

## SILVER NANOPARTICLES AS A PROMISING TREATMENT FOR *BLASTOCYSTIS HOMINIS* IN EXPERIMENTALLY INFECTED MICE

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### Abstract

*Blastocystis* is an opportunistic parasite causes gastro-intestinal symptoms including irritable bowel syndrome (IBS). This study evaluated the effect of probiotics and silver nanoparticles on *Blastocystis hominis* in mice and compared between both probiotics (*Lactobacillus*) and silver nanoparticles as compared to metronidazole. A cross-sectional study was done in which stool samples were collected from patients suffering from IBS. The stool samples were examined microscopically to detect *B. hominis* and any co-parasitism. All positive stool samples were cultured on Modified Jones' media. BALB/C mice were infected by isolated *Blastocystis*. The effect of probiotics and silver nanoparticles on blastocystosis was tested in experimentally infected mice. Cysts' mean count in stool of infected mice markedly decreased; with a decrease in serum levels of nitric oxide and glutathione antioxidants compared to drug control one. There was intestinal villi improvement of infected groups treated with probiotics and silver nanoparticles, with mild inflammation and decrease of *Blastocystis* at the intestinal surface as compared to the infected group metronidazole treated.

**Keywords:** *Blastocystis hominis*, Irritable bowel syndrome, Nanoparticles, Probiotics.

### Introduction

*Blastocystis spp.* is a common intestinal parasite in humans. Blastocystosis prevalence was high in developing countries (53.8%) than in developed ones (3.3%). Pathogenicity of *B. hominis* is unconvincing; due to contradictory reports and that the organism was found in both asymptomatic and symptomatic individuals (Khademvatan *et al*, 2017). It was considered as a commensal fecal organism (Mohamed *et al*, 2017).

*Blastocystis spp.* is a unicellular, obligate, anaerobic protozoan found in the gastrointestinal tract of humans; many other vertebrates and invertebrates act as a reservoir host (Yan *et al*, 2007). In Egypt, the prevalence has reached up to 50% and it has four main forms, which are vacuolar, granular, and amoeboid, and can be found in culture while cysts are found in the stool. Transmission of the parasite occurs through the feco-oral route mainly in people with poor envi-

ronmental sanitation with frequent exposure to animals and contaminated food also it may pass unnoticed or might be accompanied by many symptoms like nausea, vomiting, abdominal cramps, fatigue, and diarrhea especially in children and immunocompromised patients (Sari *et al*, 2018).

Diagnosis of *Blastocystis spp.* by examination of stool samples was a routine method in many laboratories, by ordinary methods, e.g. saline smear method, formalin-ether method, and trichrome stain method (Nithy-amathi *et al*, 2016). *Blastocystis spp.* was considered to stand for a direct or indirect cause of gastrointestinal symptoms including inflammatory bowel syndrome (IBS) (Scanlan, 2012).

IBS is a functional gastrointestinal disorder, with prevalence rates (35%-43%) in developing countries and (5%-24%) in developed countries (Lepczyńska *et al*, 2016). The proposed mechanism in IBS included

the low-grade inflammation caused by the constant immune activation induced by *Blastocystis* (Coyle *et al*, 2012). The IgG antibody levels to *B. hominis* were significantly higher in patients with IBS than in asymptomatic controls (Hussain *et al*, 1997). Metronidazole is the most commonly used drug for the treatment of *Blastocystis* infection. The dosage in adults ranges from 250-800mg, 3 times a day for 10 days or can be given in combination with other drugs such as paramomycin (Pasqui *et al*, 2004), cotrimoxazole (Andiran *et al*, 2006) or trimethoprim-sulfamethoxazole (Stensvold *et al*, 2008). In pediatric treatment, metronidazole is prescribed based on the patient's body weight at the dosage of 20–30 mg/kg/day (Kurt *et al*, 2016). Treatment failure of metronidazole has been reported as early as in 1976 (Zierdt and Tan, 1976). Regardless of being the first line of treatment for blastocystosis, the metronidazole efficacy ranged from 0% to 100% with varied effective dosages (Coyle *et al*, 2012). Many studied the efficacy of different drugs and respective regimens on patients (Roberts *et al*, 2014). The probiotics are living microbes feed supplement that successfully affects the host by improving its intestinal balance. Nowadays, probiotics are considered quite every day in health-promotion “functional foods” for humans. Additionally, they are therapeutic and prophylactic growth supplements in animal production and human health also other common probiotics include yeasts and spore-forming *Bacillus spp*. They have adhesion abilities; produce bio-activated molecules, which provide immune stimulation (Duc *et al*, 2004). Studies also hold an added interest in probiotics, as they can be kept in spore form and be stored on the shelf (Barbosa *et al*, 2005).

