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IN VITRO EFFECT OF SOME EGYPTIAN HERBAL EXTRACTS AGAINST *BLASTOCYSTIS HOMINIS*

By

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Abstract

Blastocystis hominis is an enteric parasite that inhabits the gastrointestinal tract of humans and many animals. This emerging parasite has a worldwide distribution. It is often identified as the most common eukaryotic organism reported in human fecal samples that showed a dramatic increase in recent years. Metronidazole is the main therapy for blastocystosis. However, frequent reports of treatment failure suggesting isolates resistance to metronidazole. This study determined the growth pattern and in vitro susceptibility of *B. hominis* to nitazoxanide (NTZ), garlic, ginger, onion and turmeric. Fecal samples positive for *Blastocystis* were collected from patients with irritable bowel syndrome (IBS), and processed for culture. Cultured samples were subjected to examination by light microscopy. Herbs' extracts was freshly prepared. Drug susceptibility assays was done using 0.1 mg/ml of NTZ, garlic, ginger, onion and turmeric. Effects assessed on parasite culture after 24 hr. and 48 hr.

Cultured fecal samples of *B. hominis* have identified several forms of the organism; vacuolar, granular, amoeboid and cyst forms within 24 hr. Nitazoxanide treatment significantly (P <0.001) lowered the parasite number after 48 hr. (mean, 337.5±17.67) /ml. The reduction rate after 48 hr. compared to PBS was 93.33%. Ginger treatment significantly (P <0.002) lowered the number of the parasite after 48 hr. (mean, 335±7.07) /ml. Moreover, garlic treatment also significantly (P < 0.002) lowered the number of the parasite after 48 hr. (mean, 382.5±10.60) /ml. The reduction rates after 48 hr. in these treated samples compared to PBS were 92.98% and 92.44% respectively. However, onion, and turmeric treatments insignificantly lowered the number of the parasite after 48 hr. (P <0.15 & <0.22 respectively).

Key words: Egyptian Herbs, *Blastocystis hominis,* Experimental study.

Introduction

Blastocystis spp. is an intestinal protozoan parasite of humans as well as many animals of worldwide distribution, especially in underdeveloped nations (Sohail and Fisher, 2005; Stensvold *et al*, 2009), in the tropical, subtropical and developing countries, the incidence for *Blastocystis spp* is about 60% (Tan, 200; 2008).

There are some major antioxidants present in herbs which considered nutrients with the demonstrated radical-scavenging abilities (Alok *et al*, 2014).

The herbs extracts used in this study are:

Garlic (*Allium sativum*): Contains a wide range of the thiosulfinates which are responsible for the antibacterial activity (Iimuro *et al*, 2002). Allicin acts by totally inhibiting RNA synthesis and partially inhibiting DNA and protein synthesis (Feldberg *et al*, 1988). The use of garlic and some of its components as anti-protozoa's has already been investigated by several studies. (Mirelman *et al,*1987) investigated the use of allicin on *E. histolytica*; (Soffar and Mohktar, 1991) assessed its use as an anti-giardial alongside its use as an anti-helminthic in a selection of patients; while Lun *et al.*(1994) looked at the effect of diallyl trisulphide (organosulphur compound of garlic) on *E. histolytica*, *G. intestinalis* and *trypanosomes*.

Ginger (*Zingiber officinale*): Is a perennial plant, cultivated in the tropics for its edible rhizome which used for a variety of purposes, including culinary and medicinal (Grant and Lutz, 2000). It occurs naturally in dif-

ferent countries as India, China, South East Asia, West Indies, Mexico and other parts of the world. It is known worldwide as a spice and flavoring agent (Ghosh *et al*, 2011). Few investigations were done upon the anthelmintic activity of ginger and its constituents. (Iqbal *et al*, 2006) showed that crude powder and crude aqueous extract of dried ginger possessed anthelmintic activity in sheep. Lin *et al.* (2010a) showed that hexahydrocurcumin, a constituent isolated from the ginger might be used as larvicidal agents against *Angiostrongylus cantonensis*. Lin *et al*. (2010b) investigated the anthelmintic activity of previous compounds against the nematode worm *Anisakis simplex;* they reported that these compounds kill or reduce spontaneous movement in *Anisakis simplex* larvae. Moreover, Osama and others demonstrated that ginger is having antischistosomal activities (Mostafa *et al*, 2011). They found that, there were a reduction in worm recovery and egg density in the hepatic tissues and feces of treated mice with ginger.

