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EFFECT OF ETHANOLIC EXTRACT OF *CHLORELLA SP.* ON *ENTAMOEBA HISTOLYTICA* PARASITE *IN VIVO*

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Abstract

The objective of this study was to conduct in vivo to detect the effect of *Chlorella* extract on *Entamoeba histolytica*. and to examine its therapeutic effect in male laboratory rats type *Mus musculus*. These rats were dosed with a concentration of (1, 1.5 and 2) mg/ml of the aforesaid extract at a daily dose of (10, 15 and 20) days and then, the rats' faeces were tested to observe the change in parasite numbers after dosing the extract. The results showed that the ethanolic extract of *Chlorella sp*. was effective in reducing the vesicated and fed stages *Entamoeba histolytica*-infected rats, where the rate of killing those stages had recorded the highest percentage at dose of 2mg/ml during a period of 20 days. Moreover, qualitative detections of chemicals for secondary metabolism of ethanolic extract of *Chlorella sp*. were carried out, which indicated the presence of chemically active compounds. The therapeutic efficiency of the extract was also calculated, which has recorded 68.70%. The *Chlorella* can be used to control infectious diseases and prevent *Entamoeba histolytica* parasite.

Key words : *Large intestine, Chlorella, Entamoeba histolytica.*

Introduction

The medical concerns of mosses were confined until 1950s to the practice of traditional folk medicine, which is usually defined by a particular geographic area, (Lincoln *et al.,* 1991) where the interest in mosses has increased as a source of bioactive compounds in the last four decades. These compounds were chemically categorized into aggregates of Carbohydrates, Proteins, Alkaloids, Phenols, Terpenes and Fitty acid (Cannell 1993) These have been used as Antivirus, Antibacterial, Antifungal, Anti-inflammatory and Antitumor (El-Baroty *et al.,* 2007). Mosses produce many bioactive compounds represent broad pharmaceutical and medicinal characteristics resulting from the secondary metabolism of these mosses, particularly cyanobacteria and green mosses. Marine mosses, including red and brown mosses contain high concentrations of these active compounds (Tajbakhsh *et al.,* 2011). Research on the use of effective compounds derived from mosses as antimicrobials are very few compared with that which have been dedicated to examine these compounds as fungal, bacterial and viral antibiotics (Santos *et al.,* 2010). Entamoeba histolytic parasite is an intestinal parasite that affects humans and causes the disease of Amoebic dysentery and is one of the public health problems in the tropics (Lejeune *et al.,* 2009). Amoebiasis is the third leading cause of death from parasitic after *Plasmodium* and *Schistosoma*. According to the World Health Organisation (WHO), Amoebiasis is responsible for 40000 – 100000 deaths annually. (Marie and Petri 2014). The parasite passes through its life cycle in two basic ways: Trophozoite phase and Cyst phase. The latter represents the infective stage where the parasite is infected by eating and drinking at that stage (Linford *et al.,* 2009). Trophozoite phase is the harmful phase and lives in the cavity of the large intestine and feeds on the mucosa of the intestines and on the red blood cells, where it releases enzymes that decompose the mucosa and deepen sway inside the intestinal wall, destroying its cells, causing painful sores and resulting in amoebic dysentery (Achers and Mirelman 2006)

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Materials and Methods

The samples of faeces for patients infected with amoebic dysentery were collected from Al-Hussein Educational Hospital, Al-Mousawi Hospital for Children

and Bint Al-Huda Hospital in Nasiriyah district for the period from November 2018 to February 2019. The green mosses were obtained from the American company Amazon in the form of powder. The animals of the experiment were obtained from the Animal House of the Department of Life Sciences at Faculty of Science-University of Dhi- Qar, which is 25 white rats. The weights ranged from 25-30 grams of males, which ranged in age from 8-10 weeks. The first, second, third group was dosed by ethanol extract with a concentration of 1, 1.5 and 2 mg/ml once a day over 10. 15. 20 days, whereas the fourth group infected with the parasite and fifth group was fed with 0.5% of the normal saline solution.

Statistical Analysis

Statistical analysis of the results was performed by one-way ANOVA using SPSS ver.23. Significant differences ($P<0.05$) among the concentrations were analyzed by Duncan test.

Results

The rats that have been orally dosed by suspend contain the parasite showed a highly recorded Susceptibility to the infection of cyst phases to *E. histolytica*, this was proved through direct microscopic examination of infected rats after ten days of the oral dosing, where the cysts of *E. histolytica* parasite were marked through their spherical shape, which contain four nuclei, while the vegetative phase has irregular shape with one nucleus and food gaps containing red blood cells. The results of the ethanol extract of moss showed a significant disincentive effect on the parasite, using concentrations of 1, 1.5 and 2 mg/ml for the period of 10, 15 and 20 days. Table 1 shows the rate of cystic phases in the parasitic faeces of a single microscopic field during the days when laboratory animals infected with the parasite were treated, treated with extract and +ve control. It was observed that the parasite was completely eliminated at the concentration of 2 mg/ml of day 20 when ethanol extract was used, this compares with the + ve control, in which the number of cystic phases continued to increase over time, with the therapeutic efficiency of the extract at 68.70%.