Nanoparticles (NPs) have intrinsic properties related to size, distribution, and morphology that can improve their optical, electronic, catalytic, and magnetic features (Silva *et al*, 2013; Bhosale *et al*, 2014). A promising mechanism of parasite growth inhibition

was by stimulating the function of the immune system; another mechanism is by producing cellular oxidative stress in microbes; also it induces morphological and structural changes in the parasites and another way is by binding to the cell membrane of the infected cell (Abaza *et al*, 2016). Thus, they have a very important role in diagnosis and treatment of diseases because of their larger surface areas in relation to the bulk material (Qasim *et al*, 2014; Akbari *et al*, 2017).

Silver nanoparticles, chitosan, and oxidized metals have growth inhibitory or cytotoxic effects on parasites as *Giardia*, *Leishmania*, *Plasmodium*, *Toxoplasma* and insect larvae (Elmi *et al*, 2013). The silver nanoparticles silver ions, and silver nanoparticles contained complexes antimicrobial behavior with a high ability to inactivate viruses and bacteria (Jebali and Kazemi, 2013; Catalano *et al*, 2016). Silver nanoparticles are excellent nano-carriers for anti-parasitic drug, but their effect on *B. hominis* has not been widely explored (Sun *et al*, 2019).

The present study aimed to assess the effect of probiotics and silver nanoparticles on *Blastocystis hominis* infected mice and compared the effect of both probiotics and silver nano-particles with the traditional metronidazole treatment.

### Patients and Methods

This is a cross-sectional study, in which stool samples were collected from patients suffering from IBS attending the Tropical medicine department, Beni-Suef University Hospital. Related demographic and clinical data were obtained. All stool samples were microscopically examined using iodine wet mount before and after formalin ethyl acetate concentration and permanent trichrome staining to detect *B. hominis* and any co-parasitism. All positive stool samples were cultured for *Blastocystis* using Modified Jones' media. Isolated *B. hominis* was used for animal experiments; mice were infected intraoesophageal with previously prepared 10,000 *Blastocystis* cyst.

Laboratory bred Swiss Albino mice, CD-I

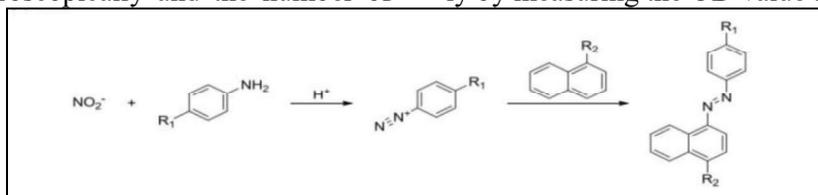
strain 3-5 weeks old, weighing 20–25g, were used and divided into 2 main groups: the control group (GI) and tested group (GII). Control group (GI) consisted of three subgroups of 8 mice each; negative control (Non-infected, non-treated) subgroup (GIA), positive control (infected, non-treated) subgroup (GIB,) and drug control (infected and treated with metronidazole as 10mg/kg/day given 3 weeks after proving *Blastocystis* infection for seven consecutive days) subgroup (GIC) (Becker *et al*, 2011). Tested group (GII) consisted of two subgroups, each containing 8 mice; *Blastocystis spp.* infected, treated with single daily dose of 10 billion probiotic *lactobacilli* for one week (GIIA) (Becker *et al*, 2011; El-Sayed *et al*, 2017) and *B. hominis* infected, treated with a single daily dose of 50 mg/kg silver nanoparticles for one week (GIIIB). All infected mice subgroups were treated 3 weeks post-infection to ensure the establishment of infection.

