academicJournals

Vol. 9(36), pp. 893-899, 29 September, 2015
DOI: 10.5897/AJPP2015. 4336
Article Number: 1BB1DC955359
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

The role of artemether as a possible drug for treatment *Blastocystis hominis* infection: *In vivo* and *in vitro* studies

Zeinab Hassanein Fahmy[1], Eman Aly[1*] and Olfat Hammam[2]

[1]Department of Parasitology, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt.
[2]Department of Pathology, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt.

Received 9 April, 2015, Accepted 25 August, 2015

The study is carried out to evaluate the *in vitro* and *in vivo* effect of treatment of artemether against *Blastocystis hominis* infection. In addition, transmission electron microscopic examination (TEM) and histopathological studies of *B. hominis* were carried out. The effect of artemether on *Blastocystis* viability showed reduction in viability 7 and 15% after 1 h in the group treated with 500 and700 µg/ml, respectively. There are reductions in living cell count (LCC) and living cell rate (LCR) activity of artemether on *B. hominis* isolates in 500 and 700 µg/ml, respectively in both counts. The highest concentration (1000 µg/ml) caused rapid death of the parasite. The combination of artemether + metronidazole (MTZ) showed remarkable improvement in histopathological picture. By TEM examination, *Blastocystis* cells treated with metronidazole (120 µg/ml) showed shrinking of the cytoplasm and appetites change. Blastocystis cells after exposure to 500 µg/ml artemether showed large cytoplasmic vacuoles which appeared empty with rupture of the cell wall. Finally, our studies showed that artemether can be useful as herbal candidate for *B. hominis* infection therapy.

Key words: Artemether**,** TEM examination, *Blastocystis* cells, metronidazole, histopathological picture.

INTRODUCTION

Blastocystis hominis is one of the most important protozoan parasite that affects the human intestinal tract. Various epidemiological surveys have recorded a prevalence of up to 10% of the population in developed countries and as much as 50 to 60% in developing countries. Tan (2008) and El-Shewy et al. (2002) reported the prevalence of *Blastocystis hominis* among school children in Egypt as 10%. In Dakahlia governorate (Egypt), the prevalence rate of *B. hominis* cysts reached 22% (El-Shazly et al., 2006). The parasite is commonly associated with gastro intestinal tract (GIT) symptoms such as watery and mucus diarrhea, vomiting, abdominal cramps and bloating (Kaya et al., 2007). Epidemiological and parasitological studies also suggested its role in irritable bowel syndrome (Ustun and Turgay, 2006; Stark et al., 2007; Eida and Edia, 2008).

*Corresponding author. E-mail: alye97107@gmail.com

Blastocystis is not only found in patients with enteric symptoms, but also in healthy and asymptomatic subjects (Tan, 2004, 2008). *Blastocystis* in the human intestine shows pathogenic potential which is controversial due to foundation of organism in both symptomatic and asymptomatic individuals (Chen et al., 2003). Pathogencitiy of *B. Hominis* has been proven experimentally on guinea-pigs, which were infected either by natural way (orally) or by rectal infection of the parasite into the cecum (Boeva-Bangyozova et al., 2008). The most commonly recommended drugs for treatment of *B. hominis* and other pathogenic intestinal protozoa is metronidazole (MTZ), which was reported to cause undesirable side effects and failures in treatment (Lemee et al., 2000). The combination of trimethoprim/sulfamethoxazole with metronidazole is effective in some individuals (Moghaddam et al., 2005). The use of metronidazole as an effective drug is not recommended in intervention. The cystic form has been shown to be resistant (up to 5 mg/ml) to the cytotoxic effect of the drug. These observations may often explain the failure of treatment (Rossignol et al., 2005).

Rossignol et al. (2005) reported the effective drug against *Blastocystis* were Nitazoxanide, a 5-nitrothiazozle broad-spectrum antiparasitic agent, and cotrimoxazole. Garavelli (1991) stated that, metronidazole, iodoquinol, or co-trimoxazole are effective in *Blastocystis* infections.

Introduction of a natural antimicrobials, such as standardized oil of oregano, black walnut, artemesinin, berberine sulphate, citrus seed extract, can be of most importance (Garavelli, 1991).

Artemisia annua (sweet annie or qinghao) was naturally occurring substances having anti-protozoan activity, a plant that yields the lactone artemisinin (qinghaosu) which is the basis for a new class of anti-malarial compounds widely used in Asia and Africa (Hien and White, 1993). The anti-protozoan effects of artemisinin to its content of endoperoxides kills parasities through oxidation. The low toxicity of artemisinin and its antibiotic activity are stimulatedmacrophages, which an important componentich are important component of the immune response to protozoan infestation (Tang, 1992). Armentia et al. (1993) studied that the eradication which depend on the successful restoration of mucosal immune will function and result in continued non exposure to repeat infection.

Different concentrations (10, 100, 500 microg/ml) of *Nigella* aqueous extract and metronidazole are an active standard drug for *B. hominis* (El-Wakil, 2007). Hence, the need to develop a safe and effective alternative antiprotozoal agent is required. Artemether is active *in vitro* against the parasitic protozoa *Entamoema histolytica* and *Giardia lamblia* (Sanmi, 2012). The present study was carried out to evaluate the *in vitro* and *in vivo* effect of treatment of artemether against *B. hominis* infection. In addition, transmission electron microscopic examination (TEM) and histopathological studies of *Blastocystis hominis* were used.

MATERIALS AND METHODS

Stool samples were subjected to:

1. Direct microscopy: Stool samples were obtained from diarrheic patients attending parasitology lathe outpatient clinictient clinic of TBRI, examined by wet smear (both unstained and iodine stained) from fresh stool (Melvin and Brooke, 1974) and merthiolate iodine formaldehyde concentration technique (MIF) (Blagg et al., 1955).
2. In-Vitro cultivation of stool samples positive for *B. hominis:* Approximately 50 mg of each faecal specimen was inoculated into 2 ml of Jones' medium (0.01% yeast extract in buffer saline) (Jones, 1946), supplemented with 20% horse serum in a screw cap tube. The tubes were, then, incubated at 37°C for 48 h and a drop of the cultured solution was examined under light microscope at 10× and 40× magnifications. *B. hominis* vascular forms were found in culture specimens and were variable in size. Culture is regarded as negative when there is failure to detect *B. hominis* after 72 h (Zierdt, 1988). Stool culture was undertaken to exclude bacterial pathogens.
3. All tubes of the different group were incubated in a humidified CO2 at 37°C for ascending time intervals (30 min and up to 2 h in some assays).

(a) Parasites were counted using a Neubauer haemocytometer (Weber, England) after treatment. Total trophozoite numbers per ml in each group were calculated from the mean of at least three haemocytometer counts.
(b) Trophoziote viabilility was assessed using Eosin brilliant cresyl blue stain. The possible effect of artemether on *B. hominnis* living cell count (LCC) was assessed 30 min, 1 and 2 h and the counts were compared with those of metronidazole for the same time intervals.

Experimental animals

Twenty laboratory bred male hamsters each weighting 100 to 110 g were used. Experimental animals were kept for 3 weeks in air conditioned rooms at 21°C, receiving food containing 24% protein. The animals were supplied and housed throughout the study in Schistosome Biological Supply Center (SBSP) at Theodor Bilharz Research Institute (TBRI), an Institution responsible in animal ethics.

Infection

B. hominis cells were obtained from diarrheic patients attending parasitology laboratory, in the outpatient clinic of TBRI. Each hamster was administered *B. hominis* orally using stainless steel esophageal in a dose of 10,000 *B. hominis* cell.

Drugs

Artemether was obtained in tablet form (Kunming Pharmaceutical Cooperation, PR China) with a documented purity of 99.6%. Artemether was suspended in 7% Tween-80 and 60% (v/v) DMSO to get a final stock solution of 1 mg/ml. Serial dilutions of both drugs were performed using RPMI-1640 medium to get the desired test concentrations. Metroindazole was used as a standard drug supplied by Rhone Poulence Rorer in dose of 120 µg/ml.

Treatment

Experimental animals were divided into four groups, each

Table 1. Effect of artemether on *Blastocystis* viability using eosin-brilliant cresyl blue supravital stain.

