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The evaluation of the Theraputic Effect of Aqueous Extract of Aloe Vera on *Entamoeba histolytica* in Vivo

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ABSTRACT: Amoebiasis caused by Entameoba spp. It is a zoonotic disease and is the most widespread. The study was conducted to evaluate the effectiveness of aqueous aloe vera extracts against amebiasis compared to metronidazole in laboratory mice. Stool samples were collected from Al Karama Hospital patients who suffered from diarrhea, and 15 samples for both sexes were examined microscopically using Lugulos iodine stain to detect, isolate and examine cysts infected with the parasitIt was purified and preserved in potassium dichromate for the purpose of using it to cause infection in laboratory mice. The experimental study was conducted on groups of 35 mice by giving the parasitic cysts orally 1000 cysts/ml, with the exception of the negative control group, which was immersed in physiological saline solution. The first group, which included 15 mice, was treated after being divided It was divided into three subgroups A, B, and C. Each secondary group had 5 mice treated with the aqueous extract of the A. vera plant at different concentrations of 50, 100, and 150 mg/ml, respectively. The second group included 10 mice that were treated with metronidazole at a concentration of 10 mg/ml, and the positive control group remained 10 mice. She was infected with the parasite and was not treated. After treatment, microscopic examination was performed by assessing the average excretion of parasitic cysts using a hemocytometer slide.

Keywords: E. histolytica, ale vera, metronidazole, therapeutic

1. INTRODUCTION

Amoebiasis is a human gastrointestinal infection caused by *E. histolytica*, a primary parasite that causes widespread mortality and morbidity, it affects 40-50 million people around the world through diarrheal disease, *E. histolytica* is able to invade the intestinal mucosa and form an abscess in tissues such as the liver and lung

The symptoms of invasive amoebiasis include amoebic colitis and extra-intestinal manifestation represented by a potentially fatal liver abscess. Notably, other species may infect humans including *E. dispar* and *E. moshkovskii* [1] *E. histolytica* has a simple, two-stage life cycle, consisting of the infective cyst and colon-invasive trophozoite forms. *E. histolytica* infections occur when cysts are ingested through contaminated food or water. In the lower intestine trophozoites emerge from cysts (a process known as excystation)[2]. As a result of unknown stimuli in the intestine, trophozoites again can differentiate into cysts (a process known as encystation), which may be excreted in feces to infect other humans. Although the cyst is the only form to transmit infections, most studies on *E. histolytica* have focused on the trophozoite form, which is the only form that can be readily cultured. The inability to encyst trophozoites in vitro has severely impaired our knowledge on the infectious stage of *E. histolytica* [2]

2.MATERIALS AND METHODS

2.2. Methods

2.2.1. Samples collection

15 stool samples were collected from patients admitted to Al-Karama Hospital suffering from diarrhea for both sexes, for the period from November 1, 2022 to February 29, 2023, and kept in potassium dichromate solution for the purpose of conducting examinations.