The anti-*Blastocystis* effect of silver nanoparticles, probiotics and metronidazole, treated mice were sacrificed after cessation of treatment was assessed by: 1 Parasitological examination of stool: Fresh fecal pellets from the large intestine of each mouse were collected separately and examined daily then the mean numbers of the cysts were calculated at the 21<sup>st</sup> day PI (to assess infection establishment) and 28<sup>th</sup> day PI (one week post-treatment) before mice scarification, according to each group. Each fecal sample was suspended in 7 % formalin and homogenized; 50 microns of the solution was examined microscopically and the number of

*Blastocystis* cysts was counted. 2- Biochemical assessment: Antioxidants level, both sera glutathione peroxidase nitric oxide (NO), were calorimetrically determined according to the kits manual from Elabsceince, Inc. 3- Histopathological studies: Jejunum, proximal 2cm of ileum, and large intestine were fixed in 10% neutral buffered formalin followed by immersion in xylene then impregnated in paraffin. 4-mm thick section was taken from each block and stained with hematoxylin and eosin for evaluation (Bancroft and Stevens, 1990).

Determination of serum glutathione peroxidase activity: Glutathione Peroxidase (GSH-Px) promoted reaction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and reduced glutathione to give H<sub>2</sub>O & oxidized glutathione (GSSG). Glutathione reductase (GR) catalyzes NADPH to reduce GSSG and produce GSH, & NADPH was oxidized to NADP<sup>+</sup> at same time. NADPH has a specific absorbance peak at 340nm, while NADP<sup>+</sup> didn't. GSH-Px activity can be calculated according to the changing rate of absorbance value. Calculation: Oxidation amount of 1nmol of NADPH catalyzed by 1ml of sample per minute was defined as 1 unit. GSH-Px activity (nmol/min/ml) =  $\Delta A \times 536$

Determination of serum nitric oxide: NO was easily oxidized to NO<sub>2</sub> - in vivo or in aqueous solution, and a reddish azo compound formed with color developing agent, and the concentration of azo compound was linearly related to the NO concentration. The concentration of NO was calculated indirectly by measuring the OD value at 550 nm.



Calculations: Nitrite in sample

$$(\mu\text{mol/L}) = \frac{A \text{ sample}}{A \text{ standard}} \times 50$$

Statistical analysis: Data were coded and entered using the statistical package for the Social Sciences (SPSS), version 26 (IBM Corp., Armonk, NY, USA). Data were done as mean and SD for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done by (ANOVA) analysis with multiple comparisons post hoc (Chan, 2003a). For comparing categorical data, Chi square test was used. Exact test was used instead when the expected frequency was less than 5 (Chan, 2003b). *P*-values less than 0.05 were significant.

### Results

Mean number of cysts in positive control non-treated subgroup (GIB) was 2810 cysts with SD=33.91, while the mean count in the infected group treated with metronidazole (GIC) was 2214 cyst with SD=32.02. The mean number of cysts detected in infected subgroup treated by probiotics (*Lactobacillus*) (GIIA) was 1999 cyst with SD=35.78 and their mean number in infected subgroup, treated by silver nanoparticles (GIIB) was 700 cysts with SD=27.39.

There was a decrease in mean parasitic count in all infected treated subgroups compared to positive infected non-treated control subgroup. This was marked and statistically significant in the infected group treated by silver nanoparticles (GIIB) and in the infected group treated by probiotics (GIIA) with ( $p < 0.001$ ) as compared to infected group treated with metronidazole (GIC).

