ROLE OF *Rosmarinus officinalis* PHENOLIC COMPOUNDS IN TREATMENT OF *Entamoeba histolytica* INFECTION

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ABSTRACT

The present study was designed to detect role of phenolic compounds against the *E. histolytica*. The study used 20 adult male rats that distributed to four groups (each group consist 5 rats); control group that received normal saline, second group rat administrated with *E. histolytica* at dose 10³ cyst/ml. third group rat administrated with *E. histolytica* at dose 10³ cyst/ml and treated with 50ug/ml of phenolic compounds for four weeks. Fourth group rat administrated with *E. histolytica* at dose 10³ cyst/ml and treated with 100ug/ml of phenolic compounds for four weeks. The results show high significant increased (P < 0.05) in levels AST, ALT and MDA with high significant decreased (P < 0.05) in levels of catalase in second group compared with control group. The results of third and fourth groups show non-significant changes (P < 0.05) 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 mononucleated inflammatory. After treatment by using phenolic compounds, tissues of liver appear semi-normal in third and fourth groups. It was concluded that phenolic compounds has a role against *E. histolytica*.

Keywords: *E. histolytica*, *Rosmarinus officinalis*, phenolic compounds, oxidative stress, liver functions.


INTRODUCTION

Amoebiasis, or amoebic dysentery, is a term used to describe an infection caused by the protozoan *Entamoeba histolytica*¹. Most infections are asymptomatic, but invasive intestinal disease may occur manifesting with several weeks of cramping, abdominal pain, watery or bloody diarrhea, and weight loss ². Infection with *E. histolytica* has been estimated to be as high as 50% in some developing countries as South and Central America, Africa and Asia ³. *Entamoeba histolytica* occurs in the following forms, the trophozoites, precyst, cyst, metacyst, and metacystictrophozoite. The trophozoite is the active stage that moves, feeds, and divides ⁴,⁵. *Rosmarinus officinalis* L. is a medicinal plant that belongs to the Lamiaceae family and is commonly known as rosemary ⁶. *R. officinalis* exerts various pharmacological activities such as antibacterial ⁷, antidiabetic ⁸, anti-inflammatory ⁹, antitumor ¹⁰ and antioxidant ¹¹. Generic terms ‘phenolic compounds’, ‘phenolics’ or ‘polyphenolics’ refer to more than 8,000 compounds found in the plant kingdom and possessing at least an aromatic ring with one or more hydroxyl substituents, including...
functional derivatives like esters, methyl ethers, glycosides \cite{12}. Phenolics derived from various natural sources are linked to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardioprotective, anti-arthritic and antimicrobial activities \cite{13}. So, the aim of this study is detect the role of \textit{R. officinalis} phenolic compounds against \textit{E. histolytica}.

**MATERIALS AND METHODS**

**Animal model**

In this study twenty adult male albino rats, (wt 200-250gm with age 4-6 month) obtained from Science College/ Kirkuk University, and kept on a standard pellet

**Culturing the parasite**

Small amount of positive stool sample was cultured on the LES-media (NIH modification of Boeck and Drbohlav, s media). Culture tube incubated vertically at 37°C for 48h. For experimental inoculation, actively growing trophozoites were sediment after chilling the culture tubes for 5min in an ice-water bath.

**Extraction and purification of phenolics**

A dried sample of \textit{R. officinalis} l0g extracted for 30 min. by stirring at 4P o PC with 200ml of cold aqueous ethanol \%65 containing 0.5% Sodium metabisulphite. The homogenate was filtered through four layers of cheesecloth, and the residue was then extracted with two additional portions (100ml each) of the same extraction solution as described above. The combined filtrate was centrifuged at 7000 rpm for 15 min. at 4P o PC and residue was discarded. Ethanol was removed from the supernatant by rotary evaporator under vacuum at 35P o PC, and the mass is measured. Pigments were eliminated by two successive extractions with petroleum ether. After addition of 20% ammonium sulphate and 2% metaphosphoric acid to the aqueous phase, the compounds were extracted three times with ethyl acetate. The extracts were combined, evaporated and then dried under vacuum at 35P o PC. The residue was redissolved in methanol (1:1) for analysis \cite{14}.

**Determination of phenolic compounds**

The phenolic compounds of the \textit{R. officinalis} were determined using High Performance Liquid Chromatography (HPLC) \cite{15}. The absorbance was monitored at 254 nm. C-18 Chromatographic column was used. The mobile phase consisted of 100 % methanol. A sample size of 5 µl from the intact phenolics was injected for the HPLC analyses.

**Experimental design**

Twenty adult male albino rats were used in this study and then divided as follow (each group consist five rats):

- **A.** Control group received standard pellet diet only for seven days and then killed.
- **B.** Second group rat administrated with \textit{E. histolytica} dose 10³ cell/ ml.
- **C.** Third group rat injected administrated with \textit{E. histolytica} dose 10³ cell/ ml and treated with 100ug/ml of phenolic compounds for four weeks.
D. Fourth group rat injected administrated with *E. histolytica* dose $10^3$ cell/ml and treated with 50ug/ml of phenolic compounds for four weeks.

**Measurements**

**ALT and AST**

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

**Plasma Peroxidation levels (MDA), Glutathione (GSH) and Catalase**

MDA (malonialdehyde), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer $^{[16]}$. Catalase was measured by using the procedure of Biovision-USA kits.

**Histology processing**

Liver were collected and fixed with 10% formalin, processed by paraffin method, cut at six micrometers in thickness by using rotary microtome and stained with Hematoxylin and Eosin (H&E) $^{[17]}$. Sections were examined with olympus Microscope (Japan).

**Statistical analysis**

The Data were analyzed using a statistical Minitab program. A statistical difference between the means of the experimental groups was analyzed using one-way analysis of variance (ANOVA).

**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).
Oxidative stress

MDA levels show significant (P<0.05) increase in second group compared 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 compared with control group as shown in figure (2).

![Figure 2: levels of MDA and catalase](image)

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.

DISCUSSION

Amoebiasis, also known amoebic dysentery, is an infection caused by any of the amoebae of the *Entamoeba* group. Symptoms are most common during infection by *E.histolytica*\(^{[18]}\). In this work, *E.histolytica* leads to increase in levels of liver enzymes (ALT & AST) and different changes in its tissues. The result is in agreement with Al-Kubaissi\(^{[19]}\) who found a high level of concentration of liver enzymes reached 90% of patients infected with dysentery. Also, Ventura-Juarez et al.,\(^{[20]}\) referred that diffusion of amoebic molecules occurs to the endothelium, and hepatocytes located further away die by necrosis. Results show *E.histolytica* lead to increased MDA levels and decreased catalase levels. *E.histolytica*\(^{[21]}\) referred induced an increased in the intracellular ROS level that explains the results of present study. After treatment, liver enzymes and its tissues back to normal status, Jassim et al.,\(^{[22]}\) referred phenols may be used in the liver detoxification resulting from drug toxicity as its ability to decrease live enzyme activity. Also saponins (which are phenolic compounds) themselves possess antioxidant activity that contributes to efficacy of the phenolic compounds to protect against liver injury\(^{[23]}\).
CONCLUSION

Phenolic compounds has been a role against the E. histolytica.

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ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES