Journal of the Egyptian Society of Parasitology, Vol. 51, No. 3, December 2021
J. Egypt. Soc. Parasitol. (JESP), 51(3), 2021: 539 - 550
(Online: 2090-2549)

COMPARISON BETWEEN THE EFFECTS OF MAGNESIUM OXIDE (MGO) NANO-PARTICLES AND MICROWAVES (MVS) IRRADIATION ON GIARDIA LAMBLIA CYSTS

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Abstract

The efficiency of the transmission of *Giardia lamblia* is assured by the presence of a cyst wall, which provides resistance to drastic osmotic and pH variations. Both magnesium oxide (MgO) nanoparticles (NPs) and microwaves (MVs) are known to inactivate many microorganisms.

This study compared the effects of MgO NPs & MVs irradiation on *Giardia lamblia* cysts in experimental mice as to treated and un-treated cysts viability, excystation, ultrastructure changes and infectivity.

The results showed that the *G. lamblia* cysts count were reduced to (LD_{90}) after 8, 16, & 24hrs of exposure to doses 100, 50, & 25mg/ml, respectively, while reduction rate of (EC_{50}) after 8, 16, &24 hrs of exposure to dose 50, 25, & 12.5mg/ml, respectively. MVs LD_{90} efficacy on cysts was detected after 30 seconds while EC_{50} after 20 seconds. Failure of excystation was 90% and 98% among MgO NPs and MVs treated cysts. MgO NPs treated cysts showed multiple cytoplasmic vesicular vacuoles while the cyst wall appeared destructive with the release of the cytoplasm outside. MVs treated cysts were filled with one big vesicular vacuole with an increase in size while some contents were decreased in size and others disappeared completely. The infectivity rate among mice inoculated with MgO NPs and microwave treated cysts was 33.3% & 8.2 % with a mild histopathological changes, with significant differences.

Keywords: Mice, *Giardia lamblia*, MgO NPs, Microwave, Infectivity, Viability, Ultrastructure.

Introduction

Giardia lamblia (=*G. duodenalis* or *G. intestinalis*) infects about 280 million people worldwide, mainly in developing countries with poor hygienic conditions (CDC, 2011). Prevalence rates were 20-30% & 2-5% in developing and developed countries, respectively (Thompson, 2000), and major cause of childhood diarrhea (Nkrumah *et al,* 2011) *Giardia* life cycle includes motile trophozoites that parasitized the small intestine upper part and in response to appropriation, trophozoites differentiated into dormant resistant thick walled cysts in the lower part that surv ive outside the host and maintain transmission cycle by fecal-oral route (Upcroft and Upcroft, 2001). The contaminated water and food with the cysts is another way of spreading even with < 10 cysts (Cernikova *et al,* 2018). El Shazly *et al.* (2007) in Egypt reported that potable water samples were contaminated with zoonotic protozoa of cryptosporidiosis and giardiasis were the pre-dominant ones. *Giardia* cyst wall resisted physical and chemical disinfectants (Arguello-Garcia *et al,* 2004). Giardiasis causes acute and/or chronic diarrhea with gastrointestinal symptoms (Feng and Xiao, 2011). Most giardiasis infected children have no symptoms at all, but few suffered from abdominal pain and watery, foul-smelling diarrhea that can lead to dehydration, also have excessive gas and bloating and could have a poor appetite, leading to weight loss, and uncommon fever. These symptoms begin 7 to 14 days after exposure to *Giardia* infection and may last,

without treatment, for up to 4 or 6 weeks (CDC, 2018).

In giardiasis the majority of drugs acted on the trophozoites to treat the patients, but infective viable cysts continuously were excreted in stools during treatment (Gardner and Hill, 2001). Effective anti-giardiasis have been intense, but recurrent infections, virulence factors, and drug resistance-imposed the effective treatment (Cernikova *et al,* 2018). *G. lamblia* genotyping showed that assemblages A & B were the commonest zoonotic ones (Feng and Xiao, 2011). However, both are completely different in variant-specific surface protein (VSP) gene (Laishram *et al,* 2012). Besides, in mice assemblage-A induces minimal infection and cleared before establishing, but assemblage B readily infects them (Ankarklev *et al,* 2010). Also, both assemblages differed in rate of in-vitro growth, encystation, biochemical activities, symptoms, susceptibility to RNA *Giardia* parasite, and epidemiologic characteristics (Karanis, 1998; Plutzer *et al,* 2010).

Synthesis, characterization, and exploration of materials in nanometer region (1-100 nm) generally confers larger surface areas compared with macro-sized ones by nanotechnology that enhanced their physicochemical and biological properties in food processing (Yadav, 2013). Large-scale one-dimensional magnesium oxide (MgO) nanowires with 6x10μm have been successfully synthesized and studied (Al-Hazmi *et al,* 2012). Although several anti-*Giardia* NPS in-vitro and in-vivo showed different validities degrees (Al-Mohammed *et al,* 2021), yet MgO has some advantages to other metals such as non-mutagenic effect, good stability, biologically synthesized in normal component of human body (Tang and Ly, 2014).

Microwaves (MVs) radiation is now popular in food industry for thawing drying, baking and inactivating microorganisms in foods with less heating and less food exposure to destructive (Rosenberg and Bögl, 1987). MV heating inactivates many pathogens as gram-positive and negative bacteria, viruses, parasites, and fungi (Rougier *et al,* 2014; Woo *et al*, 2000; Franssen *et al,* 2019). The major advantages of MVs in food industry were rapid heat transfer, selective heating, compactness of equipment, speed of switching on, and pollution-free without products of combustion that made using MVs whether thermal or non–thermal for soil disinfection (Yarahmadi *et al,* 2016). But, MVs as a household machine may be used as an alternative way for decontamination of food and water (Graczyk *et al,* 2007). Thus, treatment of infected hosts with NPs killed zoonotic infective stage (Idan and Ardalan, 2020).

This study aimed to evaluate the effects of MgO NPs & MVs radiation in treating *Giardia lamblia* cysts viability, excystation, and infectivity.

Materials and Methods

Source of *G. lamblia* cysts: Fecal samples obtained from 9 school children heavily infected with G. lamblia as shown by abundant cysts in their stools and negative to other parasites were selected from the attendances of the pediatrics clinic in Suez Canal University to be the source of G. lambilia cyst in this study. Parasite in the patients' stool was genotyped assemblage B as a part of a genotyping study for Giardia (**Hussein *et al,* 2017).** This study was conducted from January to December 2019. The stool samples were immediately processed within 1-10 hours after excretion.