Microscopic Examination of Stool

For the detection of *Entamoeba spp*. cysts, each sample was examined by smears stained with a by lugols iodine stain according to the method [3] in which a portion of feces the size of the tip of a match was taken and mixed on a clean glass slide and mixed with a drop of distilled water, then distribute it over the entire area of the slide and leave it in the open air to dry , For 10 minutes without using a flame, the swab was fixed by adding drops of 11% methyl alcohol for 5 minutes and left to dry at room temperature, after which carbol red concentrated fuchsin dye was added to the fixed swab and left for 3-5 minutes and passed over a quiet flame. The stain was washed off with a weak stream of tap water and left to air dry then shortened swab with acidified alcohol for 30 seconds, washed with tap water and left to dry. Then the swab was dyed with methylene blue dye for two minutes, washed with a weak stream of water, and dried. The stained specimens were examined with a light microscope under the objective lens of X40 and then the oil lens of X100 for the examination of *Entamoeba* spp. cysts and to confirm its presence in the stool samples. Isolation and purification of *Entamoeba* spp. cysts Parasite cysts were isolated from stool samples preserved in potassium dichromate solution in the first stage by flotation using Scheither's sugar solution according to [4] where stool samples were washed three times using phosphate-buffered saline (PBS) centrifugation at IO rpm for 5 minutes, Each time the filtrate was poured out and the precipitate was shaken until the yellow color of the potassium dichromate solution was removed. Then add 10ml of precipitated sugar sheather solution and mix well and then centrifuge at 700 rpm for 20 minutes, this process is known as flotation because the cysts float in a highly concentrated sugar solution. Then collect the floating portion containing the oval sacs using a Pasteur pipette and dilute it with distilled water in a volume ratio of 1:10 to prevent the sugar solution from affecting the cysts. Then the diluted solution was precipitated in a centrifuge at 700 rpm for 15 minutes, then the liquid was poured out, the precipitate was re-washed three times with distilled water at the same speed and time. Precipitates containing cysts were collected in conical tubes and a solution of l% sodium hypochlorite was added to it in a volume equal to the volume of the precipitate, then distilled water was gradually added to the wall of the conical tube. The Qasr product was washed several times with distilled water by rapid disposal (700 rpm for 15 min) and isolation steps were carried out at 4°C to prevent cysts from breaking. After each separation process, a drop of sediment is taken on the glass slide; the sliding cover is placed on it and examined under the microscope to ensure the presence of the cysts of the parasite. Then the cysts were counted for each millimeter of the suspension using the counting slide scale. Finally, it was used in experimental injury.

Entamoeba spp. cysts Counting

The number of cysts of the parasite that were used in animal doses was calculated using a hemocytometer slide and based on the method [5]where a drop of iodine solution was placed as a dilution agent to suspend the pure cysts to stain them and make them more visible under the microscope. The counting slide was washed with distilled water and covered with a lidcover slide, then put a drop stuck in the counting chamber to spread under the cover with the diffusion characteristic and put it under the microscope and adjust the power of the lenses to get a clear view, as the bags were counted in the eight corners on both sides of the slide according to the equation:

Number of cysts in 1 ml=**calculated cysts number 8 x** 1000 [6]

Experimental Infection Animals

The experimental study was conducted in vivo (white mice), which included (57) male mice aged (8-10) weeks and weighing (28-30) gm, divided into (5) groups and dosed with 104oocyst/ml for each mice by the oral dosing syringe [7]except for the negative control group, which was dosed with physiological saline only, The stool was examined daily using logos iodine stain to ensure that the parasite cysts shed, as infection was confirmed on the seventh day by 100%.

Microscopic Examination of Experimental Animals Feces

Stools of mice inoculated with parasite oocysts were examined microscopically with lugols iodine stain on a daily basis for parasite detection and experimental infection was achieved [5] in which a portion of the stool sample was placed on a clean glass slide and mixed with a drop of distilled water and distributed over the entire area of the slide Leave in the air for 10 minutes to dry, taking into account the numbering of stool. A few drops of 95% methanol were then placed for 1 minute for the purpose of fixation. Then Carbol Fusion red dye was added to the fixed slide and left for 15 minutes. The sample was then washed with distilled water and left to dry. Acid alcohol was added for the purpose of default and washed with tap water. Then the slide was stained with methylene blue for two minutes. The slide was washed with light water and left to dry. Examination was performed under 40X and 100 X optical microscopes.

Statistical Analysis

Statistical significance was determined by entering the obtained data into a computer database, the Statistical Package for Social Sciences (SPSS) program was used for statistical analysis, data were recorded in numbers and percentages, numbers were compared using a nova test, and $P \leq 0.05$ was considered significant [8]

3. RESULT AND DISCUSSION

1. Prevalence of *Entamoeba spp*. infection according to microscopic examination test

The current study included the examination of 15 stool samples of patients suffering from diarrhea, as they were examined microscopically, the results showed that *Entamoeba spp.* cysts were spherical, red in color with a blue background, containing four spores as in Figure (3.1). The result showed the percentage of those infected with the disease amounted to 100 % where the number of positive samples reached 15 out of 15 samples as in Table (3.1).

