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EFFECT OF ETHANOL EXTRACT OF *PANCRATIUM MAXIMUM* BULBS ON *ENTAMOEBA HISTOLYTICA IN VIVO*

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ABSTRACT
Knowledge of traditional medicine is still transmitted from generation to another in Yemen orally, *Pancratium maximum* has been used as a medicinal plant in traditional medical systems in southern Yemen. The study determined if administration of ethanolic extract *P. maximum* bulbs effects against *Entamoeba histolytica in vivo*. In this study, the antiamoebic activity of *P. maximum* was tested using experimental infections of *Entamoeba histolytica*, the most common cause of protozoa diarrhoea worldwide, in rats (*Rattus norvegicus*). Plants were extracted in ethanol. Rats were treated for ten days orally once a day, with the doses of 125, 250, and 500mg/kg of body weight. Rats' faeces were taken every day and examined by microscopy, the number of shed *E. histolytica* were counted using a haemocytometer. After rats' sacrifice and dissection, their colon were then processed for examination using histological sectioning and scanning microscopy. The results showed a significant ($P<0.05$) reduction in the numbers of parasite cells from the second day of treatment in all the groups treated with the ethanolic extract. This decrease gradually continued during the days of treatment until it reached zero on the 10th day of treatment for the dose of 500 mg/kg. The infected treated group (positive control) showed significant ($P<0.05$) increases in the *E. histolytica* numbers in the feces of rats during 10 days of infection. Histological studies of the infected rats indicate and no showed improvement after using ethanolic extract of *P. maximum* compared with the negative group (non-infected without treatment).

KEYWORDS: *Pancratium maximum, Entamoeba histolytica, Rattus norvegicus,* antiamoebic, Histopathology.

INTRODUCTION
Entamoeba histolytica is a protozoan parasite that causes a parasitic disease known as amoebiasis.[1] Which infected mammalian species, including humans, spread occurs via by the stool of infected people or animals in everywhere such as soil, water, food or surfaces, as well as by oral-anal sexual habits.[2,3] symptoms of amoebiasis include fever, stomach pain, bloody stools, and diarrhea.[4,5] In rare cases, *E. histolytica* can invade the liver causing hepatic amoebiasis and abscess. it may move to other parts of the body, such as the brain or lungs.[3] Fifty million people suffer from amoebic colitis and liver abscess caused by *E. histolytica* and it is responsible for an estimated 50,000 to 100,000 deaths yearly.[6,7] Infection with *E. histolytica* leads to excystation releases the trophozoites phase that invades the mucous layer of the large intestine which causes amoebic colitis, colonic ulceration, this deep ulcer maybe leads to peritonitis, which in turn leads to death.[8,9]

The most frequent treatment against amoebiasis are nitro-imidazoles such as metronidazole or tinidazole, or benzimidazole compounds. These treatments are effective but can produce significant undesirable side-effects such as headaches, metallic taste in the mouth, and vomiting as well as neurotoxicity.[9,10] Moreover, resistance to these treatments has been described by *E. histolytica*. Due to the side effects, the search for new, safe, and effective therapeutic alternatives to the treatment of amoebiasis has become necessary.

Pancratium maximum elongs to the family Amaryllidaceae, bulbous herb up to 20 cm long, 0.5-2 cm wide with 4-5 linear leaves, arising from a bulb. The bulb is globose, 4-6 cm in diameter, narrowed above into a cylindrical neck, covered with several layers of dark reddish-brown papery tunics. The flowers solitary, resembling a white daffodil, consisting of 6 narrowly lanceolate lobes containing a similar to the cup, the

corona of the slender tube which is 10-15 cm long, the whole borne on a short peduncle and subtended by a large sheathing scarious spathe.[11] The Arabic name, meaning (Baboons Onion) is particularly apt since the bulbs are one of the main foods of the baboons which are present in these valleys.[12] This species is endemic to southwest Arabia.[13]

The study aimed to use the ethanol extract of *P. maximum* bulbs at different doses as a therapeutic attempt against the disease of amoebic dysentery in rats and the study of histological changes after 10 days of treatment.

