THE ANTIAMOEBIC POTENTIAL OF AQUEOUS EXTRACT OF *PANCRATIUM MAXIMUM* BULBS IN RATS INFECTED WITH *ENTAMOEBA HISTOLYTICA*

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ABSTRACT

The study determined if administration of aqueous extract *Pancratium maximum* bulbs effects against *Entamoeba histolytica* in experimentally infected rats.

Eighteen rats (200-250 g) were divided into 6 groups of 3 rats each. Groups 1–5 were inoculated with *E. histolytica* (17×10^3cell/ml), followed by daily administration of 125, 250 and 500 mg/kg aqueous extract after the establishment of infection for groups 1-3. Group 4 was administered with doses of 500 mg/kg of metronidazole, while group 5 which oral with the parasite and untreated with plant extract and considered control positive while group 6 the negative control group which non-infected with the parasite and untreated with plant extract.

The results showed significant ($P<0.05$) reduction in the numbers of parasite cells from the second day of treatment in all the groups treated with aqueous extract. This decrease gradually continued during the days of treatment until it reached the lowest level on the 9th and 10th days of treatment for the dose of 500 mg/kg. Infected treated group showed significant ($P<0.05$) increases in the numbers of *E. histolytica* in the feces of rats during 10 days of infected. Histological studies of the infected rats indicate and no showed improvement after using aqueous extract of P. maximum compared with the metronidazole group.

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

1. INTRODUCTION

Entomoeba histolytica is an infection of the intestinal tract that causes the human disease known as amoebiasis, the infection with *E. histolytica* is increased in tropic and subtropic regions, particularly in suffer from poor health and living conditions sanitation areas (Shaker *et al.,* 2018). Amoebiasis comes third in terms of mortality parasitic diseases after Malaria and schistosomiasis (Samie *et al.,* 2012). It is responsible for estimated 100,000 deaths per year and at least 50 million

cases of diarrhea in the world (Herrera-Martínez *et al.,* 2016). 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 may be lead to peritonitis, which in turn leads to death (Ralston and Petri, 2011; Shannon *et al.,* 2013).

Due to the use of metronidazole, paramomycin and tinidazole against this disease, which have a clear side effects such as headaches, metallic taste in the mouth and vomiting as well as neurotoxicity, drug resistance by *E. histolytica*. In the United States proved the failed metronidazole treatment (Mehdi *et al.,* 2019a).

The study aimed to use the aqueous 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.

2. MATERIALS AND METHODS

2.1. PLANT COLLECTIONS

The plant used in this study was collected from Rdfan villages in Lahj government-Yemen (13°26′N, 44°59′W). Proper identification and authentication were done at the Department of Biology, Faculty of Science at Aden University, by Dr. Othman Saad Saeed Al-Hawshabi. Herbarium voucher with reference number (KA144/19/20/34) was already deposited at the Department of Botany, Faculty of Science at Aden University. The bulbs of *P. maximum* were dried in the laboratory in the absence of sunlight for two weeks. The bulbs were ground with the mechanical grinder until they became soft powder.

2.2. EXTRACT PREPARATION

100 g of the dried powdered material was blended with 400 ml of distilled water. The mixture was put on a magnetic stirrer for 24 hrs. It was first filtered with four layers of gauze cloth through a Buchner funnel, the extract was centrifuged at 3500 rpm for 10 min; and later filtered using Whatman number 1 filter paper, the supernatant was put in the incubator at 60 °C for drying. The percentage yield of the extract was 25.00 %.

2.3. ANIMALS

Eighteen adult female and male (*Rattus norvegicus*) weighing (200-250 g) of 2.5-3 month old were used in the present study. Rats were housed in iron boxes bedded with wooden chips. During the experimental period, three rats were kept in each box, they were housed and under standard laboratory conditions (12h light: 12hrs dark and at 26 ± 2 Cº. Rats 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 approval date 16/08/2018 and all experiments were performed according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.4. DETERMINATION OF LETHAL DOSE (LD50)

The median lethal dose (LD50) of the aqueous extract was evaluated in accordance by the method of Lorke (1983) with slight modification. Eighteen (18) rats weighing 200-250 g were divided into six groups (A–F) consisting of three animals each. Group A served as control and orally received 1ml distilled water while groups B to F received 1ml of 125, 250, 500, 750 and 1000 mg/kg body weight (b/w) of aqueous extract. All animals were observed for clinical signs including mortality and morbidity, immediately after dosing during 24 hrs.

