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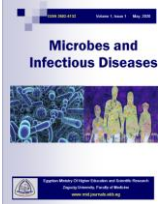

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Original article

Efficacy of olibanum and propolis medicinal extracts versus metronidazole in *Giardia lamblia* experimentally infected mice

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ABSTRACT

Background: Giardiasis is a common intestinal infection, recently included by the World Health Organization in the 'Neglected Diseases Initiative'. Despite the efficacy of nitroimidazoles; the main antigiardial chemotherapeutics, adverse effects and resistance enforced developing non-chemical alternatives. The present study aimed to assess the therapeutic efficacy of ethanol extract of olibanum (OL), propolis (PR), and their combination versus metronidazole (MTZ) against *Giardia lamblia* (*G. lamblia)* infection. **Methods:** Sixty Swiss male albino mice were randomly divided into 6 groups; 10 mice each, Group I: normal control (non-treated; non-infected). Group II: infected with *G. lamblia* cysts, non-treated. On the 6th day post infection (dPI), the remaining 4 infected groups were treated orally with: Group III: (OL). Group IV: (PR). Group V: combination of (OL+PR). Group VI: (MTZ). These mice were subjected to direct parasitological diagnosis of *Giardia* trophozoite in intestinal exudate, immunochromatographic test for antigen detection and histopathological studies. **Results:** After 7 days therapy, complete clearance of *Giardia* trophozoites were in the combination of (OL+PR) and MTZ groups therapy. Lower percentages of reduction (91%) & (83%) were recorded in PR and OL-treated groups, respectively. Histopathological examination showed marked healing of intestinal mucosa using non-chemical combination and different degrees of dysplasia using MTZ, while partial healing was observed using olibanum and propolis separately. **Conclusion:** Olibanum, PR and their combination were proved to enhance the clearance of *Giardia* trophozoites; with progressive improvement of the histopathological changes of jejunal mucosa, making good non-chemical alternative antigiardial therapeutics sidestepping the obstacles of MTZ like dysplasia and teratogenicity.

Introduction

Giardia lamblia is a very common intestinal protozoan causing diarrhea among children and affects about 200 million people per year; mainly in Africa, Asia and America [1]. Malabsorption, abdominal pain and loss of weight are the main complaints leading to delayed growth and development with mild self-limiting illness or a several months-chronic illness [2].

Microscopy using wet mount or iodine stain is the gold standard methodology for *G. lamblia*

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diagnosis [3]. However, false-negative results due to intermittence and low number of the cysts shed in fecal samples may be encountered. To overcome these obstacles, a combination of diagnostic methods is frequently indicated [4]. In the past years, several complementary, immunological diagnostic methods for giardiasis have been encountered. Immunochromatographic devices are promising tools in the diagnosis being reliable in identifying the positive and negative stool samples. Superiority of immunochromatographic method over conventional microscopy is linked to its capability of detection of minimal quantities of antigens, even with low burden of the parasite, wherever several fecal samples are required by conventional microscopy, especially with the intermittent cyst shedding in chronic infections [5], or damaged cysts with frozen fecal samples [6].

Commercial antigiardial drugs include nitroimidazoles e.g. metronidazole (MTZ); benzimidazoles, e.g. fenbendazole; and paromomycin [7]. However, most of these chemotherapeutics may cause severe side effects and are not well tolerated by human and animals [8]. Moreover, clinical failure and drug resistance were also detected [9]. Hence, many studies tried to identify new natural antigiardial alternatives [10]. In traditional medicine, olibanum (OL) or frankincense; a natural *oleogum* resin from *Boswellia* species, is often used for the treatment of variety of diseases. Animal experiments showed evidence based anti-inflammatory activity of OL [11]. The immunostimulant, immunomodulatory, and anti-leukotriene activities are added values to its use in several immune disorders [12]. **Abdalla et al.** [13] have defined the antiparasitic activity of OL in vivo and using in-vitro culture of *G. lamblia* trophozoites on TYI-S-33 medium. Propolis (PR) extracts have antiseptic, anti-inflammatory, antioxidant, and anticancer activities. Moreover, antimicrobial besides antiulcer and immunomodulatory properties could be detected [14]. Propolis was promisingly proposed to manage giardiasis with minimal side effects [15].