MATERIALS AND METHODS

Collections of plant

Bulbs of *P. maximum* (Fig. 1) (Amaryllidaceae) were collected between July and August 2017 Rdfan villages in Lahj government-Yemen (13°26′N, 44°59′W). The plant was identified by the Faculty of Science at Aden University and by Dr. Othman Saad Saeed Al-Hawshabi of the Department of Biology. A voucher specimen KA144/19/20/34 of the plant has been deposited at the herbarium of this college. The collected bulbs of *P. maximum* were air-dried in the laboratory and reduced to powder. The powder was kept in brown covered glass bottles.

Figure 1: *Pancratium maximum* bulbs.

Preparation of crude ethanolic extract

Preparation of crude ethanolic extract according to the method of Mehdi *et al.*,[14] An amount of 40 g of bulbs powder and 400 ml ethanol was extracted in a Soxhlet apparatus at 50-55C°, till the color extract disappeared to get an extract. Next, the extract was filtrated and evaporated under reduced pressure at 40-45°C by a Rotary evaporator (PT-70, India). After that, it was transferred to an incubator (BTI-05, India) for 24 hrs at 50 C°, then the percentage yields were calculated, the percentage yield of the extract amounted to 26.83%.[15]

Laboratory animals

The Eighteen female and adult male (*Rattus norvegicus*) were used in this study. 2.5-3 months old rats (approx. 200-250g) were divided into treatment groups. All animals were fed on standard rat pellets and tap water ad libitum. Ethical approval for the study was obtained from the Interfaculty Ethics Committee of the Aden University, Yemen, with an approval date of 16/08/2018 and all experiments were performed according to the Guide for the Care and Use of Laboratory Animals.[16]

Determination of lethal dose (LD50)

The median lethal dose (LD50) of the ethanolic extract was evaluated in accordance by the method of Mehdi *et al.*,[6]

Antiamoebic activity assay

In vivo antiamoebic activity of the extract was tested using the method previously described by Mehdi et al.,[6] *Rattus norvegicus* rats were used in this study, twelve of them were inoculated with *E. histolytica* (17×10^3cell/ml) obtained from the stool, and three rats were kept in the same environmental conditions as controls, after 7-10 days the feces of each rat were examined. All rats were kept in separate cages and divided into six groups (three rats in each group). The treatment period took ten days. After starting cyst shedding (post-infection), examined by microscopy.

There were five groups of one non-infected rat treated, three groups were treated intragastrically once a day for ten days with the doses of 125, 250, and 500 mg/kg of the extract respectively. The positive control received PBS once a day as well as the group non-infected

without treatment that received just PBS solution. Faeces were taken three times a day directly from the bottom of the cages. 1g of all the specimens from individual animals were put separately in test tubes. Then 10 ml of distilled water was added to each tube. Faeces were examined by microscopy. The number of *E. histolytica* were counted using a haemocytometer (number of *E. histolytica* per 1 g of faeces) to obtain the number of parasites per gram of faeces. It was contained out according to the following formula Mehdi *et al.*,[12]

N = S / (Vol × Wt)
N = the number of parasites in 1g of faeces
S = the counted number of parasites in a hemocytometer
Vol = the used volume of quantity (0.01ml)
Wt = the used weight of feces sample (1g)

The procedure of counting parasites continued period through the period of treatment which was 10 days.

Preparation of histological sections

All rats were sacrificed by chloroform and the colon was removed from each rat. Tissue sections were prepared according to the method of Mehdi *et al.*,[9]

Statistical analysis

The results of the present study were analyzed by Genstat® (Version 5.2) using general treatment structure (no blocking), factorial experiment, with 3 replications. The least significant different test (LSD) was used to test the difference between means (groups) at P≤0.05 and was considered significant.

