

## THERAPUTIC EFFICACY OF PROBIOTICS AGAINST EXPERIMENTAL GIARDIASIS: HISTOPATHOLOGICAL, HISTOCHEMICAL AND ULTRASTRUCTURAL CHANGES

By

MAGDA MOSTAFA SANAD<sup>1</sup>, DALIA ABD EL KHALIK ABO EL MAATY<sup>1</sup>,  
NAGLAA FATHY ABD EL-AAL<sup>1</sup>, ASMAA M. FAROUK AL-GHANDOUR<sup>1</sup>, AZZA  
ISMAEL FARAG<sup>2</sup>, ASMAA MOHAMMED YOUSEF<sup>1</sup> and TAHANI ISMAIL FARAG<sup>1\*</sup>  
Departments of Medical Parasitology<sup>1</sup> and Human Anatomy and Embryology<sup>2</sup>,  
Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt  
(\*Correspondence: tahaniabdelkhalik@yahoo.com)

### Abstract

*Giardia lamblia*, protozoan intestinal flagellate, is the causative agent of human giardiasis. Currently used therapeutic agents have significant side effects or contraindicated in some clinical conditions and show failure due to drug resistance. This study evaluated the probiotic *in vivo*-therapeutic efficacy; potent *Acidophilus* probiotics versus metronidazole against giardiasis in experimentally infected mice, by histopathological changes of intestinal mucosa, and their impact on the ultrastructure of the pathogenic trophozoites.

Forty seven Swiss albino mice were divided into groups: GI: 2 mice non-infected, non-treated mice. GII: 15 mice only infected with *G. lamblia* cysts, (non-treated). GIII: 15 mice infected and treated orally with potent *Acidophilus* probiotics. G IV: 15 mice infected with *G. lamblia* cysts and treated orally with metronidazole. All mice were subjected to parasitological, histo-pathological, goblet cells mucous secreting activity and trophozoite ultrastructural studies.

There was high significant difference between the infected non-treated controls and potent *Acidophilus*-treated group concerning; patent period, infection intensity, reduction percentage of cyst shedding and cure rate, but without significant difference between potent *Acidophilus*-of treated group and metronidazole-treated group.

**Keywords:** *Giardia lamblia*, Swiss albino mice, potent *Acidophilus*, Ultrastructure, Intestinal Mucus & Histopathology.

### Introduction

*Giardia lamblia* is the protozoan parasite responsible for giardiasis, a disease that is characterized by either acute or chronic intestinal malabsorption, diarrhea, dehydration, weight loss, and abdominal pain in humans particularly in children. It is one of the most common luminal parasites, with about 200-300 million annual worldwide human cases (Travers *et al*, 2016). Children suffering from giardiasis have high morbidity due to nutritional deficiencies with a very bad impact on public health mainly in developing countries (Veenemans *et al*, 2011).

Metronidazole (5-nitroimidazole) remains the drug of choice for giardiasis (Leitsch *et al*, 2011). But, many side-effects as vertigo, anorexia, nausea, vomiting, and dizziness were reported (Gardner and Hill, 2001); the existence of drug resistance and contra-indi-

cation in certain clinical situations necessitate research on the alternative therapeutic strategies (Ansell *et al*, 2015).

Probiotics are live microorganisms that if consumed in tolerable amount stimulate and regulate immune responses and enhance the mucosal barrier (Claude *et al*, 2011). This was done by competition for binding sites and available food sources of the parasite in the intestinal lumen leading to diminished-pathogen survival (Reid and Hammond, 2005). Genera *Lactobacillus* and *Bifidobacterium* are mainly the most commonly consumed probiotics. Probiotics could mend the host health by various mechanisms including; augmentation of immune function by reinforcing the mucosal barrier function, reducing mucosal transfer of the luminal organisms and metabolites to the host, cumulative mucosal antibody production, reinforc-

ement of epithelial integrity and direct antagonism of pathogenic microorganisms (Conlon and Bird, 2015). In the gut, colonization of lactic acid bacteria can control mucin production by motivating different signaling cascades and secretory chemical agents (Dharmani *et al*, 2008). Mattar *et al*. (2002) also suggested that there might be binding between *Lactobacillus* and specific receptor sites on the enterocyte that may enhance up regulation of mucin 2 (MUC2).

The present study aimed to evaluate the probiotic *in vivo*-therapeutic efficacy; potent *Acidophilus* probiotics versus metronidazole against giardiasis in experimentally infected mice,

### Materials and Methods

**Parasite:** Fresh stool samples were obtained from a heavily infected patient attending the outpatient clinic, Department of Pediatric, Zagazig University Hospital.

**Animal groups:** Weanling laboratory-bred Swiss Albino mice of either sex, aged 3-4 weeks old, weighing 15-20gm each, intestinal parasites free and protected against acquisition of any parasite were infected by 100.000 cysts/mouse (Hill *et al*, 1983) and divided into groups: GI: Two mice, non-infected, non-treated. GII: 15 mice infected, non-treated. GIII: 15 mice infected with *G. lamblia* cysts and orally treated with probiotics (potent *Acidophilus*) for 10 days from the 7<sup>th</sup> day post-infection. GIV: 15 mice infected with *G. lamblia* cysts and orally treated with metronidazole (Flagyl) for 10 days from the 7<sup>th</sup> day post-infection.

**Ethical consideration:** Mice were maintained in accordance with the research protocols of the National Institutes of Health Guide recommendations for care and use of laboratory animals, Faculty of Medicine, Zagazig University.

**Therapeutic agents:** Commercial potent *Acidophilus* capsules (GNC, Saudi Arabia) each contain a mixture of 50 million live probiotic bacteria of five species: *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus sylvarius*, *Lactobacillus brevis*

and *Bifidobacterium bifidum* were given orally as aqueous suspension in a single daily dose of 130.000bacteria/0.2ml/mouse (Paget and Barnes, 1964). Metronidazole (Flagyl) tablets 500mg (EPICO, Egypt) was given orally as aqueous preparation, in a daily dose of 1.37mg/mouse divided in 2 doses. Both therapeutic agents were given for 10 days starting from 7<sup>th</sup> day post-infection according to drug table of Paget and Barnes (1964). All mice were subjected to:

**Parasitological Study:** For the determination of patent period, intensity of infection, percent-age of reduction in cyst shedding at 10, 13 & 17<sup>th</sup> post infection days and cure rate (Blagburn, 1998).

**Transmission Electron Microscopy:** Infected groups were subjected for TEM study in the Electron Microscopy Unit at Al-Azhar University, Cairo. One mouse from each infected treated and non-treated group was sacrificed on 10<sup>th</sup> post-infection day under ether anesthesia. Specimens from duodenum and jejunum were rapidly dissected out, cut into 1 mm blocks, fixed in 2.5% cold buffered glutaraldehyde solution; then processed for electron microscopy. Specimens were washed twice in PBS fixative and post fixed in 1% osmium tetroxide in phosphate buffer for 3 hrs at 4°C and washed twice in water and transferred to 1% uranyl acetate in 50% ethanol for 1 hr. Specimens were dehydrated in ascending grades of ethanol up to 100% and finally embedded in Ebon 812 resin. Polymerization of resin was achieved at 70°C over a period of 12 h. Sections for TEM were cut on ultra-microtome using a diamond knife and were loaded onto 200 mesh copper grids. They were stained with uranyl acetate and lead citrate (Smith and Croft, 1991), and examined with a JEOL-JEM 1010 TEM at accelerating voltage 80 KV.

**Histopathological Study:** On the 10<sup>th</sup>&17<sup>th</sup> post infection days, the jejunum upper part of sacrificed mice from infected treated and non-treated groups was dissected, fixed in 10% formalin, for 48 hrs, dehydrated by graded ethanol and embedded in paraffin. Tran-

sverse sections of 5µm thickness were obtained from all specimens stained with hematoxylin and eosin and examined under light microscopy. Other sections were stained with Periodic acid schiff (PAS) to demonstrate mucous secretory activity of goblet cells (Bancroft, 1975).

Statistical analysis: Data were presented as mean ±SD. Analysis was done by paired "t" test for differences within same group. Differences between groups were determined by a one-way ANOVA and correlation coefficient (r). P>0.05 was not significant, P <0.05 significant and P<0.01 highly significant. SPSS version (14) program for Windows (SPSS Inc. Chicago, IL, USA) was used.

Inoculation of 45 mice with 100,000 living *G. lamblia* cysts/mouse caused 100% infection by the presence of cysts and/or trophozoites in stool by direct iodine stained smears. *Giardia* cysts shedding started 4-6 days post-infection.

## Results

Parasitological patent period: Mice of probiotics (potent *Acidophilus*) treated group had short duration of excretion of *Giardia* cysts (12.8±3.6) compared to control infected one (30.1±4.6) with highly significant difference (P<0.01) in between, without significant difference (p>0.05) compared with metronidazole treated group patent period (11.5±3.4).

Table 1: Patent period in treated and non-treated infected groups.

| Groups               | Patent period (days)  |
|----------------------|-----------------------|
| GII                  | 30.1±4.6 <sup>a</sup> |
| GIII                 | 12.8±3.6 <sup>b</sup> |
| GIV                  | 11.5±3.4 <sup>b</sup> |
| Statistical analysis | P <0.001**            |

SD: Standard deviation. \*\*Highly significant (P<0.01)

Intensity of infection: Mice of control-infected (GII) without significant (p>0.05) reduction in mean count of cyst shedding at 10, 13 & 17<sup>th</sup> post infection days. Mean count of cyst shedding (X40) of control infected groups at 10, 13 & 17<sup>th</sup> post infection days was 15.60±1.92, 13.00±1.49 & 12.20±1.78 respectively. Probiotics treated (GIII) showed significant reduction in mean count of cyst shedding at 10, 13 & 17<sup>th</sup> post infection days. Mean count of shedding

cysts (X40 microscopic field) of probiotics treated group at 10, 13 & 17<sup>th</sup> post infection days was 5.2±1.06, 3.3±1.201 & 1.32±0.38 respectively with highly significant difference (P<0.01). Metronidazole treated (GIV) showed significant reduction in mean count of shedding cysts at 10, 13 & 17<sup>th</sup> post infection days. Mean count of shedding cysts (in X40 microscopic field) at 10, 13 & 17<sup>th</sup> post infection days was 4.90±1.6, 2.90±1.77 & 1.17±0.40 respectively.

Table 2: Intensity of infection in non-treated and treated infected groups at different post infection periods.

| Post-infection days<br>Groups | Number of cyst shedding |                         |                         | P <sup>^</sup>  |
|-------------------------------|-------------------------|-------------------------|-------------------------|---|
|                               | 10 <sup>th</sup>        | 13 <sup>th</sup>        | 17 <sup>th</sup>        |   |
| GII                           | 15.60±1.92 <sup>a</sup> | 13.00±1.49 <sup>a</sup> | 12.20±1.78 <sup>a</sup> | >0.05 (NS) <sup>1</sup><br>>0.05 (NS) <sup>2</sup><br>>0.05 (NS) <sup>3</sup> |
| GIII                          | 5.2±1.06 <sup>b</sup>   | 3.3 ±1.201 <sup>b</sup> | 1.32±0.38 <sup>b</sup>  | <0.001** <sup>1</sup><br><0.001** <sup>2</sup><br><0.001** <sup>3</sup>       |
| GIV                           | 4.90±1.6 <sup>b</sup>   | 2.90±1.77 <sup>b</sup>  | 1.17 ±0.40 <sup>b</sup> | <0.001** <sup>1</sup><br><0.001** <sup>2</sup><br><0.001** <sup>3</sup>       |
| Statistical analysis          | P <0.001**              | P <0.001**              | P <0.001**              |   |

