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EFFECTS OF ALTERNATIVE NATURAL AND NANOTHERAPIES VERSUS METRONIDAZOLE ON GIARDIASIS INFECTED HAMSTERS

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Abstract

Giardia lamblia is one the most common human zoonotic protozoan affecting about 200 million people annually, but recurrent symptoms and drug resistance were reported in giardiasis patients. The present study assessed the therapeutic effect of Tomex® and probiotics against giardiasis as well as evaluated efficacy of chitosan nanoparticles and metronidazole. Sixty healthy male hamsters were divided into 6 groups. Each group consists of 10 hamsters as following: GI non-infected hamsters, GII infected control, GIII, GIV, GV & GVI infected with *G. lamblia* and treated with tomex, probiotics, chitosan nanoparticles (CsNPs), and metronidazole (MTZ) respectively. Hamsters were orally infected with 10,000 *Giardia* cysts. After 3 weeks stool samples were examined for *Giardia* samples and contents for intestinal histopathology and electron microscopy of *Giardia* trophozoite ultrastructure. The highest reduction in *Giardia* cyst and trophozoite were in hamsters received MTZ but with natural alternative therapies chitosan nanoparticles showed a therapeutic effect with highest reduction in cyst and trophozoite counts (80.59% & 76.55%) respectively and best recovery in intestinal pathology. MTZ gave higher reduction rates (91.04% & 90.75%) respectively with normal intestinal histopathology.
Key words: Chitosan, Probiotics, Metronidazole, *Giardia lamblia*, Tomex®.

Introduction

Giardia lamblia was firstly reported in 1681 by Van Leeuwenhoek (Magill *et al*, 2013), since it was considered the commonest human zoonotic protozoan affecting about 200 million people annually (Pecková *et al*, 2018). Giardiasis patients may be asymptomatic or present with dehydration-causing diarrhea and abdominal discomfort, producing chronic diarrhea in malabsorption, and weight loss contributed to increased mortality of malnourished and immune deficient children especially on first 3 years of life (Cotton *et al*, 2011). *G. lamblia* presented in one of two forms, either infective quadrinucleated cysts or trophozoites attached to mucosal epithelium of small intestine causing different mucosal inflammations (Ankarklev *et al*, 2010). Giardiasis patients must be treated with anti-giardial drugs and to stop passage of infective cysts in the environmental (Dyab *et al*, 2016).

Update metronidazole (1- β-hydroxyethyl-2-methyl-5-nitroimidazole) is widely used to treat; *G. lamblia, Entamoeba histolytica, Trichomonas vaginalis* and *Blastocystis* spp. (Mirza *et al,* 2011; Leitsch *et al*, 2011). Recurrent symptoms and drug resistance were reported with them (Bansal *et al,* 2006; Tejman-Yarden *et al*, 2011). Great efforts were done to developdrug against giardiasis with least side effects (Muller *et al*, 2018).

Chitosan, is a natural amino polysaccharide (cationic polysaccharide), produced by alkaline deacetylation of chitin present in the shells of insects and murine crustacean, with a wide range of antimicrobial and antifungal activity plus reducing toxicity to mammalian cells (Kumar, 2000; Said *et al*, 2012). Polymeric nanoparticles of synthetic and natural polymers have received the majority of value due to the stability and facility of surface modified, chitosan nanoparticles (CsNPs) produced mild protection in giardiasis treatment (Said *et al*, 2012).

Argüello-García *et al*. (2018) found that *Allium sativum* (garlic) proved effective in treated giardiasis. Its efficacy was due to anti-oxidant effect (Prasd *et al*, 1995).

Probiotics therapy also have attracted attention as a potential substitute alone or as combined therapy against Giardiasis due to their powerful activity, stability and low toxicity to humans and other mammal hosts (Hagel *et al*, 2011). Tangtrongsup and Scorza (2010) approved that, Probiotics interfere

with *G. lamblia* infection through different mechanisms, including competition for limited adhesion sites, competition for nutrients with *G. lamblia***.** Perrucci *et al*. (2019) suggested blending the nutritional interventions like probiotics in treating and minimizing giardiasis signs and symptoms.

