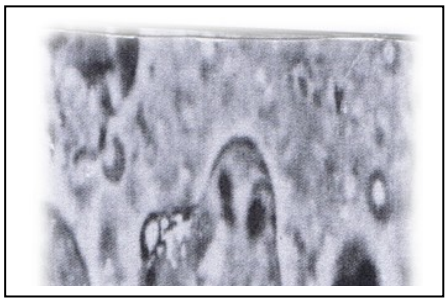

Fig.1: Demonstrate *E. histolytica* inside the Liver parenchyma.

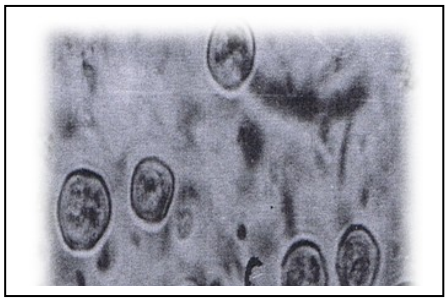

Fig. 2: Demonstrate the cystic form of *E. histolytica* in the excrements of infected mice.

4. Conclusion

Extract from *C. Perciscum* herbal plant prepared especially from the leaves can promote an inhibitory effect on protozoon parasite *E. histolytica* in the intestinal mucosa of mice after infection and treatment. The experimental work is suggesting further detailed study to make the utmost beneficiary from this type of herbs.

References

Cheesbrough M. (2000)"District laboratory practice in tropical countries", part 2.Cambridge university press.UK.

Young L (2011). Common Parasite Potentially Increases Risk of Brain Cancers. USGS. Retrieved 8 August.younglandis.wordpress.com/tag/usgs/

DuPont HL (1998). "Interventions in diarrheas of infants and young children". J. Am. Vet. Med. Assoc. 173 (5 Pt 2): 649

Lvie, G. (2007)."The chemistry of plant and their medical toxicology, environmental and convolutionary significance", 4nd ed. Academic press. Canada .

Ryan KJ; Ray CG (2004). Sherris Medical Microbiology (4th ed). pp. 723.

WHO (1998). "Amoebiasis. Report of a WHO Expert Committee". Technical Report Series 421.

Facchetti, S. & Geiss, F., (2005). “Isotopic lead experiment”: Luxembourg, Commission of the European Communities (Publication No. EUR 83523 EN).