F: ANOVA test P<sup>^</sup>: Paired t test, \*\*: Highly significant (P<0.01), NS: Non significant (P>0.05), (\*\*<sup>1</sup>): 10<sup>th</sup> day versus 13<sup>th</sup> day paired t test, (\*\*<sup>2</sup>): 10<sup>th</sup> day versus 17<sup>th</sup> day paired t test (\*\*<sup>3</sup>): 13<sup>th</sup> day versus 17<sup>th</sup> day paired t test.

Reduction % in probiotics treated group was 66.66±11.97, 74.61±6.98 & 89.18±8.14 at 10, 13 & 17<sup>th</sup> post infection respectively with highly significant difference (P<0.01).

Reduction % in metronidazole treated group was 68.95±8.8, 77.69±5.09 & 90.4±3.18 respectively with highly significant difference (P<0.01). Cure rate of probiotics treated gro-

up was (84.61%), metronidazole treated mice cured (100%). Cure rate was measured on 13 mice of each treated groups by absence of cysts/trophozoites in stool for 3 consecutive days after treatment on the 17<sup>th</sup>-19<sup>th</sup>

days post-infection and absence of luminal trophozoites in H&E stained jejunal sections of 3mice/group sacrificed on 19<sup>th</sup> day post-infection.

Table 3: Percentage of reduction in cyst shedding among different treated groups at different post-infection days.

| Post-infection days<br>Groups | Percentage of reduction in cyst shedding (post infection days) |                         |                          | P <sup>^</sup>  |
|-------------------------------|--|-------------------------|--------------------------|---|
|                               | 10 <sup>th</sup>   | 13 <sup>th</sup>        | 17 <sup>th</sup>         |   |
| III                           | 66.66±11.97 <sup>b</sup>                                       | 74.61±6.98 <sup>b</sup> | 89.18 ±8.14 <sup>b</sup> | <0.001 <sup>**1</sup><br><0.001 <sup>**2</sup><br><0.01 <sup>**3</sup>  |
| IV                            | 68.95±8.8 <sup>b</sup>   | 77.69±5.09 <sup>b</sup> | 90.4 ±3.18 <sup>b</sup>  | <0.001 <sup>**1</sup><br><0.001 <sup>**2</sup><br><0.001 <sup>**3</sup> |
| Statistical analysis          | P >0.05 (NS)   | P >0.05 (NS)            | P >0.05 (NS)             |   |

P<sup>^</sup>: Paired t test, \*\*: Highly significant, NS: Non significant (P>0.05). <sup>\*\*1</sup>:10<sup>th</sup> day versus 13<sup>th</sup> day paired t test, <sup>\*\*2</sup>:10<sup>th</sup> day versus 17<sup>th</sup> day paired t test <sup>\*\*3</sup>:13<sup>th</sup> day versus 17<sup>th</sup> day paired t test.

TEM: Ultrastructure of infected control luminal trophozoite (Figs. 1, & 2) was normal. Trophozoite showed a lot of peripheral vesicles beneath cell membrane mainly in dorsal surface and naked area. Such vacuoles were absent beneath the ventral disk and the ventro-lateral flange. Cytoplasm contained also, endoplasmic reticulum and randomly distributed microtubules. Two ovoid nuclei lacked nucleoli. Four pairs of flagellae were found, run in their intra-cytoplasmic course as axonemes consisting of nine pairs of peripheral microtubules encircling one central pair (9+2). The part of the ventro-lateral flange surrounding ventral disk was supported by striated marginal plates. The ventral disk composed of microtubules layer underlying the plasma membrane. Changes were in the overall morphology and cytoplasm of luminal trophozoites treated with probiotics at 10<sup>th</sup> day post infection. These changes (Figs. 3, 4, 5 & 6) included decreased number of peripheral vesicles beneath dorsal plasma membrane and nuclear changes. The loss of normal integrity of flagellae, ventral disk, ventro-lateral flange and lateral crest were evident. Deformed shape with vacuoles was inside cytoplasm of luminal trophozoite treated with metronidazole (Figs. 7, 8 & 9) at 10<sup>th</sup> day post infection, disappearance of peripheral vesicles beneath dorsal plasma membrane and the contents of cytoplasm were depleted, appearance of cyt-

oplasmic protrusions on cell surface. Deformed nuclei with heavy electron dense deposits were seen in the cytoplasm. Gross of endoplasmic reticulum and vacuolization of cytoplasm were seen. Microtubules associated with ventral disk, flagellae, ventro-lateral flange and lateral crest were notably altered so the parasite appeared as luminal ghost.