Table 1: Rate of cysts in the faeces of rats infected with *E. histolytica* parasite.

Concentration mg/mL				Time (day)
Control	2	1.5	1	
14	8	10	13	10
20	3	5	8	15
27	0	2	6	20

[a-c] Different letters within each column indicate significant difference ($P< 0.05$).

Table 2: Effect of chlorella on *E. histolytica* cysts after 10, 15 and 20 days after infection.

Time (day)	Concentration mg/mL			
	1	1.5	2	Control
10	9.80 a	7.60 + 1.14 a	5.00 + 1.00 a	14.00
15	7.40 a	1.40 + 4.40 a	3.00 + 1.00 a	19.80 b
20	5.40 b	1.60 c	0.00 + 0.00 c	27.80 a

[a-c] Different letters within each column indicate significant difference ($P< 0.05$).

Histological study of large intestine

Microscopic examination of the histological sections of the present study showed the normal state of the large intestines tissue in rats of the -ve control, as the Mucosa was visible with a profusion in Goblet cells. Submucosa looked thin, and Muscularis externa contained clear, tidy and fusiform muscle fibres inside the muscle fibre. (Fig1). Some histological changes in the tissue of the large intestine were observed in rats infected with the parasite, when compared with the tissue of the large intestine for negative control, where the mucus layer is thin and suffers from erosion in the form of degenerative foci caused by parasite entry, with a significant infiltration of inflammatory cells, vasodilatation atrophy on intestinal villi and Goblet cells breakdown in the submucosal layer, along with noting epithelial cells, that is, hyperplasia and thickening of the muscular layer. (Fig. 2). In the histological changes, the tissue was resorted to its normal state, where the mucous glands appear to be light coloured, which indicates that it returns to its normal state with mucus secretion, as well as we have not observed hyperplasia or dilation of blood vessels as well as the return of the muscle layer to its normal thickness. (Fig. 3)

Discussion

Effective chemical compounds derived from natural sources such as plants are widely used in the treatment of many diseases. This is because it is safe compared to common synthetic chemical compounds that may have mutagenic properties making natural alternatives required, as it contains effective molecules in processing with the least chance of developing resistance against it, where its effects on the host are few (Rani 2011). The extraction pattern and type of solvent used demonstrate these success of isolating bioactive compounds, (De Almeida *et al.*, 2011) so ethanol alcohol has been used at a concentration of 70%, as a globally known solvent and as the healthiest safe solvent for the preparation of extracts (De Gives *et al.*, 2012). The results of the present study showed that the extract has a distinct inhibitory effect to varying degrees (depending on the concentration) on the appearance of cysts in the faeces

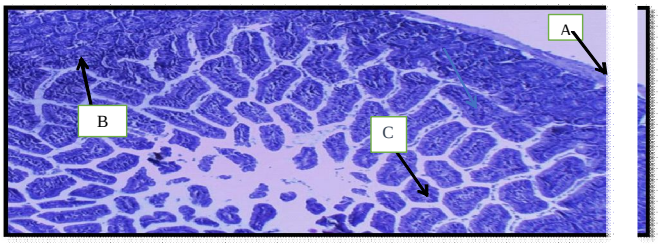

Fig. 1: Cross section of the large intestine of a group of -ve control rats treated with normal saline showing the muscular layers (A), mucous glands (B) and Goblet cells (C)x100 (H&E).

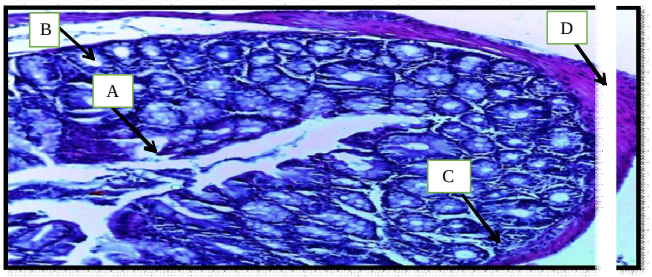

Fig. 2: Cross-section of the tissue of the large intestine for +ve control infected with the parasite *Entamoeba histolytica* shows the erosion of the mucus layer, and the degeneration of its cells (A) degeneration of the mucus cells (B) and the infiltration units (C) and thickening of the smooth muscular layer (D) (100X) (H&E).

