

## EFFICIENCY OF LENTINUS EDODES ON ENTAMOEBIA HISTOLYTICA IN LABORATORY MICE

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### ABSTRACT

The principal objective for this study was to evaluate the efficacy of *Lentinus edodes* against *Entamoeba histolytica* infection compared with metronidazole by using mice models. A single oral dose of *Lentinus edodes* water extracts of up to (0.1ml /mice/day) reduced parasite appearance in feces and disappear completely at the (8<sup>th</sup>) day compared with metronidazole the cysts parasite continued till (10<sup>th</sup>) day. The histomorphological study findings showed in the group which was given *Lentinus edodes* the majority of tissue intestine return to normal shape. These findings indicated that *Lentinus edodes* may be effective in treating *Entamoeba histolytica*.

**KEYWORDS:** *Entamoeba histolytica*, *Lentinus edodes*, aqueous extract, metronidazole, intestinal tissue.

### 1. INTRODUCTION

*Entamoeba histolytica* is an anaerobic parasitic protozoan, part of the genus *Entamoeba*.<sup>[1]</sup> Predominantly infecting humans and other primates, *E. histolytica* is estimated to infect about (50) million people worldwide. Previously, it was thought that (10%) of the world population was infected, but these figures predate the recognition that at least (90%) of these infections were due to a second species, *E. dispar*. Mammals such as dogs and cats can become infected transiently, but are not thought to contribute significantly to transmission.<sup>[2]</sup> The disease caused by this parasite is known as amebiasis or entamoebiasis.<sup>[3]</sup> Amoebiasis can present with no, mild, or severe symptoms.<sup>[4]</sup> Symptoms may include abdominal pain, mild diarrhoea,

bloody diarrhea or severe colitis with tissue death and perforation, this last complication may cause peritonitis, and people affected may develop anemia due to loss of blood.<sup>[4]</sup>

Invasion of the intestinal lining causes amoebic bloody diarrhea or amoebic colitis. If the parasite reaches the blood stream it can spread through the body, most frequently ending up in the liver where it causes amoebic liver abscesses.<sup>[4]</sup> Liver abscesses can occur without previous diarrhea. Cysts of *Entamoeba* can survive for up to a month in soil or for up to (45) minutes under fingernails.<sup>[4]</sup>

*Lentinus edodes* is the first medicinal macrofungus to enter the realm of modern biotechnology. It is the second most popular edible mushroom in the global market which is attributed not only to its nutritional value but also to possible potential for therapeutic applications. *Lentinus edodes* is used medicinally for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistencies.<sup>[5]</sup> It has also antiviral, antibacterial, and antiparasitic effects.<sup>[6,7,8,9]</sup>

In the last (15-20) year, *Lentinus edodes* mushroom has been subject to various clinical studies in humans and is thought to be beneficial for a wide variety of disorders including different types of cancer, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, and hepatitis. The mushroom is used medicinally for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal (especially *Candida*) infection, frequent flu and colds, bronchial inflammation, and regulating urinary incontinence.<sup>[10]</sup> Shiitake mushrooms have excellent nutritional value. Their raw fruit bodies include (88-92%) water, protein, lipids, carbohydrates as well as vitamins and minerals. It should be noted that amounts of nutrients and biologically active compounds differ in various strains and are affected by substrate, fruiting conditions, and methods of cultivation. On a dry weight basis, they have a relatively high nutritional value when compared to commonly consumed vegetables. Dried shiitake mushrooms are rich in carbohydrates and protein. They contain (58-60%) carbohydrates, (20-23%) protein (digestibility of (80-87%), (9-10%) fiber, (3-4%) lipids.<sup>[11]</sup>

## 2. MATERIAL AND METHODS

### 2.1 Feces Samples

The present study included (30) stool samples were collected from patients infected with amoebiasis, in laboratories of Baghdad city, directly a wet slide prepared by using logols iodine stain to search to *E. histolytica*.<sup>[12]</sup> The positive samples saved in cool containers, transferred to laboratories of parasitology in Baghdad university.