Onion (*Allium cepa*): The most important chemical compounds are the Organosulphur compound including allicin which was thought to be responsible for its properties (Koch, 1996). Onion is also rich in flavonoids (Dixon *et al,* 1983; Augusti, 1996; Griffiths *et al*, 2002) which are known to be synthesized by plants in response to antimicrobial infection (Dixon *et al*, 1983). Hence, it is not surprising that they have been found to be effective antimicrobial substances. Also, plant flavonoids have antiamoebic and anti-giardial activity (Calzada *et al*, 1999). Furthermore, *A. cepa* oils have anti-helminthic effect in rats infected with *Trichinella spiralis* and resulted in increased production of antibodies generated during life cycle of this parasite (Abu El Ezz, 2005).

Turmeric (*Curcuma longa*): Is a plant common in South Asia and it is the key ingredient in curry powder. Its' medicinal properties is due to its active ingredient cur cumin. Curcumin has demonstrated antioxidant, antiviral, anti-inflammatory, antibacterial, and antifungal activity in animals (Kumar and Sakhya, 2013). In addition, it inhibits the growth of various parasites. Moderate anti *Plasmodium falciparum* and *Leishmania major* activity has been found (Akram *et al*, 2010).

Various antimicrobials such as metronidazole, furazolidone, nitazoxanide, have been previously used for the treatment of diarrhea and enteritis associated with *B. hominis* as the sole identified pathogen in children and adults (Dunn and Boreham, 1991). Nitazoxanide (Alinia; Romark Pharmaceuticals, Tampa, FL) is indicated for treatment of persistent diarrhea caused by *Giardia* and *Cryptosporidium* (Rossignol *et al,* 2005).

This study aimed to investigate the effect of some Egyptian herbal antioxidant (Garlic, Ginger, Onion and Turmeric) against *Blastocystis hominis* as compared to Nitazoxanide.

Material and Methods

The study was conducted in the period from August 2014 to December 2014 in the Parasitology Department, Faculty of Medicine, Minia University**.**

Strains of *B. hominis* were isolated from the stool specimens obtained from irritable bowel syndrome (IBS) patients (Rossignol *et al,* 2005; Yakoob *et al,* 2010b). Patients were selected from out and inpatients in Tropical Medicine Department, Minia University. They were diagnosed as irritable bowel syndrome (IBS) patients (Poirier *et al,* 2012; Nagel *et al,* 2014). Stool samples were collected in clean sterile cups and immediately subjected to examination: a- Direct wet smear methods, namely saline wet mount and iodine wet mount (Zaman and Khan, 1994), by both low (×10) and high (×40), when indicated Giemsa stain was used. Positive samples were kept at 4ºC to be used in culture (Garcia, 2001). b- Stool cultivation: Approximately, 50 mg of positively detected stool sample for *B. hominis* were cultivated immediately in 5ml screw

caped tube with LE media (Saksirisampant *et al*, 2010) supplemented with 10% horse serum (Invitrogen, Groningen, The Netherlands), 100 UI/ml penicillin and 100µg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 2-3 days. The cultures were screened by light microscopy every 12 hr. and sub-cultured for an additional 2-3 days in fresh medium. Subcultivation was performed every 72 hr. The organism could be maintained for more than three months (Saksirisampant *et al*, 2010).

Preparation of Herbs Extracts: 1- Fresh bulbs of garlic and onion were carefully peeled and frozen. Before preparation 100 ml of distilled water were added per 50gm of garlic or onion, and crushed in a mixer machine. The resultant slurry was squeezed and filtered through sterile fine cloth and the filtrate was frozen (-20°C) until used (Suru, 2008). Concentration of Garlic and Onion was prepared and added to media to give a final concentration of 0.1 mg/ml (Yakoob *et al*, 2011). 2- Ginger and Turmeric: These two spices were measured (50gm of each), and soaked in distilled water for about 48 h at room temperature to have a stock solution of 100 mg/ml. Solution was filtered through Grade 1 filter paper (Whatman, UK). Infusions were neutralized to pH 7.0. Extracts were stored in the dark at -20°C until use. Concentration of Ginger and Turmeric was prepared and added to media to give a final concentration of 0.1 mg/ml (Yakoob *et al*, 2011).