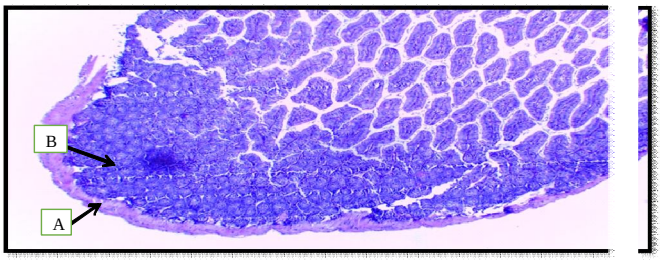

Fig. 3: Cross section of tissue of the large intestine of the group rats infected with *Entamoeba histolytica* and treated with ethanolic extract of *chlorellasp*. It shows the muscular layer in its natural form (A) intestinal glands in its normal form (B) (100X) (H&E).

of the experiment animals, which is due to the effect of the extract on the parasite in the intestine, compared with the +ve control in which the incidence continued to increase throughout the trial period, qualitative tests of moss showed. This study proved that Berberine sulphate showed high efficacy against parasites *E. histolytica*, *Giardia lamblia*, *Trichomonas vagainalis*. It caused the clumping of chromatin in the vegetative nucleus of the *E. histolytica* parasite with the formation of Autophagic gaps and small gaps in the cytoplasm (Almeidi *et al*., 2002). As for phenolic compounds, it leads to loss of permeability of the cellular membrane of the parasite and thus the entry and exit of substance to and from the parasite without regulation and thus the death of the parasite (Kandale *et al*., 2011). Some studies have shown that phenolic compounds in moss extracts have the ability to bind to lipids in the cell membrane and cell membranes of organelles and then change their functional structure and lastly the death of the living cell (Naguleswaran *et al*., 2006). As for the histological study of the large intestine infected with the parasite, it was observed that the Trophozoite stage of the histolytic mutant has the ability to live in the large intestine of the host, where it was found to have 60% of ulcers in the large intestine caused by the parasite, (Gilchrist and Petri 1999) resulting in diarrhoea accompanied by blood, mucus and several other changes, and these activists have the ability to live and proliferate for a long time within the hinds of the mucosa of the large intestine, as it depends in its nutrition on starches and mucous secretions and metabolic interaction with intestinal bacteria, nonetheless, these activists begin to infect tissues and then begin to disunite the cells of the mucous membrane. At this stage the metamorphosis tissue no longer requires the presence of bacteria to meet the nutritional needs (Roberts *et al*., 2009). The inflammatory response process is a physiological process of tissue protection, as it represents a complex overlap between vascular system, immune system and repair mechanism, as it changes the normal flora environment located inside the intestines as a result of parasitic infection may turn it into a pathological, (William and Sodeman 2000) and sometimes the mutant with the help of bacteria may penetrate the muscular down to muscles layers and Serosa, this in turn leads to the movement of activists to other sites throughout the body through blood and lymph, caused by secondary lesions, this will be accompanied by perforated colons and peritonitis, which in turn is deemed to be one of the leading causes of death, while repairing the holes has become very difficult because the strongly ulcerated colon has become very sensitive and of these are caused by amoebic dysentery (Lebbad 2010). Regarding the histological changes of the infected parasite group, and the treatment with ethanolic extract of the moss, it showed the relative improvement in the tissue of the large intestine when compared with +ve control, which was represented by the thickness of the mucous layer with elongation of the crypts, which were characterized by the presence of clear and dilated goblet cells, and a slight infiltration of inflammatory cells, with a slight bleeding in the submucosa layer, the smooth muscular layer appears in its natural shape with an arranged and containing spindle cores, and the serous layer looked normal and thin. This may be due to the effective therapeutic role of the extract that led to the eradication of the parasite, which allowed the tissues to compensate their cells and be healed. This consistent with the findings of a study (Al-Oqabi 20014) dedicated to test the efficiency of moss Cladofora

crispate in the elimination of the parasite *E. histolytica*. And also consistent with study (Al-Jaber 2016) on the *Echinococcus granulosus* parasite that has been treated by using *Enteromorpha intestinalis*. Histopathological changes in the tissue of large intestine -infected by parasite and treated with moss extracts- have receded with reversible changes consisted in the inflammation in those organs, infiltration of inflammatory cells accompanied by bleeding and congestion, as after the removal of the cause the tissue can return to normal state and function (Kumar *et al.*, 2011).

Conclusion

A marked anti- parasite activity of *Chlorella* extract was observed which may be attributed to the presence of bioactive compounds and other phytochemicals. The *Chlorella* can be used to control infectious diseases and prevent *Entamoeba histolytica* parasite.

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