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Original Article

Ginger and Cinnamon: Can This Household Remedy Treat Giardiasis? Parasitological and Histopathological Studies

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Abstract

Background: *Giardia lamblia* is one of the most common protozoal infections in human especially children. Metronidazol (MTZ) is the drug of choice for treatment of giardiasis; its chemical composition possesses major threats and is becoming less sensitive. This study aimed to search for natural extracts alternative to MTZ.

Methods: In-vivo effects of dichloromethane extracts of ginger and cinnamon in doses of 10 and 20 mg/kg/day separately were studied on 30 experimentally infected albino rats divided into 6 groups (5 rats each). Plant extracts were started on the 6th day post infection for 7 successive days. The study was evaluated by fecal cyst and intestinal trophozoite counts, histopathology, scanning and transmission electron microscopic examinations of the small intestinal mucosa.

Results: Ginger and cinnamon caused reduction of fecal cyst and trophozoites counts. Histopathology, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) after exposure to each extract revealed evident improvement of intestinal mucosal damage produced by *G. lamblia* infection and direct structural injury to the trophozoites. However, these results were more obvious after exposure to cinnamon extracts.

Conclusion: We confirmed the potential therapeutic effects of ginger and cinnamon extracts on *G. lamblia* infection in albino rats as a promising alternative therapy to the commonly used antigiardial drugs.

Introduction

Worldwide, *Giardia lamblia* infection is a major cause of diarrheal illness in humans (1). The prevalence rates of the disease range between 2-7 in developed countries and 20-30% in developing countries where overpopulation, poor water supply and poor hygienic conditions are associated with the feco-oral infection (2). In general, *G. lamblia* causes inflammation and shortening of the villi in the small intestine without penetration of the intestinal wall but the presence of extreme numbers of trophozoites lead to a direct, physical blockage of nutrient uptake (3). Infection can be asymptomatic but in acute and more frequently in chronic symptomatic infections especially in children and immune-compromised patients, major morbidity is marked resulting in malabsorption, sever diarrhea and weight loss (4).

Metronidazole and other nitroimidazoles like tinidazole have been used as drugs of choice against giardiasis; however, unpleasant side effects, failures of treatment and multidrug resistance have been reported (1, 5). The appearance of parasites resistant to current therapies highlights the need for new alternative ones and draws attention towards the use of medicinal plants, many of which have shown promise in the treatment of giardiasis; however, scientific proof to confirm the use of plants remains restricted (6). *Zingiber officinale*, commonly known as ginger, belonging to the family Zingiberaceae is a familiar dietary spice having several medicinal properties. It has been widely used as a common household remedy from ancient times, as a flavoring agent, an antiemetic and a digestive aid (7). The crude extract and the essential oil of *Zingiber officinale* contains alkaloids, saponins, tannins, glycosides, flavonoids which are responsible for anti-inflammatory, antidiarrheal, antibacterial, antiviral, antifungal, spasmolytic action and antioxidant properties (8, 9). In vitro studies reported that herbs rich in flavonoids exhibited antigiardial activities (10). Significant antiparasitic activities of *Z. officinale* against *Schistosoma* adults, *Trichinella spiralis* intestinal and muscular phases, microfilarie of *Dirofilaria immitis* in dogs and protoscolices of hydatid cyst were observed (11-14).

Cinnamon zeylanicum, commonly known as cinnamon is grown in almost every tropical region of the world. It has been used in traditional systems of medicine for the treatment of various diseases. Cinnamon contains terpenoids, flavonoids and polyphenolic compounds which are responsible for antioxidant activity improve the health of the colon and benefit the immune system (15, 16). Cinnamon aromatic oil has been used for thousands of years and possessed antibacterial, antifungal, antiviral, anti-inflammatory activities (17). Pharmacological investigations suggested that cinnamon oil has anti-parasitic activity against flagellates, *Trichomonas gallinarum* and *Histomonas meleagridis* in chicken (18) and against *Cryptosporidium parvum* in murine models (19).

This study aimed to evaluate the in-vivo efficacy of dichloromethane extracts of ginger and cinnamon as natural therapeutic herbs for giardiasis and assessment of structural injury produced to *G. lamblia* trophozoites.

Materials and Methods

Plant materials and preparation of extracts

The dried rhizomes of *Z. officinale* and stem bark of *C. zylancium* were purchased from the local market in Assiut Governorate, Egypt. Botanical identification was done at the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Both of the two plants were crushed separately to a fine powder, sieved and stored in well-closed dark glass containers until used. Two hundred and fifty g of each extract were macerated in 1 L dichloro- methane (Merck) for two days with frequent stirring, followed by filtration; and this process was repeated three times. The combined dichloromethane extract was fil-

tered using Whatman filter paper No. 1. The filtrate was evaporated to dryness under vacuum at 40 ^{0}C using a rotary evaporator. The solvent free residue of each plant was used for preparation of the required doses (20).