The present work aimed to assess the therapeutic effect of garlic (Tomex tablets), prob-iotics and chitosan nanoparticles versus Metronidazole (Flagyl tablets) in giardiasis by evaluating *Giardia* infection intensity, jejunum histopathological changes and ultrastructural changes of *Giardia* trophozoites in experimentally infected hamsters.

Material and Methods

Experimental animals: Sixty healthy laboratory bred male Syrian hamsters (*Mesocricetus auratus*) weighted 100-110gm were used. They were kept on standard diet of 24% protein, 4% fat and about 4-5% fiber, water ad-libidum and under a temperature of 24^{o}C in animal house of Theodor Bilharz research institute (TBRI).

Ethical consideration: Approval was taken from the institutional review board (IRB), Faculty of Medicine Zagazig University, Official permission from Authorities of Pediatrics Department of Zagazig University and Informed consents were taken from parents of all the children.

Hamsters were maintained following the research protocols following the NIH guide for care and use of laboratory animals approved by TBRI ethics committee.

Giardia lamblia and hamsters infection: Fresh stool samples containing at least 3-5 *G. lamblia* cysts and free from other parasites, were obtained from patients attended outpatient clinic, Pediatric Department of Zagazig University Hospital.

Stool samples were processed (Aly *et al*, 2013) to obtain the infecting dose 10,000 cysts/ml. Each hamster except those of GI was orally infected with 1ml of *G. lamblia* cyst suspension containing 10,000 cysts, using esophageal tube.

Experimental design: Hamsters were divided into six groups of 10 hamsters each as followed: GI: non-infected and non-treated, GII: infected and non-treatment, GIII: infected and treated with *Allium sativum* (Garlic) Tomex tablets 200mg given orally in aqueous suspension, as a single daily dose of 1.04mg/0.2ml/hamster for 14 days started from 7th day post infection (Paget and Barnes, 1964), GIV (Probiotic): infected and treated with commercial potent Acidophilus capsules (GNC, Saudia Arabia) each contained a mixture of 50 million live probiotic bacteria: *Lactobacillus acidophilus, L. bulgaricus, L. sylvarius, L. brevis* and *Bifidobacterium bifidum* were given orally in aqueous suspension in a single daily dose of 130.000 bacteria/0.2ml/hamster for 14 days started on 7th day post infection (Paget and Barnes, 1964). GV (CsNPs): infected and treated with chitosan nanoparticles suspension in a dose of 50µg/hamster/day for 7 consecutive days (Said *et al*, 2012). GVI: infected and treated with full dose of metronidazole (MTZ) suspension 125mg (Sanofiaventis Pharmaceutical, Cairo) purchased in a dose (7ml/ hamster/day) for 7 consecutive days (Ammar *et al*, 2014).

CsNPs preparation and characterization: CsNPs were prepared using ionic gelation technique. Chitosan (Cs) was dissolved in 1% acetic acid solution with sonication, until solution appeared transparent. Sodium hydroxide was added to raise PH to get 4.6-4.8. Tripolyphosphate (TPP) as 1mg/ml was prepared by dissolving of TPP in distilled water. The acidic chitosan solution (0.3%) was added to an equal volume of TPP solution, with magnetic stirring at room temperature, and formation of CsNPs was spontaneously obtained. Nanoparticles were purified by centrifugation at 10.000rpm for 30min at 4°C, followed by supernatant removal and CsNPs were rinsed via distilled water for removal of any sodium hydroxide. These nanoparticles were at 4-8°C till used (Elzatahry and Mohy-Eldin, 2008).

Using photon correlation spectroscopy and laser Doppler measurements of size of nano-

particles and zeta potential were done on fresh samples with an average size of 20±5nm and zeta potential + 42.8 mV.

Half the hamsters were sacrificed on 10th day PI to study ultrastructure changes, and second half was sacrificed on 21st day PI. Euthanasia was done by intraperitoneal anesthesia using thiopental sodium as 1gm in 20 ml distilled water/hamster (Ammar *et al*, 2014).

Parasitological drug evaluation: Three weeks post infection feces were collected for copro-parasitological examination using direct smear method (unstained and Lugol's iodine stained) to detect *G. lamblia* cysts. Two weeks treatment, feces were collected from hamsters for cyst counting using merthiolate iodine formaldehyde (MIF) as 1gm for parasites number by a hemocytometer (Aly *et al*, 2013).