Nitazoxanide was used as a positive control for its known anti-*B. hominis* effect, work solution of 0.1 mg/ml was prepared.

Growth inhibition assays*:* In vitro susceptibility assays were performed (Zaman and Zaki, 1996). In each of the12 media tube, 50µl of cultures containing $1X10^6$ *B. hominis*. These tubes were incubated for, 24 hr. and 48 hr. at 37°C with the different herbs. Nitazoxanide was used as a positive control. Phosphate buffer saline (PBS) was used a negative control. The effects were assessed after allowing the *B. hominis* to grow for 24 hs and for 48 hs. By the end of the culture periods, the supernatant from each tube was carefully removed without disturbing the pellet at the bottom. The sediment was then gently agitated to obtain a uniform distribution of the *B. hominis*, the percentage of increase or decrease in growth between the control and the test tubes was calculated. The experiment was done twice.

Results

By light microscopy, *Blastocystis* vacuolar form was the commonest in the stool samples. Culture-fecal samples of *B. hominis* were characteristically identified within 24 hr. (Fig.1).

Nitazoxanide (NTZ) treatment significantly ($P < 0.001$) lowered the parasite number after 48 hr. (337.5±17.67) /ml (Figs. 2&3). The reduction rate after 48 hr. compared to PBS was 93.33%. Ginger treatment significantly ($P < 0.002$) lowered the parasite number after 48 hr. (335±7.07) /ml, and also garlic treatment significantly ($P < 0.002$) lowered the parasite number after 48 hr. (382.5±10.60) /ml. The reduction rate after 48 hr. in treated samples compared to PBS was 92.98% & 92.44% respectively. But, onion, and turmeric treatments insignificantly lowered the number parasite after 48 hr. ($P < 0.15$ & < 0.22 respectively).

Microscopically, the death features included distortion of parasite-shape, nuclear condensation and arrest of binary fission, which were more observable after nitazoxanide, ginger and garlic treatment.

Discussion

Blastocystis hominis, is one of the most common intestinal protozoan found in humans. It shows multiple morphologies and reproductive processes (Govind *et al*, 2002). *B. hominis* has been associated with diseases in the immunocompetent and immunocompromised individuals (Dunn and Boreham, 1991; Jonkers *et al*, 1999). Metronidazole is the main therapy for *Blastocystis* infections. On the other hand, varying isolates resistance to metronidazole, suggesting an efficient drug, or drug combinations, at cor-

rect doses must be established to eliminate *B. hominis*. In addition to susceptibility of blastocystosis to the standard antimicrobials was not markedly clear (Moghaddam *et al,* 2005).

In the present study, *in vitro* effect of dietary herbs; garlic, ginger, onion, and turmeric on the *B. hominis* counts at 24 hr. & 48 hr. showed different results. Nitazoxanide was used as a control for its known anti-*B. hominis* effect instead of metronidazole that developed drug-resistant (Haresh *et al,* 1999; Zaman and Zaki, 1996; Khajuria *et al*, 2002; Yakoob *et al*, 2010a,b) and in experimental studies metronidazole induced carcinogenicity (WHO, 2010). The efficacy of NTZ on treating *B. hominis* was proved (Rossignol *et al,* 2005). However, Dinleyici *et al*. *(2011)* demonstrated clinical efficacy of both NTZ and *S. boulardii* in symptomatic *B. hominis* infected children. Similarly to Moghaddam *et al*. (2005) in severe *B. hominis* infections, it appeared that metronidazole and TMP/SMX were effective in some individuals but not in all (Moghaddam *et al*, 2005). In contrast, Nagel *et al.* (2012) reported failure of NTZ or trimethoprim/ sulfamethoxazole treatment of symptomatic cases. As none of 11 symptomatic patients were completely treated after 14-day course of metronidazole or trimethoprim/sulfamethoxazole (Nagel *et al,* 2012)