Drug/contraction (µg/ml)	% Viable cells (1/2 h)	% Viable cells (1 h)	% Viable cells (2 h)
1% DMSO	100	100	100
Metronidazole 120	63.0	63.0	63.0
200	70.1	60	60
500	30	15	7
700	14	7	0
1000	0	0	0

Table 2. Effect of artemether (Art) on *Blastocystis* cell concentration in culture.

Drug/contraction (µg/ml)	Cells/ml±SEM (1/2 h)	Cells /ml±SEM (1 h)	Cells /ml±SEM) (2 h)
Control	100×104±4.06	100×104±4.06	100×104±4.06
1% DMSO	72.55×104±1.55	72.66×104±1.65	74.65×104±1.55
Metronidazole 120	60.72×104±1.23*	65.75×104±1.70*	52.75×104±1.90*
200	52.52×104±0.86*$^{+}$	35.35×104±1.41*$^{+}$	32.35×104±1.27*$^{+}$
500	40.5×104±2.02*	30.25×104±1.25*$^{+}$	21.25×104±1.20*$^{+}$
700	25.5×104±0.44*$^{+}$	13.25×104±2.35*$^{+}$	0
1000	0	0	0

*Significant difference between treated group and control groups ($P < 0.05$). $^{+}$Significant difference between treated group(Contraction 200) and control groups ($P < 0.05$)

comprising 3 hamsters. Animals were administered artemether orally using stainless steel oesophogeal for 3 consecutive days in a dose of 400 mg/kg/day. Animals received metronioazole (supplied by Rhone Poulence Rorer) in a dose of 400 µg/kg body weight. Animals of all four groups were sacrificed 3 week post infection.

Experimental design

Group 1: infected untreated group (control) group.
Group II: hamsters receiving metronioazole (supplied by Rhone Poulence Rorer) in a dose of 400 µg/kg body weight. Group III: hamsters were given Artemether in a dose of 400 mg/kg/day for 3 consecutive days.
Group VI: Infected-treated with a combination of 1/3 dose of metronioazole that is, 100 µg/kg body weight) plus artemether a dose of 300 mg/kg/day for 3 consecutive days.

Parasiological analysis

Three weeks following infection, treatment was given. Two weeks later stool analysis was performed by the merthiolate iodine formaldhyde concentration (MIFC) technique (Blagg et al., 1955) *Blastocystis* cell which is vacuolated from cells/ml were counted.

Transmission electron microscopic examination (TEM)

From culture sediment after receiving different drugs was done as a trial to discuss the mode of action of these drugs. Samples from different groups were fixed in 4% glutaraldehyde in 0.1 sodium cacodylate buffer to be studied by TEM according to Grimaud et al. (1980). TEM examination was performed in the electron microscopy lab at TBRI.

Histopathological studies

After scarification of hamsters, the small bowel was removed. A 1 cm segment was excised 5, 15 and 25 cm from the gastroduodenal junction. The excised segment was opened longitudinally and finally embedded in paraffin wax. Transverse sections of paraffin blocks were cut by microtome and mounted on glass slides. Thickness 5 µm deparaffinization sections were performed by dipping slides in 100% xylene and descending grades (100, 95, 80 and 70%) of ethanol for rehydration. Finally, sections were stained with Enrlich's hematoxylin and counter stained with eosin. Five slides/animal and 3 sections/slide in each group were prepared. Histological section (3 to 5 µm) were stained with hematoxylin-eosin lymph nodes (Peyer's patches) and spleens were also examined (Bancroft and Stevens, 1975).

Statistical analysis

Statistical analysis of the results was carried out using one-way analysis of variance (ANOVA) according to Campbell (1989). Comparison between two groups was done by the Student' test. The data were considered significant if $P < 0.05$.