2.5. EXPERIMENTAL ANIMALS

Eighteen white albino rats *Rattus norvegicus*, aged 2.5-3 months, weighing 200-250 g. The stool of them was examined before the beginning of the experiment to make sure that the rats are free from any intestinal parasites. Fifteen 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 kept in separate cages and divided into six groups (three rats in each group), all rats kept in separate cages.

The groups 1, 2 and 3 were administered with doses 125, 250 and 500 mg/kg respectively for ten days by aqueous extract of *P. maximum* while group 4 was administered with doses 500 mg/kg of metronidazole. Group 5 which oral with the parasite and untreated with plant extract and considered control positive while group 6 the negative control group which non-infected with the parasite and untreated with plant extract. After starting the experiment, all the faeces were checked by light microscopy every day and enumerate the number of the parasite, after reading the slide, accounting of *E. histolytica* by using a hemocytometer. All indicted groups ware determined through parasites accounts number (by eosin stain) to obtain the number of parasites per gram of faeces. It was contained out according to the following formula Mehdi *et al.,* (2019b):

$N = S /(Vol \times Wt)$

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 parasite continued period through the period of treatment which was 10 days.

2.6. 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.*, (2019b)

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. Least significant different test (LSD) was used to test the difference between means (groups) at $P \leq 0.05$ and was considered significant.

3. RESULTS

3.1. ACUTE TOXICITY OF PLANT EXTRACTS

To study acute toxicity tests for plant extracts in this study albino rats (*Rattus norvegicus*) were used. The result showed no sign of toxicity and death after the aqueous extract of *P. maximum* was orally administered to the rats to the level dose of 1000 mg/kg.

3.2. EFFECT OF AQUEOUS EXTRACT OF *P. MAXIMUM* BULBS ON *E. HISTOLYTICA IN VIVO*

In the present investigation, the effect of aqueous extract of *P. maximum* bulbs on *E. histolytica* infections was investigated as an anti-amoebic material. As shown in Figure (1), it was observed that 10 days following infection by *E. histolytica*, the production of *E. histolytica* differed between aqueous extract of *P. maximum* treated and untreated rats. *E. histolytica* excretion was reduced in all doses during the experimental treatment. The dose of 500 mg/kg treated group caused gradually significant reducing ($P<0.05$) in the number of parasites during the treatment days, the rates of the number of *E. histolytica* reached 0.80, 0.60 and 0.10 $\times 10^3$ in those treated by does 125, 250 and 500 mg/kg after 10 days of treatment, respectively Table (1).

Table 1: The effect aqueous extract of *P. maximum* bulb against *E. histolytica in vivo* ($\times 10^3$)

	Concentration	Days										Means
		1	2	3	4	5	6	7	8	9	10	
Aqueous Extracts	Control	2.10	2.10	2.20	2.39	2.78	2.88	3.58	3.60	2.80	2.59	2.70
	500mg/kg	2.28	1.63	1.43	1.17	1.00	0.93	0.87	0.77	0.55	0.10	1.07
	250mg/kg	2.10	1.87	1.48	1.25	1.13	1.03	1.03	0.97	0.73	0.60	1.22
	125mg/kg	2.07	1.93	1.67	1.43	1.25	1.23	1.13	1.10	0.90	0.80	1.35
Means		2.14	1.88	1.70	1.56	1.54	1.52	1.65	1.61	1.25	1.02	1.59
LSD 5%		Extract = 0.0482, Concentration = 0.0681, Days = 0.1077										
CV%		11.7										