### 2.2 Parasite Purification

Method of Bingham and Meyer 1979.<sup>[13]</sup> Has been used to purify the parasite (cyst), cysts were suspended in phosphate buffer saline (PBS-7.2) to a final concentration of (1×10<sup>6</sup> cysts/ 0.1 ml).

### 2.3 Preparation of aqueous extract of *Lentinusedodes*

*Lentinus edodes* was obtained from department of biology in Baghdad University, The aqueous extract of *Lentinula edodes* was obtained as follows. Ground basideocarp was resuspended with distilled water, heated at (60) °C for 1 h and centrifuged at (3000 × g for 5 min). The supernatant was pre-filtered and submitted to ultrafiltration in (0.2) µm pore size membrane, and stored at (-20) °C.<sup>[14]</sup> *Lentinusedodes* routinely maintained on (malt extract agar) slants, then kept in refrigerated at (5) °C and sub culturing at least monthly.

### 2.4 Animals

To evaluate the histological and pathological changes induced by *Entamoeba histolytica* parasite in the intestine of experimental mice, a total number of (40) albino mice males with ages range between (8-10 weeks), were obtained from National Control Center for Drugs and Researches (NCCDR). Mice were put in plastic clean cages, And feces of them was examined before the beginning of the experiment to make sure of clearance mice from any intestinal parasites.

### 2.5 Experimental Design

Immunosuppressed of (40) mice by dexamethasone (0.1ml) with doze of (2 mg/ml) according to Zhongguoetal. (2006).<sup>[15]</sup> After (5 days), only (30) mice were inoculated orally by micropipette with (0.1 ml) of prepared inoculum of *Entamoeba histolytica*.

In the next day all mice feces was examined to confirm the presence of the parasite in the stool and occurrence of the infection ,then the infected mice were divided into (3) groups

(10) mice each, the remaining ten mice which were not infected, kept as a negative control group.

**Then each group was inoculated as follows**

**Group one:** mice given orally (0.1ml) of *Lentinus edodes* mold ( $1 \times 10^7$  cell/ml) as a single dose per day.

**Group two:** mice given orally (0.1ml) of metronidazole (30 mg/kg/day) as a single dose per day.

**Group three:** mice were given orally (0.1ml) of normal saline and considered as a Positive control group.

**Group four:** mice were given orally (0.1ml) of normal saline. This group considered as a negative control group.

## 2.6 Enumeration of *Entamoeba histolytica* cysts in faeces

Cysts in the fecal samples of mice were enumerated as per Shukla et al. 2008.<sup>[13]</sup> Briefly, one gram of freshly passed fecal sample was dissolved in 10 ml of normal saline, homogenized using pestle and mortar. Cysts stained with iodine were counted on every third day using hemocytometer and were expressed as cysts.

## 2-7 Histological study

Mice were sacrificed by cervical dislocation and large intestine was removed aseptically, fixed in (10%) buffered formalin, processed, stained with haematoxylin and eosin and were examined under the light microscope.

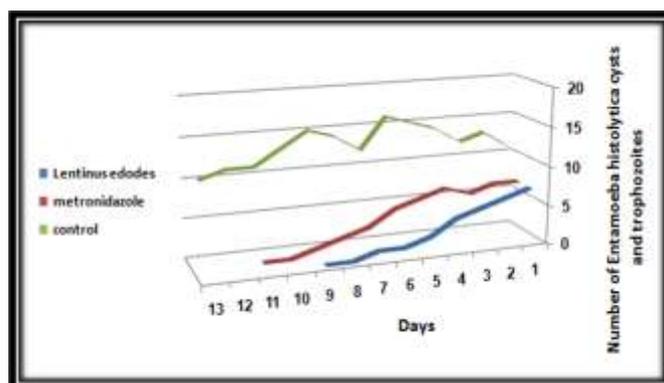
## 2.8 Statistical Analysis

Data are reported as mean  $\pm$  standard deviation and the inter group variation performed by t-test.