RESULTS

Table 1 shows the effect of artemether on *Blastocystis* viability using eosin-brilliant cresyl blue supravital stain. It shows reduction in viability 7 and 15% after 1 h in the group treated with 500 and700 µg/ml, respectively. Table 2 shows living cell count (LCC) and living cell rat (LCR) activity of artemether showing reduction in LCC of *B.*

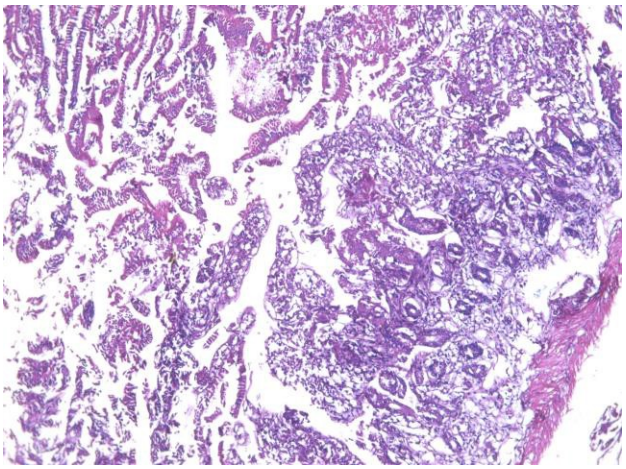

Figure 1. Intestine of control infected group, lined by columnar mucin secreting cells with partial loss of villouus pattern showing erosions and ulcers . Lamina and core of villi are infiltrated by large number of chronic inflammatory cells (H&E ×100).

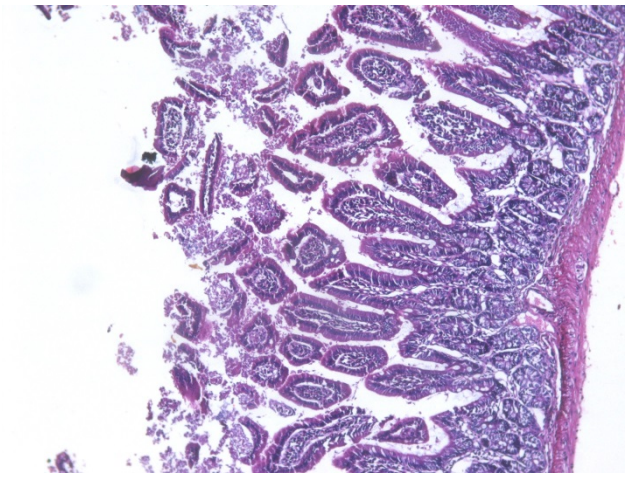

Figure 3. Intestine of treated group with Artmether lined by columnar mucin secreting cells with presentation of normal villous pattern showing focal superficial erosions (short arrow). The lamina and core of villi are infiltrated with moderate number of chronic inflammatory cells (H&E ×200).

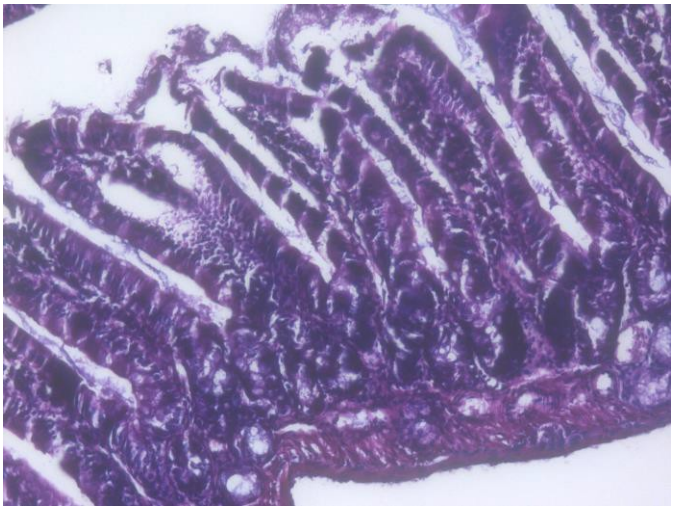

Figure 2. Intestine of treated group with Metronidazole lined by columnar mucin secreting cells with presentation of villous pattern showing focal minute ulceration of the lining epithelium. lamina and core of villi connective tissue is infiltrated by mild number of chronic inflammatory cells (H&E ×100).