G. lamblia cysts isolation, concentration, and purification: Stools were mixed thoroughly with an equal volume of distilled water and the suspension was filtered on a sieve of 4 gauze layers to remove coarse materials. Then, the filtrate was centrifuged at 800g for 5min., the deposit was re-suspended in distilled water and washed 3 times (Alvarado and Wasserman, 2006). A sucrose solution of 1.18 density was prepared (455g sucrose in 355ml distilled water), and then a sucrose solution of 1.9 density was prepared by mixing a known volume sucrose solution 1.18 density in an equal volume of distilled water. In a round bottom test tube, the two

different sucrose gradients were introduced carefully without mixing them up. About 1ml of stool suspension was poured on the surface of the test tube containing sucrose solution was centrifuged at 600g for 10min. The *G. lamblia* cysts were aspirated with a Pasteur pipette from the interface between the two gradients. Purified cysts were re-suspended in distilled water and stored at 4°C with antibiotics (Penicillin® and Streptomycin®, 1,000 units and 1mg/ml, respectively) for a maximum of 3 days before use (Mohsen and Abdel Hamid, 1993). These cysts were used for in vitro and in vivo studies.

Giardia lamblia cysts *in-vitro* MgO NPs experiments: The large-scale one-dimensional MgO nanowires with diameters of 6nm x lengths of 10μm and a surface area of about 461m2/g, were successfully synthesized and prepared (Al-Hazmi *et al,* 2012). The doses ranged from 3, 6.25, 12.5, 25, 50, & 100mg were incubated with *Giardia* cysts in a Pyrex test tube (1ml of purified viable 1X 105 cysts) at 37°C in PBS (PH 7.2) and examined 3 times daily until reached to the lethal dose. For each experiment, the activity was determined against time viable cysts counts were studied.

MVs irradiations: By placing three Petridishes containing 1ml of purified viable (1X 105) cysts previously washed three times by BPS. MV energy disintegration was done with Panasonic MVs (915 MHz) at 1000 W) and applied at 5. 15, 30, 45, & 60 seconds, respectively. Three experiments/time exposure were used (Hussain, 2015).

Assessment of *Giardia* cyst viability *in-vitro*: It was done by using trypan blue vital dye 0.4% (Strober, 2015). Cyst exclusion of the dye means viability. The lethal concentration dose (LD_{90}) was defined as the lowest concentrations of MgO_2 lethal to 90 % of cysts after exposure and effective concentration (EC_{50}) was defined as median effective concentrations of MgO_2 NPs lethal to 50% cyst after exposures (Hussein *et al,* 2018). Viability of cyst/ml was calculated before and after microwaving (Escobedo and Cimerman, 2007). The cytocidal effect (IC_{90}) was defined as lowest time of MV exposure lethal to 90% *Giardia* cyst was confirmed by a non-stained cyst. Inhibitory concentrations (IC_{50}) were defined as lowest time of MV exposure lethal to 50 % Giardia cyst. In addition, encystationof the cysts were assessed, and the number of cysts in each group at each indicated time was determined from the representative three culture tubes.

TEM: Treated and non-treated cysts were examined for any changes in the wall, morphology, & ultrastructure (Speer *et al,* 1979). One ml of each treated cyst with lethal dose and non-treated cysts were washed twice in 0.1M sodium cacodylate buffer (pH 7.2) with 5% sucrose. Each sample was fixed in the following fixatives: 1% glutaraldehyde in 0.1M sodium cacodylate buffer (pH7.2) for 3hrs, followed by two washes in the buffered 2% Osmium tetraoxide (OsO_4) in 1% Potassium Ferro-cyanide solution for one hr. and two washes in 0.5M sodium cacodylate buffer. After processing, slides were microscopic examined by Joel 1210 TEM.

Excystation rate: One ml containing $1x10^5$ of purified *G. lamblia* cysts suspension incubated in 0.1M potassium phosphate (pH 7.0) & 0.3M sodium bicarbonate for 30 min at 37°C in culture tubes. Cysts treated with MgO_2 and MV irradiation LD_{90} were incubated separately for 30 minutes in bicarbonate then for 1.5hrs at 37°C in culture medium (TYI-S-33) after Ward *et al.* (1997).

For induction of excystation, assessment of total, partial, or failure excitation was determined by counting the cysts treated compared to cysts untreated using a hemocytometer under the microscope.

In vivo experiments: For assessment of the infectivity of treated cysts, 3 groups of *G. lamblia* infected (A, B & C) of 6-week-old Albino mice (12/each) were intra-esophageal inoculated with 5x103 cysts. GA & GB were inoculated with MgO_2 & MV LD_{90} doses, respectively while GC was inoculated with non-treated cysts. Besides, twelve healthy mice were used as control. Cysts output

was counted by fecal stool examinations of Logul's iodine stained smears daily up to 12 days post-infection (PI). Mice were put individually in separate cages and feces excreted over 2hrs was collected. Scoring of infection intensity was estimated by counting cysts /10 high power fields (HPF) and 1-5 (1+) = mild infection, 6-10 (2+) = moderate infection, and >10 cysts (3+) + heavy infection (Qin *et al,* 2020).

Histopathological examination: Gradings were after Williamson *et al.* (2000**)**. Grade 0: Majority of villi architecture and epithelium cells was normal and villous/ crypt ratio about 3:1. Grade I: Most villi were finger-shaped, little broadened due to edema or mi-ld plasma cell infiltration. Epithelium of so-me villi usually with nuclear shifted from basal to cell superficial part, vesiculation in basal part, and infiltration of a few lymphocytes. Crypts normal and villous/crypt ratio was 2.5:1. Grade II: Most of villi had broadened, shortened, and heavy infiltration predominately plasma cells. Epithelium was often cuboidal disorganized by a lymphocytic infiltrate and sometimes marked vesiculation. Crypts length and villous/crypt ratio was 2:1. Grade III: Villi fused or bow-shaped with a heavy infiltrate in lamina propria, and epithelial changes similar to Grade II. Villous/crypt ratio was 1.5:1. Grade IV: Mucosa almost flat except for some indentations and crypts opened into surface. Epithel ium was heavily infiltrated and disorganized. Crypts had numerous mitosis and occasional crypts abscess.

Statistical analysis: IC_{50} & IC_{90} were evaluated (drug concentration in X-axis & inhibition percentage in Y-axis) using office XP (SDAS) software with linear regression as M±SD. ANOVA test (one-way analysis) and Chi-square/Fisher exact test were also used.

Significant levels were at $P < 0.05$. Cysts reduction and excystation rate were counted by following equation: Reduction rate/ excitation rate = (a-b/a) x 100. a = mean number of intact/excyst cysts in control experiments, and b = mean number of intact /excyst cysts in treated experiments.