Histopathological examination of jejuna sections of control non-infected G1 showed normal villous architecture (Fig.10). The control infected (GII) (Fig. 11) showed shortening, fusion, and crypts hyperplasia. It was noticed that probiotics when given as a therapeutic supplementation to *Giardia* infected mice (GIII) at 3<sup>rd</sup> day post treatment (10<sup>th</sup> day post infection), broadening, fusion and shortening of villi were noticed (Fig. 12). At day 17p.i. there was improvement in pathologic mucosal changes with restoration of normal villous architecture and mild inflammatory cell infiltrate in lamina propria (Fig.13). The supplementation of metronidazole to *Giardia* infected mice (GIV) at 10<sup>th</sup> day post infection showed abnormal villous architecture as shortening and fusion of villi (Fig.14). At 17<sup>th</sup> day post infection, histopathologic examination (GIV) treated with metronidazole, had almost normal mucosal morphology (Fig.15). Jejunal mucosa of control non-infected (GI) showed intense PAS reaction in villi (Fig.16). Control infected non-treated (GII), at 10<sup>th</sup> day post infection redu-

ced PAS reaction in villi (Fig.17). At day 17 post infection (Fig. 18) showed more reduction of PAS reaction in villi. GIII treated by probiotics at 10<sup>th</sup> day p.i. showed increase in mucous content in goblet cells with intense reaction in villi (Fig.19). At 17day p.i. or end of probiotics treatment, intense PAS reaction in villi became normal (Fig. 20). GIV, metronidazole treated at 10<sup>th</sup> day p.i. showed slight increase of PAS reaction in villi (Fig. 21). At day 17 p.i. or end of metronidazole treatment, villi reaction was normal (Fig. 22).

### Discussion

Great attention was paid to probiotics as potential substitutes, or as combined therapy to currently used anti-*Giardia* drugs. This was due to their influential action, stability and low toxicity to mammalian hosts including man (Hagel *et al.*, 2011). Many mechanisms were employed by probiotics in prohibiting *G. lamblia* infection, including competition for limited adhesion sites, competition for nutrients that would otherwise be utilized by *G. lamblia*, stimulation of host immune response and by producing substances to inhibit infection (Tangtrongsup and Scorza, 2010). Gupta and Garg (2009) and Shukla and Sidhu (2011) stated that *Lactobacillus* and *Bifidobacterium* species are the most widely used to prevent and treat giardiasis. This agreed with the present study where potent *Acidophilus* contained a mixture of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus sylvarius*, *Lactobacillus brevis* and *Bifidobacterium bifidum* showed significantly less patent period, decrease in infection intensity and significant increase in reduction of cyst shedding at the 10, 13 & 17<sup>th</sup> post infection days as compared to control infected one (P<0.01). But, no significant difference between probiotics treated group compared with metronidazole (P>0.05). The end of probiotics-treated group achieved 84.61% cure rate, without significant difference with metronidazole that showed complete cure (100%).

The present study agreed with Benyacoub

*et al.* (2005) who found that number of trophozoites recovered from intestine of *Enterococcus faecium* 68-fed mice (EF68) was lower than that from control infected ones. Clinically, Besirbellioglu *et al.* (2006) found that *Saccharomyces boulardii* decreased *G. lamblia* cysts number in feces from adult patients treated with a combination of *S. boulardii* and metronidazole as compared with patients only treated with metronidazole. The results agreed with Shukla *et al.* (2008), Shukla and Sidhu (2011) and Shukla *et al.* (2013) who found that *Giardia* cysts count in stool was significantly lower in mice from *Giardia-L. casei* probiotic group than that of control infected group. This finding may be due to better colonization of *L. casei* with an adjuvant effect on immune responses (both specific & nonspecific), enhancing anti-parasitic activity or interference with pathogen-enterocyte interaction, both acts synergistically to limit giardiasis (Madsen, 2006). Ribeiro *et al.* (2018) reported that treating *Giardia* infected gerbils with *Saccharomyces boulardii* probiotics (ITSB) and continued until the end of experiment (22<sup>nd</sup> days) caused drop of 70% of parasite load, suggesting its use as a co-adjuvant in giardiasis treatment. Also, Shukla *et al.* (2019) found that *Giardia* infected mice treated with probiotic protein of *Lactobacillus rhamnosus* GG showed significant reduction in cyst count compared with *Giardia*-infected mice.

In the present study, probiotics treated trophozoite, ultrastructural changes at 3<sup>rd</sup> day of treatment, showed the least number or disappearance of peripheral vesicles beneath dorsal plasma membrane of t treated groups that reflected inhibition of pinocytosis or complete digestion of its content. Vannier-Santos and de Castro (2009) reported that the size of these vesicles differed due to their activity for obtaining soluble substances in intestinal lumen. Grossing of endoplasmic reticulum of metronidazole might reflect its hyperactivity as a response against injurious toxic effect of treating agents.

Flagellae and ventral disk showed lethal

effect of probiotics, misshaping of nuclei reflected DNA affection. Electron dense deposits were in cytoplasm of metronidazole, indicating a lethal anti-giardial effect. In metronidazole treated group, electron dense deposits on nuclear membrane that agreed with Campanti and Monteiroleal (2002). These results agreed with Perez *et al.* (2001) who found that trophozoites treated with *Lactobacillus acidophilus* culture supernatants *in vitro* had a cellular damage that was observed by SEM, taking into account that damaged trophozoites might be unable to grow. Also, these data agreed with Amer *et al.* (2014) who found that oral inoculation of bacteriocins derived from *L. acidophilus* & *L. plantarum* probiotic strains in a dose of 50µg/mouse for 5 successive days resulted in marked changes in cellular architecture of trophozoites with evident disorganization of cell membrane, adhesive disk and cytoplasmic components. Perrucci *et al.* (2019) reported that *G. lamblia* trophozoites treated with commercial Slab51 supernatant probiotics for 24 hours *ex-vivo* (culture of intestinal cell line of control normal mice with *G. lamblia* trophozoites) gave variations of cellular membrane and with a vacuolar degenerative form, damaged nuclei and ventral disk rupture.