This study was conducted to assess the therapeutic efficacy of OL, PR, and their combination versus MTZ as non-chemical therapeutic alternatives for control of *G. lamblia* infection in experimentally infected mice.

Material and Methods

Parasites purification

Fresh stool samples containing at least five *G. lamblia* cysts by high power field in a routine saline smear and free from other parasites, were obtained from 3 heavily infected patients attending the Outpatient Clinic of the Pediatric Department of Zagazig University Hospital. Emulsification of the samples in saline and sieving were done to remove the large particles. The cysts were concentrated by repeated centrifugation (2000 r.p.m for 5 min.) and washing in saline. After the last washing, the deposit was mixed thoroughly with normal saline and the number of cysts in the suspension was adjusted by the haemocytometer to be 500,000 cysts/ ml [16].

Herbal preparation and extraction

Olibanum was purchased from Egyptian market as solid whitish masses. Chemical identification was carried out according to **Abdallah et al.** [13]. Ten grams were extracted with 50 ml absolute ethanol. The combined ethanol extracts were filtered and evaporation using a rotator evaporator and freeze dryer. Ethanol (70%) was added to the dried extract to obtain a concentration of 200 µg /ml solvent. Propolis (Biopropolis) tablets 400mg [Sigma, Egypt]: The drug was given orally in the form of aqueous suspension in a single daily dose of 1.04 mg/0.2ml/mouse (Paget and Barnes, 1964). Chemotherapy: Metronidazole (Flagyl) tablets 500mg [EPICO, Egypt]: The drug was given orally in the form of aqueous suspension in a daily dose 1.37 mg/0.2ml/mouse [17].

This experimental study was carried out at Medical Parasitology Department, Faculty of Medicine, Zagazig University and Theodor Bilharz research institute (TBRI), on 60 apparently healthy laboratory bred male Swiss albino mice, with a weight range of 20-25grams, aged six to eight weeks old. Mice were kept in numbered clean cages, maintained on stock diet and kept under fixed appropriate conditions of housing and handling in animal house of TBRI. Every day, food and water containers, wire inserts were thoroughly washed by hot water. All mice were maintained according to the research protocols following the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals, and as approved by ethics committee of TBRI. Mice were treated for three consecutive days with metronidazole solution (10 mg/mouse/day), which was administered orally. One week after treatment, three consecutive fecal examinations were performed prior to experimental infections. This treatment ensured that mice were free from all possible protozoan infections.

Out of the current prepared suspension, containing 500,000 *G. lamblia* cysts, one inoculum of 100.000 cysts/0.2ml/mouse was aspirated in a tuberculin

syringe to which a blunt tipped needle was applied. The mouse was held by the left hand slightly bent backwards. The curved needle was gently and carefully introduced into the pharyngeal opening and the inoculum dose was slowly administered into the stomach [16]. Successive stool samples from each mouse were collected in a dry labeled, wide-mouth plastic container with tight fitting cover to be immediately examined by iodine-stained smears either directly or by concentration method. To confirm the induction of infection, stool samples from animals were checked daily for the presence of *G. lamblia* cysts by iodine-stained smears.

Treatment with the two medicinal extracts and metronidazole was started on the 6th day post-infection (dPI); [peak of intestinal colonization], for seven consecutive days. Mice were randomly divided into six groups (10 mice each): GI (Control normal): Normal control (non-treated and non-infected mice). GII (Control infected): Infected without receiving any treatment. GIII (OL): Infected and treated intragastrically with 25µl of the olibanum extract, dissolved in 70% ethanol, was given in a single daily dose. Its chemical identification was performed by **Mikhaeil et al.** [12]. GIV (PR): Mice infected and treated with 1.04 mg/0.2ml/mouse, was given in a single daily dose. GV (Combination of OL&PR): Full doses of both olibanum and propolis extracts, were given in a single daily dose [18]. GVI (MTZ): Metronidazole was given orally in the form of aqueous suspension in a single daily dose (1.37 mg/0.2ml/mouse) [17].