Histopathological evaluation: After hamsters' scarification, three segments of 1cm each were cut at a distance of 5, 15 & 25cm from gastroduodenal junction, stained with hematoxylin-eosin and examined for changes and degree of mucosal healing (Slaoui and Fiette, 2011).

TEM: This was done at electron-microscope unit Mansoura University. Intra-intestinal trophozoites of all groups were collected on 10th day PI. Specimens of jejunum upper part were fixed in 2.5% Glutaraldehyde (Polysciences), 4% sucrose in 0.1 mol/L sodium cacodylate buffer (pH 7.2) for 1hr, post-fixed in 1% Osmium tetroxide (Polysciences) & 0.8% Potassium ferricynide in same buffer for 40min, dehydrated in acetone series, and embedded in Polybed resin (Polysciences). Blocks were sectioned by ultra-microtome using diamond knives and examined at accelerating voltage 80KV (Smith and Croft, 1991).

Statistical analysis: Data were triplicated 3 times, and analyzed (ANOVA) P values below 0.05 were significant. Percentage reduction (%R) in parasite count was calculated as: %R= 100 (C-E)/ C, where C: control hamsters and E: experimental ones (Penido *et al*, 1994).

Results

Mean number and percentage reduction of *Giardia* cysts and trophozoites per gram stool showed a significant difference among groups ($P < 0.001$) as in (Tab. 1).

Table 1: Percentage reduction of *Giardia* trophozoite and cyst count per gram stool among groups

Items	Trophozoite count/ H.P.F in small intestine		Cyst count/ H.P.F per gram stool	
	Mean ± SD	R%	Mean ± SD	R%
GII	362.5±16	-	13400 ± 1300	-
G III	168.5 ± 30.8	53.51%	6250 ± 1075	53.35%
G IV	196 ± 45	45.9%	6800 ± 985.6	49.25%
GV	85 ± 9.75	76.55%	2600 ± 873.3	80.59%
GVI	33.5 ± 5.6	90.75%	1200 ± 410.3	91.04%
F	1396.7		283.06	
P	< 0.001		< 0.001	

SD: Standard deviation F: ANOVA test (P<0.001): Highly significant

Table 2: Percentage of goblet cell count and reduction in inflammatory cell count / H.P.F in groups

Items	Inflammatory cell count/H.P.F	(R%)	Goblet cell count/H.P.F
G I	22.25 ± 3.5		12.4 ± 1.5
GII	90.2 ± 4.25		3.8 ± 2.2
GIII	48.7 ± 6.2	46%	6.2 ± 2.5
GIV	58.4 ± 3.6	35.25%	4.7 ± 1.4
GV	40.5 ± 5.0	55.1%	4.7 ± 2.3
GVI	20.75 ± 2.5	77%	8.5 ±1.5
F	238.86		280.37
P	< 0.001		< 0.001

Histopathological examination of small intestinal sections showed significant difference ($P < 0.001$) in goblet cell count/ H.P.F among groups, with significant difference ($P < 0.001$) in inflammatory cell counts/H.P.F in small intestinal sections (Fig 1- 6). In normal intestinal histopathology (Fig.1), GII showed marked changes in form of villous shortening and degeneration, decreased ratio of villous height to crypt length and diffuse loss of brush border (Fig. 2a,b).

After therapeutic treatment, GIII & GIV

showed mild improvement in histopathological changes, as intestinal mucosa was partial healed with mild villous shortening and no marked decrease in ratio of villous height to crypt length (Fig. 3a, b & Fig. 4a, b). GV showed a moderate degree of improvement in form of mild mucosal ulceration and preservation of brush border (Fig.5a, b). GVI showed highest improvement degree as marked healing intestinal mucosa, no mucosal ulceration, preservation of brush border, average villous architecture and preserved ratio of villous height to crypt length (Fig. 6a,b).

TEM: Infected control luminal trophozoite (Fig.7a) was normal shaped with a lot of peripheral vesicles beneath cell membrane mainly in dorsal surface. Cytoplasm contained two nuclei. Flagellae consisted of nine pairs of peripheral microtubules encircling one central pair (9+2). Ventral disk composed of microtubules layer underlying plasma membrane. Part of ventro-lateral flange and lateral crest surrounding ventral disk was supported by striated marginal plates.