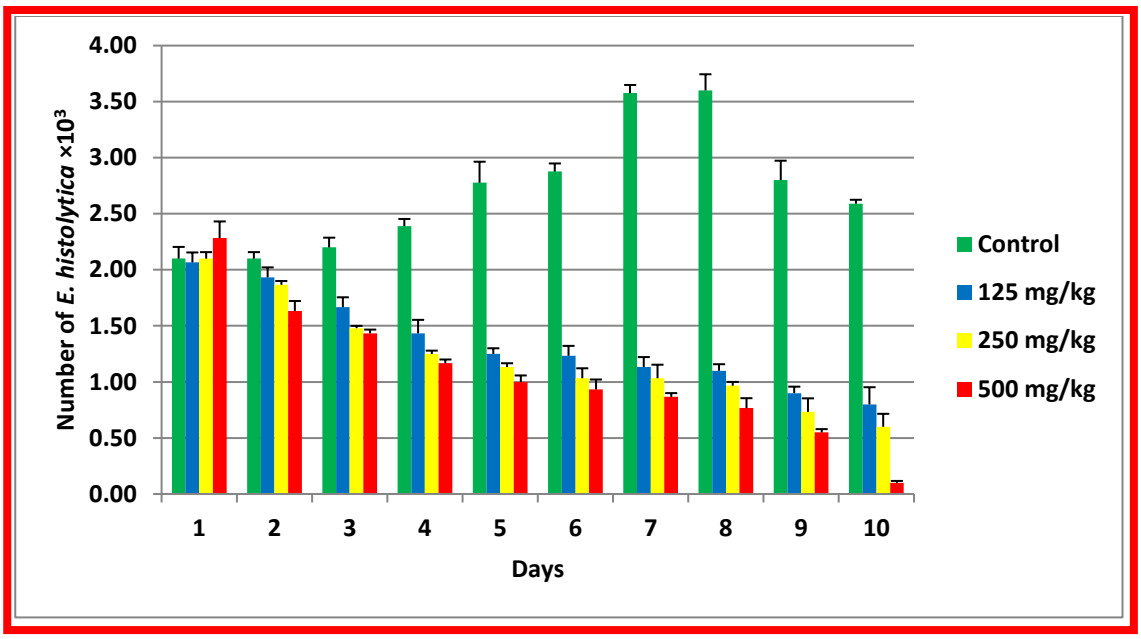

Figure 1: Effect of the aqueous extract of *P. maximum* leaves on *E. histolytic in vivo*.

3.3. EFFECT OF AQUEOUS EXTRACT *P. MAXIMUM* ON HISTOPATHOLOGICAL IN ALBINO RATS INFECTED WITH *E. HISTOLYTICA*

large intestine histopathological examination showed difference in the appearance of villi of the infected rats with *E. histolytica* when it was treated with aqueous extract of *P. maximum* compared with the negative control, the positive control and the metronidazole group, in this experiment through 10 days (Figures 2 a, b, c, d and e). The intestine of rats treated with aqueous extract at a dose of 125 mg kg-day showed abnormal with clear changes including mucosal desquamation in most villi, blood hemorrhage, mucosal necrosis and mucosal infiltration of inflammatory cells in the lamina propria of mucosa for large intestinal and submucosal atrophy (Figure 6-12d). On the other hand, the rats administered for 10 days with aqueous extract at a dose

of 250 mg/kg-day had less intestinal damage than the positive control, in which they, showed mucosal necrosis, server hemorrhage in the mucosa, mucosal infiltration and blood congestion in the submucosa (Figure 6-12e). While the treated group at the dose of 500 mg kg-day showed lower histopathological changes than low doses of the same extract but less than of the metronidazole group (Figure 6-12f) where showed slight necrosis in the villi, decrease in inflammatory cells, few hemorrhages and a few submucosal atrophy.