The mean level of serum NO was 58.72 with SD= 7.74 in infected positive non-treated control (GIB) and 41.26 with SD= 5.3 in drug control subgroup treated with metronidazole (GIC). The mean NO was 37.86 with SD= 2.17 in infected group treated with *Lactobacillus* (GIIA); and was 30.70 with SD=1.03 in infected subgroup treated with silver nanoparticles (GIIB). A significant decrease of mean level of serum NO of infected treated mice with silver nanoparticles and probiotics compared to infected mice metronidazole treated ( $P < 0.001$ ).

Mean titer of serum glutathione in non-treat-

ed mice (GIB) was 209.6 with SD= 31.21 & reduced to 148.82 with SD=19.40 in serum of metronidazole control treated (GIC). Cysts count decreased by 28.86%, & 75.09% in GI-IA (treated by probiotics) and GIIB (treated by silver nanoparticles) respectively; compared to 21.21% of GIC (treated by metronidazole). Activity of serum NO was significantly decreased by 35.54%, & 47.73% in GIIA & GIIB, respectively compared to 29.75% in GIC.

Serum glutathione level was reduced by 36.78% in GIIA & 39.44% in GIIB compared to 29% in GIC. Decrease serum glutathione titer was more in infected mice treated with *Lactobacillus* (GIIA) & silver nanoparticles (GIIB) 132.5 & 126.93, respectively compared to infected mice treated with metronidazole with significance ( $p < 0.001$ ).

Microscopic examination of small and large intestine sections from negative control mice (GIA) showed normal mucosa, while that of positive control mice (GIB) showed variable degree of villous atrophy with moderate congestion of submucosal capillaries associated with moderate edema, heavy infections of intestinal lumen with vacuolated form of *B. hominis* and marked inflammatory cells infiltrates with predominant eosinophils. Large intestinal mucosa of GIB showed moderate capillaries congestion, edema with inflammatory cell infiltrates; more large intestinal lumen showed heavy *B. hominis* infections surrounded by inflammatory cells.

Section from small intestine of GIC (metronidazole) showed villous atrophy with short broad villi distended with moderate inflammatory cell infiltrates without *B. hominis*. By microscopy large intestinal section showed mild inflammatory cell infiltrated with mild submucosal edema and congested capillaries without *B. hominis*. Small intestine of infected treated with probiotics showed normal intestinal villi with edema and mild inflammatory cell infiltrates associated with mild congestion with absence of *B. hominis* while large intestine showed moderate edema and mild congestion with the absence of *B. hominis*. Small intestine of infected, treated with silver nanoparticles showed normal intestinal villi without

inflammation, congestion, edema or *B. hominis*, but large intestine showed normal intestinal mucosa with the absence of inflammation, congestion, edema and *B. hominis*. Details were given in tables (1, 2, 3, 4, 5, & 6) and figures (1, 2, 3, 4, 5, 6, 7, & 8).

Table 1: Comparison between mean number of cysts of *Blastocystis hominis* in stool and standard deviation in groups:

<i>Blastocystis hominis</i> in stool	(GIB)		(GIC)		(GIIA)		(GIIB)		P Value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
	2810.00	33.91	2214.00	23.02	1999.00	35.78	700.00	27.39	<0.001*

\*Significant ( $p < 0.05$ )

Table 2: Correlation of *Blastocystis hominis* cysts count in stool between groups (Post hoc pair wise comparisons)

	(GIB)	(GIC)	(GIIA)	(GIIB)
(GIB)		< 0.001	< 0.001	< 0.001
(GIC)	< 0.001		< 0.001	< 0.001
(GIIA)	< 0.001	< 0.001		< 0.001
(GIIB)	< 0.001	< 0.001	< 0.001	

\*Significant ( $p < 0.05$ )

Table 3: Mean level and standard deviation of nitric oxide and glutathione peroxidase in sera of different groups.