GIII showed evident changes in all morphology and cytoplasm of luminal trophozoite treated with tomex, as shape distortion with irregular appearance, smaller peripheral vesicles number beneath dorsal plasma membrane, nuclei completely disintegrated, and adhesive disk was seriously affected with some flagellae (Fig. 7b). GIV showed changes in all morphology and cytoplasm of luminal trophozoites treated with probiotics, as peripheral vesicles disappearance beneath dorsal plasma membrane and nuclear changes, disruption of *Giardia* cell membrane, loss of flagellar normal integrity, ventral disk, ventro-lateral flange and lateral crest microtubules (Fig.7c). GV showed luminal trophozoites treated with chitosan nanoparticles changes in all morphology and cytoplasm, as smaller peripheral vesicles number beneath dorsal plasma membrane and nuclear changes, loss of flagellar normal integrity and ventral disk microtubules, ventro-lateral flange and lateral crest microtubules were notably altered (Fig.7d).

GIV showed evident changes in all morphology and cytoplasm of luminal trophozoite treated with metronidazole, as distortion shape with ovoid and irregular appearance, lack of peripheral vesicles beneath dorsal plasma membrane, complete disintegration of nuclei, and adhesive disk and fagellae was bad affected with parasite as luminal ghost appearance (Fig.7e).

Discussion

Giardia lamblia is one of the commonest zoonotic protozoa, causing diarrhea in millions of people worldwide (Tian *et al*, 2010). Giardiasis infection in man was an increase ng problem especially in developing countries (Gholami *et al*, 2014).

Metronidazole, albendazole and mebendazole were used for giardiasis (Plummer *et al*, 2001), with metronidazole as drug of choice in the last decades (Buret 2008). But, failure due to gastrointestinal disturbances, headache and neurotoxic effects increased parasite resistance **(**Andrade *et al*, 2010). Srivastava *et al*. (2009) declared that medicinal plants or herbal remedies were alternative drugs.

In the present study, among the natural studied agents chitosan nanoparticles treated group showed the highest percentage reduction in both cyst and trophozoite counts in hamsters (80.59% & 76.55%) respectively. Goy *et al*. (2009) reported that chitosan nanoparticles only hindered *Giardia* parasites growth rather than killing them. Divya *et al*. (2017) found that chitosan nanoparticles have a higher anti-giardiasis activity due to the interaction between polycationic chitosan and negatively charged parasite surface, and the large surface area of chitosan nanoparticles facilitated its tight adherence to its surface. Yarahmadi *et al*. (2016) and Chabra *et al*. (2019) assessed the validity of chitosan nanoparticles in treating experimental *G. lamblia* infection, found reduction of *Giardia* cysts and trophozoites number in stool compared to infected untreated control, and recommended CsNPs as a safe, effective alternative therapy for giardiasis.

In the present study, tomex® treated hams-

ters showed high reduction percentages in cyst and trophozoite counts in infected intestines (53.35% & 53.51%) respectively as compared to control infected ones. The mechanism by which garlic inhibited cyst shedding was due to the sulphur-containing compounds especially allicin, that showed a variety of antigiardial activities (Ankri and Mirelman 2009). Sanad and Al-Ghabban (2011) found that *Giardia* infected mice treated with fresh crushed garlic cloves caused significant reduction ($P<0.001$) in infection intensity and reduced cyst shedding. Increased doses of garlic cloves alcoholic extract gave more active in reduction of trophozoites in stool of mice (Abid Al-Khfaji, 2017).

In the present study, probiotics treated hamsters showed (49.25% & 45.9%) reduction of cyst and trophozoite counts respectively. This was attributed to interference with parasite enterocyte interactions (Bibiloni *et al*, 1999), modulation of immune response (Haller *et al*. 2000), probiotics bacteria exocellular factors (Lievin-Le *et al*, 2002), gave a better intestinal environment to inhibit trophozoites adhesion to the mucus epithelium (Koji, 2005).

Shukla *et al*. (2008) found that daily administration of *Lactobacillus casei* simultaneously with a single dose of 5×10^6 trophozoites in mice for 30 days significantly reduced their number in gut. Shukla *et al*. (2019) found that given *Lactobacillus casei,* and albendazole significantly reduced cysts and trophozoite in counts of intestinal fluid.