The histopathological results showed that probiotic agreed with Shukla *et al.* (2008), Shukla and Sidhu (2011) and Shukla *et al.* (2013) who reported that daily administration of probiotics *Lactobacillus casei* to *Giardia* infected mice reduced atrophied villi and infiltrating cells in small intestinal mucosa compared to severe microvillus atrophy, oedematous, vacuolated epithelial cells and ileitis in control-infected mice. Shukla *et al.* (2016) found that inulin supplementation either before or concurrently with prebiotic total nourished mice-infected with *Giardia*, caused less mucosal damage in microvilli compared with severely damaged and blunted villi of the malnourished *Giardia* infected mice. Also, Ribeiro *et al.* (2018) reported that treating *Giardia* infected gerbils with

*Saccharomyces boulardii* probiotic (ITSB) 15 days prior to infection with *G. lamblia* and continued until the end of experiment (22<sup>nd</sup> days), improved damaged intestinal villi and crypt compared to the *Giardia*-infected and non-treated gerbils. Also, Shukla *et al.* (2019) found that treating *Giardia* infected mice with probiotic protein of *Lactobacillus rhamnosus* GG, probiotic had intact mucosal epithelium lining, basal crypts with normal villi compared with severe microvillus atrophy, vacuolated epithelial cells and ileitis in *Giardia*-infected non-treated mice.

Periodic acid–Schiff (PAS) staining could be used to validate if there was alteration in secretion of polysaccharides upon *Giardia* infection and treatment with probiotics. Secretion of mucus by intestinal cells generally increased due to presence of pathogenic microorganisms (Yu *et al.*, 2008). Control infected group at 10<sup>th</sup> day p.i. showed reduction of PAS reaction in villi. At day 17 post infection, mucous secreting activity showed more reduction of PAS reaction in villi. This agreed with Shukla *et al.* (2012) and Shukla *et al.* (2019) who found that *Giardia* infected bulb/c mice had reduced number of goblet cells as with advanced *Giardia* infection, marked micro-villus damage led to reduce of goblet cells number and severe ileitis. Also, Aly *et al.* (2013) and Ammar *et al.* (2014) found that *Giardia* infected hamsters had reduced number of goblet cells with depletion of its contents. Zenian and Gillin (1985) and Kim and Khan (2013) attributed the depletion to *Giardia's* mucin-degrading enzymes, which may disrupt mucin-2 (MUC2) integrity to produce less viscous physical barrier to aid movement of the parasite and penetration of the host mucous barrier.

In the present study, at 10<sup>th</sup> day post infection (3<sup>rd</sup> day of treatment), there was increase in intensity of PAS reaction of goblet cells in the villi and at the brush border. At day 17p.i. by the end of treatment, intensity of PAS reaction returned to normal. This agreed with Ribeiro *et al.* (2018) who reported

that treatment of *Giardia* infected gerbils with *Saccharomyces boulardii* (ITSB) probiotic to the trial end (22<sup>nd</sup> days), caused increasing in goblet cells and mucus production of the ITSB groups as compared with *Giardia*-infected non-treated gerbils. Fonseca *et al.* (2019) supposed that *Bifidobacterium longum* enhanced mucus production in *Giardia*-infected gerbils hindering adherence of *Giardia* to luminal epithelium. This mucus layer gave a negative feedback, especially for parasite luminal adhesion (Roskens and Erlandsen, 2002). Shukla *et al.* (2019) found that *Giardia* infected mice treated with probiotic protein of *Lactobacillus rhamnosus* GG and killed probiotics increased goblet cells number compared to severe microvillus atrophy, vacuolated epithelial cells, reduced goblet cells number and ileitis in *Giardia*-infected non-treated mice. Babinska *et al.* (2005) found that the supplementation of diets containing *Bifidobacterium* and *Lactobacillus acidophilus* bacteria to piglets increased goblet cells number. Aliakbarpour *et al.* (2012) found that *L.* probiotics induced mucins secretion (MUC2 & MUC3) and prevented enteric pathogens adherence, indicating that enriched mucous layers overlying intestinal epithelium gave protection against pathogens invasion. Also, Smirnov *et al.* (2005) found that probiotic supplementation increased the expression of MUC2 gene in rat jejunum & colon (Caballero-Franco *et al.*, 2006). Mucin is the major component of mucous layer (Iwashita *et al.*, 2003) defends intestinal absorptive surface areas against injurious microbes and acts as a lubricant between brush border and luminal contents (Deplanck and Gaskins, 2001; Uni *et al.*, 2003). So, mucous layer is the first line of host luminal defense that powers digestion & nutrient absorption.

### Conclusion

The potent *Acidophilus* caused morphological changes and improved histopathological changes of intestinal mucosa with rapid parasite clearance. Probiotics diffuse to trophozoite membrane and seriously affected

its nucleus, ventral disk and flagellae. Adherence of trophozoite to mucosa thus caused parasite rapid clearance and giardiasis control. More studies to exploit probiotics specific strains role in treating giardiasis, other intestinal protozoa, and possible combination with chemotherapeutic agents are ongoing and will be published in due time elsewhere.