The NTZ was used as a control for its well-known anti-*B. hominis* effect (Rossignol *et al*, 2005) instead of metronidazole. This demonstrated that the *B. hominis* clinical isolates was sensitivity to ginger was proportional to nitazoxanide in suppressing the growth of *B. hominis*. The reduction rates after 48 hr compared to PBS was 93.33% and 92.98% respectively. This was contradictory with Yakoob *et al*. (2011) who found that *B. hominis* isolates were not sensitive to ginger compared to NTZ. These differences can be explained by the differences in stains isolates. Also, an aqueous extract of garlic showed a potent lethal effect on these isolates of *B. hominis*. This sensitivity was also proportional to NTZ in suppressing the growth of *B. hominis*. The reduction rate after 48 hr. compared to PBS was 92.44%, which agreed with Yakoob *et al*. (2011). There was no significant difference between the inhibitory effect of ginger, garlic and NTZ on *B. hominis* living cell count. Conversely, *B. hominis* isolates were not sensitive to onion, and turmeric compared to ginger, garlic and nitazoxanide they were unable to suppress *B. hominis* growth. These tested herbs (onion, and turmeric) had no or very little effect on *B. hominis* isolates. Nevertheless, the active ingredients might demonstrate their activity against *B. hominis* when obtained by another method (Khajuria *et al*, 2002).

Generally, many clinicians usually have low awareness about *B. hominis* is a cause of human disease. The number of infections appears to be high in most populations; however, the frequency is grossly underestimated (Barahona *et al*, 2003). Asymptomatic shedding of *B. hominis* provides an appropriate environment for its transmission to other subjects (El-Shazly *et al*, 2005). *B. hominis* is now considered as potential parasitic pathogen (Al-Fellani *et al*, 2007). Also, *B. hominis* infection contributes to the development of iron deficiency anemia in the Egyptian pregnant women (El Deeb *et al*, 2012).

Conclusion

Blastocystis hominis should be considered pathogenic intestinal parasite particularly when present alone in large numbers in symptomatic patients as well as in immunosuppressed children. In such cases treatment is a must. The natural herbs as an alternative for blastocystosis treatment not only reduce drug resistance but also reduce its side effects and the cost of the treatment especially in developing countries. *In vivo* studies are ongoing for evaluation of therapeutic potential for ginger and garlic.

The therapeutic effect of ginger and garlic, afford another treatment options for metronidazole treatment failures.