In the present study, MTZ treated hamsters showed the highest reduction in both cyst and trophozoite counts (91.04% & 90.75%) respectively. This agreed with Fahmy *et al*. (2014) who gave metronidazole in a dose of 120µg/kg, twice per day for 5 days to infected hamsters and found 92.15% reduction in trophozoite count as compared to infected control ones. Dyab *et al*. (2016) reported that using of metronidazole in a dose of 15 mg/kg/day to infected mice for 7 days gave complete disappearance of cyst and trophozoites from small intestinal content. So, metronidazole acted against giardiasis by DNA damage, strand breaking and crosslinking or by causing many genes modification that participated in mitosis completion (Plummer *et al*, 2001) or inhibited parasite oxygen consumption (Gardner and Hill 2001).

On the contrary, this disagreed with Lemée *et al*. (2000) in France assessed metronidazole susceptibility of 11 clinical isolates *G. lamblia* using a neonatal mouse model and found clinical resistant to metronidazole. Nash *et al*. (2001) reported that metronidazole failed in some cases, in spite of receiving successive treatment courses.

In the present study, histopathological changes were improved in treated hamsters as compared to infected untreated control ones. Besides, the use of chitosan nanoparticles and metronidazole showed best cure evidence as compared to other natural agents.

In the present study, infected control hamsters *G. lamblia* affected the architecture of intestinal mucosa in the form of villi shortening, broadening and blunting with altered villous/crypt ratio and lamina propria infiltration with inflammatory cells. Mahmoud *et al*. (2014) reported that villi of *G. lamblia* infected and untreated hosts showed shortening, atrophy and villi fusion led to blunting with desquamation of most villi, heavily infiltrated lamina propria and aggregated lymphocytes with necrosis of some enterocytes.

In the present tomex® treated hamsters, jejunal mucosa showed thickening of villi with flattening of enterocytes surface, reduction (46%) of infiltrating inflammatory cells in lamina propria. An ultimate immune function mediator within cell was toxic to *Giardia* (Eckmann *et al*, 2000). Capasso (2013) added that the anti-giardial efficacy of garlic *in vivo* was due to stimulated nitric oxide synthase (NOS).

In the probiotics treated ones, histopathological examination of jejunal mucosa showed marked broadening, shortening and villi fusion with villous/crypt ratio 2/1 in some, and decrease in inflammatory cell infiltrate in lamina propria but a least of 35.25% was

among them. Arvola *et al*. (1999) found that probiotics modulated the cytokines release that played a central role in the defense mechanism. Also, Shukla *et al*. (2019) and Perrucci *et al*. (2019) reported that in *Giardia* infected mice, *Lactobacillus casei* reduced villous atrophy and infiltrating inflammatory cells in the small intestinal tissue. These results showed the antigiardial effect of probiotics particularly *L. casei,* by modulating the gut cells inhibited *in-vivo* colonization and multiplication of trophozoites and reduced pathogenicity. In the present study, histopathologically chitosan nanoparticles treated hamsters sho-wed partial healing of mucosa with mild villous shortening, without marked decrease in villous height ratio to crypt length and mild improvement of goblet cell count with 55.1% reduction of inflammatory cellular infiltration.

Ahmed and Aljaeid (2016) reported that CsNPs showed significant effect against local GIT diseases and intestinal disinfection. Wardani *et al*. (2018) found that chitosan nanoparticles improved GIT mucosal epithelial cells necrosis caused by toxic agents. Hu *et al*. (2011) rep-orted that chitosan nanoparticles produced cellular oxidative stress by increasing reactive oxygen compounds associated with cytotoxicity to various cells including mucosal epithelium.

In the present study, metronidazole showed histopathological improvement in partially healed small intestinal mucosa with mild improvement goblet cells count mild shortening of villi and mild decrease in the ratio of villous height to crypt length. Also, lamina propria showed reduction of inflammatory cellular count (77%). This agreed with Leffler and Lamont (2009) reported that metronidazole has anti-inflammatory, anti-oxidant and immunomodulatory effect especially in intestinal lumen. Ammar *et al*. (2014) who found healing of small intestinal mucosa and mild inflammatory reaction in lamina propria after metronidazole administration to infected hamsters. Besides, many ultra-structural changes occurred in trophozoites in all treated hamsters at the 10^{th} day post infection with evident distortion in its shape indicating diffusion of treating agents through the cell surface, flagellae and ventral disk was affected with misshaping nuclei by DNA affection.