### References

- Ansell, BR, McConville, MJ, Ma'ayeh, SY, Dagley, MJ, Gasser, RB, et al, 2015:** Drug resistance in *Giardia duodenalis*. Biotechnol. Adv. 33, 6 Pt1:888-901.
- Aliakbarpour, HR, Chamani, M, Rahimi, G, Sadeghi, AA, Qujeq, D, 2012:** The *Bacillus subtilis* and lactic acid bacteria probiotics influences intestinal mucin gene expression, histomorphology and growth performance in broilers. Asian-Austral. J. Anim. Sci. 25, 9:1285-9.
- Aly, MM, Shalaby, MA, Attia, SS, El Sayed, SH, Mahmoud, SS, 2013:** Therapeutic effect of Lauric acid, a medium chain saturated fatty acid on *Giardia lamblia* in experimentally infected hamsters. PUJ 6, 1:89-98.
- Amer, EI, Mossallam, SF, Mahrous, H, 2014:** Therapeutic enhancement of newly derived bacteriocins against *Giardia lamblia*. Exp. Parasitol. 146:52-63.
- Ammar, AIA, Mahmoud, SSM, El Hefnawy, NN, 2014:** Effect of ginger on hamsters infected by *Giardia lamblia*. J. Environ. Stud. Res. 11:45-56.
- Bancroft, JD, 1975:** Histochemical Techniques. 2<sup>nd</sup> Edition, Butterworth, London, and Boston.
- Babinska, I, Rotkiewicz, T, Otrocka-Domaga, I, 2005:** The effect of *Lactobacillus acidophilus* and *Bifidobacterium* spp. Administration on the morphology of the gastrointestinal tract, liver and pancreas in piglets. Pol. J. Vet. Sci. 8:29-35.
- Benyacoub, J, Pérez, PF, Rochat, F, 2005:** *Enterococcus faecium* SF68 enhances the immune response to *Giardia intestinalis* in mice. J. Nutr. 135, 5:1171-6.
- Besirbellioglu, B, Ulcay, A, Can, M, 2006:** *Saccharomyces boulardii* and infection due to *Giardia lamblia*. Scandinavian J. Infect. Dis. 38, 6/7:479-81.
- Blagburn, BL, Drain, KL, Land, TM, Kinard, RG, Moore, PH, et al, 1998:** Comparative efficacy evolution of dicationic corbrazole compounds, nitazoxanide, paromomycin against *Crypt-*

- osporidium parvum* infection in a neonatal mouse model. Antimicrob. Agents Chemother. 24: 2877-82.
- Caballero-Franco, C, Keller, K, De Simone, C, Chadee, K, 2006:** The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am. J. Physiol. Gastrointest. Liver Physiol. 292:315-22.
- Campanati, L, Monteiro-Leal, L, 2002:** The effects of the antiprotozoal drugs metronidazole and furazolidone on trophozoites of *Giardia lamblia* (P1 strain). Parasitol. Res. 88:80-5.
- Claude, PR, Champagne, P, Ross, M, Saarela, K, Hansen, F, et al, 2011:** Recommendations for viability assessment of probiotics as concentrated cultures and in food matrices. Int. J. Food Microbiol. 149, 3:185-93.
- Conlon, MA, Bird, AR, 2015:** The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7, 1:17-44.
- Deplancke, B, Gaskins, HR, 2001:** Microbial modulation of innate defense: Goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73: 1131-41.
- Dharmani, P, Srivastava, V, Kissoon-Singh, V, Chadee, K, 2008:** Role of Intestinal mucins in innate host defense mechanisms against pathogens. J. Inna. Immun. 8:123-35.
- Fonseca, JF, Alvim, LB, Nunes, AC, Oliveira, FMS, da Silva Amaral, R, et al 2019:** Probiotic effect of *Bifidobacteria longum* 51A and *Weissella paramesenteroides* WpK4 on gerbils infected with *Giardia lamblia*. J. Appl. Microbiol. 127, 4:953-1279.
- Gardner, TB, Hill, DR, 2001:** Treatment of giardiasis. Clin. Microbiol. Rev.14:114-28.
- Gupta, V, Garg, R, 2009:** Probiotics. Ind. J. Med. Microbiol. 27, 3: 202-9.
- Hagel, I, Cabrera, M, Puccio, F, Santaella, C, Buvat, E, et al, 2011:** Coinfection with *Ascaris lumbricoides* modulates protective immune responses against *Giardia duodenalis* in school Venezuelan Rural Children. Acta Trop. 117, 3:189-95.
- Hill, DR, Guerrant, RL, Pearson, RD, 1983:** *Giardia lamblia* infection of suckling mice. J. Infect. Dis. 147:217-21.
- Iwashita, J, Yukita, S, Hiroko, S, Nagatomo, T, Hiroshi, S, et al, 2003:** mRNA of MUC2 is stimulated by IL-4, IL-13 or TNF- $\alpha$  through a mitogen-activated protein kinase pathway in human colon cancer cells. Immunol. Cell Biol. 81: 275-82.
- Kim, J, Khan, W, 2013:** Goblet cells and mucins: Role in innate defense in enteric infections. Pathogens 2, 1:55-70.
- Leitsch, D, Burgess, AG, Dunn, LA, Krauer, KG, Tan, K, et al, 2011:** Pyruvate: ferredoxin oxidoreductase and thioredoxin reductase are involved in 5-nitroimidazole activation while flavin metabolism is linked to 5-nitroimidazole resistance in *Giardia lamblia*. J. Antimicrob. Chemother. 66: 1756-65.
- Madsen, K, 2006:** Probiotics and the immune response. J. Clin. Gastroenterol. 40, 3:232-4.
- Mattar, AF, Daniel, H, Teitelbaum, RA, Drogowski, F, Yongy, CM, et al, 2002:** Probiotics up-regulate MUC-2 mucin gene expression in a caco-2 cell-culture model. Pediatr. Surg. Int. 18:586-90.
- Paget, GE, Barnes, JM, 1964:** Evaluation of results: Qualitative application in different species. In: Laurence DR, Backarach AL (eds.), Evaluation of Drug Activities. Pharmacom. Academy Press, London & New York.
- Perez, PF, 2001:** Inhibition of *Giardia intestinalis* by extracellular factors from *Lactobacilli*: an in vitro study. Appl. Environ. Microbiol. 67: 5037-42.
- Perrucci, S, Fichi, G, Ricci, E, Galosi, L, Lalle, M, et al, 2019:** In vitro and ex vivo evaluation of the anti-*Giardia duodenalis* activity of the supernatant of Slab51 (SivoMixx). PloS one 14, 3: e0213385.
- Reid, G, Hammond, JA, 2005:** Probiotics; Some evidence of their effectiveness. Can. Fam. Phys. 51, 11:1487-93.
- Ribeiro, MRS, Oliveira, DR, Oliveira, FMS, Caliari, MV, Martins, FS, et al, 2018:** Effect of probiotic *Saccharomyces boulardii* in experimental giardiasis. Beneficial Microbes 9, 5:789-97.
- Roskens, H, Erlandsen, SL, 2002:** Inhibition of in vitro attachment of *Giardia* trophozoites by mucin. J. Parasitol. 88:869-73.
- Shukla, G, Sidhu, RK, 2011:** *Lactobacillus casei* as a probiotic in malnourished *Giardia lamblia*-infected mice: A biochemical and histopathological study. Canad. J. Microbiol. 57, 2:127-35.
- Shukla, G, Devi, P, Sehgal, R, 2008:** Effect of *Lactobacillus casei* as a probiotic on modulation of giardiasis. Digest. Dis. Sci. 53, 10:2671-9.
- Shukla, G, Sidhu, RK, Verma, A, 2012:** Restoration of anthropometric, biochemical and histopathological alterations by *Lactobacillus casei* supplementation in *Giardia intestinalis* infected