Parasitological assay: Mice were sacrificed, and intestinal washes were examined on the following different (8th, 12th and 15^{h} dPI). The proximal 1 cm segment of each mouse jejunum was removed, placed in 1 ml of chilled sterile Phosphate buffered (PBS) saline, and after 15 minutes on ice was vortexed for 30 seconds to release trophozoites from intestinal wall. Trophozoites were counted on a haemocytometer; at least four separate quadrants (grids) on the haemocytometer were counted for each mouse since a single parasite on one grid corresponds to X 10^4/ml [19]. The percentage reduction (%R) in the parasite count was calculated according to the following equation: %R= 100 (C-E)/C, where C: infected control group and E: Experimental groups of mice [20].

Detection of *G. lamblia* antigen in stool samples of the mice on the 8th, 12th and 15th dPI was done by a quick immunochromatographic test using commercially available RIDA-Quick (R-BiopharmGmb H, Darmstadt, Germany) kit. The method was done according to manufacturer's instructions. Stool samples were considered positive if *red* and *blue* bands were seen in the strips and were considered negative if only the *blue* band was visible in the test strips. The test is considered invalid if no bands appear or a combination other than the one described above or other changes in band colour. Likewise, changes in band colour which appear after 10 minutes or later are also without any diagnostic value and must not be used for evaluation [21].

Histopathological examination: Duodenal and jejunal specimens of sacrificed mice, on the 12th and 15th dPI, were fixed in 10% formalin and kept as paraffin blocks. Microtome sections were cut at a thickness of 4 µm, stained with (H&E), then experienced histopathological examination to assess the histopathological changes that occurred during the infection and detect the degree of affection of mucosa after treatment [22].

Data are presented as mean and standard deviations (± SD). Statistical significance was determined by two-way ANOVA, followed by a post hoc Bonferroni test, one-way ANOVA with Tukey's Multiple Comparison Test, unpaired Student’s t-test for selected pairs of data using Graph Pad Prism version 5 (Graph Pad Software). *P values >0.05* are considered non-significant. Significant *p value<0.05* and highly significant *P value <0.001*.

Results

In comparison to control infected group (GII), there was a high significant difference among all studied groups (*P<0.001),* and a significant decrease in the mean *Giardia* trophozoite count in all treated groups (*P<0.05*) on the entire 8th, 12th and 15th dPI. Also, (GIII & GIV) showed significant decrease in trophozoite count [Reference drug] (*P<0.05*), but (GV) showed significant difference (*P>0.05*) on the 8th and 12th dPI but significantly different (*P>0.05*) on the 15th dPI in comparison to (GVI) **(Table 1**). Percentage reduction of trophozoite count in intestinal washes showed that (GIII & GIV) showed significant increase (*P<0.05*) in comparison to GVI on the entire 8th, 12th and 15th dPI. But, no significant difference *(P>0.05*) in percentage of reduction was found between (GV&GVI) **(Table 2**).

On the entire 8th, 12th and 15th dPI, all the tested treated groups showed significant decrease in number of positive Immunochromatographic Test (ICT) in comparison to control infected group (*P<0.05*). Also, there was significant difference in

number of positive ICT+ve of GIII&GIV in comparison to GVI (*P<0.05*). On the other hand, no significant difference (*P>0.05*) in ICT +ve test number was found between (GV&GVI) **(Table 3)**.

Inflammatory cell infiltrate count, on the entire 8th, 12th and 15th dPI showed highly significant difference between all the studied groups (*P<0.001*), and a significant decrease in all tested treated groups in comparison to GII (*P<0.05*). Also, (GIII & GIV) showed significant decrease in comparison to (GVI); (*P<0.05*), but (GV) showed no significant difference (*P>0.05*) in comparison to (GVI). Percentage reduction of inflammatory cell infiltrate count showed that (GIII & GIV) showed significant increase (*P<0.05*) in comparison to (GVI) on the entire 8th, 12th and 15th dPI. But, no significant difference (*P>0.05*) in percentage of reduction was found between (GV&GVI) **(Table 4)**.

