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Effect of *Equisetum Arvense* Phenolic Extract in Treatment of *Entamoeba Histolytica* Infection

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Abstract

Study aimed to detect the activity of phenolic compounds against the *E. histolytica* parasite. 20 rats divided as following; control group. Second group rat infected with (10^3 cyst/ml) *E. histolytica*. Third group infected rat were treated with (50ug/ml) phenolic for four weeks. Fourth group infected rat were treated with (50ug/ml) phenolic for four weeks. The findings demonstrated significant elevate ($P < 0.05$) in levels AST, ALT and MDA with significant ($P < 0.05$) reduce in levels of catalase in an infected rats compared with control group. The results of treated rats show non-significant ($P < 0.05$) changes in all parameters compare with control group when using phenolic compounds. About the histological changes, second group show degeneration of hepatocytes with thickening wall of central vein and infiltration of mono-nucleated inflammatory. After treatment by using phenolic compounds, tissues of liver appear semi-normal in third and fourth groups. So, phenolic show a high efficacy to treatment *E. histolytica* infection.

Keywords: Dysentery, anti-oxidant enzymes; *E. arvense*.

Introduction

Disease of amoebiasis is define as *E. histolytica* infection lead to watery or bloody diarrhea [1]. Infections mostly are without sign and asymptomatic, but intestinal disease may happen featuring including various symptoms such as pain of abdominal region, dysentery diarrhea and resulting in weight loss [2]. *E. histolytica* Infection has been assess about more than 50% in several countries as America continent, Africa continent and Asia continent [3]. *E. histolytica* happens in different stages include trophozoite stage, precyst stage, cyst stage, metacyst stage, and metacystic trophozoite stage. The trophozoite stage cans moves, feeds, and has ability to divides [4-5]. *E. arvense* (family : Equisetaceae) contained different compound such as various forms of carbohydrate, alkaloids with different types of proteins and amino acids, saponin types, various acids such as ascorbic and silicic acids, phenol compounds, tannin, flavonoid types, triterpenoids, volatile oils and different other biological active substances [6-8]. Phenolic obtain from different sources are linked to different activities such as anti-oxidant activity, anti-carcinogenic activity, antihypertensive activity, cardio-protective activity, anti-arthritic activity, anti-allergic and antimicrobial efficacy [9].

Materials and Methods

Animal model

Twenty rats were used in this work, (wt 200-250 gm with age 4-6 month) get from Education College for Pure Science / Samaraa University.

Culturing the parasite

Fecal sample that contain E. histolytica was cultured on

the LES-media. Incubation of culture tube was done vertically (37 C0 for 48h).

Phenolic extraction and purification

l0 g of *E. arvense* extracted for 30 min. The mixture was filtered by using three layers of gauze, and remain part was then extracted by two additional parts of the similar solution. The final solution was centrifuged at 5000 rpm for 15 min. Ethanol in filtrate was removed by using rotary evaporator device under optimum condition. After addition of ammonium sulphate (20%) and acid of metaphosphoric (2%) to the aqueous phase of filtrate, the phenolic compounds were extracted by using ethyl acetate three times [10].

Determination of phenolic compounds

E. arvense phenolic was determined by using High HPLC technique [11]. The absorbance was observed at 254 nm. The mobile phase composed of methanol (100 %). A size of sample is 5 µl was used with HPLC.

Experimental design

Twenty rats were used in this work and after that divided as follow:

A. Control group received standard diet.
B. Second group rat administrated with (dose 10^3 cell/ml.) *E. histolytica*
C. Infected rat were treated with 100ug/ml of phenolic for month.
D. Infected rat were treated with 50ug/ml of phenolic compounds for month.

Measurements

ALT and AST

ALT and AST were measured by technique according to the instructions of manufacturer company kit (Randox).

Oxidative agents

MDA (malonedialdehyied) was measured in this stusy according to colorimetric reaction by using thiobarbituric acid (TBA) [12]. Catalase was determined by using the Biovision-USA kits procedure. All parameters were done on intestines extract.

Processing of histology

Liver species were obtained from rats and fixed with formalin (10%), processed by paraffin method, cut at 6µm in thickness by microtome device and stain step done by using Hematoxylin and Eosin (H&E) [13]. Sections were diagnosed by using Olumpis microscope.

Statistical analysis

Current data were analyzed by using program known as Minitab (statistical program). A statistical change between the groups means were analyzed using one-way analysis of variance.