## 3. RESULTS

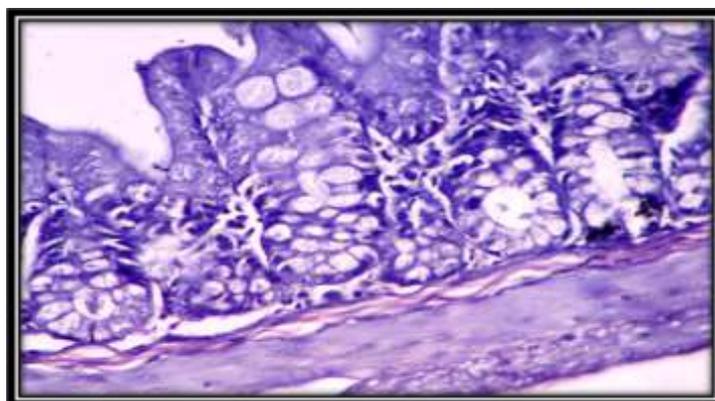
The present study was designed to assess the effect of aqueous extract of *Lentinus edodes* supplementation on *Entamoeba histolytica* in albino mice and compared it with metronidazole. It was found that orally administered *E. histolytica* cyst in mice could transiently colonize the gut, and infection was occur between (2-3) days, the infected mice beginning cyst excretion on day 3ed post inoculation . It was noticed that orally inoculation of this mold in infected mice led to reduce the shedding of this parasite in feces of mice since

the first day of treatment, and continue to decrease gradually with days till stopped shedding of parasite and became (zero) in day (8<sup>th</sup>) post inoculation , compared with metronidazole showed a decrease occurred after a first day post inoculation , then shedding of parasite start decrease gradually till reach to zero at day (10<sup>th</sup>).In compare with control positive group that maintain cyst excretion reach to 12 cyst/gm in 7th day and decrease to 10 cyst /gm in 13<sup>th</sup> day, as shown in figure (1).

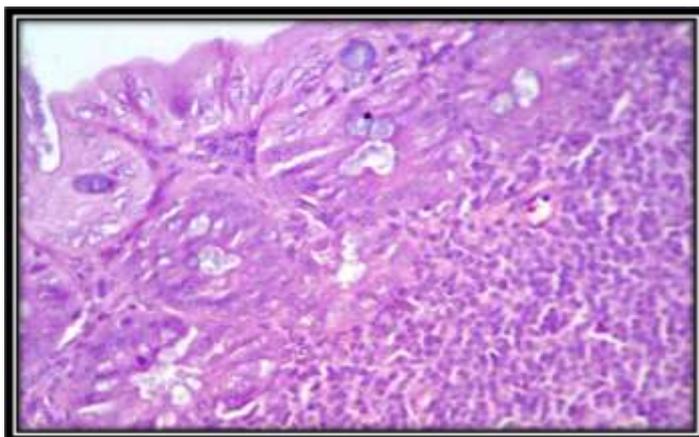


**Figure (1): Number of Entamoeba histolytica parasites in treatment and control groups.**

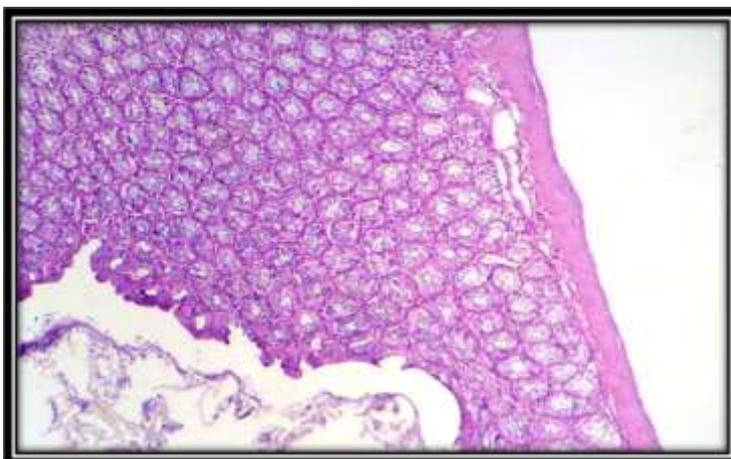
Slides of histological examination of the cecum and colon of positive control group showed intense inflammatory-cell infiltration, mucosal damage, inflammation or increased infiltration of lymphocytes in the mucosa compared with negative control group, as shown in figure (2), figure (3). The histological study showed that the aqueous extract of *Lentinus edodes* mushroom was able to re-structure of mice intestinal tissue, (figure4) when compared with control group of mice (figure2), while antiparasitic metronidazole caused damage in the tissues of large intestine which noticed through the infiltration of lymphocytes occurred. Figure (5).