Colormetry	(GIA)		(GIB)		(GIC)		(GIIA)		(GIIB)		P Value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Nitric Oxide	25.78	0.65	58.73	7.74	41.26	5.3	37.86	2.17	30.70	1.03	< 0.001
Glutathione peroxidase	112.31	11.91	209.60	31.21	148.82	19.4	132.5	9.80	126.93	14.39	< 0.001

\*Significant ( $p < 0.05$ )

Table 4: Correlation of antioxidants serum levels between all groups

Colormetry		(GIA)	(GIB)	(GIC)	(GIIA)	(GIIB)
Nitric Oxide (NO)( $\mu\text{mol/l}$ )	(GIA)		< 0.001	0.011	0.069	1.000
	(GIB)	< 0.001		< 0.001	< 0.001	< 0.001
	(GIC)	0.011	< 0.001		1.000	0.024
	(GIIA)	0.069	< 0.001	1.000		0.276
	(GIIB)	1.000	< 0.001	0.024	0.0276	
Glutathione peroxidase (nmol/min/m)	(GIA)		< 0.001	0.428	1.000	1.000
	(GIB)	< 0.001		0.002	< 0.001	< 0.001
	(GIC)	0.428	0.002		1.000	1.000
	(GIIA)	1.000	< 0.001	1.000		1.000
	(GIIB)	1.000	< 0.001	1.000	1.000	

\*Significant ( $p < 0.05$ )

Table 5: *B. hominis* reduction count in stool of infected treated groups and mean level of serum NO and glutathione peroxidase in all infected groups compared to control positive group

Variables	(GIC)	(GIIA)	(GIIB)
Number of <i>Blastocystis hominis</i> cysts in stool	21.21%	28.86%	75.09%
Colormetry Nitric Oxide (NO)( $\mu\text{mol/l}$ )	29.75%	35.54%	47.73%
Colormetry Glutathione peroxidase (nmol/min/ml)	29.00%	36.78%	39.44%

\*Significant ( $p < 0.05$ )

Table 6: Degree of congestion, inflammation, oedema and count of *Blastocystis* cysts in intestine of test groups and control positive subgroup and Silver nanoparticles infected treated and probiotics compared to infected Metronidazole treated

Variables		(GIB)		(GIC)		(GIIA)		(GIIB)		P value
		Count	%	Count	%	Count	%	Count	%	
Congestion	Absent	0	0.0	0	0.0	1	20	5	100	< 0.001
	Mild	0	0.0	3	60	2	40	0	0.0	
	Moderate	4	80	2	40	2	40	0	0.0	
	Sever	1	20	0	0.0	0	0.0	0	0.0	
Lymphocytic Infiltration	Absent	0	0.0	0	0.0	2	20	4	80.	-----
	Mild	0	0.0	2	40	1	20	1	20	
	Moderate	4	80	2	40	2	40	0	0.0	
	Sever	1	20	1	20	0	0.0	0	0.0	
Inflammatory Edema	Absent	0	0.0	1	20	2	20	5	100	< 0.001
	Mild	1	20	2	40	1	20	0	0.0	
	Moderate	4	80	2	40	2	40	0	0.0	
	Sever	0	0%	0	0.0	0	0.0	0	0.0	
Number of <i>Blastocystis Hominis</i>	Absent	0	0.0	3	60	4	80	5	100	< 0.001
	Little	0	0.0	2	40	1	20	0	0.0	
	Moderate	1	20	0	0.0	0	0.0	0	0.0	
	Heavy	4	80	0	0.0	0	0.0	0	0.0	

\*Significant ( $p < 0.05$ )

## Discussion

Blastocystosis is an unusual enteric protozoan parasite of humans and many animals of worldwide distribution (Tan, 2008). It has been reported in Jordan (Nimri and Batchoun, 1994), Kuwait (Zaki *et al.*, 1991), Qatar (Abu-Madi *et al.*, 2015), UAE (Abu-Odeh *et al.*, 2019), Saudi Arabia (Mohamed *et al.*, 2017), as well as many Egyptian authors (Khalifa, 1999; Mahmoud and Saleh, 2003; El Shazly *et al.*, 2006; Fouad *et al.*, 2011; El-ghareeb *et al.*, 2015; El Saftawy *et al.*, 2019). Metronidazole was the first golden *B. hominis* treatment (Sekar and Shanthi, 2013), but, with side effects, treatment failure, and parasitic resistance (Rajamanikam *et al.*, 2019).