RESULTS

Acute toxicity of the ethanolic extract

Ethanolic extract of *P. maximum* was found to be toxic as it did cause the death of one rat at the highest single oral dose of 1000 mg/kg body weight. Hence lethal dose (LD50) was estimated to be ≥ 1000 mg/kg.

Effect of ethanolic extracts of *P. maximum* Bulbs on *E. histolytica in vivo*

In this study, data of the number of *E. histolytica* from 12 rats divided into four groups of three was evaluated. The statistical evaluation of the course of *E. histolytica* number in the tats infected Table 1.

Rats treated with ethanolic extract by dose 125 and 250mg/kg showed a slight decline in the number of *E. histolytica* but this result was statistically significant at p<0.05 for *E. histolytica* in comparison with the control (there was a decrease in the number of *E. histolytica* from day 1 to day 10) (Fig. 1).

A significant decline in *E. histolytica* numbers was found in the group which was treated by dose 500 mg/kg of ethanolic extract of *P. maximum*, the rats' feces became free of the *E. histolytica* parasite on the10th day of treatment (Fig. 1).

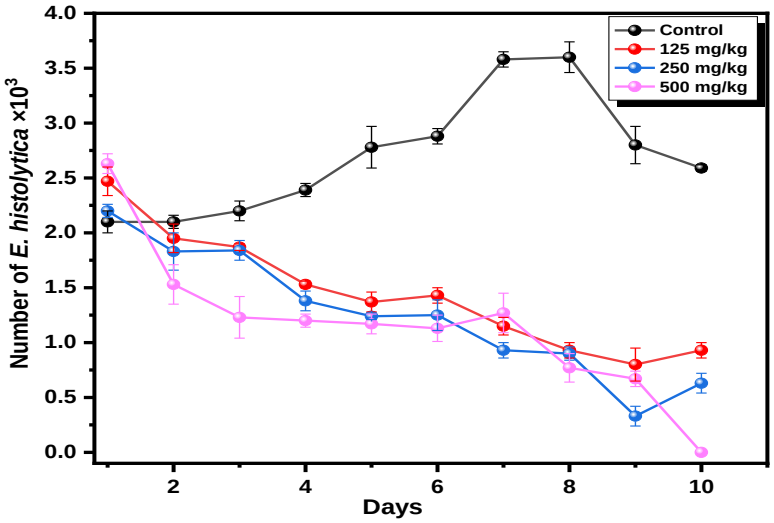

Figure 2: Effect of the ethanolic extract of *P. maximum* leaves on *E. histolytic in vivo*.

Table (1): The effect of ethanolic extract of *P. maximum* bulb against *E. histolytica in vivo* (×10³)

Extract	Dose	Experimental Period (Days) (Number of *E. histolytica* in 1 g × 10³)										Means
		1	2	3	4	5	6	7	8	9	10	
Ethanolic	Control	2.10	2.10	2.20	2.39	2.78	2.88	3.58	3.60	2.80	2.59	2.70
	125 mg/kg	2.47	1.95	1.87	1.53	1.37	1.43	1.15	0.93	0.80	0.93	1.44
	250 mg/kg	2.20	1.83	1.84	1.38	1.24	1.25	0.93	0.90	0.33	0.63	1.25
	500 mg/kg	2.63	1.53	1.23	1.20	1.17	1.13	1.27	0.77	0.67	0.00	1.16
Means		2.35	1.85	1.79	1.63	1.64	1.67	1.73	1.55	1.15	1.04	1.64
LSD 5%		Dose (Ds)= 0.0681, Days(D) = 0.1077, Ds*D=0.2154										
CV%		11.7										

Effect of ethanolic extract of *P. maximum* on histopathological in albino rats infected with *E. histolytica*

The effect of the ethanolic extract of *P. maximum* bulbs on histopathological of the large intestine is shown in Fig. (3). The histological study showed that the ethanolic extract of *P. maximum* bulb was not able to repair the structure of large intestine tissue of the infected rats with *E. histolytica* when it was treated with extract in comparison with the negative control. In contrast, the large intestine tissue of the untreated rats (positive control) showed shortening, atrophy, and villi fusion and desquamation of most villi with necrosis of some enterocytes in the intestine tissue (Fig. 3b).