Comparing the groups to normal intestinal histopathology, GII showed marked changes in the form of marked infiltration of lamina propria with inflammatory cells with fusion of villi, goblet cell depletion and heavy *Giardia* trophozoite infection on the 12th and 15th dPI **(Figures 3, 4,5)**. After treatment, histopathological improvement on the 15th dPI appeared in sections of the small intestine from mice of (GIII, IV, V&VI) compared to GII. GIII showed mild improvement in the histopathological changes on the 15th dPI, as intestinal mucosa showed mild to moderate inflammatory cell infiltrate, normal goblet cells and no *Giardia* trophozoite attached to the villi (**Figures 7, 8**). GIV showed a moderate improvement on the 15th dPI in the form of moderate inflammation with no organism in between the villi and normal goblet cells **(Figure 10)**. GV showed high degree of improvement on the 15th dPI in the form of marked healing of intestinal mucosa with clearance of *Giardia* infection and nearly intact villi (**Figure 12**). GVI showed the highest degree of improvement on the 15th dPI in the form of marked healing of intestinal mucosa, preservation of brush border, with clearance of *Giardia* infection and nearly intact villi but with mild cell dysplasia **(Figure 14,15)**, and moderate degree of dysplasia with mitotic figures **(Figure 16)**.

Table 1. Mean counts of *G. lamblia* trophozoites (x 10^4/ml) from intestinal washes of infected mice treated with different drugs at different post-infection days (dPI).

Groups	Days Post infection		
	8th dPI	**12th dPI**	**15th dPI**
Infected control	32.21±0.45	64.40±0.96	79.12±0.10
Olibanum (OL)	22.21±0.31 # @	19.50±0.40 # @	13.13±0.13 # @
Propolis (PR)	16.65±0.25 # @	11.21±0.65 # @	6.77±0.10 # @
Combined OL & PR	5.64±0.15# @	2.16±0.15 # @	00 #
MTZ	2.10±0.05 #	0.09±0.03 #	00 #
F	19490	22604	1.530e+006
P	*< 0.001*	*< 0.001*	*< 0.001*

(@) = *p< 0.05* compared to MTZ group. (#) = *p< 0.05* compared to infected control group. Data are presented as mean ± SD, and analyzed by 2-way ANOVA, followed by a post hoc Bonferroni test.

Table 2. Percentage of reduction in *Giardia* trophozoite in intestinal wash among different treated groups on different post-infection days (dPI).

Groups	Percentage of reduction		
	8^{th}dPI	**12th dPI**	**15th dPI**
Olibanum	31 $^{@\#}$	70 $^{@\#}$	83 $^{@\#}$
Propolis	48 $^{@\#}$	83 $^{@\#}$	91 $^{@\#}$
Combined OL&PR	82	97	100
MTZ	93	100	100

(@) =*p< 0.05* compared to MTZ-group. (#) = *p< 0.05* compared to combination group. Data are analyzed by Chi-square test.

Table 3. Percentage of Immunochromatography positive mice (ICT +ve) for *Giardia* copro-antigen on different days of assessment.

Study groups	Percentage (%) of ICT +ve mice		
	8^{th}dPI	**12th dPI**	**15th dPI.**
Infected control	100	100	100
OL	60 $^{@\ \#}$	40 $^{@\ \#}$	30 $^{@\ \#}$
PR	40 $^{@\ \#}$	30 $^{@\ \#}$	20 $^{@\ \#}$
Combined OL&PR	20 $^{\#}$	10 $^{\#}$	0 $^{\#}$
MTZ	10 $^{\#}$	10 $^{\#}$	0 $^{\#}$

(@) = *p<0.05* compared to (MTZ). (#) = *p<0.05* compared to infected control. Data are analyzed by Chi-square test.

Table 4. Mean count and percentage reduction (R%) of inflammatory cell count/ H.P.F in duodenum and jejunum of sacrificed mice on the 12th and 15th days PI stained with hematoxylin and eosin (H&E) stain.

Groups	**12th day post infection**		**15th day post infection**	
	inflammatory cell count	**R%**	**R%**	**inflammatory cell count**
Normal control	25.9 ± 3.5			
Infected control	93.9±2.7			102.4 ± 4.25
OL	82.1±4.3 $^{\#\ @}$	12.57	37.99	63.5 ± 6.2 $^{\#\ @}$
PR	77.4±3.1 $^{\#@}$	17.57	43.75	57.6 ± 3.6 $^{\#@}$
Combined OL& PRRP	57.8±6.2 $^{\#}$	38.45	74.41	26..2 ± 5.0 $^{\#}$
MTZ	53.4±3.9 $^{\#}$	43.13	72.36	28.3 ± 2.5 $^{\#}$
F	355.0	1.323		551.9
P	*< 0.001*	*0.3838*		*< 0.001*

(@) = *p< 0.05* compared to MTZ group. (#) = *p< 0.05* compared to infected control group. *Data are analyzed by 2-way ANOVA, followed by a post hoc Bonferroni test.