Ethical approval: Verbal consent was obtained from the parents of patients, and all of the procedures were conducted according to the ethical standard approved by the Institutional Human Ethics Committee, Faculty of Medicine, Suez Canal University. All procedures related to animals were performed according to the ethical standards approved after Declaration of Helsinki (1964).

Results

In the present study, during 24hrs exposure to different doses of MgO NPs, in vitro, MgO NPs ≤6.25 doses did not affect *Giardia* cysts. But, cysts count reduced to 90% (LD_{90}) after 8, 16, & 24hrs of exposure to doses 10, 50, & 25mg/ml, respectively, but reduction rate reached 50% (EC_{50}) after 8, 16, & 24hrs of exposure to dose 50, 25, & 12.5mg/ml, respectively. As to MVs irradiation on *Giardia* cyst, LD_{90} for treated cysts was after 30 seconds, and EC_{50} was after 20 seconds as compared to control count 1x105 cysts, with significant differences

Reduction in excystation was 85% among cysts treated with MgO NPs LD90 while it was 98% in (LD_{90}) MV treated cysts compared to 10% in the untreated control, with significant differences ($P = 0.0001$). Viable cyst was unstained while non-viable appeared stained blue by light microscopy.

TEM ultrastructure changes included size, shape, morphology, and appearance. Most of MgO NPs treated cysts were filled with multiple vesicular vacuoles that led to an increase in cell volume and size, but others were with decrease in cytoplasmic contents, lacerating cyst wall, with evacuated cellular material due to rupture. As to *Giardia* cysts the MV irradiations many changes were detected. Most of the treated cysts were filled with one vesicular vacuole that led to an increase in cell volume and size. Other contents decreased in size and shrinkage or sometimes disappeared completely with a small rim of granular cytoplasm surrounding cyst wall.

In vivo infection rate in mice inoculated with MgO_2 NPs treated cyst were 33.3% with mild degree pathological changes, and infectivity rate was 8.2% in mice inoculated with microwave treated cysts. In control, 91.8% infection rate with grade degree III of pathological changes was among mice inoculated with untreated cysts, with significant differences. After 6-12 days of PI, stool analysis showed treated mice had a mild degree of infection intensity versus mice inoculated by untreated cysts that showed heavy infection intensity. Details were given in tables (1, 2, 3, & 4) and figures (1, 2, & 3)

Table 1: Counts of viable *Giardia* cyst after exposure to MgO NPs different doses as to time of exposure,

Doses of MgO2 NPs	8 hr.		16 hr.		24 hr.	
	Mean ± S.D.	%	Mean ± S.D.	%	Mean ± S.D.	%
.6.5 mg/ml	99000 ±923.953	1	98000±923.95	2	90000±4619.764	10
12.5 mg/ml	90000±4619.7	10	80000±9239.52	20	50000±18479.057	50*
25 mg/ml	80000 ±4619.7	20	50000±923.95	50*	10000±1847.906	90**
50 mg/ml	50000 ±923.953	50*	9833.33±705.6	90**	7000±923.953	93
100 mg/ml	10000 ±923.953	90**	5000±923.9	95	4000±923.953	96
F, P. value	443.390, 0.000		289.732, 0.000		289.732, 0.000	

Control count= 1x105 cysts, with significant differences * EC50, **LC90

Table 2: Effect of MVs *Giardia* cysts irradiation as to time exposures.

Seconds	Mean ± S.D. of viable cysts	%
10	99000 ±461.976	1
15	74366.66±115.507	25
20	50366.00±241.992	50*
25	31033.3±188.037	70
30	9966.6±465.046	90**
F, P. value	27,918.331, 0.000	Significant

Control count= 1x105 cysts, with significant differences *EC50, **LC90

Table 3: Excystation rate among MgO NPs &MVs treated *Giradia* cysts compared to untreated.

	Complete		Partial		Failure	
	Mean± S.D.	%	Mean± S.D.	%	Mean± S.D.	%
MVs	250 ±9.24	0.13%	1850 ±18.479	1.87	98000±40.62	98
MgO2	10500 ±461.97	10%	5000 ± 561.9	5	85000±459.61	85
Untreated cysts	90000 ±45.619	90	7000 ± 450.6	7	3000±370.3	3
F, P. value	100,752.784, 0.000		116.943, 0.000		68,217.383, 0.000	

Significant differences

Table 4: Infectivity rates and grades of histopathology in mice infected with MgO NPs and MVs treated Giardia cysts compared to untreated. The differences were statistically significant

	Infected		No-infected		Total	Fisher exact p.value
	No.	%	No.	%		
MVs	1	8.2*	11	91.8	12	17.775
MgO2	4	33.4*	8	33.3	12	0.00013
Untreated cysts	11	91.8***	1	8.2	12	

*Grade I, with mild infection intensity, ***Grade III with heavy infection intensity

Discussion

In the present in vitro study, *G. lamblia* cysts count reduced up to 90% (LC_{90}) after 8, 16, & 24hrs of exposure to doses 100, 50, & 25mg/ml, respectively, and reduction rate reached 50% (EC_{50}) after 8, 16, & 24hrs of exposure to dose 50, 25, & 12.5mg/ml, respectively.

This present data agreed with in agreement with the present study, as Chitosan (Cs) NPs at dose of400ug/ml after 180 minutes exposure killed all *Giardia* cysts (Yarahmadi *et al,* 2016). Whereas, Elmi *et al.* (2013) in Iran reported that Cs NPs was at 33.3% reduction rate of nanachitosan particles on *Giardia* cysts at dose of 50ug/ml after 180 minutes exposure. On the other hand, Malekifard and Tavassoli (2020) in Iran found that Cs NPs had multiple cytotoxic effects in vitro and vivo. Gold (G) NPs is another metal that had 96% anti-*Giardia* cysts effect with a concentration of 0.3mg/ml in 180 minutes (Buzea *et al,* 2007). Unfortunately, Gold NPs toxicity caused the *in-vitro* and *in-vivo* to damage several host tissues (Barathmanikanth *et al,* 2010; Abdelhalim *et al*, 2013). Subsequent-

ly, Selenium (Se) and copper oxide (CuO) NPs at concentrations 0.6 & 0.3mg/ml gave similar effects to metronidazole on *Giardia* cysts (Malekifard and Tavassoli, 2020). On the same time, Se NPs toxicity was contributed to the thyroid gland (Avery and Hoffmann, 2018), and slight-to-severe pathologic al lesions were found among animals (Whanger *et al*, 1996) and humans even with the sub-lethal doses (Urbankova *et al*, 2021). Nevertheless, CuO-NPs induced oxidative stress in human lymphocytes (Assadian *et al*, 2018). Consequently, MgO_2 is a normal component of the human body, so its toxicity was minimal when compared to other metals (Jin and He, 2011). MgO_2 NPs triggered an inflammatory response via anti-apoptotic and antioxidant pathways, and simultaneously alter related hematologic factors particularly with the higher doses (Mazaheri *et al,* 2019). This toxicity was due to the small size and larger surface areas to volume ratio of NPs and thus, their biological reaction proved to be more than the bulk material (Lanone and Boczkowski, 2006). However, the nontoxic effects of NPs were reported in other studies (Buzea *et al,* 2007). The differences in anti-*Giardia* effect in the present study and others might be due to the origin of NPs themselves (Chithrani and Chan, 2007).