Table (3-1) Prevalence of *Entamoeba* spp. infection according to microscopic examination test

Microscope examination of e*ntamoeba spp*	Results	
	No	**%**
Specimen positive for *Entamoeba spp*	**15**	**100%**
Specimen negative for *Entamoeba spp*	**0**	**0**
Total	**15**	**100**

The present study disagrees with Alyousefi, (2012) [9] findings in Sana'a city, Yemen. When analyzing 503 positive diarrheic stool samples, the study revealed a prevalence of 17.1% for *E. histolytica* [9].

The result of current study disagrees with some previous studies, including the study of Ihsan *et al*., (2017) [10] in Najaf, Wasit, Basra and Diwaniya provinces, showed the lowed prevalence rates (18.6%, 10.4%, 10.2% and 10.3% respectively) [10]. Khder (2018) in Saladin, Thi-qar, Wasit and Baghdad showed the prevalence rates of *E. histolytica* was (18.09%, 17.5%, 16.78% and 15.77% respectively) which agree with our result [11].

Also present results was disagree with Al-Sultany and Al-Morshidy that showed the rates of *E. histolytica* was (10.54%) [12]. Our study findings are consistent with the research conducted by Al-Waaly *et al*. (2020) in Diwaniyah City. Other study reported a prevalence of 15.06% for *E. histolytica*, which aligns with our own results [13]. The results of the current study disagree with Shakir and Hussein (2014) in Baghdad province they registered the rate of *E. histolytica* (41.25%) [14].

In addition to disagree with Akram (2018) in ThiQar he reported *E. histolytica* rate (67.7%) [15]. Also disagree with study by Ibrahim *et al*. (2019)[16] in Egypt who mentioned *E. histolytica* (3.5%). The elevated occurrence of *E.histolytica* detected in microscopic examinations may be linked to the extended viability of cysts in the environment, as indicated by Nath *et al*. in their study conducted in 2015, and its direct mode of transmission, which does not necessitate an intermediate host.

Additionally, factors such as inadequate emphasis on aseptic water treatment, scarcity of materials essential for water purification, and the use of pesticides contribute to the increased prevalence of *E. histolytica* [17].

This prevalence of present study can be considered very high compared to other similar studies conducted in other country, for instance, Obadiah (2012)[18] in his study, reported a high prevalence of 37.9% in Kaduna state. [19] also obtained as high as 45% prevalence in his study in Kano metropolis. [19] in Abeokuta, Ogun state obtained a prevalence of 19.4%. The value 6.5% is also lower than what Mbagwu, Abioye, & Seye (2019)[20] obtained in Karachi, Pakistan. These variations may be due to use of transmission and pathogenesis as well as other risk factors which favor the persistence of this infection that may contribute to high prevalence of *Entamoeba* infection [21].

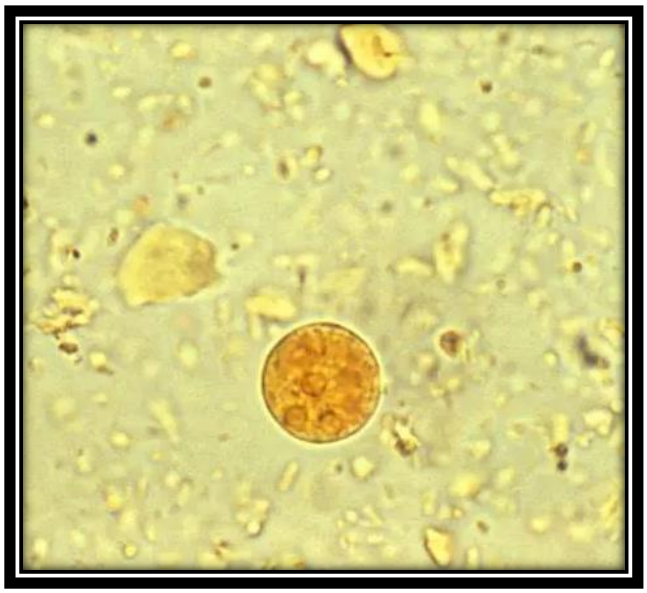

Figure (3-1) Direct examination of *Entamoeba* spp. cyst by Lugol's Iodine method. Cysts have round shape with one nucleus 40x.