In the present study, Tomex caused trophozoite mild swelling with loss of flagellae. This agreed with Miron *et al*. (2000) who found that disk fragmentation was due to direct allicin action on parasite DNA and Harris *et al*. (2001) who found in vitro effect of whole garlic on *G. lamblia* trophozoites. Argüello-García *et al*. (2018) reported that in vitro incubation of trophozoites with several thioallyl compounds from fresh crushed aqueous garlic extracts led to a complete integrity loss of plasma membrane, marked destruction of cytoplasmic contents, but ventral disk cytoskeletal elements and flagellae remained structurally unaltered.

In this study, probiotics showed little changes in cellular architecture of trophozoites with less cell membrane distortion. This partially agreed with Perez (2001) who found that trophozoites in vitro treated with *L. acidophilus* had a cellular damage by SEM, and that trophozoites were unable to grow. Amer *et al*. (2014) found that oral inoculation of *L. acidophilus* & *L. plantarum* probiotic strains in a dose of 50µg/mouse for 5 successive days caused marked changes in cellular architecture of trophozoites with adhesive disk & cytoplasmic components distortion. Perrucci *et al*. (2019) found that *G. lamblia* trophozoites treated with commercial Slab51 supernatant probiotics for 24hrs ex-vivo gave variations of cellular membrane and with a vacuolar degenerative form, damaged nuclei and ventral disk rupture.

In the present study, chitosan nanoparticles treated hamsters showed marked trophozoites ultrastructural changes. This agreed with Choi and Hu (2008) who found nanoparticles interaction with parasites surface. Saad *et al*. (2015) by SEM found that *Cryptosporidium parvum* cysts treated with silver & copper oxide nanoparticles showed many struct-

ural changes led to loss oocysts viability.

In the present study, by ultra-structure metronidazole showed complete trophozoite distortion with ghost appearance by nitro-group reduction in drug by ferrodoxins electrons with subsequent drug activation due to the DNA molecules of *Giardia* trophozoite. This agreed with Campanti and Montero-Leal (2002) who found that the trophozoite cytoplasm membranous was more rounded appearance when they were incubated for 6hrs in-vitro with metronidazole 1μ/ml.

Conclusion

Among the natural alternative therapies against giardiasis chitosan nanoparticles showed a therapeutic effect against *Giardia* infection in hamsters with highest percentage reduction in both cyst and trophozoite counts (80.59% & 76.55%) respectively as well as the best recovery in intestinal pathology but metronidazole gave high reduction rates (91.04% & 90.75%), respectively with nearly normal intestinal histopathology.

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Explanation of figures

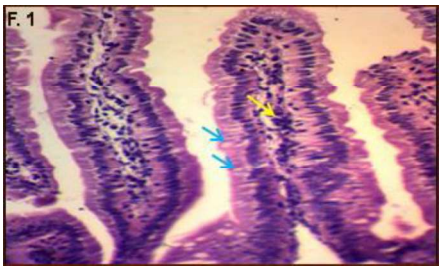
Fig. 1: Intestinal cut section in GI (normal control) showed normal villi with average number of goblet cells and in flammatory cells in lamina propria (H&E, 400x).

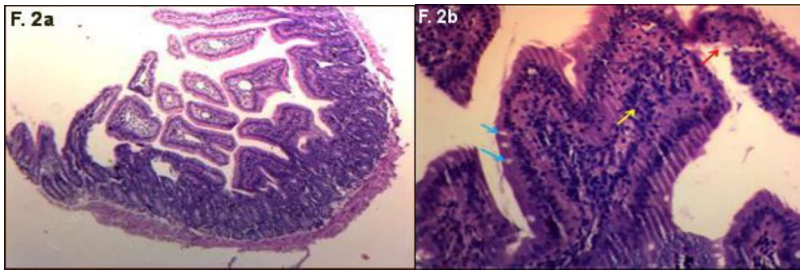
Fig. 2: H&E stained intestinal cut section in GII showed shortened and fused broad villi (black arrow) with crypt hyperplasia (red arrow)(fig.2a 100x). Fig.2b (400x) showed marked inflammatory cells infiltration in lamina propria (yellow arrow) with goblet cells depletion (blue arrow) and ulceration of intestinal epithelium (red arrow).