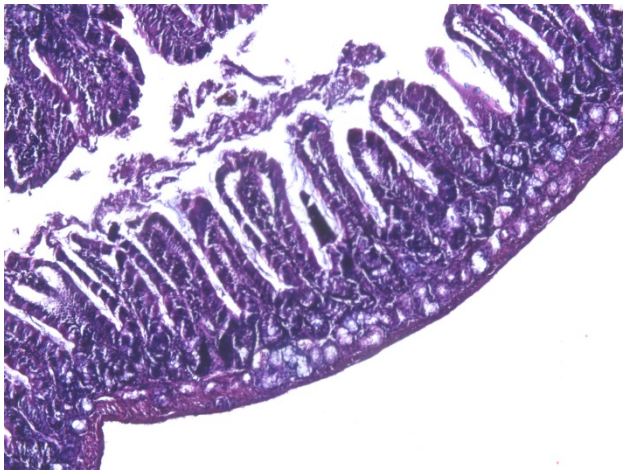

Figure 4. Artmether + Metronidazole: Intestinal mucosa showed, normal architecture . The villa are lined by mucin secreting cells with no erosions & ulcers. The lamina and villi cores are infiltrated by mild number of chronic inflammatory cells (H&E, ×200).

hominis isolates in 500 and 700 µg/ml, respectively. The highest concentration (1000 µg/ml) caused rapid death of the parasite. Metronidazole produced less reduction in mean number of count 52.75 cells/ml of *Blastocystis* cells in culture which was after 2 h.

Histopathological study

Figure 1 shows intestine of control infected group, lined by columnar mucin secreting cells with partial loss of villouus pattern showing erosions and ulcers. Lamina and core of villi are infiltrated by a large number of chronic inflammatory cells (H&E ×100). Figure 2 shows intestine of the treated group with metronidazole lined by columnar mucin secreting cells with presentation of villous pattern showing focal minute ulceration of the lining epithelium. Lamina and core of villi connective tissue are infiltrated by mild number of chronic inflammatory cells, these lead to partial hilling of intestine (H&E ×100). Figure 3 shows intestine of the treated group with artmether lined by columnar mucin secreting cells with presentation of normal villous pattern showing focal superficial erosions. The lamina and core of villi are infiltrated with moderate number of chronic inflammatory cells, these lead to partial hilling of intestine (H&E ×200). Figure 4 shows

Figure 5. (a) Control Ultrastructure of *B. hominis*. TEM showing *B. hominis* cell displaying normal morphology and DNA chromatin seen as a crescentic mass (×14000) Control. (b) Ultrastructure of *B. hominis*. TEM showing *B. hominis* cell displaying normal morphology and DNA chromatin seen as a crescentic mass (×14000) Control.

artmether + metronidazole. Intestinal mucosa showed normal architecture. The villa is lined by mucin secreting cells with no erosions and ulcers. The lamina and villi cores are infiltrated by mild number of chronic inflammatory cells (H&E ×200). It shows remarkable improvement of histopathological study.

Transmission electron microscopic (TEM) studies

Figure 5 *shows* TEM *B. hominis* cell displaying normal morphology and DNA chromatin seen as a crescentic mass (×14000) control. Figure 6 shows TEM *Blastocystis* cell treated with metronidazole (120 µg/ml) showing

concentration (200, 500, 700 and 1000 µg/ml) at different time intervals 1/2, 1 and 2 h) were studied. In this study, artemther at 500 and 700 µg/ml induced highly significant reduction in the number of concentrations of *Blastocystis* cells after 2 h incubation ($p < 0.01$). Higher concentration (1000 µg/ml) caused rapid death of the parasite and no viable cells were detected after 30 min. Our results agreed with El-Wakil (2007) who proved that *N. sativa* aqueous extract could be useful in the treatment of *B. hominis.* This study is an arrangement with Hassan et al. (2010) who stated that, dodecanoic acid (monolaurin) induced highly significant reduction of viability of *B. hominis* cells, 30 and 20% in culture after 2 h incubation at 500 and 700 µg/ml concentration, respectively at ($p < 0.01$). The highest concentration (1000 µg/ml) caused rapid death of the parasite and no viable cells were detected after 30 min incubation. Shrinking of *B. hominis* cells were observed using direct microscopy ×100 following incubation with monolaurin. TEM studies of *B. hominis* cells displayed key morphological features of programmed cell death, viz., nuclear condensation and DNA in nucleus showed reduced cytoplasmic volume with maintenance of plasma membrane integrity.

