The effect of phytase on the liver tissue after infection with Entamoeba histolytica in adult albino male rat

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Summary

The objective of current study was designed to show the activity of phytase enzymes against *E. histolytica* and its effectiveness to recovery liver tissue after infection. 24 adult male rats (with any diseases) were used and divided into four groups (6 rats in each group); control group feeding on standard pellet diet, second group administrated with *E. histolytica* dose $10^3$ cyst/ml. Third group: administrated with ($10^3$ cyst/ml) *E. histolytica* and feeding with special diet content (25mg/kg) phytase for four weeks. Fourth group: administrated with ($10^3$ cyst/ml) *E. histolytica* and feeding with special diet content (50mg/kg) phytase for four weeks. About oxidative stress, Levels of Malondialdehyde (MDA) and catalase (antioxidant enzyme) show high significant changes ($P < 0.05$) in an infected (with *E. histolytica*) group compare with control. After using phytase, levels of MDA and catalase in third and fourth groups show non-significant changes ($P < 0.05$) compare with control. The sections of infected group degeneration and necrosis of hepatocytes, with picknotic nuclei of some hepatocytes. The section of (25mg/kg) phytase group shows show thickening wall of some central veins. The sections of (50mg/kg) phytase group show no difference between this group and control group. It was concluded that phytase have been potential role against *E. histolytica*.

Keywords: phytase enzyme, *E. histolytica*, oxidative stress, liver tissue


Introduction

Phytase define as an enzyme that phytate molecule with dephosphorylates, leading to the release of phosphorus ion (P) and other cations that used by broiler. the Increased in process of phytate availability lead to decreases the need for inorganic P to fills the requirement of broiler’s P, and available P increase lead to decreases P elimination by progressing the use of phytate-bound P [1-2]. Although different studies have referred improvements in

rendering parameters that linked to incorporation of phytase enzymes to the diets of human, only certain studies referred to subjoin supplement of phytase enzymes at level more than 1000 (FTU/kg) and reach to 10 000 FTU/kg [3-4].

*Entamoeba histolytica* define as a unicellular organism, pathogenic protozoan from the family Entamoebidae [5-6]. The host cells will becomes infected after feeding (oral ingestion) food or water polluted with protozoan cysts (the so called fecal-oral transmitted way) [7]. After invasion of parasite in the lining of intestines lead to amoebic bloody diarrhea or/and amoebic colitis. If *E. histolytica* extend to blood stream it can diffusion through the host body, most considerably ending up in the liver where it causes abscesses in host liver [8]. Abscesses and nodules in liver can appear without previous diarrhea. Entamoeba cysts can survive and still for more than month in soil or more then (45) minutes under fingernails [9]. Different drugs are used in the therapy process of amoebiasis, most type of drugs that used in treatment is metronidazole [10], but many studied were reported different side effects like disorders of stomach and intestine, nausea (stomach discomfort) and metallic taste [11]. So, the aim of current study was show the effect of phytase enzymes against *E. histolytica* and its effectiveness to recovery liver tissue after infection.

**Materials and Methods**

**Samples Collection**

Stool samples, that content cysts and trophozoites of *E. histolytica*, were collected from privet laboratory in Kirkuk city. The samples were examined to ensure that contain the phases of *E. histolytica*.

**Culturing the parasite**

Positive stool sample (content *E. histolytica*) was cultured on special media called LES-media [12]. Tube of test was incubated vertically at 37°C for 48h. For experiential inoculation, trophozoites were deposit after intestine tubes that content culture for 5 min in an ice-water bath.

**Animal model**

Twenty four adult rats, (weight: 200-250 gm with age 4-6 mon.), were used in the current study and its acquired from Science College/ Kirkuk University, and kept for week before experiment.

**Phytase**

Phytase enzyme that used in the present experiment was Denmark (phytase Feed-Bio) Novozymes by add 500mg/kg in rat food.