In the present study, the ultrastructure changes that occurred in *Giardia* cyst after incubation with MgO_2 NPs LD_{90} were matched with several studies that reported the ability of MgO_2 NPs to deactivate several pathogens through the cell wall distortion (Jin and He, 2011; Rafiei *et al*, 2013). The ultrastructure changes shown in *Cyclospora cayetanensis* oocysts when incubated with the MgO_2 NPs (Matica *et al*, 20017) were similar to that detected in the present study. The MgO_2 NPs destructed the cyst wall barrier by the formation of reactive oxygen species that reacted with the carbonyl group in the peptide linkages in the parasite wall, leading to lipid peroxidation (Tang *et al,* 2020). Subsequently, destruction of the lipids and proteins of the membrane by MgO_2 NPs led to increased permeability and flow of cytoplasmic contents out of the cell (Jin and He, 2011). Moreover, the strong electrostatic interaction between the cyst surface and the MgO_2 NPs led to degradation of the protein (Xing and Bin-Fing, 2014), with the high pH (alkaline effect) that damaged the parasite's wall (Siles-Lucas *et al,* 2018)

In the present study, experimental results showed that the rate of infection among the mice inoculated with MgO_2 NPs treated cyst was 33,3% with a mild degree of histologic changes. Low histologic grade among mice inoculated with LD_{90} of MgO_2 NPs alternated DNA replication and protein synthesis of cysts during their penetration (Ramanujam and Sundrarajan, 2014). Thus, the ability of 10% viable cysts to induce infection was weak when compared to grade III in controls. In coordination with the present data, the oral administration of Cs NPs at the dose of 100ug/kg reduced the mean of excreted cysts after 3 days up to 10 times in infected *G. lamblia* BALB mice (Chabra *et al*, 2019). Moreover, Cs NPs 50ug/Syrian hamster/day in combination with metronidazole significantly reduced the cysts and trophozoites counts from 63.3% to 94.6% (El-Gendy *et al,* 2012). With *Giardia*-infected rats treated with Curcumin NPs, the cysts and trophozoites count reduced to 54.6% & %51.7% respectively (Said *et al,* 2013). Silver (Ag) NPs alone reduced the Giardia cyst and trophozoites counted up to 72.7 and 81.1%in rats infected with *Giardia lamblia* after 8 days treatment while in combination with metronidazole, the effects reached 83.3% after 3 days treatment of infected BALBS mice while it was 66.6% after one day with Silver NPs treatment alone (Jarrell and Sener, 2003). Although, Ag NPs anti-*Giardia* effect was efficient and induced size-dependent cytotoxicity in human lung cells (Gliga *et al,* 2014). But, the combination of Zinc oxide NPs and metronidazole gave 100% lethal effect in experimentally infected mice and induced neurotoxicity among treated mice ((Brakat *et al,* 2019). The presence of infection among mi-

ce in the present study may be referred to as the smallest infective dose (10 cysts) of giardiasis that matched with the count of completely excysted cysts among treated groups. Also, all of the previous in vivo work concerned the treatment of infected hosts with NPs and few studies involved on NPs effect on the cyst as the infective stage (Bavand *et al,* 2017).

In the present study, LD90 for treated cysts was demonstrated after 30 seconds while EC_{50} was shown after 20 seconds. The differences were statistically significant. At the same time, the infectivity rate was very low (8.2%) among mice inoculated with MV-treated cysts. The present results were supported with other research as MVs were effective against multiple foods borne human parasites such as *Cryptosporidium parvum* (Duhain *et al,* 2012), *Cyclospora cayetanensis* (Ortega and Liao, 2006), *Anisakis simplex* (Vidaček *et al,* 2011). *Microsporidium* spores (Graczyk *et al,* 2007) and *Heterophyes heterophyes* encysted metacercaria (El-Zawawy, 2008). MV irradiations were lethal to the microorganisms in the range of 1-350 MHz and the peak lethal effects were at 60 GHz (Leonelli and Mason, 2010; Carole *et al,* 2014). The ability of MVs to affect proteins is highly dependent on the bound water content of molecules. Absorbed energy is converted into heat energy within the medium resulting in increased temperature (Rosenberg and Bögl, 1987). However, sanitization of sewage sludge and solid waste landfill leachates by MVs radiation <1000 Watt was not efficient for inactivation of the *Giardia lamblia* cyst up to 7.5 minutes' exposure (Park *et al,* 2006), and it was significant in reduction of the cysts number with Mvs with 1000 watt (Graczyk *et al,* 2008). The controversy between the present result and the previous one may be due to the differences in the origin and size of samples used. As in the present study, only human samples with *Giardia lamblia* assemblage B were used, but animals may contaminate sewage sludge and solid waste landfill leachates samples with all *Giardia* assemblages. In addition, MV was not effective against *Toxoplasma gondii* cysts (El-Nawawi *et al,* 2008). Although the destructive effect of thermal exposure by MV to the microorganism was the main, some bacteria had metabolic imbalance only after exposure (Mudhoo and Sharma, 2019). This explained the presence of 10% MV treated cysts as viable by the dye and at the same time their abilities to excyst or to induce infection in the present which was very low.

In the present study, MV irradiations on *Giardia* cysts showed failure of excystation was 98% in LD_{90} treated cysts compared to 10% in untreated control, as well as multiple structures changes showed by TEM. Most of the treated cysts were filled with one vesicular vacuole, but other contents were decreased in size, and shrinkage and sometimes disappeared completely with a small rim of granular cytoplasm surrounding the cyst wall. The reduction in number of viable *Giardia* cysts and ultrastructural changes agreed with Woo *et al.* (2000), they reported that the MV electromagnetic radiations that have a non-ionizing range with the high biological effects. Moreover, MV high thermal effect within the irradiating tissues and non-thermal effects in the absence of significant heating (Baniks *et al,* 2003), manifested at the cellular lvel (Morozov and Petin, 19980 particularly and partially at the molecular part; (Duhain *et al,* 2012). These effects were determined by the amount of penetrating and absorbed electromagnetic energy that is significantly converted into heat and other parts lead to the oscillation of ions and dipolar water molecules in tissue leading to irreversible destruction of the organisms (Rosenberg and Bögl, 1987; Yarahmadi *et al,* 2016). The biophysical processes induced in the microorganisms or parasites by thermal and non-thermal effects led to denaturation of proteins, nucleic acids, or other vital components, as well as disruption of membranes (Melgunov *et al,* 2019). At the same time, MVs did not reduce the nutri-

tional value of fresh vegetables such as vitamins and minerals any more than conventional cooking (Hoz *et al,* 2007; Duhain *et al* 2012). Although the MVs possessed the advantage of fast heating without any radioactivity (Pan *et al,* 2007), yet they require the technical expertise than the other methods of heating (Carole *et al,* 2014).