Experimental animals

The present study was carried out on 30 laboratory bred, parasite free and weaning male albino rats 3-4 weeks old, weighing 150-200 gm. They were obtained and maintained in the animal house, Faculty of Medicine, Assiut University, Assiut, Egypt. Animals were equally divided into 6 groups, 5 for each group: GI non-infected non-treated, GII infected non-treated, GIII and GIV infected and Ginger 10 and 20 mg/kg/day, respectively. GV and GVI infected and treated with Cinnamon 10 and 20 mg/kg/day respectively. Animals of all groups except GI were infected orally each with 200,000 *G. lamblia* cyst suspended in 1 ml saline, from heavily infected fresh human stool containing no other parasites. Rats were subjected to a parasitological assay by stool examination for detection of cysts from third day post infection. Plant extract regimens were started on the 6th day post infection; peak of intestinal colonization; for 7 successive days. All animals were sacrificed one day after treatment regimen (10, 21, 22).

Assessment of ginger and cinnamon effects

Parasitological examination

Stool samples were collected from each rat for 3 days before scarification; due to intermittent discharge of cysts, ten high power fields were examined for each sample and the mean number of cysts/HPF (high power field) was calculated (22).

Intestinal wash

Intestinal wash was done on the 8th day post treatment. The duodenum and proximal jejunum of each rat were removed, placed in a petri dish containing 1ml sterile PBS (phosphate buffered saline). This dish was placed on ice for 15 min and vortexed for 30 sec to release the trophozoites from the intestinal wall. Trophozoites were counted using a haemocytometer; at least 4 separate grids were counted for each mouse since a single parasite on one grid corresponds to 10^4 trophozoites/ml and colonization was expressed as the number $\times 10^4$ trophozoites/ml wash (10).

Histopathological examination

Specimens of 2-5 cm from the proximal part of the small intestine (duodenum and jejunum) removed from rats sacrificed on the 8th day post treatment were fixed in 10% formalin and embedded in paraffin, and sections at 5 microns were stained with haematxylin and eosin (H&E) for examination (23).

Scanning and transmission electron microscopy (SEM, TEM)

Small pieces from the duodenum and proximal jejunum of the sacrificed rats on the 8th day post treatment were immediately fixed in cold glutaraldehyde for 2 hours. After that, they were fixed in 2% osmium tetroxide for 2 hours, dehydrated in serial ascending ethanol, dried using liquid CO_2. For SEM, samples were mounted on stainless steel holders, sputter-coated with a thin layer of gold (22) and examined by JOEL (JSM-5400LV) SEM. For TEM, samples were cleared in propylene oxide and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue and examined with light microscope. Ultrathin sections were double stained with uranyl acetate and led citrate (24), and photographed by JOEL (JEM-100cx) TEM. Both SEM and TEM examinations were carried out in the Electron Microscopic Unit, Assiut University, Assiut, Egypt.

Statistical analysis

The collected data were analyzed by the program (SPSS; Statistical Package for Social Sciences) version 20 for windows. All values were expressed as mean ± standard deviation. The significance of differences between the groups was calculated using Student's *t*-test. The goblet cell count was made from 25 villus: crypt unit per slide. Quantitative histomor-

phometry was performed to measure the number of cells, villus height and crypt depth in H&E stained sections. Both of them were done using image analyzing system software (Leica Q 500 MCO) in the Histology Department, Faculty of Medicine, Assiut University, Assiut, Egypt. The mean value of the thirty samples from villus/crypt ratio was calculated (24).

Ethical considerations

The experimental animal studies were conducted in accordance with the international valid guidelines and they were maintained under convenient conditions. The local Committee Ethics approved the study.

Results

Parasitological examination of fecal cysts

Parasitological examination following treatment with ginger and cinnamon caused highly significant reduction of fecal cyst counts in all groups in comparison to the infected non-treated group. This reduction was higher in cinnamon than in ginger.

The percentage of reductions (%r) reached up to 100% following exposure to cinnamon 20 mg/kg/day (Table 1). Most of the recovered cysts were swollen and distorted in shape.