Histological examinations of sections of the large intestine in the infected rats with *E. histolytica*, which was administered with the ethanolic extract of *P. maximum* bulbs at a dose of 125 mg/kg, showed still cyst of *E. histolytica* in the mucosa, mucosal desquamation, mucosal infiltration, necrosis in mucosal, mucosal atrophy, blood hemorrhage in the mucosa and submucosal atrophy (Fig. 3c).

Large Intestine histology in *E. histolytica*-infected rats administered with 250 mg/kg body weight, also, showed slight necrosis in mucosal, blood hemorrhage, inflammation of the mucosa, and submucosal atrophy (Fig. 3d). On the other hand, it showed changes in the villi such as necrosis, atrophy, in addition to inflammation, blood hemorrhage in the mucosa, and trophozoites attached to the brush border of villi in *E. histolytica*-infected rats administered with a dose of 500 mg/kg (Fig. 3e).

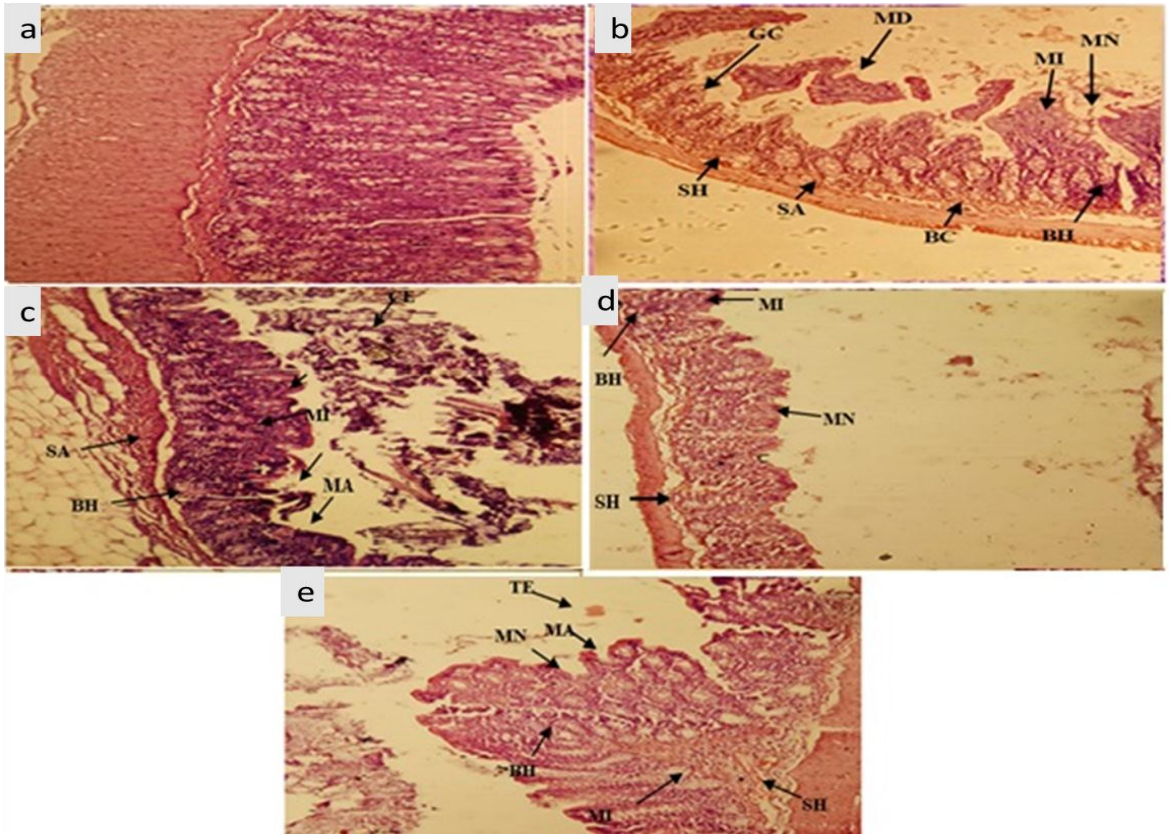

Figure 3: Photomicrographs of large intestine sections stained with Hematoxylin & Eosin. (a) control of negative, (b) control of positive, (c) group treated by 125 mg/kg of ethanolic extract of *P. maximum*, (d) group treated by 250 mg/kg of ethanolic extract of *P. maximum*, (e) group treated by 500 mg/kg of ethanolic extract of *P. maximum*.

Whereas: GC= Goblet Cells, MN= Mucousal Necrosis, MI= Mucosal Infiltration, MD= Mucosal Desquamation, MA= Mucosal Atrotpy, BH= Blood Hemorrhage, BC= Blood Congestion, SA= Submucosal Atrophy, SH= Severe Blood, CE= Cyst of *E. histolytica*, TE= Trophozoite of *E. histolytica*.

DISCUSSION

Amoebiasis is the most common form of protozoa diarrhea, a disease that affects humans worldwide. Because of the available treatment, i.e. metronidazole has toxic side effects like nausea, upset stomach, optic nerve atrophy, bitter taste, and possibly skin-causing dermatitis. In addition, metronidazole causes carcinogenic mutations in rats.[6,17] Because of drug resistance by these parasites, it necessitates the search for new therapeutic agents from natural sources.

In this study, we tested plant extract from *P. maximum* bulbs extracted by ethanol to potential anti amoebic

activity in vivo. This plant has already been studied for its biological and pharmacological effects but there is poor evidence existing of the impact that *P. maximum* has on *E. histolytica*.