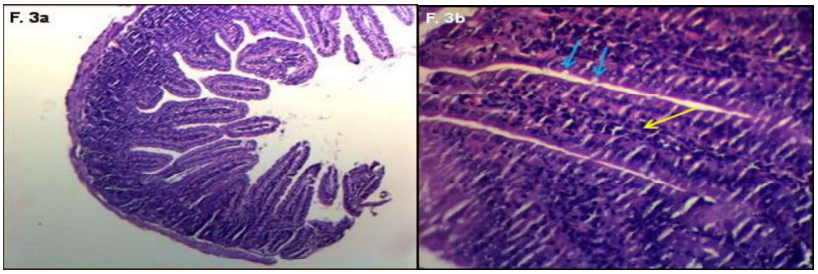
Fig. 3: H&E stained intestinal cut section in GIII showed shortened broad villi (fig.3a 100x) and mild inflammatory cells infiltration (yellow arrow) and mild goblet cells depletion (blue arrow) (fig.3b 400x).

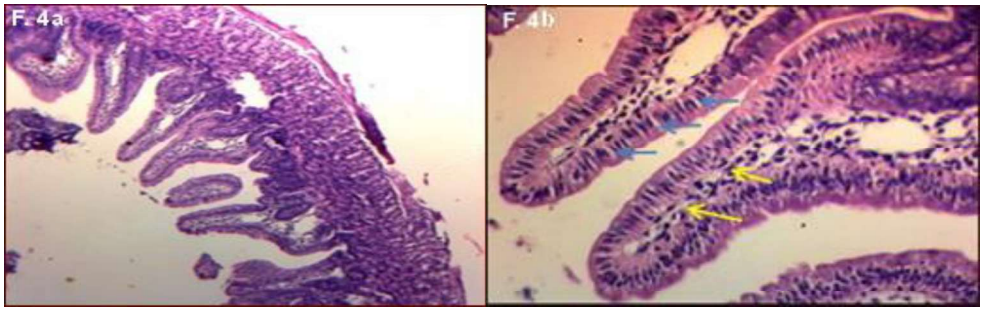
Fig. 4: H&E stained intestinal cut section in GIV showed partial healing of intestinal villi (fig.4a 100x) and mild goblet cell depletion (blue arrow) with mild inflammatory reaction (yellow arrow) (fig.4b 400x).