Conclusion

The excitation and infectivity rates were higher among cysts treated with MgO_2 NPs (10% & 33.4%) than MV treated ones, but the intensity of infection and pathological grades were similar. Despite infectious doses of giardiasis < 10 cysts but, treated cysts were hardly induce infection among mice. As to anti-*Giardia* effects, the microwave was superior to MgO NPs.

Author contributions: All authors equally contributed in this study.

Conflict of interest: The authors declare that they neither have any conflict of interest nor received fund

References

Abdelhalim, MNK, Abdelmottaleb, A, Moussa, SA, 2013: The gold nanoparticle size and exposure duration effect on the liver and kidney function of rats: In vivo. Saudi J. Biol. Sci. 20:177-81.

Al-Hazmi, F, Alnowaiser, F, Al-Ghamdi, AA, Al-Ghamdi, MM, Tuwirqi, A, *et al* 2012: A new large scales synthesis of magnesium oxide nanowires structural and antibacterial properties. Superlattices Microstructures 52, 2:200-9.

Al-Mohammed, HI, Albalawi, AE, Al Sadoun, H, Bakhitiari, N, Amraei, M, *et al,* 2021: Applying nanoparticles for treating *Giardia* infection: A systematic review. Int. J. Appl. Pharm. 13, 5:15-19.

Alvarado, A, Wasserman, M, 2006: Quick and efficient purification of *Giardia intestinalis* cysts from fecal samples. Parasitol. Res. 99:300-2.

Ankarklev, J, Jerlstrom-Hultqvist, J, Ringqvist, E, Troell, K, Sv, SG, 2010: Behind the smile: Cell biology and disease mechanisms of *Giardia* species. Nat. Rev. Microbiol. 8:413-22.

Arguello-Garcia, R, Cruz-Soto, M, Romero-Montoya, L, Ortega-Pierres, G, 2004: Variability and variation in drug susceptibility among *Giardia duodenalis* isolates and clones exposed to 5-nitroimidazoles and benzimidazoles in vitro. J. Antimicrob. Chemother. 54: 711-21.

Assadian, E, Zarei, MH, Gilani, AG, Farshin, M, Degampanah, H, *et al,* 2018: Toxicity of copper oxide (CuO) nanoparticles on human blood lymphocytes. Biol. Trace Elem. Res. 184: 350-7.

Avery, JC, Hoffmann, PR, 2018: Selenium, selenoproteins, and immunity. Nutrients 10: 1203. Doi: 10.3390/nu10091203

Baniks, S, Bandyopadhy, S, Ganguly, S, 2003: Bio-effects of microwave: A brief review. Bioresour. Technol. 87:155-9.

Barathmanikanth, S, Kalishwaralal, K, Sriram, M, Pandian, SRK, Hyung-Seop, Y**, H, *et al,* 2010:** Anti-oxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J. Nanobiotechnol. 8:16. Doi: 10.1186/1477-3155-8-16.

Bavand, Z, Gholami, S, Honari, S, Rahimi, E B, Torabi, N, *et al,* 2017: Effect of gold nanoparticles on *Giardia lamblia* cyst stage in vitro. Arak Med. Univ. J. 16:27-37.

Brakat, RM, Sharaf El-Deen, SA, Abd-Elhafiz HI, 2019: Zinc oxide nanoparticles kill *Giardia* species and protect against intestinal damage. Egypt. J. Med. Microbiol. 28:95-103.

Buzea, C, Pacheco, II, Robbie, K, 2007: Nanomaterials and nanoparticles: sources and toxicity. Biointerphases 2, 4:MR17-71.

CDC, 2011: Giardiasis case definition. Atlanta, GA: US Department of Health and Human Services, At http://wwwn.cdc.gov/nndss/script/ casedef. aspx?CondYrID=685&DatePub =1/1/2011

CDC, 2018: Giardiasis NNDSS Summary Repo rt for 2018. https://www.cdc.gov/healthywater/surveillance/giardiasis/giardiasis-2018.html#:~:text=Giardiasis%20is%20a%20nationally%20notifiable,local%20or%20state%20health%20departments

Cernikova, L, Faso, C, Hehl, AB, 2018: Five facts about *Giardia lamblia*. PloS Path. 14, 9: e1007250. Doi: 10.1371/journal.pp.1007250.

Chabra, A, Rahimi-Esboei, B, Habibi, M, Monadi, T, Azadbakht, M, *et al,* 2019: Effects of some natural products from fungal and herbal sources on *Giardia lamblia* in vivo. Parasitology 146:1188-98.

Chithrani, BD, Chan, WC, 2007: Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. Nano Letters 7:1542-50.

Duhain, GL, Minnaar, A, Buys, EM, 2012: Effect of chlorine, blanching, freezing, and microwave heating on *Cryptosporidium parvum* viability inoculated on green peppers. J. Food Prot. 75, 5:936-41.

El Shazly, AM, Elsheikha, HM, Soltan, DM, Mohammad, KA, Morsy, TA, 2007: Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. J. Egypt. Soc. Parasitol.37, 1:55-64.

El-Zawawy, LA, 2008: Impact of several control measures on the encysted metacercariae of heterophyids, J. Egypt. Soc. Parasitol. 38, 3:945-56.

El-Gendy, MA, Hammed, MP, Ghallab, M, Abdel Aziz, MO, Ibrahim, SM, 2021: Therapeutic effect of chitosan nanoparticles and metronidazole in treatment of experimentally giardiasis infected hamsters. Iran. J. Parasitol. 161: 32-42.

Elmi, T, Gholsmi, S, Fakhar, M, Azizi, F, 2013: A review on the use of nanoparticles in the treatment of parasitic infections. J. Mazand. Univ. Med. Sci. 23, 102:126-33.

El-Nawawi, FA, Tawfik, MA, Shaapan, RM, 2008: Methods for inactivation of *Toxoplasma gondii* cysts in meat and tissues of experimentally infected sheep. Foodborne Pathogol. Dis. 5, 5:687-90.