Figure 1. Jejunal section of non-infected, non-treated control mouse (GI) on the 12th dPI showing normal intact villi with normal inflammatory cell infiltrate with normal goblet cell content (X100, H&E). **Figure 2.** Jejunal section of non- infected non-treated control mouse (GI) on the 15th dPI showing normal intact villi with normal inflammatory cell infiltrate with normal goblet cell content (X 400, H&E).

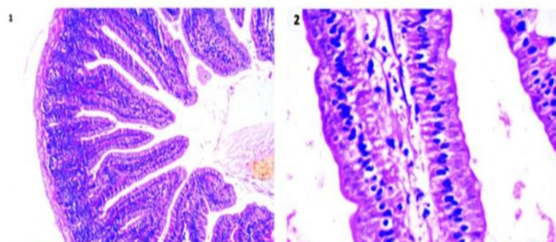


Figure 3. Jejunal section of an infected non-treated mouse (GII) on the 12th dPI showing with goblet cell depletion with heavy giardial infection, Arrow to *Giardia* Trophozoite (X1000 H&E). **Figure 4.** Jejunal section of an infected non-treated mouse (GII) on the 12th day PI showing marked infiltration of lamina propria with inflammatory cells with goblet cell depletion with heavy *Giardia* trophozoite infection (X400, H&E). **Figure 5.** Jejunal section of an infected non-treated mouse (GII) on the 15th dPI showing marked infiltration of lamina propria with inflammatory cells with fusion of villi and goblet cell depletion with heavy giardial trophozoite infection; arrow (X400, H&E).

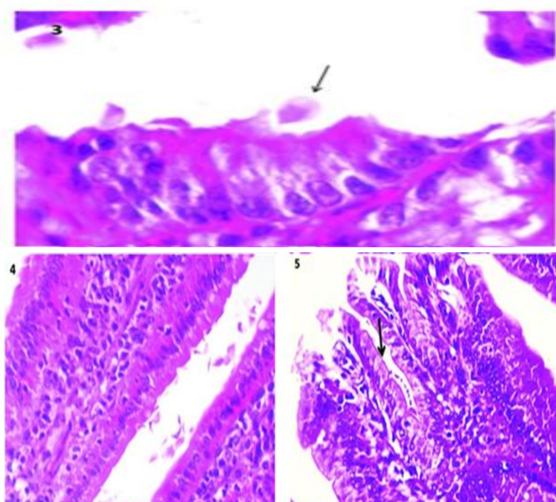


Figure 6. Jejunal section of an infected olibanum-treated mouse (GIII) on the 12th dPI showing moderate infiltration of lamina propria with inflammatory cells with goblet cell depletion and *Giardia* trophozoites in between villi (X400, H&E).

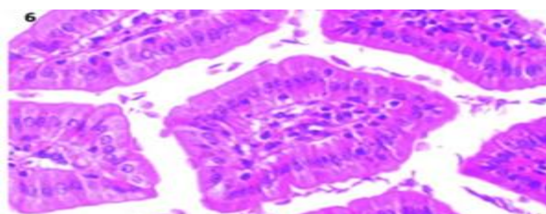


Figure 7. Jejunal section of an infected olibanum-treated mouse GIII) on the 15th dPI showing mild infiltration of lamina propria with inflammatory cells, goblet cells begin to be restored, no *Giardia* trophozoites in between villi (X200, H&E). **Figure 8.** Jejunal section of an infected olibanum-treated mouse (GIII) on the 15th dPI showing mild infiltration of lamina propria with inflammatory cells, goblet cells begin to be restored, no *Giardia* trophozoites in between villi (X400, H&E).