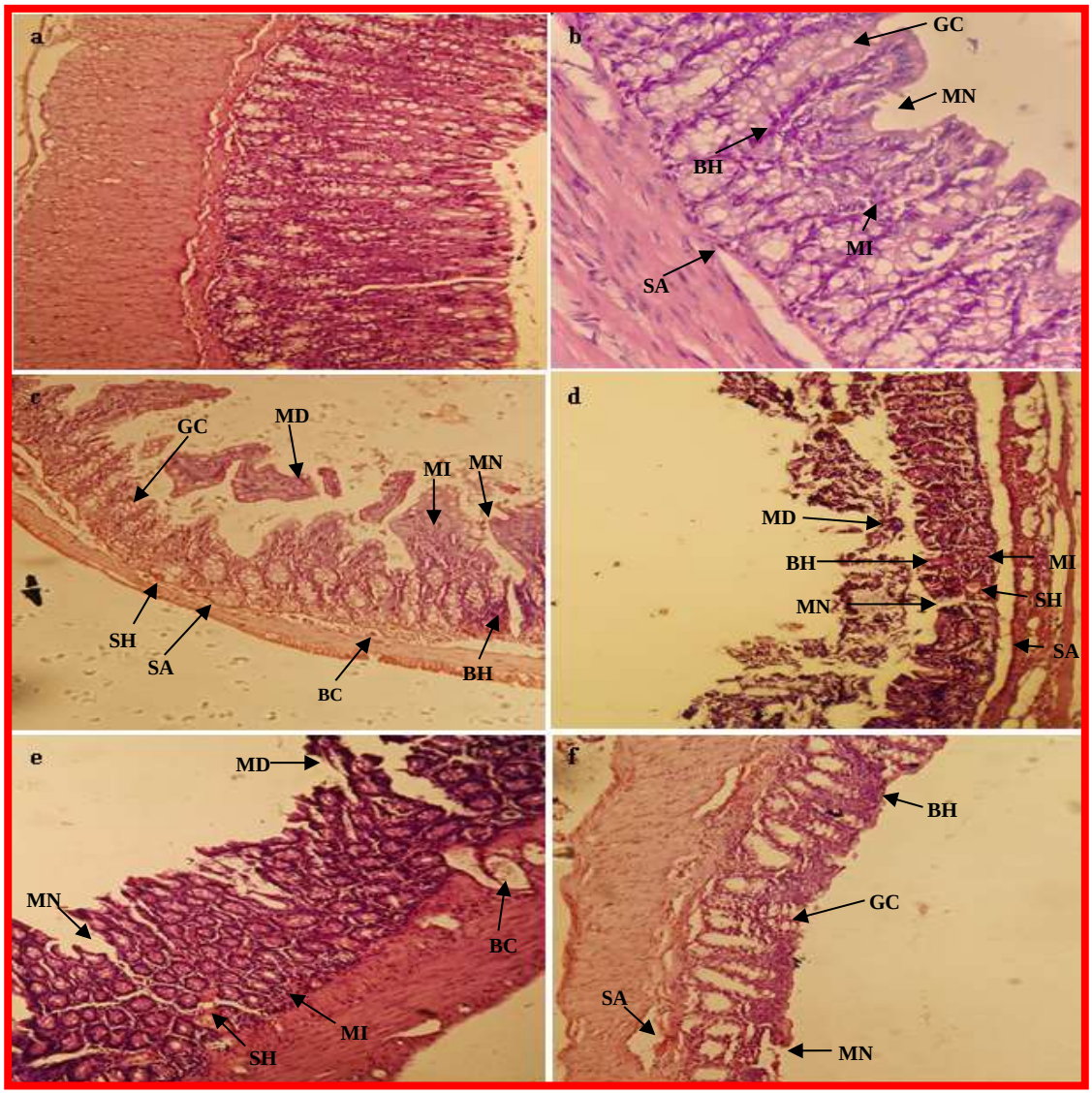

Figure (2): Photomicrographs of large intestine sections stained with Hematoxylin & Eosin. (a) control negative, (b) metronidazole, (c) control positive, (d) group treated by 125 mg/kg of aqueous extract of *P. maximum*, (e) group treated by 250 mg/kg of aqueous extract of *P. maximum*, (f) group treated by 500 mg/kg of aqueous extract of *P. maximum*.

Whereas: GC= Goblet Cells, MN= Mucousal Necrosis, MI= Mucosal Infiltration, MD= Mucosal Desquamation, MA= Mucosal Atrophy, BH= Blood Hemorrhage, BC= Blood Congestion, SA= Submucosal Atrophy, SH= Severe Blood.