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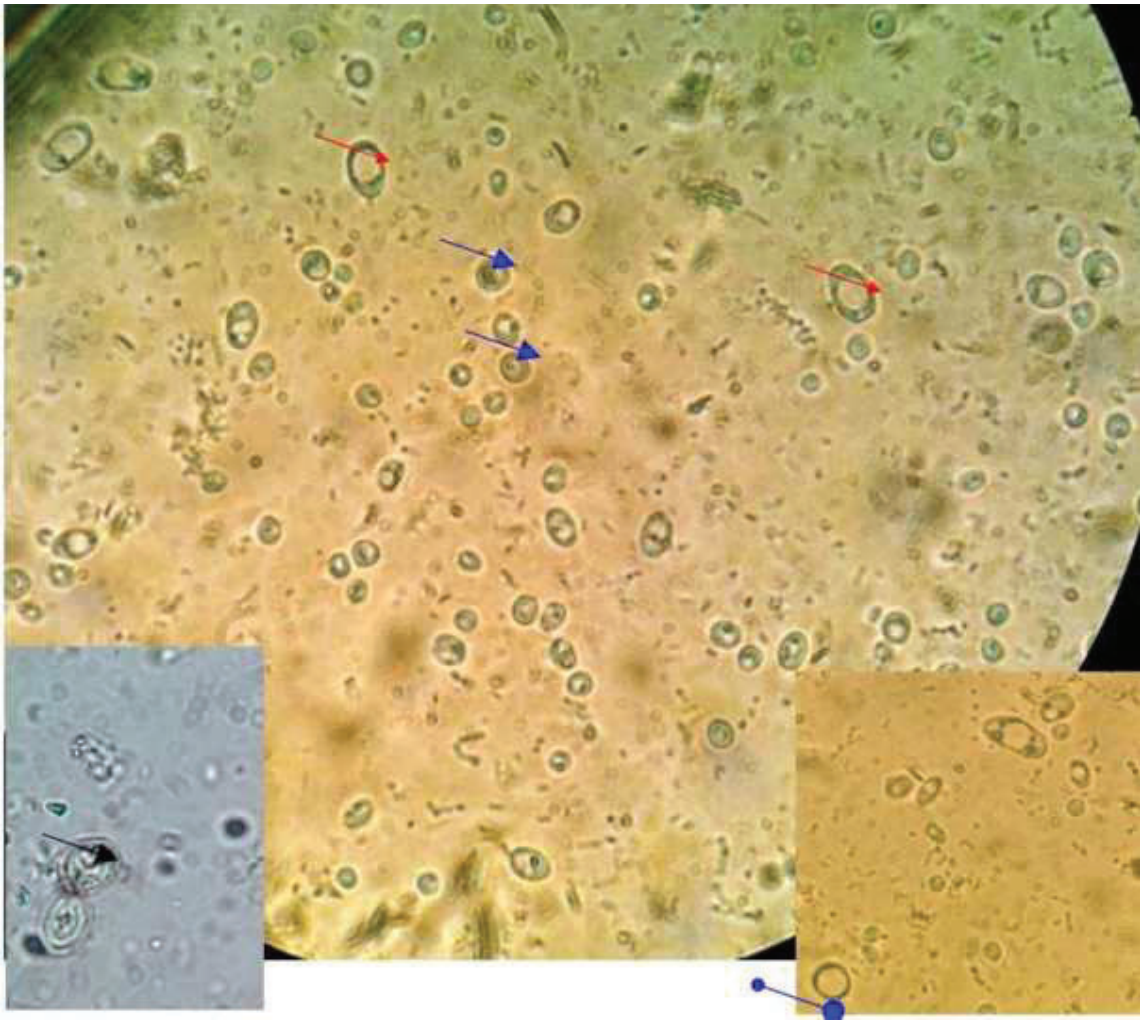

Fig. 1: Vacuolar forms of *B. hominis* commonest ones seen.

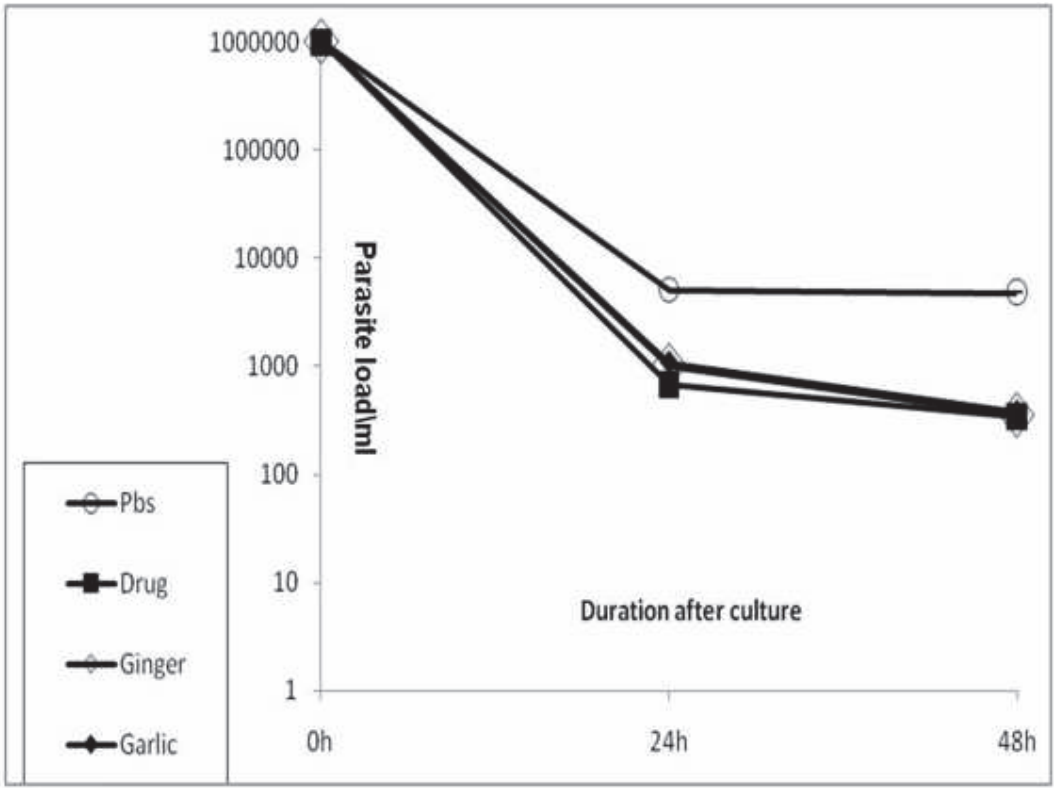
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Fig. 2