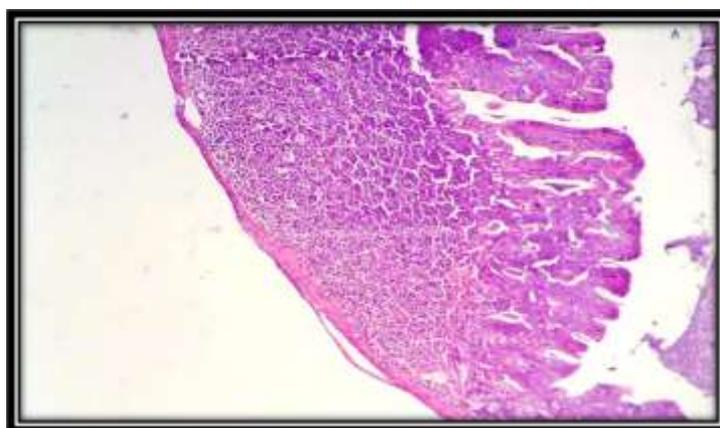
**Figure (2): Section of large Intestine in mice of negative control group showing normal structure appearance of normal colonic mucosa tissue. (H&E), 400x.**



**Figure (3):** Section of large intestine in mice of positive control group, showing damage in mucosa tissue with heavy inflammatory cells infiltration and hydropic degeneration. (H&E), 400 x.



**Figure (4):** Section of large intestine in mice treated with aqueous extract of *Lentinus edodes*, showing nearly normal appearance of colonic mucosa. (H&E), 100x.



**Figure (5):** Section of large intestine in mice treated with mitronidazole, showing infiltration of lymphocytes and short length of villi. (H&E), 200x.

*Lentinus* is effective against various bacterial, viral (including AIDS), and parasitic infections.<sup>[16]</sup> The aqueous extracts administered in this study caused significant reduction of the parasite, this reduction observed with the aqueous extracts of *Lentinusedodes* may be attributed to the polysaccharides  $\beta$ -glucans occur as a principal component of the cellular walls. Some microorganisms, such as yeast and mushrooms, are of economic interest because they contain large amounts of  $\beta$ -glucans. These substances stimulate the immune system, modulating humoral and cellular immunity, and thereby have beneficial effect in fighting infections (bacterial, viral, fungal and parasitic infection).<sup>[17]</sup>

The administration of  $\beta$ -glucan to mice infected with *Eimeria* infection vermiformes showed increased resistance to due to immunomodulation, which involved non-specific as well as specific response.<sup>[18]</sup>  $\beta$ -Glucans partially restored T and B cell response to the mitogen in mice infected by *Toxocaracanis*, reducing the larval number found in the muscles of the animals that received  $\beta$ -glucans.<sup>[19]</sup>

## CONCLUSION

*Lentinus edodes* is effective against various parasitic infections includes *Entamoeba histolytica* parasites. Many fungal species are used as foods and dietary supplements to promote good health, as well as to prevent or treat many diseases. Relatively nontoxic herbal products are particularly desirable for alleviating chronic health effects. The therapeutic applications of this macrofungus *Lentinus edodes* is the source of several well-studied preparations with proven pharmacological properties.

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