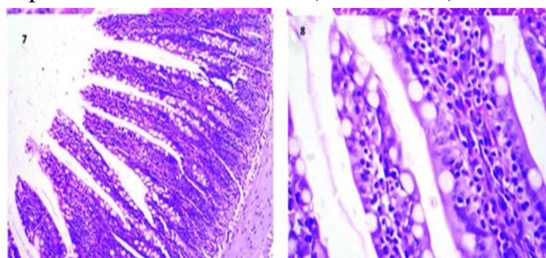


Figure 9. Jejunal section of an infected propolis-treated mouse (GIV) on the 12th day PI showing mild to moderate infiltration of lamina propria with inflammatory cells with goblet cell depletion with *Giardia* trophozoites in between villi (X400, H&E).

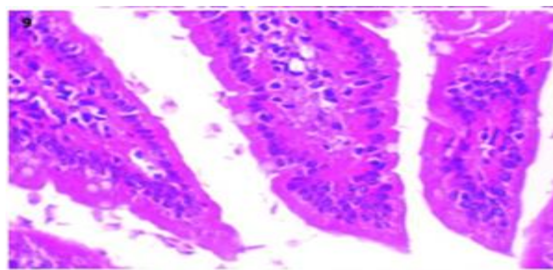


Figure 10. Jejunal section of an infected propolis-treated mouse (GIV) on the 15th dPI showing mild infiltration of lamina propria with inflammatory cells with goblet cells begin to be restored, no *Giardia* trophozoites in between villi (X400, H&E). **Figure 11.** Jejunal section of an infected combination-treated mouse (GV) on the 12th dPI showing mild infiltration of lamina propria with inflammatory cells with goblet cell depletion with *Giardia* trophozoites in between villi (X400, H&E).

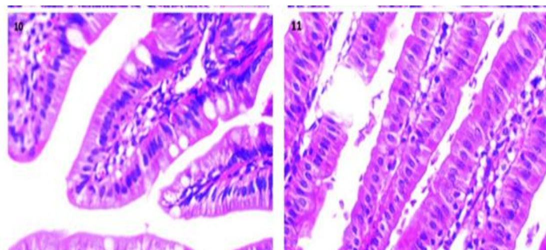


Figure 12. Jejunal section of an infected combination-treated mouse (GV) on the 15th dPI showing normal infiltration of lamina propria with inflammatory cells with goblet cells restored to be normal, no *Giardia* trophozoites in between villi (X400, H&E). **Figure 13.** Jejunal section of an infected MTZ-treated mouse (GVI) on the 12th day PI showing mild infiltration of lamina propria with inflammatory cells with goblet cell depletion and *Giardia* trophozoites in between villi (X400, H&E).

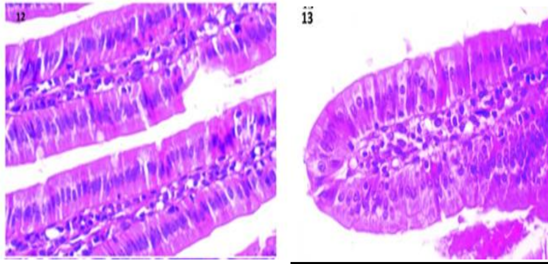


Figure 14. Jejunal section of an infected MTZ -treated mouse (GVI) on the 15th dPI showing with mild cell dysplasia, normal infiltration of lamina propria with inflammatory cells with goblet cells restored to be normal, no *Giardia* trophozoites in between villi (X400, H&E). **Figure 15.** Jejunal section of an infected MTZ-treated mouse (GVI) on the 12th dPI revealing mild cell dysplasia, (X1000, H&E).

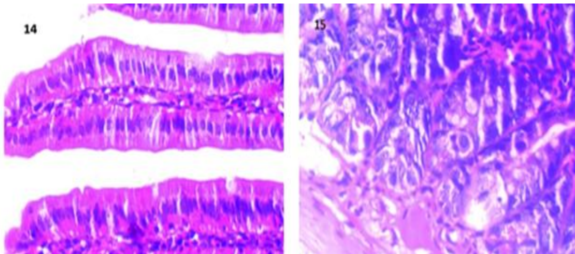
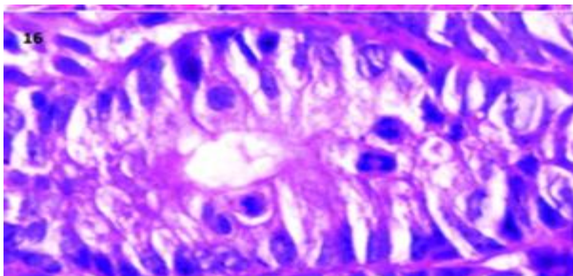


Figure 16. Jejunal section of an infected MTZ-treated mouse (GVI) on the 15th dPI revealing moderate cell dysplasia with mitotic figures, (X1000, H&E).