In the present study, the probiotics and silver nanoparticles compared to metronidazole on in experimentally infected mice showed a decrease in mean parasitic count in all infected & treated groups compared to infected, non-treated, positive control group. This was marked and significant in the infected subgroups treated by probiotics or silver nanoparticles in compared to the infected metronidazole treated ones ( $p < 0.001$ ). This agreed with Boorom *et al.* (2008) who reported of metronidazole failure treatment of symptomatic patients and recommended the natural plant extracts alone or combined with metronidazole.

Consequently, search for bioactive plants which can be used as non-conventional anti-parasitic treatment received attention in recent times due to the increasing worldwide development of resistance to chemical drugs in parasitic populations (Abouel-Nour *et al.*, 2016), particularly those with phenolic contents (Abdel-Hady *et al.*, 2014). Sawangjaroen and Sawangjaroen (2005) in Thailand found that 3/7 plants extracts, the dichloromethane and methanol extracts from the *Brucea javanica* seed and a methanol extract from *Quercus infectoria* nut gall gave the highest activity at a concentration of 2000 microg/mL, they killed 82, 75 & 67% of the *B. hominis* samples and inhibited 94, 100 & 76% of them, respectively, and the metha-

nol extracts from the *Brucea javanica* seed and a methanol extract from *Quercus infectoria* nut gall showed highest activity at a concentration of 2000microg/mL, they killed 82, 75 & 67% of the *B. hominis* samples and inhibited 94, 100 & 76%, respectively. Bremer *et al.* (2015) in Denmark found that six ethanolic out of 24-plant extracts showed significant anti-*Blastocystis* activity. *Mallothus oppositifolius* gave good activity as the reference anti-protozoal drug MTZ. El-Bali *et al.* (2020) in Egypt used *Salvadora persica* to treat three virulent subtypes of *Blastocystis* sp. and recommended their evaluation in vivo.

The probiotics (viable microbial organisms) in treatment of specific infections was evolved into a fundamental option, with immune-modulating properties and enhance mucosal barrier, they can have a significant influence on the treatment and prevention of disease (Termmathurapoj *et al.*, 2004).

In the current study, the *Lactobacillus* was combatable to metronidazole and *Saccharomyces boulardii* probiotics have potential beneficial effects on *B. hominis* symptoms and presence. This agreed with Dinleyici *et al.* (2011). Also, when lauric acid was given alone or combined with metronidazole on experimental giardiasis with its anti-giardial activity was better compared to metronidazole (Rayan *et al.*, 2007). Thus, nano-biotechnology in treating parasites was due to the unique properties of nanoparticles as they reached nanomaterials size (10-100nm) as the size of most biological structures, so enhance the penetration of the drug to various body tissues (Norouzi, 2017). It was best used in treatment of malaria (Barabadi *et al.*, 2019).

Others investigated the inhibitory activity of various concentrations of silver nanoparticles and curcumin nanoparticles on *B. hominis* in vitro (Ahmed *et al.*, 2015). The effect of silver nanoparticles and curcumin nanoparticles was significantly higher than metronidazole through different incubation periods, and that the effect of the single