El-Gayar and Soliman (2011) studied the effect of Quillaja saponaria (QS) against cultures of *B. hominis* and demonstrated its implementation on ultrastructure changes. They found that the QS (1000 µg/ml) produced a high significant reduction in both living cell counts (LCC) and living cell rate (LCR) on day 1 compared to the control. Zierdt (1991) stated that an active drug (metronidazole) concentration should inhibit the growth of *B. hominis.* Sawangjaroena and Sawangjaroen (2005) recorded that at a concentration of 2000 µg/ml the three extracts *Acacia catechu* (Fabaceae) resin, *Amaranthus spinosus* (Amaranthaceaae) and *Brucea javanica* (simaroubaceae) killed 82, 75 and 67% of *B. homninis* parasites tested, respectively and was used as ant diarrheic agents against *B. homninis*.

Mahmod et al. (2006) studied the effect of the antimalarial drug artesunate, in intestinal giardiasis. He found that, the double dose of artesunate (100 mg/kg) gives the lowest mean number of Giardia (0.7 ± 1.1) and completed regeneration of endothelial cells in intestinal villi. This could be of utmost help in endemic areas like Egypt, where *Giardia lamblia* intestinalis is a common parasitic infection and resistance to the commonly used antigiardial drugs commonly known as supervenes. In our histopathological study, best results were obtained in the group receiving Artemether + MTZ. In Figure 4, intestinal mucosa showed normal architecture. The villa is lived by rows of early epithelial cell with no erosions and alces. The lamina and villi cones are unfiltered by needle under number of chronic inflammatory cells . This means remarkable improvement in histopathological picture. El-Gayar and Soliman (2011) found a remarkable improvement in the intestinal histopathological findings, the mucosa was infiltrated with few acute inflammatory cells, the lining columnar epithelial cells were intact and the submucosa was free from inflammation, in infected rats receiving 500 µg/ml. Quillaja saponaria (QS) was observed in comparison with those receiving MTZ.

Zhang et al. (2006) showed that when metronidazole was used (120 µg/ml), TEM observation indicated a reduction of microvilli on the surface of absorptive cells. Mitochondrial edema, rough endoplasmic reticulum dilatation and degranulation were found on absorptive cells and goblet cells. Lymphocyte infiltration and eosinophilia were found in intercellular stroma. These results agree with our results which indicated that *Blastocystis* cell treated with metronidazole (120 µg/ml) showed shrinking of the cytoplasm and apoptotic change (Figure 6). In this study, blastocystis cell after exposure to 500 µg/ml artemether showed large cytoplasmic vacuoles that appeared empty and a ruptured cell wall (Figure 7).

This agreed with El-Gayar and Soliman (2011), in which their study on QS proved that *B. hominis* programmed cell death with appotic-like features and concluded that QS is a promising new herbal therapeutic agent against *B. hominis* infection.

In this study, histopathological studies showed significant improvement in the experimental hamster receiving artemether comparable to MTZ. The effect of artemether on *Blastocystis* viability showed reduction in viability 7 and 15% after 1 h in the group treated with 500 and700 µg/ml, respectively. There is reduction in living cell count (LCC) and living cell rate (LCR) activity of artemether on *B. hominis* isolates in 500 and 700 µg/ml, respectively. The highest concentration (1000 µg/ml) caused rapid death of the parasite. The combination of artemether + MTZ showed remarkable improvement in histopathological picture. By TEM examination, *Blastocystis* cell treated with metronidazole (120 µg/ml) showed shrinking of the cytoplasm and apoptotic change.

Blastocystis cell after exposure to 500 µg/ml artemether showed large cytoplasmic vacuoles which appeared empty in ruptured cell wall.

Conclusion

Artemether can be useful as herbal candidate therapy for *B. hominis* infection therapy.

ACKNOWLEDGMENT

We are really grateful to Amira H. Mohamed, Department Electron Microscopy (Haematology) , for reading Electron Microscopy and made a comment on picture in Theodor Billarz Research Institute.

Conflict of interest

Authors have none to declare.

REFERENCES

Armentia A, Mendez J, Gomez A, Sanchis E, Fernandez A, De la Fuente R, Sanchez P (1993). Urticaria by <I>*Blastocystis hominis*</I>. Succe ssful treatment with paromomycin. Allergologia et Immunopathologia 21:149-151.

Bancroft JD, Stevens A (1975). Histopathological Stains and their Diagnostic Uses. Churchill Livingstone, Edunberg, London and New York pp. 25-46.