**Experimental design**

Twenty four rats were used in this current study and then distributed as follow (six rats in each group):

A. Control group: male rat feeding on standard diet only for four weeks and then killed.
B. Male rat administrated with *E. histolytica* at dose $10^3$ cysts/ml.
C. Male rat administrated with $(10^3$ cyst/ ml) *E. histolytica* and feeding with special diet content (25mg/kg) phytase for four weeks.
D. Male rat administrated with $(10^3$ cyst/ ml) *E. histolytica* and feeding with special diet content (50mg/kg) phytase for four weeks.

**Plasma Peroxidation levels (MDA) and Catalase**

MDA (also called lipid peroxidation marker) was measured according to the reaction with thiobarbituric acid (TBA) by using technic of spectrophotometer [13]. Catalase was measured according to standard procedure of Biovision-USA kits.

**Histology processing**

Liver pieces were collected from rats and fixed by using 10% formalin, processed by paraffin method, cut at six micrometers in thickness by using rotary microtome and stained with histological stains called Hematoxylin and Eosin (H&E) [14]. Sections were examined by using Optica Microscope (Italy).

**Statistical analysis**

The data of MDA and catalase were analyzed by using a program called Minitab (statistical program). The difference between the experimental group's means was analyzed by ANOVA.

**Results**

**MDA and catalase**

MDA (increased) and catalase (decreased) levels in an infected rats show significant changes ($P<0.05$) compare with control. MDA and catalase enzyme in treated (C and D) groups show no significant changes ($P < 0.05$) compare with control as shown in figures (1-2).
Liver tissue

The section of control group show normal form of hepatocytes and central vein with normal size of sinusoids (fig: 3). The section of infected group show degeneration and necrosis of hepatocytes, with picknotic nuclei of some hepatocytes (fig: 4). The section of (25mg/kg) phytase group show thickening wall of some central veins (fig: 5). The sections of (50mg/kg) phytase group show no difference between this group and control group (fig: 6).

Figure (3): liver section control group show normal form of central vein, hepatocytes (HC) and sinusoids (S) H&E X400.

Figure (4): liver section infected group show degeneration (D) and necrosis (N) of hepatocytes, with picknotic nuclei (P) of some hepatocytes H&E X400.
The results of the present study show different changes in liver tissue and oxidative stress. The power of *E. histolytica* to induce damage in host tissue and survive within liver is amid a robust proteins regulation and adaptive response, like *E. histolytica* virulence factors [15]. Ventura-Juarez [16] who indicated that dissemination of *E. histolytica* happens to the epithelium cells, and liver cells found further amazing rot, they propose that cytotoxicity can happen because of the emission of amoebic particles which will cause dangerous impacts a ways off, in any event, when there's not close contact between the trophozoites and liver cells, that is in concurrence with consequences of present examination and clarify the impact of *E. histolytica* during this investigation predictable with [17], amoebic liver canker arrangement after intraportal vaccination of harmful *E. histolytica* trophozoites in experimental animals (hamster) includes three back to back phases: acute inflammation (mono-

**Figure (5):** Liver section third group show central vein with thickening wall (TW), hepatocytes (HC) and sinusoids (S) H&E X400.

**Figure (6):** Liver section fourth group show central vein, hepatocytes (HC) and sinusoids (S) H&E X400.
nucleated cells), abscess formation (contain pus cells), and damage. Otherwise, the results agreement with [18] that referred that the patients with \textit{E. histolytica} show increased in levels of MDA and decreased in GSH compared to regulate group. Suggest that the increased of MDA levels and decreased in GSH back to the power of parasite to extend the radical which induces cytological changes. About the role of phytase during this study, its show important recovery in liver tissue and oxidative state, phytic acid inhibits radical generation in vitro [19] which will improve the liver oxidant and cancer prevention agent status in rodents of present investigation. Be that as it may, it’s been accounted for that characteristic phytate in two types of plant (maize and soybean) was defensive against oxidative degradation of lipids inside the pigs colon with a reasonably significant dietary iron admission level [20]. it had been likewise announced [21] that phytase supplementation of poultry eats less expanded the hepatic convergence of coenzyme Q10 proposing an amelioration inside the oxidative status of phytase took care of winged animals, which will explain the role of phytase within the present study.

Reference