therapy was better than the combined silver nanoparticles and curcumin nanoparticles (El-Sayed *et al.*, 2017). Also, silver nanoparticles have many inhibitory and cytotoxic effects on many parasites such as *Giardia*, *Toxoplasma*, *Leishmania*, *Plasmodium*, and even insect larvae (Elmi *et al.*, 2013). As to disinfection ability of silver nanoparticles on *Cryptosporidium parvum* oocysts in vitro confirmed the in vivo results study, and the magnitude reduction in oocyst shedding in mice fed on silver nanoparticles treated oocysts when compared with the control (Su *et al.*, 2014). Besides, both silver nanoparticles and silver ions significantly decreased *Cryptosporidium* oocysts viability, in a dose-dependent manner, between concentrations of 0.005 & 500 µg/ml, as assessed by an excystation assay and the shell/sporozoite ratio, were statistically significant at 500 & 5000 µg/ml (Cameron *et al.*, 2015).

In the present study; there was a significant decrease in mean level of both serum nitric oxide and glutathione peroxidase in all infected treated mice compared to infected not treated ones (<0.001). A significant decrease in nitric oxide and glutathione peroxidase mean level was reported in the infected mice treated with probiotics *Lactobacillus* and silver nanoparticles compared to the infected mice treated with metronidazole (<0.001).

This agreed with Eida *et al.* (2008) who in in vivo study detected high levels of NO in mice infected with *B. hominis* isolated from symptomatic patients compared to low changes in the control. Also, this agreed with Saran *et al.* (2004) who studied *Trichomonas vaginalis* and found that the mean level of NO was significantly higher in severe symptomatic than moderate and mild symptomatic ones. Steinert *et al.* (2010) found that noninvasive, lumen-dwelling, extracellular pathogens as *Giardia* and *Blastocystis* rarely come in contact with macrophages, but in close immediacy to NO producing enterocytes, and thus survived the

hostile environment, and suppressed intestinal epithelial NO production.

These suggested the diverse pathogenic roles of *Blastocystis* spp. isolates from different clinical presentations, with variation in growth kinetics and metronidazole sensitivity of *Blastocystis* spp. isolated from different clinical asymptomatic and symptomatic patients (Ahmed *et al.*, 2018). There was a higher oxidative protein damage intensified the antioxidant processes associated with blastocystosis in improving host immune-modulating strategies (Tan *et al.*, 2013).

Undoubtedly, oxidative stress was increasingly relevant as a significant clinical and biochemical mechanism of pathogenicity that oxidative stress functions against the pathogens was the first line of defense and immune response, with high metabolic rate of the rapidly growing, multiplying parasites producing high free radicals by-products (Becker *et al.*, 2004). Parasitic infection and IBS contributed to oxidative stress independently and when present together, the oxidative stress burden may be increased (Elnekave *et al.*, 2003).

In the present histopathological examination of jejunum, ileum, and large intestine of *Blastocystis* infected positive controls showed a profound effect on the structure of intestinal mucosa. This effect was in the form of moderate numbers of *Blastocystis* cysts with a moderate degree of inflammation, congestion, and lymphocytic infiltration metronidazole treated mice. But, mild numbers of *Blastocystis* cysts with a mild degree of inflammation, congestion and lymphocytic infiltration were in infected mice treated with probiotics. This effect was nearly absent in infected mice silver nanoparticles treated seen as some epithelial damage and mucosal sloughing. So, *B. hominis* infected mice exhibited severe goblet cell hyperplasia. This agreed with Santos and Rivera (2009) who found that goblet cells were one of the potential host antiparasitic defense mechanisms against blastocystosis.

Yason *et al.* (2019) in Singapore found that particular isolates of *Blastocystis* ST7 caused changes in microbiota populations and potentially lead to an imbalance of the gut microbiota. This study suggested that certain isolates of *Blastocystis* exert their pathogenic effects through disruption of the gut microbiota and provides a counterpoint to the increasing reports indicating the commensal nature of this ubiquitous parasite. Also, a study on experimental giardiasis, which found that treatment with metronidazole, lauric acid, and *Lactobacillus* improved pathology in the form of partial healing of intestinal mucosa, mild shortening, and thickening of the villi, with few *Giardia* trophozoites in the lumen (Amer *et al.*, 2007).