Basuldo JA, Cordoba MA, de Luca MM, Ciarmela ML, Pezzani BC, Grenovero MS, Minvielle MC (2007). Intestinal parasitoses and environmental factors in a rural population of Argentina, 2002-2003. Rev. Inst. Med. Trop Sao Paulo 49:251-255.

Blagg W, Schloegel EL, Mansour NS, Khalaf GJ (1955). A new concentration technic for the demonstration of protozoa and Helminth eggs in feces. Am. J. Trop. Med. Hyg. 4:23-28.

Boeva-Bangyozova V, Panayotova M, Chakarova B (2008). Blastocystosis: Pathogenesis, clinical course, diagnostics and treatment. Trakia J. Sci. 6:94-97.

Calzada F, Yepez-Mulia L, Tapia-Contreras A (2007). Effect of Mexican medicinal plant used to treat trichomoniasis on Trichomonas vaginalis trophozoites. J. Ethnopharmacol. 113(2):248-51.

Campbell RC (1989). Statistics for Biologists. 3rd Edn., Cambridge University Press,. Cambridge, UK p 446.

Chen TL, Chan CC, Chen HP, Fung CP, Lin CP, Chan WL, Liu CY (2003). Clinical Characteristics and endoscopic findings associated with <I>Blastocystis hominis</I> in healthy adults. Am. J. Trop. Med. Hyg. 69:213-216.

Dinleyici EC, Eren M, Dogan N, Reyhanioglu S, Yargic ZA, Vandenplas Y (2011). Clinical efficacy of <I>*Saccharomyces boulardii*</I> or metronidazole in symptomatic children with <I>*Blastocystis hominis*</I> infection. Parasitol. Res. 108:541-545.

Eida AM, Eida MM (2008). Identification of <I>*Blastocystis hominis*</I> in patients with irritable bowel syndrome using microscopy and culture compared to PCR. Parasitol. United J. 1:87-92.

El-Shewy KA, El-Hamshary EM, Abaza SM, Eida AM (2002). Prevalence and clinical significance of *Blastocystis hominis* among school children in Ismailia city. Egypt. J. Med. Sci. 23:31-40.

El-Wakil SS (2007). Evaluation of the in vitro effect of Nigella sativa aqueous extract on Blastocystis hominis isolates. J. Egypt Soc. Parasitol. 37:801-813.

El-Gayar EK, Soliman RH (2011). *In vitro* and *in vivo* effects of Quillaja on *Blastocystis hominis* isolates. PUJ 4:101-108.

El-Shazly AM, Awad SE, Sultan DM, Sadek GS, Khalil HH, Morsy TA (2006). Intestinal parasites in <I>*Dakahlia governorate*</I>, with different techniques in diagnosing protozoa. J. Egypt. Soc. Parasitol. 36:1023-1034.

Garavelli PL (1991). The therapy of blastocystosis. J. Chemother. 3:245-246.

Grimaud JA, Druget M, Peyrol S, Chevalier D (1980). Collageimmuno-typing in human liver: Light and electron microscope study. J. Histochem. Cytol. 28:1145-1151.

Hassan SI, Moussa HME, Fahmy ZH, Mohamed AM (2010). In Vitro the effect of medium-chain saturated fatty acid (dodecanoic acid) on *Blastocytsis* spp. Int. J. Infect. Dis. 14:584.

Hien TT, White NJ (1993). Qinghaosu. Lancet 341:603-608.

Hussien A, Virmami OP, Popli SP (2008). Dictionary of Indian Medicinal Plants. CIAP, Lucknow, India p 2384.

Jones WR (1946). The experimental infection of rats with Entamoeba histolytica; with a method for evaluating the anti-amoebic properties of new compounds.. Ann. Trop. Med. Parasitol. 40:130-140.

Kaya S, Cetin E, Aridogan BC, Arikan S, Demirci M (2007). Pathogenicity of <I>*Blastocystis hominis*</I>, a clinical reevaluation. Turkiye Parazitol. Derg 31:184-187.

Lemee V, Zaharia I, Nevez G, Rabodonirina M, Brasseur P, Ballet JJ, Favennec L (2000). Metronidazole and albendazole susceptibility of 11 clinical isolates of <I>*Giardia duodenalis*</I> from France. J. Antimicrob. Chemother. 46:819-821.