renourished BALB/c mice. *Antonie Van Leeuwenhoek* 102, 1:61-72.

**Shukla, G, Kaur, H, Sharma, L, 2013:** Comparative therapeutic effect of probiotic *Lactobacillus casei* alone and in conjunction with anti-protozoal drugs in murine giardiasis. *Parasitol. Res.* 112, 6: 2143-9.

**Shukla, G, Bhatia, R, Sharma, A, 2016:** Prebiotic inulin supplementation modulates the immune response and restores gut morphology in *Giardia duodenalis* infected mal-nourished mice. *Parasitol. Res.* 115, 11: 4189-98.

**Shukla, G, Sharma, A, Bhatia, R, Shama, M, 2019:** Prophylactic potential of synbiotic (*Lactobacillus casei* & Inulin) in malnourished murine giardiasis: An immunological and ultra-structural study. *Prob. Antimicrobial. Proteins* 11, 1:165-74.

**Smirnov, A, Perez, R, Amit-Romach, E, Sklan, D, Uni, Z, 2005:** Mucin dynamics and microbial population in chicken small intestine are changed by dietary probiotic & antibiotic growth promoter supplementation. *J. Nutr.* 135:187-92.

**Smith, M. Croft, LS, 1991:** Embedding and thin section preparation. In: *Electron Microscopy in Biology: Practical Approach*, Rickwood, D. & Harris B.D. IRL Press, Oxford.

**Tangtrongsup, S, Scorza, V, 2010:** Update on the diagnosis and management of *Giardia* spp. infections in dogs and cats. *Top. Comp. Anim. Med.* 25:155-62.

**Travers, MA, Sow, C, Zirah, S, Deregnacourt, C, Chaouch, S, et al, 2016:** Deconjugated bile salts produced by extracellular bile-salt hydrolase-like activities from probiotic *Lactobacillus johnsonii* La1 inhibit *Giardia duodenalis* in vitro growth. *Front. Microbiol.* 7: 1453.

**Uni, Z, Smirnov, A, Sklan, D, 2003:** Pre- and post-hatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. *Poult. Sci.* 82:320-7.

**Vannier-Santos, MA, De Castro, SL, 2009:** Electron microscopy in antiparasitic chemotherapy: A (close) view to a kill. *Curr. Drug Targets* 10, 3:246-60.

**Veenemans, J, Mank, T, Ottenhof, M, Baidjoe, A, Mbugi, E, et al, 2011:** Protection against diarrhea associated with *Giardia intestinalis* is lost with multi-nutrient supplementation: a study in Tanzanian children. *PLoS Negl. Trop. Dis.* 5, 6:e1158.

**Yu, LCH, Huang, C, Kuo, WT, Sayer, H, Turner, JR, 2008:** SGLT-1-mediated glucose up take protects human intestinal epithelial cells against *Giardia duodenalis*-induced apoptosis. *Int. J. Parasitol.* 38: 923-34.

**Zenian, A, Gillin, FD, 1985:** Interactions of *Giardia lamblia* with human intestinal mucus: Enhancement of trophozoite attachment to glass 1. *J. Protozool.* 32, 4:664-8.

#### Explanation of figures

Fig. 1: TEM section of luminal *G. lamblia* trophozoite of control non-treated group (II) showed a normal convex dorsal surface with normally distributed peripheral vesicles (PV), normal appearance of cytoplasmic vacuoles (CV), shape of nucleus (N), pattern of flagellar microtubules (9+2) and ventral disk microtubules (VD) with preserved caudal edge (CE) (Scale bar = 1  $\mu$ m).

Fig. 2: TEM section of luminal *G. lamblia* trophozoite of control non-treated (GII) showed a normal size of endoplasmic reticulum (ER)

Fig. 3: TEM section of luminal *G. lamblia* trophozoite of probiotics treated (GIII) at 10<sup>th</sup> day post infection showing smaller number of peripheral vesicles (PV) beneath dorsal plasma membrane, no changes of endoplasmic reticulum (ER), misshaping of nuclei (N), loss of normal integrity of flagellae (F) and destructed microtubules of ventral disk (VD). Intestinal microvilli (Iv) covering intestinal epithelium (Ie), (15000X, Scale bar = 500 nm).