Escobedo AA, Cimerman S, 2007: Giardiasis: a pharmacotherapy review. Expert. Opin. Pharmacother. 8, 12:1885-902.

Feng, Y, Xiao, L, 2011: Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin. Microbiol. Rev. 24:110-40.

Franssen, F, Gerard, C, Cozma-Petruţ**, A, Vieira-Pinto, M, Jambrak A, *et al,* 2019:** Inactivation of parasite transmission stages: Efficacy of treatments on food of animal origin. Trends Food Sci. Technol. 83:114-28.

Gardner, TB, Hill, DR, 2001: Treatment of giardiasis. Clin. Microbiol. Rev. 14, 1:114-28**.**

Gliga, AR, Skoglund, S, Wallinder, IO, Fadeel, B, Karlsson, H, 2014: Size-dependent cytotoxicity of silver nanoparticles in human lung cells: The role of cellular uptake, agglomeration and Ag release. Part. Fib. Toxicol. 11:11. https: //doi.org/10.1186/1743-8977-11-11.

Graczyk, TK, Kacprzak, M, Neczaj, E, Tamang, L, Graczyk, H, *et al,* 2007: Human-virulent microsporidian spores in solid waste landfill leachate and sewage sludge, and effects of sanitization treatments on their inactivation. Parasitol. Res. 101, 3:569-75.

Graczyk, TK, Kacprzak, M, Neczaj, E, Tamang, L, Graczyk, H, *et al,* 2008: Occurrence of *Cryptosporidium* and *Giardia i*n sewage sludge and solid waste landfill leachate and quantitative comparative analysis of sanitization treatments on pathogen inactivation. Environ. Res. 106, 1: 27-33.

Hoz, A, Díaz-Ortiz, A, Moreno, A, 2007: Review on non-thermal effects of microwave irradiation in organic synthesis. J. Microw. Power Electromagn Energy 41:44-64.

Hussain, HT, 2015: Study the effect of microwaves radiation in the growth of some isolates fungi, J. Coll. Bas. Educ. 21, 89:173-84.

Hussein, EM, Ahmed, SA, Mokhtar, AB, Elzagawy, SM, Yahi, SH, *et al,* 2018: Antiprotozoal activity of magnesium oxide (MgO) nanoparticles against *Cyclospora cayetanensis* oocysts. Parasitol. Inter. 67:666-674.

Hussein, EM, Ismail, OA, Mokhtar, AB, Mohamed, SE, Saad, RM, 2017: Nested PCR targeting intergenic spacer (IGS) in genotyping of *Giardia duodenalis* isolated from symptomatic and asympto- matic infected Egyptian School Children. Parasitol. Res. 1165:763-71.

Idan, EM, Ardalan, NM, 2020: Introducing silver nanoparticles as anti-giardial in experimentally infected mice: Therapy versus toxicity Sys. Rev. Pharm. 11, 4:701-8.

Jarrell, E, Sener, K, 2003: Potential drug targets in cyst-wall biosynthesis by intestinal protozoa: Drug resistance updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy 6, 5:239-46

Jin, T, He, Y, 2011: Antibacterial activities of magnesium oxide (MgO_2) nanoparticles against food-borne pathogens, J. Nanoparticle Res.13: 6877-85.

Karanis, P, Ey, P, 1998: Characterization of axenic isolates of *Giardia intestinalis* established from humans and animals in Germany. Parasitol Res 84:442-9.

Laishram, S, Kang, G, Ajjampur, SSR, 2012: Giardiasis: A review on assemblage distribution and epidemiology in India. Indian J. Gastroenterol. 31, 1:3-12.

Lanone, S, Boczkowski, J, 2006: Biomedical applications and potential health risks of nanomaterials: Molecular mechanisms. Curr. Mol. Med. 6:651-63.

Leonelli C, Mason TJ, 2010: Microwave and ultrasonic processing: Now a realistic option for

industry: Chemical engineering and processing. Process. Intensif**.** 49: 885-900.

Malekifard, F, Tavassoli, KV M, 2020: In vitro assessment antiparasitic effect of selenium and copper nanoparticles on *Giardia deodenalis* cyst. Iran. Soc. Parasitol. 15:411-47.

Matica, A, Menghiu, G, Ostafe, V, 2017: Toxicity of chitosan based products. New Front Chem. 26, 1:65-74.

Mazaheri, N, Naghsh, N, Karimi, A, Salavati, H, 2019: In vivo toxicity investigation of magnesium oxide nanoparticles in rat for environmental and biomedical applications. Iran. J. Biotechnol. Biotech. 17, 1:e1543. Doi: 10.21859/ijb.1543.eCollection 2019 Jan

Melgunov, AN, Odoevsky, IM, Khrustalev, A V, Kurnosova OP, 2019: The Effect of microwave radiation on metacercaria of *Opisthorchuis* and larvae of *Trichinella spiralis*. Biol. Bull. 46, 3:251-7.

Mohsen, D, Abdel Hamid, M, 1993: Purification of *Cryptosporidium* oocysts and *G. lamblia* cysts using sucrose density gradients centrifugation. N. Egypt. J. Med. 9:888-9.

Morozov, II, Petin VG, 1998: Features of modifications of cytotoxic consequences of microwave and thermal heating. Radiatsionnaya Biol. Radioekol. 38:232-7.

Mudhoo, A, Sharma, SK, 2019: Microwave irradiation technology in waste sludge and wastewater treatment research. Crit. Rev. Environ. Sci. Technol. 41:999-1066.

Nkrumah, B, Nguah, SB, Nkrumah, B, *et al,* 2011: *Giardia lamblia*: A major parasitic cause of childhood diarrhea in patients attending a district hospital in Ghana. Parasit. Vectors 22, 4: 163

Nygard, K, Schimmer, B, Søbstad, Ø, Walde, A, Tveit, I, *et al,* 2006: A large community outbreak of waterborne giardiasis, delayed detection in a non-endemic urban area. BMC Public Health 6: 141. Doi: 10.1186/1471-2458-6-141.

Ortega, Y, Liao, J, 2006: Microwave inactivation of *Cyclospora cayetanensis* sporulation and viability of *Cryptosporidium parvum* oocysts. J. Food Prot. 69, 8:1957-60.

Pan, Y, Neuss, S, Leifert, A, Fischler, M, Wen, F, *et al,* 2007: Size-dependent cytotoxicity of gold nanoparticles. Small 3:1941-9.

Park, DK, Bitton, G, Melker, R, 2006: Microbial inactivation by microwave radiation in the home environment. J. Environ. Hlth. 69: 17-24. Quiz 39-40.

Plutzer J, Ongerth J, Karanis P, 2010: *Giardia* taxonomy, phylogeny, and epidemiology: Facts and open questions. Int. J. Hyg. Environ. Hlth. 213:321-3.