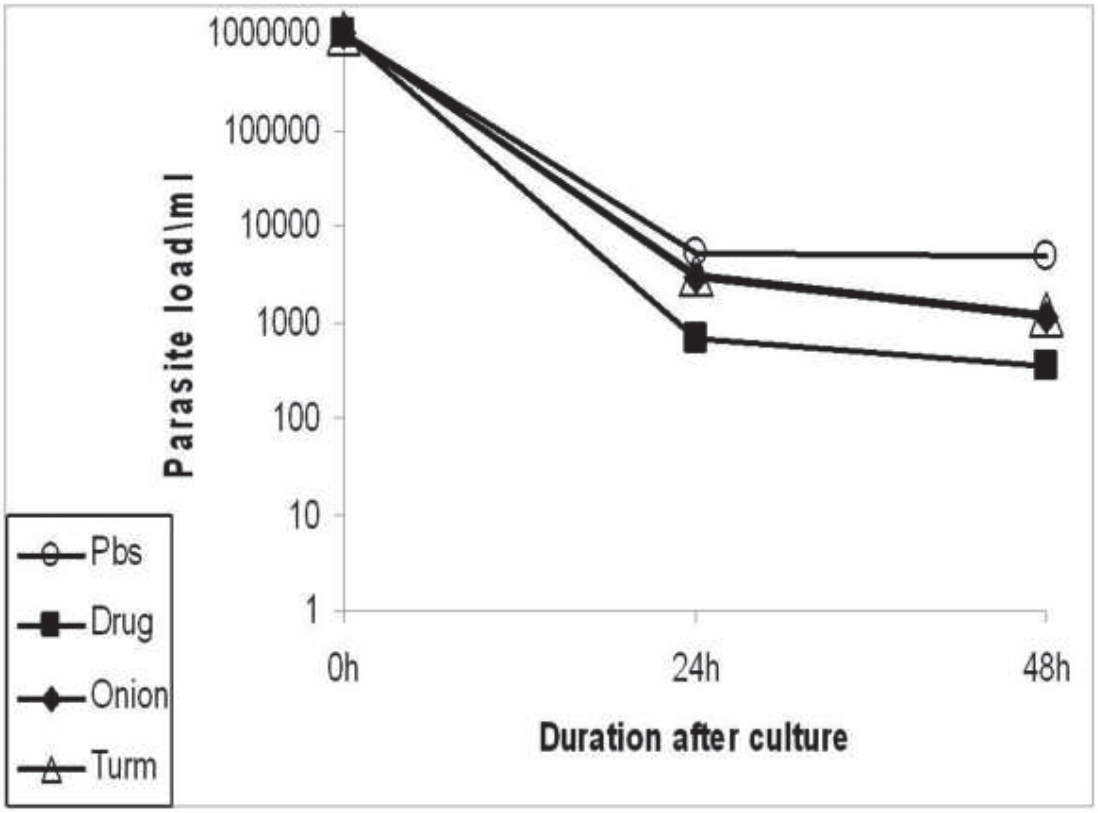
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Fig 3