3.3 Evaluation of the effect of metronidazole on cyst number of *E. histolytica* in mice

In the current study, when metronidazole was used to treat of *Entamoeba*, the number of cysts decreased significantly with positive group this led to effectively of this substance to treating of *E. histolytica*, the result of present study showed decrease of number of cysts of *E. histolytica* after 6 day of treatment (1228.80 ±50.75) this differ significantly compared with positive group (P≤0.05) (4169.00 ±319.20) as showed in table (3-1).

After 18 days of treatment of mice with metronidazole we showed highly differ significant with positive group (P≤0.05) (93.40 ±28.42, 3663.57 ±412.49) respectively as showed in table (3-1).

The present study showed highly significant (P≤0.05) percentage of cyst after treated with metronidazole in 21 day (60.20 ±10.46 b). As showed in the table (3-1).

Table (3-1): Evaluation of the effect of metronidazole on cyst number of *E. histolytica* in mice

Group	Mean ±SE							LSD value
	Before treated	After 6 day	After 9 day	After 12 day	After 15 day	After 18 day	After 21 day	
Positive	4101.00 ±528.82 A a	4169.00 ±319.20 A a	3933.71 ±418.63 A a	3686.00 ±351.25 A a	4096.86 ±597.64 A a	3725.14 ±489.32 A a	3663.57 ±412.49 A a	**607.45 NS**
Infected and treated with the Metronidazole 10mg/kg	1660.80 ±120.29 D a	1228.80 ±50.75 C ab	1007.40 ±168.78 B bc	594.60 ±114.78 B cd	270.40 ±100.54 B d	93.40 ±28.42 B d	60.20 ±10.46 B d	**581.29 ***
LSD value	970.68 *	601.40 *	633.21 *	487.03 *	799.27 *	649.27 *	546.73 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).								

Present study was agreements with [22] that obtain Mice were infected with *E. histolytica*, and infection was confirmed after 7 days by laparotomy or imaging. Mice were then administered a dose of metronidazole (10 mg/kg per day).

The current study disagree with Usuda *et al.*, (2022)[23] that conducted that of metronidazole therapy are more effective in resolving infections, issues of incomplete clearance against intestinal *E. histolytica* and tolerability by

patients remain. Additionally, metronidazole resistance has been documented in clinical isolates of *Giardia intestinalis* and *Trichomonas vaginalis* and has been artificially induced in a laboratory strain of *E. histolytica* [24]. Furthermore, transmission of metronidazole-resistant *amebiasis* has been reported [25].

Our Studies conducted in-vivo on infected mice revealed that treatment with metronidazole considerably reduced the size and number of the largest cysts when compared to treatment with un metronidazole [26]. In terms of the potential of metronidazole to treat *E. histolytica* infection, this validates the findings of the present investigation [27] ;[28].

3.4 Evaluation of the effect of group and period in cysts number of *E.histolytica* in mice treated with aqueous extract of *A.vera* compared with metronidazole 10mg/kg

In present study made comparting between *A.ver*a extract and metronidazole on mice infected with *E. histolytica*. The result showed differ significantly between aqueous extract of *A.vera* 50mg/L with metronidazole 10mg/kg after 21 day of treatment (P≤0.05) (111.80 ±9.81d, 60.20 ±10.46d) respectively as showed in table (3-2).

Additionally, metronidazole resistance has been documented in clinical isolates of *Giardia intestinalis* and *Trichomonas vaginalis* and has been artificially induced in a laboratory strain of *E. histolytica* [24]Furthermore, transmission of metronidazole-refractory *amebiasis* has been reported [25]

Table 3.2: Evaluation of the effect of group and period in cysts number of *E.histolytica* in mice treated with aqueous extract of *A. vera* (50)mg/L compared with metronidazole 10mg/kg