Younis *et al.* (2020) in Egypt assessed the efficacy of silver nanoparticles (Ag Nps) alone and combined with metronidazole (Ag Nps + MTZ) as potential alternative therapeutic agents for *B. hominis*. They found There was a statistically significant difference ( $P < 0.05$ ) in the in vitro growth inhibition of the parasite over the different time intervals when using the tested drugs against the control drug.

The present histopathological examination of infected controls showed a profound effect on intestinal mucosa, in the form of villous atrophy, and infiltration of lamina propria with inflammatory cells. Also, *B. hominis* vegetative forms were detected in intestinal lumen between villi. Pathology was in some epithelial damage and mucosal sloughing that agreed with Phillips and Zierdt (1976). Also, *Blastocystis* infected mice showed severe goblet cell hyperplasia, one of the host anti-*Blastocystis* defense mechanisms (Santos and Rivera, 2009).

### Conclusion

Detection of *B. hominis* in patient's stools complained of IBS played an important role in pathogenesis.

Silver nanoparticles proved better than probiotics and metronidazole in treatment of *B. hominis* and both of them were better than metronidazole alone.

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#### Explanation of figures

Fig. 1: Number of *Blastocystis hominis* in stool of all groups.

Fig. 2: Mean level of serum Nitric Oxide (NO) nmol/min/ml in all groups.

Fig. 3: Mean level of serum glutathione peroxidase nmol/min/ml in all groups.

Fig. 4: Reduction in *B. hominis* count in stool of infected treated groups and percentage of reduction in mean level of serum NO and glutathione peroxidase in all infected groups in comparison to control positive group.

Fig. 5: Light microscopy of small intestinal section from (A) control group showed normal mucosa, with overview insert box (H&E stain x 200). (B) *B. hominis* infected mice showed variable degree of villous atrophy (red arrows) with moderate congestion of submucosal capillaries (black arrows) associated with moderate edema (H&E stain x100). (C) Intestinal lumen showed heavy infestations of vacuolated form of *B. hominis* (red arrow) (H&E stain x400). (D) Marked inflammatory cells infiltrates with predominant eosinophils (red arrows) (H&E stain x200).

Fig. 6: Light microscopy of small intestinal section (A) metronidazole treated group showed villous atrophy with short broad villi distended with moderate inflammatory cell infiltrates (red arrows) with absence of *Blastocystis hominis* (H&E stain x100). (B) Probiotic lactobacilli treated group showed normal intestinal villi with edema and mild inflammatory cell infiltrates (red arrows) associated with mild congestion (black arrows) without *B. hominis* (H&E stain x200). (C & D) Silver nanoparticles treated group showed normal intestinal villi with absence of inflammation, congestion, edema and *B. hominis* (H&E stain, x100& x200).

Fig. 7: Light microscopy of large intestinal section from (A) control group showed normal mucosa, with overview insert box (H&E stain x100). (B) *B. hominis* infected group, intestinal mucosal showed moderated capillaries congestion (black arrows), edema (red star) with inflammatory cell infiltrates (red arrow) (H&E stain x100). (C) The mucosa of infected group was disrupted by marked inflammatory cell infiltrates (red arrow) associated with capillaries congestion (black arrow) (H&E stain x200), and the intestinal lumen showed heavy infestations of vacuolated form of *B. hominis* surrounded by inflammatory cells (red arrows) (D) (H&E stain x400).

Fig. 8: Light microscopy of large intestinal section from metronidazole treated group showed mild inflammatory cell infiltrates (red arrows) with mild submucosal edema & congested capillaries (black arrows) without *B. hominis*. (A, B) Probiotic lactobacilli treated group, mucosa showed moderate edema (red stars) and mild congestion (black arrows) with absence of *B. hominis*. (C) Silver nanoparticles treated group showed normal intestinal mucosa with absence of inflammation, congestion, edema and *B. hominis* (A, B, C H&E stain x100)