Results

Liver functions

ALT and AST levels show significant (P<0.05) increase in second group compare with control group as shown in figure (1). After treatment with phenolic compounds, ALT and AST levels show non-significant (P<0.05) changes in third and fourth groups compare with control group as shown in figure (1).

Figure 1. levels of ALT and AST

Oxidative stress

MDA levels show significant (P<0.05) increase in second group compare with control group as shown in figure (2). Where, catalase levels show significant (P<0.05) decrease in same group. After treatment with phenolic compounds, MDA and catalase levels show non-significant (P<0.05) changes in third and fourth groups compare with control group as shown in figure (2).

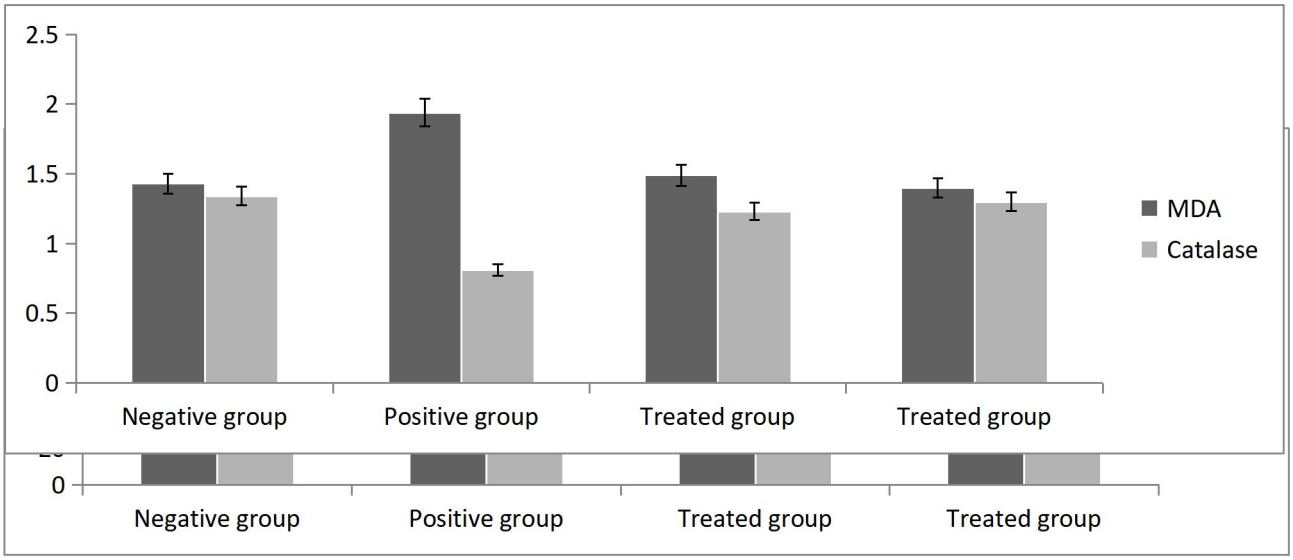

Figure 2. levels of MDA and catalase

Histological study

Sections of control group show normal form of hepatocytes with radial arrangement around central vein with normal form and size of sinusoids. In second group, liver sections show degeneration of hepatocytes with thickening wall of central vein and infiltration of mono-nucleated inflammatory. After treatment by using phenolic compounds, tissues of liver appear semi-normal in third and fourth groups as shown in table (1).

Groups\Lesions	A	B	C	D
Thickening wall	Nil	++	Nil	Nil
Degeneration	Trace	+++	Trace	Trace

Necrosis	Nil	+	Trace	Nil
Inflammation	Nil	++	Trace	Trace

Discussion

Diasease of amoebiasis is defining as *E. histolytica* infection lead to watery or bloody diarrhea [14]. In this work, *E. histolytica* leads to elevate in levels of liver enzymes (ALT & AST) and different changes in its tissues. The result is in agreement with Al-Kubaissi [15] who found increased in liver enzymes levels reached 90 % of patients infected with *E. histolytica* and suffering dysentery. Also, Ventura-Juarez et al, [16] indicate that invasive of amoebic molecules happens to layer of endothelium, and liver cells found further away die by necrosis change. Results show *E. histolytica* lead to elevated MDA levels and reduce catalase. Pineda [17] indicated that *E. histolytica* induced an elevated ROS level in cells that explains the results of current study. After treatment, liver enzymes and its tissues back to normal status, Jassim et al, [18] referred that phenols may be utilized in the detoxification of liver resulting from toxicity of drug as its ability to reduce activity of live enzyme. Also saponins themselves have been activity of antioxidant that contributes to phenolic compounds efficacy to protect and support against liver injury [19].

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