Group	Mean ±SE							LSD value
	Before treated	After 6 day	After 9 day	After 12 day	After 15 day	After 18 day	After 21 day	
Positive	4101.00 ±528.82 A a	4169.00 ±319.20 A a	3933.71 ±418.63 A a	3686.00 ±351.25 A a	4096.86 ±597.64 A a	3725.14 ±489.32 A a	3663.57 ±412.49 A a	**607.45 NS**
Infected and treated with the Metronidazole 10mg/kg	1660.80 ±120.29 D a	1228.80 ±50.75 C ab	1007.40 ±168.78 B bc	594.60 ±114.78 B cd	270.40 ±100.54 B d	93.40 ±28.42 B d	60.20 ±10.46 B d	**581.29 ***
Treated with aqueous extract of Aloe vera (50)	2277.60 ±118.46 BCD a	1628.20 ±112.29 BC b	1012.80 ±64.56 B c	604.20 ±31.75 B cd	316.60 ±39.92 B d	198.60 ±17.81 B d	111.80 ±9.81 B d	**537.41 ***
LSD value	970.68 *	601.40 *	633.21 *	487.03 *	799.27 *	649.27 *	546.73 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).								

The result of current study showed alcoholic extract of *A. vera* (50)mg/L more effective than aqueous extract of *A. vera* (50)mg/L in treated of *E. histolytica* that been reported decrease of cyst number significantly with untreated group (P≤0.05). In this study after 6 day of treatment we recorded slightly decrease number of cyst of *Entamoeba* that we showed highly significant between two extract (1628.20 ±112.29, 1275.60 ±67.59) respectively significantly differ compared with positive group (P≤0.05) (4169.00 ±319.20) as showed in table (3-2).

Then highly decrease the number of cyst after day 15 and 18 (84.60 ±14.68 , 60.40 ±10.81) respectively when using of alcoholic extract of *A.vera* 50mg/L while significantly differ compared with aqueous extract of *A.vera* 50mg/L after day 15 and 18 of treatment (316.60 ±39.92 , 198.60 ±17.81) respectively as showed in table (3-2).

The results of current study were agreement with our study that demonstrated that the alcoholic extract of *A.vera* induced significant concentration and time-dependent effect on the viability of *entamoeba* cysts. The best effect was observed at the concentration of 50 mg/L after 21 day where the inhibitory growth percentage was 97.9%, The recorded cysticidal effect of *A. vera* may be attributed to *A.vera* secondary metabolites like flavonoids, anthraquinones, tannins, coumarins, terpenes, saponins and alkaloids that responsible for its biological actions, Furthermore, *A. vera* contains phenolic compounds which possess anti-inflammatory, anti-oxidant and anti-parasitic activities [29]; [30].

3.5 Evaluation of the effect of group and period in cysts number of *E.histolytica* in mice treated with aqueous extract of *A. vera* (100)mg/L compared with metronidazole 10mg/kg

In present study made comparting between *A. vera* extract compared with metronidazole on mice infected with *E. histolytica*. The result showed differ significantly between aqueous extract of *A. vera* 100mg/L with metronidazole

10mg/kg after 21 days of treatment statistically significant different in level (P≤0.05) (32.60 ±14.94, 60.20 ±10.46) respectively as showed in table (3-3).

Additionally, metronidazole resistance has been documented in clinical isolates of *Giardia intestinalis* and *Trichomonas vaginalis* and has been artificially induced in a laboratory strain of *E. histolytica* [24]Furthermore, transmission of metronidazole-resistant *amoebiasis* has been reported [25].

In present study we showed highly decrease of cyst of *Entamoeba* when treated of mice by aqueous extract of *A.vera* 100 mg/L this change of number appear after 6 day of treatment (1308.40 ±58.28, 1905.20 ±221.65) respectively as showed in table (3-3).

Table 3.3: Evaluation of the effect of group and period in cysts number of *E. histolytica* in mice treated with aqueous extract of *A. vera* (100) mg/L compared with metronidazole 10mg/kg