Discussion

Giardia lamblia is a flagellated protozoan parasite inhabiting the small intestine. It contributes to global waterborne outbreaks of acute and persistent fatty diarrhea of all ages. Additionally, it may be complicated with sugars and fats malabsorption, chloride hypersecretion and increased intestinal transit [23]. Chemotherapeutics like MTZ, as a first-line treatment, nitroimidazoles, benzimidazoles, and their derivatives have been also used for giardiasis [24]. Despite their efficacy; relapses and proved carcinogenicity in experimental animals are still obstacles in use [25]. Dangerous adverse reactions e.g. leukopenia and neurotoxic effects as ataxia, seizures and vertigo, sometimes lead to chemotherapeutic discontinuation [26]. Treatment failures may also occur Thus longer repeated courses, higher doses, or even changing drug-class could be helpful to avoid the potential cross-resistance. In this context, combination therapy (CT) is emerging as a valuable option against refractory giardiasis [27], but increased parasite resistance to such drugs rendered urgent identification of novel, effective, non-chemical and safe agents for control of giardiasis [10].

With the hope of shedding some light on the natural alternative medicine in treatment of giardiasis, the present work was designed to study the effect of OL (*Boswellia serrate)*, and PR (Biopropolis tablets) on experimental *Giardia* infection and their combined effect versus metronidazole therapy.

Olibanum or frankincense, the natural oleo-gum-resin, is a term to describe oleo (oily in nature) gum (partly soluble in water) resin (partly or wholly soluble in alcohol) [12]. It comprises an acid resin (Boswellic acid) (56-60%), gum (30-36%), and volatile oil (3-8%). Ether soluble resin can be extracted from OL-gum [28]. **Borrelli et al.** [29] observed the OL-predominant anti-inflammatory activity to alleviate the gut functional troubles by improving motility, inhibiting diarrhea without constipation, inhibiting contraction of intestinal smooth muscles and control acetylcholine and barium chloride induced diarrhea [30]. So, OL showed an evidence in ulcerative colitis [31], Crohn's disease [32], and collagenous colitis [33]. *Boswellia*'s antioxidant activity is the mechanism involved in the physiologic maintenance of the integrity and function of the enterocytes [34], where the epithelial barrier is involved in *Giardia* infection [35] and OL presents benefits in this track [36].

Propolis was promisingly proposed to manage giardiasis as a natural source with the benefit of minimal level of side effects. Its crucial anti-giardial efficacy could be endorsed by the antioxidant effect of flavonoids concentrated in PR [37], or immunomodulatory properties via augmenting nonspecific host defense mechanisms after macrophage activation [38, 39]. Also, **Soufy et**

al. [40] showed the benefits of ethanolic and water extracts of Egyptian PR at a dose of 50 mg/kg against immunosuppressed *Cryptosporidium* infected rats, resulting in significant reduction of oocysts count in stool. The maximal reduction reached 89% on the 7th dPI. Its antiparasitic effect may be attributed to its phenolic compounds, which could enhance the oxidative defense mechanisms [41] or stimulate the immune system [42] leading to increased antibody titers with the reduction of oocysts shedding [43].