In studies concerning this impact, we observed that the administration of ethanolic bulbs extract of *P. maximum* significantly decreased ($p<0.05$) in parasite numbers in the infected-rats and this caused the decrease of the parasite during the treatment days, where it reached zero at a dose of 500 mg/kg after 10 days of treatment. This may be due to the bioactive constituents present extract. This study agrees with the one found by Mehdi et al.,[12] which noticed that crude extract of *P. maximum* has decreased *E. histolytica* numbers shedding in *E. histolytica*-infected rats.

However, some reports have shown that flavonoids, tannins, and saponins phytoconstituents may play a decrease of *E. histolytica* in infected rats.[18-20] Also, the decrease of numbers of *E. histolytica* is attributed to flavonoids, which can reduce sugars, leading to a reduction of carbohydrate metabolism and thus a decrease in ATP.[21]

In the present study, histological examinations of the large intestine tissues of the infected rats with *E. histolytica* which were treated with the ethanolic extract of *P. maximum* bulbs revealed the existence of changes in mucosal architecture, especially in the case of positive control, 125, and 250mg/kg groups with a high number of present trophozoites compared to their control negative group.

These changes may be due to the presence of the parasite in the large intestine which has many effects on mucosal in the large intestine, such as inflammatory, necrosis, desquamation, and the loss of goblet cells. These pathological changes in the mucosa of the large intestine were described in several studies.[6,22]

Nevertheless, we showed no improvement in large intestine tissue in the rats treated with 500 mg/kg-day for ethanolic extract. Histological changes observed in this study might be due to the continued presence of the parasite. These confirm the results with Mehdi et al.,[22] they observed that the continued of trophozoites in the large intestine of *E. histolytica*-infection rats are found like these histological changes.

The results obtained in this study indicate antiamoebic activity of ethanolic *P. maximum* bulb extract. For a greater understanding of this issue, *in vivo* assays should be performed, optimally with a prolonged treatment period.

CONCLUSIONS

In conclusion, the present study suggests that extract of *P. maximum* bulbs is sensitive to *E. histolytica in vivo*, but more studies, optimally with a prolonged treatment period, are required.

Ethics approval

Institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted date 16/08/2018.

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Conflict of interest

There was no conflict of interest among the authors in presenting this article for publication.

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