Rafiei, A, Roointan, ES, Samarbafzadeh, AR, Shayesteh, AA, Shamsizadeh, A, *et al,* 2013: Investigation of possible correlation between *Giardia duodenalis* genotypes and clinical symptoms in southwest of Iran. Iran. J. Parasitol. 8, 3: 389-95.

Raghupathi, KR, Koodali, RT, Manna, AC, 2011: Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir. 5:4020-8.

Ramanujam, K, Sundrarajan, M, 2014: Antibacterial effects of biosynthesized MgO_2 nanoparticles using ethanolic fruit extract of *Emblica Officinalis*. J. Photochem. Photobiol. 141:B296-300.

Rosenberg, U, Bögl, W, 1987: Microwave pasteurization, sterilization, blanching, and pest control in the food industry. Food Technol. 41, 6: 92-9.

Rougier, C, Prorot, A, Chazal, P, Leveque, P, Leprat, P, 2014: Thermal and non-thermal effects of discontinuous microwave exposure (2.45 gigahertz) on the cell membrane of *Escherichia coli*. Appl. Environ. Microbiol. 80, 16:4832-41.

Said, EF, El Samad, LM, YM, 2013: Viability of silver, chitosan, and curcumin nanoparticles as anti-*Giardia* agents. Parasitol. Res. 111:545-554.

Siles-Lucas, M, Casulli, A, Cirilli, R, Camena, D, 2018: Progress in the pharmacological treatm ent of human cystic and alveolar echinococosis: Compounds and therapeutic targets. PLoS-Negl. Trop. Dis. 12, 4:e0006422.

Speer, EC, Marchiondo, A, Mueller, B, Duszynski, W, 1979: Scanning and transmission electron microscopy of the oocyst wall of *Isospora lacazei*. Z. Parasitenkd. 59:219-25.

Strober, W, 2015: Trypan blue exclusion test for the cell viability. Current protocol in immunology 11: A3B 1-3.

Tang, Q, Jiang, X, Zhang, J, Wang, B, Liu, X, *et al,* 2020: Zinc oxide nanoparticles induce ferroptotic neuronal cell death in vitro and in vivo. Inter. J. Nanomed.15:5299-315.

Thompson, RC 2000: Giardiasis as a re-emerging infectious disease and its zoonotic potential. Int. J. Parasitol. 30: 1259-67.

Upcroft, P, Upcroft, A, 2001: Drug targets and

mechanisms of resistance in anaerobic protozoa. Clin. Microbiol. Rev. 14, 1:150-64.

Urbankova, L, Skalickova, S, Pribilova, M, Ridoskova, A, Pelcova, P, *et al,* 2021: Effects of sub-lethal doses of selenium nanoparticles on the health status of rats. Toxics 9:28. https://doi. org/ 10.3390/toxics 9020028.

Vidaček, S, De Las Heras, C, Solas, MIT, García, ML, Mendizábal, A, *et al,* 2011: Viability and antigenicity of *Anisakis simplex* after conventional and microwave heating at fixed temperatures. J. Food Prot. 74, 12:2119-26.

Ward, W, Alvarado, L, Rawlings, N, Engel, J, Franklin. C, *et al,* 1997: A primitive enzyme for a primitive cell: The protease required for excystation of *Giardia*. Cell 89:437-440.

Whanger, P, Vendeland, S, Park, YC, Xia, Y, 1996: Metabolism of sub-toxic levels of selenium in animals and humans. Ann. Clin. Lab. Sci. 26, 2:99-113.

Williamson A, O'Donoghue P, Upcroft P, 2000: Immune and patho-physiological response to different strains of *G. duodenalis* in neonatal mice. Int. J. Parasitol. 30:1259-67.

Woo, S, Rheeet, I, Park, H, 2000: Differential Damage in bacterial cells by microwave radiation on the basis of cell wall structure. Appl. Environ. Microbiol. 66,5:2243-7.

Xing, T, Bin-Fing, Lv, 2014: MgO nanoparticles as antibacterial agent: preparation and activity. Braz. J. Chem. Eng. 31:591-601.

Yadav, V, 2013: Nanotechnology, big things from a tiny world: A review, AEEE 3: 771-8.

Yarahmadi, M, Fakhar, M, Ebrahimzadeh, MA, Chabra, A, Rahimiesboei, B, 2016: The anti-giardial effectiveness of fungal and commercial chitosan against *Giardia intestinalis* cysts in vitro. J Parasit Dis 40:75–80.