4. DISCUSSION

4.1. ACUTE TOXICITY OF PLANT EXTRACT

To study acute toxicity test for aqueous extract of *P. maximum* in this study albino rats (*Rattus norvegicus*) were used. The result showed no sign of toxicity and death after the aqueous extract of *P. maximum* was orally administered to the rats up to the level of 1000 mg/kg.

4.2. EFFECTIVENESS OF AQUEOUS EXTRACT OF *P. MAXIMUM* BULBS AGAINST *E. HISTOLYTICA IN VIVO*

This is the first study about using *P. maximum* bulbs aqueous extract and the effects on *E. histolytica in vivo* have not been documented in the past. Therefore, the present study aimed at evaluating the effects of extracts of *P. maximum* bulbs in *E. histolytca*-infected rats.

The results clearly indicate that the administration of aqueous 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 to level low at a dose of 500 mg/kg. This may be due to the bioactive constituents present extract and hence, at present, it is not certain, which are responsible for the observed effects. However, some reports have shown that tannins, flavonoids and saponins phytoconstituents may play some roles in the inhibition of *E. histolytica* in infected rats (Udensi *et al.,* 2002; Okpe *et al.,* 2016). Also, the decrease of *E. histolytica* numbers is attributed to flavonoids, which can reduce sugars, leading to a reduction of carbohydrate metabolism and thus a decrease in ATP (Sarkar *et al.,* 1996). The decrease of *E. histolytica* numbers by of the when treated it with aqueous extract of *P. maximum* bulbs can also be attributed to the tannins which may penetrate the cell membrane and block the active sites of some enzymes inside the cell which are necessary for the growth of parasites (Inabo and Fathuddin 2011). However, That the present the alkaloids, glycosides, phenols, resins flavonoids, tannins, saponins and other phytoconstituents may play some roles in the inhibition of *E. histolytica* parasites in the infected rats (Otshudi *et al.,* 2000; Elizondo-Luévano *et al.,* 2018).

4.3. EFFECT OF AQUEOUS EXTRACT OF *P. MAXIMUM* BULBS ON HISTOLOGY OF LARGE INTESTINE

In the present study, histological examinations of the large intestine tissues of the infected rats with *E. histolytica* which were treated with the aqueous extract of *P. maximum* bulbs revealed the existence of changes in mucosal architecture compared to their control negative and metronidazole group.

Most of cysts and trophozoites were found attached in mucosal, distortion of villi cells, increased cellular infiltration and server hemorrhage in the mucosal layer. Also, blood congestion, hemorrhage and atrophy in the submucosa in the rats treated with 125, 250 mg/kg-day for both extracts were found. These changes may be due to the presence of the parasite in large intestine which has many effects on mucosal in large intestine, such as inflammatory, necrosis, desquamation and the loss of goblet cells. This finding is in line with reports of the studies on the effects of *E. histolytica* in rats (Chachain and Jamil, 2017; Mehdi *et al.*, 2019b).

Moreover, aqueous extract of *P. maximum* bulbs showed no improvement in large intestine tissue in the rats treated with 500 mg/kg-day. Changes observed in low doses have continued in these sections. Although the rats treated with aqueous extract showed a slight improvement in intestine tissue. Histological changes which observe in this study might be due to the continued presence of the parasite or the extract containing some toxic substances. This might be due to the presence of *E. histolytica* parasite inside of the large intestine, which secretes toxins leading to damage in intestine mucosal. This was in agreement with the study by Niculescu, (2016) who reported that the amoebic toxins leading to irritation, inflammation and damage of the mucosal. Also, this might be due to found some toxic substances in the extract. This was in agreement with the study by Lakshmanan *et al.*, (2016) who reported that the extracts of *Hericium erinaceus* contained some toxic substances which lead to damage to tissue in the treat rats.

5. CONCLUSIONS

This study shows the decreased in the numbers of *E. histolytica* in infected rats and treated by aqueous extract of *P. maximum* bulbs. However, the tissue sections no showed improvement after using aqueous extract of *P. maximum* compared with the metronidazole group.

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.

Financial support

None.

Conflict of interest

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

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Mazahar Farooqui principal of Maulana Azad College of Arts, Science and Commerce for his constant support and cooperation, also, thank Dr. Omar Bin Shuaib-Yemen for his make statistical analysis for this paper.

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