Fig. 4: TEM section of luminal *G. lamblia* trophozoites of probiotics treated (GIII) at 10<sup>th</sup> day post infection showing smaller number of peripheral vesicles (PV), loss of normal integrity of ventral disk (VD), ventro-lateral flange (VLF), lateral crest (LC) & flagellae (F). (20000X, Scale bar = 500 nm).

Fig. 5: TEM section of luminal *G. lamblia* trophozoites of probiotics treated (GIII) at 10<sup>th</sup> day post infection showing abnormal appearance of *Giardia* trophozoite, smaller number of peripheral vesicles (PV), loss of normal integrity of ventral disk (VD) and flagellae (F) with appearance of electron dense deposits (EDD). Intestinal microvilli (Iv) covering intestinal epithelium (Ie) (10000X, Scale bar = 2  $\mu$ m).

Fig. 6: TEM section of luminal *G. lamblia* trophozoites of probiotics treated group at 10<sup>th</sup> day post infection showing abnormal appearance of *Giardia* trophozoite, smaller number of peripheral vesicles (PV), loss of normal integrity of ventral disk (VD) and flagellae (F), intestinal microvilli (Iv) covering intestinal epithelium (Ie) (12000X, Scale bar = 2  $\mu$ m).

Fig. 7: TEM section of luminal *G. lamblia* trophozoites of metronidazole treated (GIV) at 10<sup>th</sup> day post infection showing disruption in cell shape, appearance of cytoplasmic protrusions (CP) together with appearance of intra-cytoplasmic vacuoles (V), misshaping of nuclei (N), electron dense deposits (EDD) on cell surface, destruction of microtubules of flagellae (F) and distension of endoplasmic reticulum (ER). Parasite appeared as luminal ghost (10000X, Scale bar = 2  $\mu$ m).

Fig. 8: TEM section of luminal *G. lamblia* trophozoites of metronidazole treated (GIV) at 10<sup>th</sup> day post infection showing distortion of cell shape together with disruption of cell membrane, destruction of microtubules of ventral disk (VD), destructed shape of ventro-lateral flange (VLF) and lateral crest (LC) with smaller number of peripheral vesicles (PV). (15000X, Scale bar = 500 nm).

Fig. 9: TEM section of luminal (Lu) *G. lamblia* trophozoites of metronidazole treated (GIV) at 10<sup>th</sup> day post infection showing distortion of cell shape together with disruption of cell membrane, swollen axoneme (A), destruction of microtubules of ventro-lateral flange (VLF) and flagellae (F) with disappearance of peripheral vesicles (PV). Intestinal microvilli (Iv) covering intestinal epithelium (Ie) (20000X, Scale bar = 500 nm).

Fig. 10: Jejunal section of a non-infected non-treated (GI) mouse showing normal villous architecture (V) with brush border, lamina propria (LP) and intestinal glands (IG) (X 200, H&E).

Fig. 11: Jejunal section of an infected non-treated mouse (GII) showing shortening, fusion (red arrow) and crypts hyperplasia (black arrows) (X 200, H&E).

Fig. 12: Jejunal section of an infected mouse, 10<sup>th</sup> day post infection and 3<sup>rd</sup> day of treatment by probiotics (GIII) showing shortening and fusion of villi (black arrows) (X200, H&E).

Fig. 13: Jejunal section of an infected mouse, 17<sup>th</sup> day post infection and 10<sup>th</sup> day of treatment by probiotics (GIII) showing improvement of pathological mucosal changes with restoration of villous appearance (V) and mild inflammatory cell infiltrate (double headed arrow) (X 200, H&E).

Fig. 14: Jejunal section of an infected mouse, 10<sup>th</sup> day post infection and 3<sup>rd</sup> day of treatment by metronidazole (GIV) showing shortening (black arrow) broadening and fusion of villi (red arrow) and abnormal shaped villi (double headed arrow) (X200, H&E).

Fig. 15: Jejunal section of an infected mouse, 17<sup>th</sup> day post infection and 10<sup>th</sup> day of treatment by metronidazole (GIV) showing apparent improvement of villous architecture (black arrow), (X 200, H&E).

Fig. 16: Jejunal section of non- infected non-treated control mouse (GI) normal mucous content and intense reaction of mucopolysaccharides (PAS +ve) at goblet cells (G), brush border of intestinal villi (V) and intestinal glands (IG) (X 200, PAS stain).

Fig. 17: Jejunal section (transverse section) of an infected non-treated mouse (GII) at 10<sup>th</sup> day post infection showing less intense PAS reaction in goblet cells (black arrow) and intestinal glands (IG) (X250, PAS).

Fig. 18: Jejunal section of an infected non-treated mouse (GII), at 17<sup>th</sup> day post- infection showing more decrease in intensity of PAS reaction in the villi and at brush border (black arrows) (X250, PAS).

Fig. 19: Jejunal section of an infected mouse, 10<sup>th</sup> day post infection and 3<sup>rd</sup> day of treatment by probiotics (GIII) showing increase in mucous content in goblet cells (black arrows) with intense reaction in villi (X200, PAS).

Fig. 20: Jejunal section of an infected mouse, 17<sup>th</sup> day post infection and 10<sup>th</sup> day of treatment by probiotics (GIII) showing intense PAS reaction in villi (black arrow) (X200, PAS stain).

Fig. 21: Jejunal section of an infected mouse, 10<sup>th</sup> day post infection and 3<sup>rd</sup> day of treatment by metronidazole (GIV) showing slight increase of PAS reaction in villi (black arrows) (X200, PAS stain).

Fig. 22: Jejunal section of an infected mouse 17<sup>th</sup> day post infection and 10<sup>th</sup> day post treatment by metronidazole (GIV) showing normal intensity of PAS reaction in villi (black arrows) (X200, PAS stain).