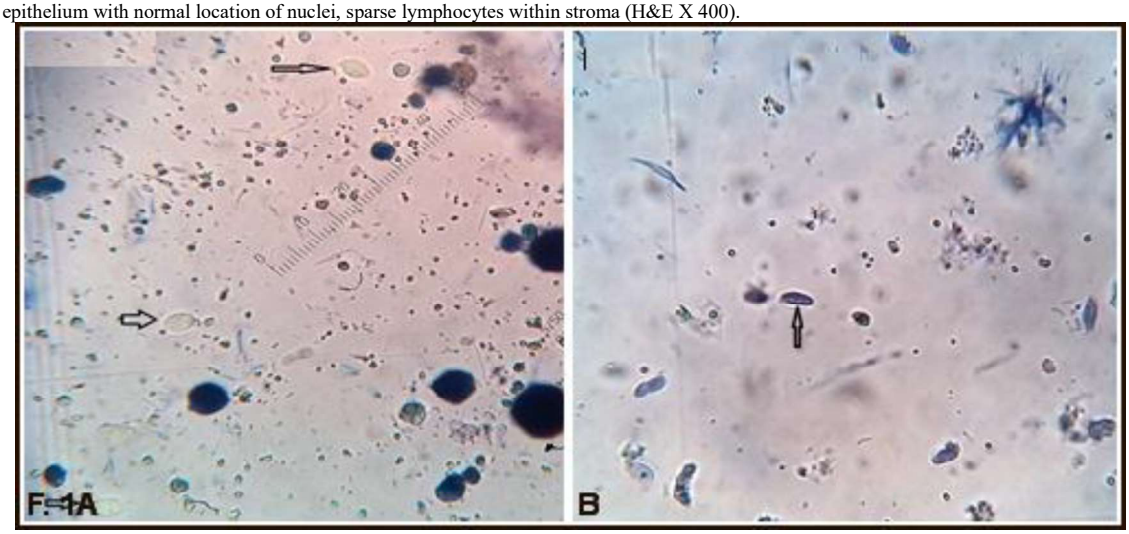
Explanation of figures

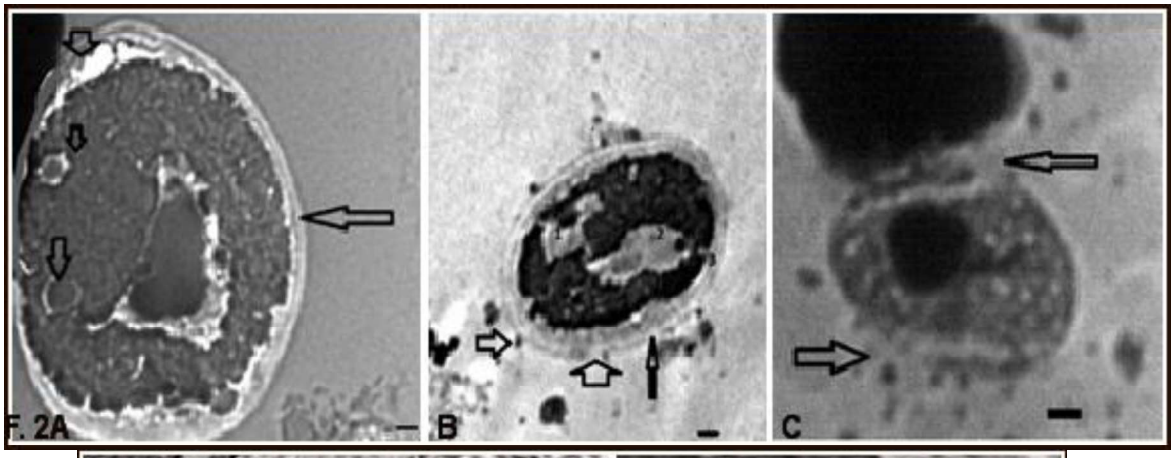
Fig. 1: A & B showed unstained viable and stained (non-viable) with Trypan blue X400.

Fig. 2: TEM of untreated and treated cyst; A: untreated cyst, long arrow showed intact cyst wall, short two arrows of two nuclei, and short and thick arrow showed white intra-cystic extra-cytoplasmic fluid. B: MgO NPs treated cyst containing two intra-cytoplasmic big vacuoles (1 &2) and injury of cell wall leads to leaking content inside cytoplasm (3), C: Injury of cyst wall in two sides with releasing of contents outside and highly vacuolated cytoplasm. D: Microwave treated cysts increase in size intact cell wall with decrease in thickness with big cytoplasmic vacuolated and shrinkages of content. E: Cyst treated with microwave showed cytoplasmic destruction into small peripheral fragments (two black arrows) and central part.

Fig. 3: A: Control negative of duodenal mucosa showed normal villous architecture with normal location of nuclei within lining columnar epithelium, sparse lymphocytes within stroma. B: small intestine of mice inoculated with untreated cyst showed duodenal mucosa with villous deformity & hyperplastic elongated crypts in epithelium with frequent mitotic figures, lamina propria with marked inflammatory infiltrate formed mainly of neutrophils, some plasma cells and lymphocytes, some neutrophils attacked glands. C: MVs treated cyst infected mouse with histopathologic Grade 1changes, villi finger like, with little oedema, lymphocytic infiltration (H&E, X 400).

Fig. 3: D Mice inoculated with nano-treated grade 1: Duodenal mucosa showed blunting of villi and increased crypts. Cores of villi show mild inflammatory infiltrate. E: MVs treated mouse with grade I: Duodenal mucosa showed blunting of villi, lining epithelium with scattered nuclei transferred to more superficial location. Cores of villi with mild inflammatory infiltrate mainly of lymphocytes and plasma cells. F: Majority of m ice inoculated with microwave treated cysts (98%), duodenal mucosa showed normal villous architecture, lining columnar epithelium with normal location of nuclei, sparse lymphocytes within stroma (H&E X 400).




F. 2A
B
C

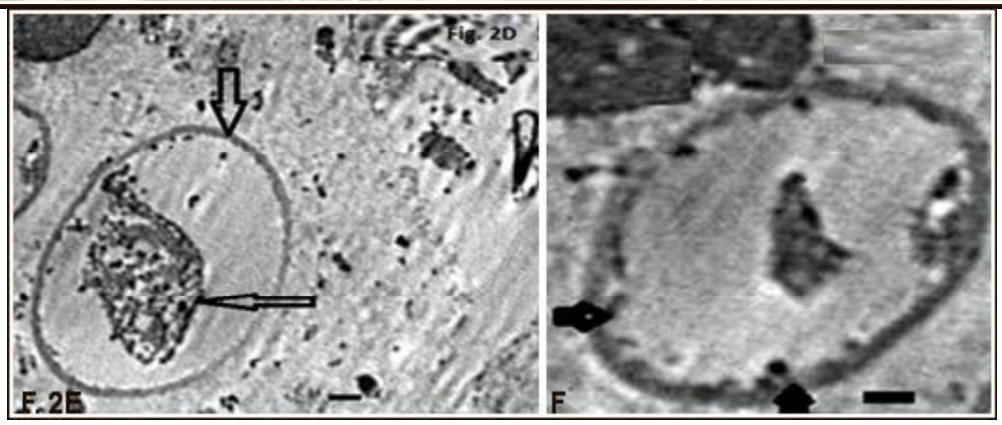
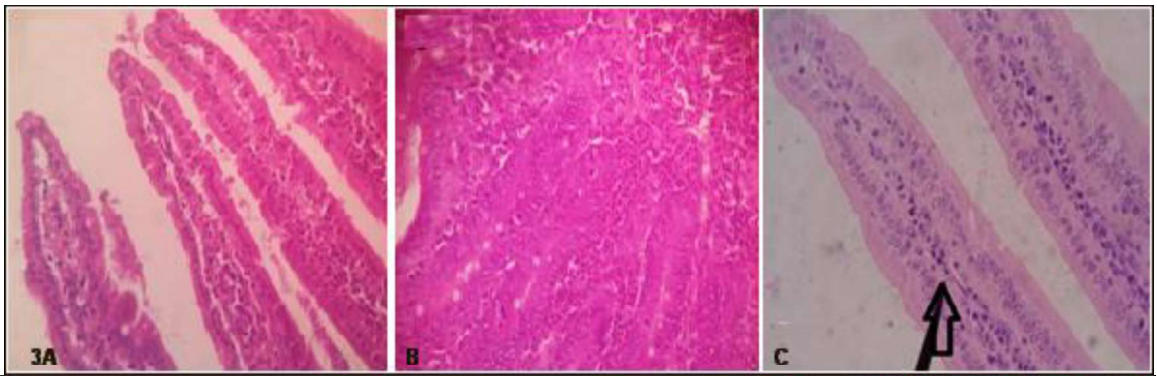


Fig. 2D
F. 2E
F


3A
B
C


F. 3
D
E
F