Group	Mean ±SE							LSD value
	Before treated	After 6 day	After 9 day	After 12 day	After 15 day	After 18 day	After 21 day	
Positive	4101.00 ±528.82 A a	4169.00 ±319.20 A a	3933.71 ±418.63 A a	3686.00 ±351.25 A a	4096.86 ±597.64 A a	3725.14 ±489.32 A a	3663.57 ±412.49 A a	**607.45 NS**
Infected and treated with the Metronidazole 10mg/kg	1660.80 ±120.29 D a	1228.80 ±50.75 C ab	1007.40 ±168.78 B bc	594.60 ±114.78 B cd	270.40 ±100.54 B d	93.40 ±28.42 B d	60.20 ±10.46 B d	**581.29 ***
treated with aqueous extract of Aloe vera (100)	1862.20 ±85.04 CD a	1308.40 ±58.28 BC b	845.20 ±37.26 BC bc	357.60 ±48.84 BC cd	127.20 ±34.70 B d	60.80 ±10.72 B d	32.60 ±14.94 B d	**512.96**
LSD value	970.68 *	601.40 *	633.21 *	487.03 *	799.27 *	649.27 *	546.73 *	---

Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).

The result of present study was agreement with (Kadry *et al*., 2021)[32] that showed *A. vera* aqueous extract in the concentration (100mg/L) reduced the mean number of viable *Entamoeba* cysts. Effects of all concentrations showed time-dependent statistically significant differences compared to non-treated controls.

3.6 Evaluation of the effect of group and period in cysts number of *E. histolytica* in mice treated with aqueous extract of Aloe vera (150) mg/L

In present study made comparting between *A. vera* extract and metronidazole on mice infected with *E. histolytica*. The result showed high differ significantly between aqueous extract of *A.vera* 150mg/L with metronidazole 10mg/kg after 21 day of treatment highly significant (P≤0.05) (0.00 ±0.00, 60.20 ±10.46) respectively as showed in table (3-4).

In present study we showed the number of cyst *Entamoeba* decrease graduate after treated with extract of *A. vera*, the changes that appear after 6 day of treatment from aqueous extract of *A.vera* significantly differed compared with positive group (P≤0.05) (1136.80 ±55.27 , 4169.00 ±319.20) respectively.

The result of this study indicate the more effect of aqueous extract of *A. vera* 150 mg/L on the number of cyst of *Entamoeba*, after 21 day of treatment this highly significant of extract and very high differ significant with positive group (P≤0.05)(3663.57 ±412.49 , 0.00 ±0.00) as showed in table (3-4).

The present study agreement with previous study that recorded when excessive concentration of Aloe vera that give more effective result for treatment of *Entamoeba histolytica* [34].

Table 3-4: evaluation of the effect of group and period in cysts number of *E.histolytica* in mice treated with aqueous extract of Aloe vera (150)mg/L

Group	Mean ±SE							LSD value
	Before treated	After 6 day	After 9 day	After 12 day	After 15 day	After 18 day	After 21 day	
Positive	4101.00 ±528.82 A a	4169.00 ±319.20 A a	3933.71 ±418.63 A a	3686.00 ±351.25 A a	4096.86 ±597.64 A a	3725.14 ±489.32 A a	3663.57 ±412.49 A a	**607.45 NS**
Infected and treated with the Metronidazole 10mg/kg	1660.80 ±120.29 D a	1228.80 ±50.75 C ab	1007.40 ±168.78 B bc	594.60 ±114.78 B cd	270.40 ±100.54 B d	93.40 ±28.42 B d	60.20 ±10.46 B d	**581.29 ***
treated with aqueous extract of Aloe vera (150)	1968.80 ±83.43 CD a	1136.80 ±55.27 C b	573.60 ±105.06 BC c	190.00 ±46.04 BC cd	18.20 ±14.61 B d	0.200 ±0.10 B d	0.00 ±0.00 B d	**548.60 ***
LSD value	970.68 *	601.40 *	633.21 *	487.03 *	799.27 *	649.27 *	546.73 *	---
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).								

4. CONCLUSIONS

The aqueous extract of Aloe vera showed an effective and safe therapeutic effect for Ameobiasis. Increasing the therapeutic efficacy of the aqueous extract of A. vera by increasing the concentration, as high concentrations showed greater therapeutic efficacy than low concentrations of extract. The extracts of A. vera have an important effect on the parasite, and when compared with the drug metronidazole it was found that the aqueous extract of A. vera with the highest concentration it has a therapeutic efficacy comparable to that of metronidazole.

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