Mahmod SS, Guirgus N, Helmy A, Sabry H (2006). Effect of a novel antimalarial drug on different pathological and parasitological parameters in experimental intestinal giardiasis. New Egypt. J. Med. 35:38-43.

Melvin DM, Brooke MM (1974). Laboratory Procedures for the Diagnosis of Intestinal Parasites. 2nd Edn., U.S. Department of Health, Education and Welfare, Washington, DC., USA p 199.

Moghaddam DD, Ghadirian E, Azami M (2005). <I>*Blastocystis hominis*</I> and the evaluation of efficacy of metronidazole and trimethoprim/sulfamethoxazole. Parasitol. Res. 96:273-275.

Nigro LL, Larocca L, Massarelli I, Patamia I, Minniti S, Palermo F, Cacopardo B (2003). A placebo-controlled treatment trial of *Blastocystis hominis* infection with metronidazole. J. Travel Med. 10:128-130.

Ozcakir OS, Gureser S, Erguven YA, Yilniaz R, Topaloglu R, Hascelik G (2007). Characteristics of *Blastocystis hominis* infection in a Turkish university hospital. Turkiye Parazitol. Derg. 31:277-282.

Rayan HZ, Ismail OA, El Gayar EK (2007). Prevalence and clinical features of *Dientamoeba fragilis* infections in patients suspected to have intestinal parasitic infection. J. Egypt. Soc. Parasitol. 37:599-608.

Rohner K, Demuth D (1994). Adverse effects of veterinary drugs. Schweiz Arch. Tierheilkd. 136:309-312.

Rossignol JF, Kabil SM, Said M, Samir H, Younis AM (2005). Effect of nitazoxanide in persistent diarrhea and enteritis associated with *Blastocystis hominis*. Clin. Gastroenterol. Hepatol. 3:987-991.

Sanmi AA (2012). Oxidative stress in biochemical, seminological and histological alterations due to acute administration of intramuscular artemether in mice. Researcher 4:9-17.

Sawangjaroena N, Sawangjaroen B (2005). The effect of extracts from anti-diarrheic Thai Medicinal plants on the *in vitro* growth of the intestinal protozoa parasite: Blastocystis hominis. J. Ethnopharmacol. 98:67-72.

Stark D, van Hal S, Marriott D, Ellis J, Harkness J (2007). Irritable bowel syndrome: A review on the role of intestinal protozoa and the importance of their detection and diagnosis. Int. Parasitol. 37:11-20.

Stensvold CR, Smith HV, Nagel R, Olsen KE, Traub RJ (2010). Eradication of *Blastocystis* carriage with antimicrobials: Reality or delusion? J. Clin. Gastroenterol. 44:85-90.

Tan KSW (2004). <I>*Blastocystis*</I> in humans and animals: New insights using modern methodologies. Vet. Parasitol. 126:121-144.

Tan KSW (2008). New Insights on classification, identification and clinical relevance of *Blastocystis* spp. Clin. Microbiol. Rev. 21:639-665.

Tang W (1992). Chinese Drugs of Plant Origin. Springer Verlag. Berlin. pp. 160-174.

Tasova Y, Sahin B, Koltas S, Paydas S (2000). Clinical significance and frequency of *Blastocystis hominis* in Turkish patients with hematological malignancy. Acta Medica Okayama 54:133-136.

Ustun S, Turgay N (2006). *Blastocystis hominis* and bowel diseases. Turkiye Parazitol. Derg. 30:72-76.

Valsecchi R, Leghissa P, Greco V (2004). Cutaneous lesions in <I>*Blastocystis hominis*</I> infection. Acta Derm. Venereol. 84:322-323.

Zhang HW, Li W, Yan QY, He LJ, Su YP (2006). Impact of *Blastocystis hominis* infection on ultrastructure of intestinal mucosa in mice. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi, 24:187-191.

Zierdt CH (1988). *Blastocystis hominis*, a long-misunderstood intestinal parasite. Parasitol. Today 4:15-17.

Zierdt CH (1991). *Blastocystis hominis* past and future. Clin. Microbiol. Rev. 4:61-79.