In the current study, OL treated group showed significant decrease in mean count of *Giardia* trophozoites in intestinal wash in comparison to control infected group. Similar results were obtained by **Abdallah et al.** [13] who found that OL of 10, 15, and 20 mg/kg/day inhibited *G. lamblia* multiplication *in vivo* in a dose-dependent manner. It improved the histopathological changes caused by infection in the duodenum and jejunum. These results confirm the antiparasitic effects of these medicinal herbs on *G. lamblia*, as a promising alternative to MTZ. Moreover, all treated groups showed highly significant decline in *G. lamblia* trophozoites from intestinal washes of infected mice treated with different drugs in comparison to GII with successive improvement in trophozoite reduction on the 8th, 12th and 15th dPI, after completing all therapeutic doses of all regimens. Similar results were reported by **Abd El Fattah and Nada** [44] who found PR gave a highly significant decrease in *Giardia* trophozoite count than that obtained by MTZ on the 6th dPI, but the efficacy was almost equal to that detected on the 12th dPI. However, **Freitas et al.** [45] stated that Brazilian PR triggered inhibition of *Giardia* trophozoites growth *in vitro*, the level of which varied according to its concentration and incubation times. The lowest concentrations of PR (7.81 and 15.63 mg/ml) failed to affect parasites growth, while higher ones (31.25 mg/ml) showed an effect and the highest giardial growth reduction was in cultures exposed to 125, 250 and 500 mg/ml of extract at all incubation periods, with the highest reduction of trophozoite adherence to 67.9% and 69.5% of 250 and 500 mg/ml concentrations respectively, while MTZ reduced trophozoite adherence to 100%. These findings agreed with **Thabet and Abdel-Fattah** [46] who studied the inhibitory effect of Egyptian PR extract on *G. lamblia* trophozoite *in vitro*, where 250 µg/ml concentration of it showed growth reduction by (90.7%); after 72h, while on increasing its dose, reached 100% compared to (83.3%) reduction obtained by MTZ, while its inhibitory effect on parasite adherence was the same as that obtained by MTZ. They also showed that PR in a dose 500µg/ml exerted 100% adherence reduction after 72h.

The previous results were confirmed by Immunochromatographic Test (ICT) which revealed that all treated groups showed significant decrease in number of positive ICT on the entire 8th, 12th and 15th dPI in comparison to control infected group. On the other hand, no significant difference found between the tested natural therapeutic medicine (GIII&GIV) in comparison to each other and in comparison, with MTZ. While (PR & OL) combination produced results close to MTZ with the benefits of minimal level of side effects during treatment.

Experimental giardiasis eventually creates a physical barrier between the enterocytes and the lumen, villus atrophy, crypt hyperplasia, further interfering with nutrient absorption, intestinal hyperpermeability, and brush border damage [47], goblet cell depletion and shortening of the microvilli [48] with the resultant diminished absorptive surface area [49]. In the present study, non-chemical therapeutic trials by OL and PR for giardiasis resulted in enhanced brush border surface area. On the 12th dPI, GIV revealed destruction of the villi due to desquamation, sloughing of epithelial cells and complete cell necrosis. Broadening, fusion and flattening to tips of villi, crypt hyperplasia with abnormal villous/crypt ratio were also noticed with inflammatory cell infiltrate within the lamina propria. However, on the 15th dPI, improvement in villous architecture was noticed with clearance of *Giardia* trophozoites. This was in accordance with **Buret** [50], who detected also increased intestinal disacharidase activity and decreased intraepithelial lymphocytes. **Buret** [51] also added that shortening of the microvilli could aggravate the deficiency in enzymatic and absorptive capability of enterocytes. However, **Chin et al.** [47] detected apoptotic changes of enterocytes in giardiasis infection. Close results were reported by **Abdel-Fattah and Nada** [44] who reported that combined use of MTZ and PR caused an immunological balance that saved normal architecture of villi. In this study, GVI; on the 12th dPI showed mild shortening, blunting and fusion of the villi with focal cellular infiltration within the lamina propria. Then, on the 15th dPI, there was pronounced improvement of mucosal

changes with normal villous architecture, with increasing degrees of dysplatic changes and mitotic figures. Close reports were recorded by **Eissa and Amer** [52] described MTZ treated mice with lamina propria mild inflammatory infiltrate, focal shortening of the villi and the presence with few *G. lamblia* trophozoites. Moreover, **Ammar et al.** [53] observed partial healing of the intestinal mucosa and villous crypts ratio of *Giardia* infected hamsters treated with MTZ.

Collectively, combination of propolis and olibanum gave better results than using them individually and near-by results to MTZ, confirmed by the used parameters. These results may be attributed to the augmented anti-inflammatory, antioxidant and immune-stimulant effects of both of them. Furthermore, their combination may be involved in the physiologic maintenance of the integrity and function of the intestinal epithelium. This enhances the use of non-chemical safe alternatives as antigiardial agents, mainly for pediatrics and pregnants.

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