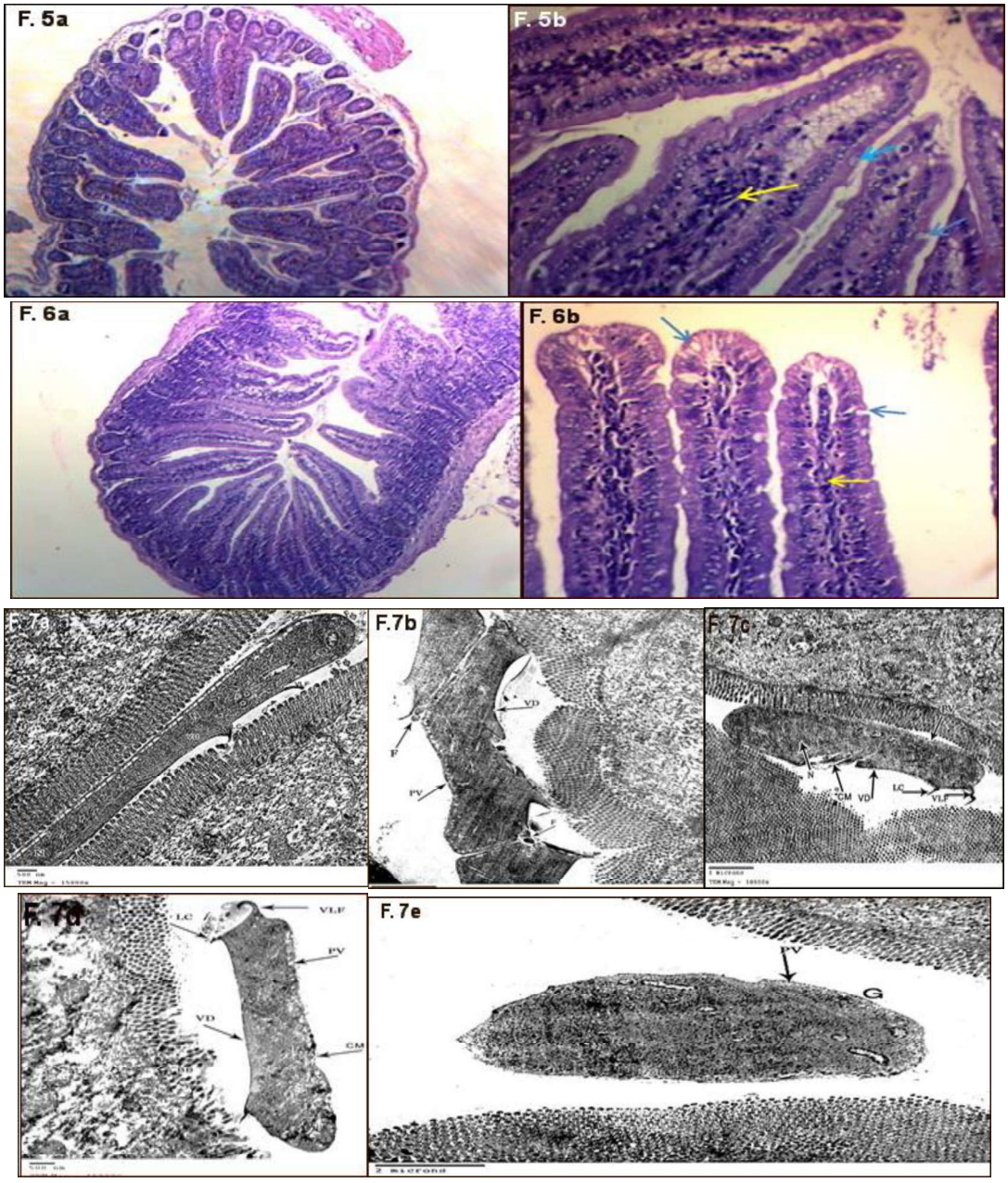
Fig. 5: H&E stained intestinal cut section in GV showed nearly normal villi (fig.5a 100x) and mild inflammatory cells infiltration (yellow arrow) with average number of goblet cells in lamina propria (fig.5b 400x).
Fig. 6: H&E stained intestinal cut section in GVI showed marked improvement of villi (fig.6a 100x) with patchy inflammation (yellow arrow) and minimal depletion of goblet cells (blue arrow) (fig.6b 400x).
Fig. 7a: TEM section of luminal *G. lamblia* trophozoite of control non-treated (GII) showed intact cell membrane, normal flagellar (F) microtubules (9+2) ,a normal shape of nucleus (N) and ventral disk microtubules (VD) with preserved lateral crest (LC)and ventro- lateral flange .
Fig. 7b: TEM section of luminal *G. lamblia* trophozoites of tomex treated (GIII) showed distortion of cell shape, destruction of ventral disk (VD) and flagellar (F) microtubules, Smaller peripheral vesicles (PV) number, complete nuclear disintegration.
Fig. 7c: TEM section of luminal *G. lamblia* trophozoites of probiotics treated (GIV) showed distortion of cell shape together with disruption of cell membrane, destruction of flagellae (F), Destruction of microtubules of ventral disk (VD), ventro-lateral flange (VLF) and lateral crest (LC), Disappearance of peripheral vesicles (PV) beneath dorsal plasma membrane and mild nuclear changes.
Fig. 7d: TEM section of luminal *G. lamblia* trophozoites treated by chitosan nanoparticles (GV) showed distortion of cell shape together with disruption of cell membrane (CM), destruction of flagellar (F) and ventral disk (VD) microtubules. Smaller peripheral vesicles (PV) number, oNuclei (N) completely distorted. Ventro-lateral flange (VLF) and lateral crest (LC) altered. Brush border (BB) destructed beneath trophozoite.
Fig. 7e: TEM section of luminal *G. lamblia* trophozoites of Metronidazole treated (GVI) showed distortion of cell shape with ovoid and irregular appearance, appeared as luminal ghost (G). Complete destruction of microtubules of ventral disk and flagellar microtubules. Nuclei were completely distorted, Disappearance of peripheral vesicles (PV).










F. 5a
F. 5b
F. 6a
F. 6b
F.7b
F. 7c
LC
VLF
PV
VD
CM
F.7e
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