Phenolic Compounds Role in Rat Immunity Changes that Caused by Entamoeba Histolytica

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Abstract

The present study was designed to show the potential role of phenolic compounds against the toxicity of Entamoeba 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 injected intraperitoneal with E. histolytica at dose 10³ cyst/ml. third group rat injected intraperitoneal with E. histolytica at dose 10³ cyst/ml and treated with 100ug/ml of phenolic compounds for four weeks. Fourth group rat injected intraperitoneal with E. histolytica at dose 10³ cyst/ml and treated with 250ug/ml of phenolic compounds for four weeks. The results show high significant increased (P < 0.05) in levels of Interferon gamma (INF-γ) and Tumor necrosis factor-α (TNFα) in group that injected with E. histolytica compared with control group. About oxidative stress, Levels of Malondialdehyde (MDA), Glutathione (GSH) and catalase show high significant changes (P < 0.05) in group that injected with E. histolytica compared with control group. After using phenolic compounds, levels of INF-γ, TNF-α MDA, GSH and catalase in treated groups show non-significant changes (P < 0.05) compared with control group. It was concluded that phenolic compounds have been potential role against E. histolytica.

Keywords: E. histolytica; phenolic compounds; oxidative stress.

Introduction

Amebiasis, 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]. Amebiasis has been defined as the pathological conditions arising from harboring the protozoan parasite E. histolytica, with or without clinical manifestation[3]. Amebiasis is a major cause of morbidity and mortality worldwide, mostly in tropical and sub-tropical countries characterized by inadequate health services and sanitation infrastructure. The majority of deaths are a consequence of severe complications associated with intestinal or extra-intestinal invasive disease [4]. Momordica is a genus of about 60 species of annual or perennial herbaceous climbers belonging to family Cucurbitaceae [5]. Cucurbitacins are reported to be the main active constituents of M. charantia that have anti-hyperglycemic[6], anti-hyperlipidemic[5], hepatoprotective [7], anti-obesity, anti-cancer[8] and anti-viral activities [9]. Phenolic compounds or polyphenols are one of the most frequent and widespread groups of substances in the world of plants, with more than 8000 identified phenolic structures [10-11]. Phenolic compounds are among the health-promoting phytochemicals. Phenolic compounds are receiving much attention because of their antioxidant properties [12]. Epidemiological studies have related dietary intake of phenolic-rich food with lower incidence in the appearance of several chronic diseases[13-14].

Material and Method

Samples Collection: Stool samples were collected from patients with diarrhea from privet laboratory randomly. Small amount of sample was examined on direct microscopic examination of feces to ensure that contain the parasite.

Culturing the Parasite: Small amount of positive stool sample was cultured on the LES-media (NIH
modification of Boeck and Drboh lav, s media) [15]. 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.

**Animal Model:** Twenty adult male albino rats in this study, (wt 200-250 gm with age 4-6 month) obtained from Veterinary college/Kirkuk University, and kept on a standard pellet diet for two weeks to ensure its normal and there isn’t any infection.

**Extraction and purification of phenolics:** A dried sample of bitter melon 10 g extracted for 30 min. by stirring at 4°C with 200 ml 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 (100 ml each) of the same extraction solution as described above. The combined filtrate was centrifuged at 7000 rpm for 15 min. at 4°C and residue was discarded. Ethanol was removed from the supernatant by rotary evaporator under vacuum at 35°C, 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 35°C. The residue was re-dissolved in methanol (1:1) for analysis [16].

**Determination of phenolic compounds:** The phenolic compounds of the bitter melon were determined using High Performance Liquid Chromatography (HPLC) [17]. 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 six rats):

**A.** Control group received standard pellet diet only.

**B.** Male rat injected (intraperitoneal) with *E. histolytica* dose 10^3 cyst/ml.

**C.** Male rat injected (intraperitoneal) with *E. histolytica* dose 10^3 cyst/ml and treated with 100ug/ml of phenolic compounds for four weeks.

**D.** Fifty Male rat injected (intraperitoneal) with *E. histolytica* dose 10^3 cyst/ml and treated with 250ug/ml of phenolic compounds for four weeks.

**Measurements:**

**INF-γ and TNF-α in serum:** Blood of the mice was withdrawn from all groups and were subjected for separation of sera. INF-γ and TNF-α Concentrations were determined by commercially available ELIS Kit.

**Plasma Peroxidation levels (MDA), Glutathione (GSH) and Catalase:** MDA (malondialdehyde), was measured based on the colorimetric reaction with thiobarbituric acid (TBA) using spectrophotometer [18]. GSH level estimated by mixed 2.3 ml buffer with 0.2ml of the sample and then added 0.5ml of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). The mixture was analyzed by spectrophotometer [19]. Catalase was measured by using the procedure of Biovision-USA kits.

**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**

**INF-γ and TNF-α:** INF-γ and TNF-α in rats injected with *E. histolytica* show significant increased (P<0.05) compared with control rats. INF-γ and TNF-α levels in third and fourth groups show no significant changes (P < 0.05) compared with control rats as shown in figures (1-2).

**MDA, GSH and catalase:** MDA, GSH and catalase in rats injected with *E. histolytica* show significant increased (P<0.05) compared with control rats. MDA, GSH and catalase levels in third and fourth groups show no significant changes (P < 0.05) compared with control rats as shown in figures (1-2).
Figure (2): Levels of TNF-alpha in all groups

Figure (1): Levels of INF-cama in all groups

Figure (3): Levels of MDA in all groups

Figure (4): Levels of GSH in all groups

Figure (5): Levels of catalase in all groups

**Discussion**

The present study shows a toxicity effect of *E. histolytica* on immunity of mice, where results show increases the levels of INF-γ and TNF-α. Mieh[20] referred that the infection with *E. histolytica* lead to increase the levels of TNF-α and suggest that TNF-α have a role in the defense against *E. histolytica* infection. Otherwise, Exogenous IFN-γ can activates neutrophils and macrophages for killing *E. histolytica* in vitro [21], Seydel et al. [22] suggested that IFN-γ and nitric oxide (NO) are important in host defense against the protozoan parasite *E. histolytica*, also it able to activate host neutrophils and macrophages to kill amoebic trophozoites in vitro and may play similar function in the murine model of amebic liver abscess, which explains the high levels of INF-γ in the present study. Also, results show increased levels of MDA and decrease GSH and catalase levels in rats injected with *E. histolytica*, Al-Kaky [23] who referred that the patients with *E. histolytica* show increased in levels of MDA and decreased in GSH compared to control group. Suggest that the increased of MDA levels and decreased in GSH back to the ability of parasite
to increase the free radical which induce cytological changes. On the other hand, phenolic compounds in this study show improved immunity of mice and its role against *E. histolytica*, polyphenols are known for their antioxidant activities; they scavenge a wide-ranging selection of ROS. Polyphenols can scavenge radicals and chelate metal ions, for example quercetin chelates iron ion [24]. They also inhibit multiple enzymes responsible of ROS generation [25]. phenolic compounds stimulate antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione (GSH) peroxidase (Px) which lead to ROS detoxification [26].

**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.

**Funding:** Self-funding

**Reference**