Table 1: Fecal cyst counts in stool and trophozoite count in intestinal wash /HPF of infected control and treated groups

Group	Fecal cyst counts /HPF in stool		trophozoite count /HPF in intestinal wash	
	Mean± Std. Deviation	% of reduction	Mean± Std. Deviation	% of reduction
Infected non-treated (GII)	11.82±4.62	-	50.4±9.39	
Ginger 10 mg (G III)	1.71±0.49**	85.5	27.4±3.21**	45.6
Ginger 20 mg (G IV)	1.17±0.41**	90.1	12.4±2.07**	75.4
Cinnamon 10 mg (GV)	1.00±0.00**	91.5	36.6±5.73*	27.4
Cinnamon 20 mg (GVI)	0.00±0.00**	100.0	33.2±9.86*	34.1

P-value represents the relationship between GII and all treated groups/* Significant at ($P \leq 0.05$), ** highly significant at ($P \leq 0.001$)

Intestinal wash

Significant reduction of trophozoite counts recovered from the intestinal wash of both extracts was detected. However, ginger showed more reduction than cinnamon especially in a dose of 20mg /kg/day (Table 1).

All the main distinguishing features of normal *G. lamblia* trophozoites were visible in the infected non-treated group. Following exposure to ginger and cinnamon (10 mg/kg/day), *G. lamblia* trophozoites appeared distorted, elongated and swollen with indistinct nuclei. More distortion, ballooning and sometimes destruction of trophozoites were detected following exposure to ginger and cinnamon (20 mg/kg/day). These results were more obvious with ginger.

Histopathological examination

The villi of non-infected non-treated group (GI) had normal appearance without showing any villus atrophy or fusion, and no inflammatory response in the lamina propria was observed (Fig. 1-A).

On the other hand, the appearance of the villi of the infected non-treated group (G II) showed evident changes including shortening, atrophy and villi fusion together leading to blunting with desquamation of most villi, heavily infiltrated lamina propria and aggregated lymphocytes with necrosis of some enterocytes (Fig. 1-B). After exposure to ginger 10 mg/kg/day (GIII) desquamation of most villi with flat epithelial cell surface were observed and lamina propria was infiltrated with numerous inflammatory cells while the muco-

sa of G IV (treated with ginger 20 mg/kg/day) showed restoration of normal villous architecture and decrease in inflammatory cells in the lamina propria (Fig. 1-C). The mucosa of G V and G VI after treatment with cinnamon 10 mg and 20 mg/kg/day, respectively showed slight or no histopathological changes and nearly regained their normal appearance resembling those in the non-infected non-treated group; however, this improvement was pronounced in G VI. (Fig. 1-D).

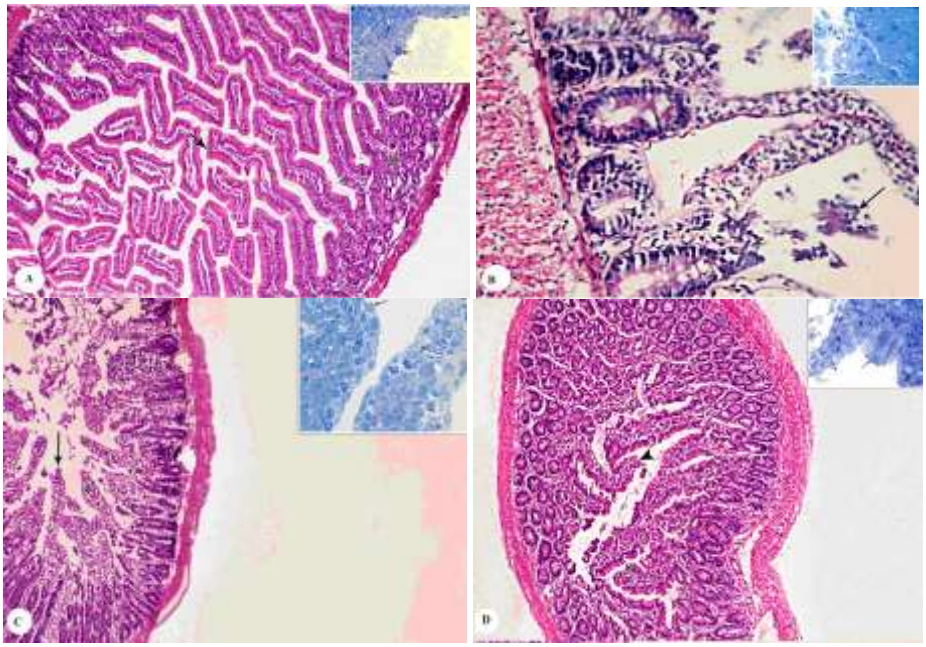

Fig. 1: Sections in the small intestinal mucosa of control and treated rat groups. (A) From GI showing upright villi (arrow) and invaginated crypts (astrik). (H&E. X 100). Inset of a semithin section in the same group showing regular continuous brush border with tall columnar enterocytes (arrow) and goblet cells in-between (two arrows) (T.B (Toludine Blue) X1000). (B) From GII showing disorganized villi with pyknotic villous membrane and necrosis of some enterocytes with pyknotic nuclei (arrow), heavily infiltrated lamina propria with aggregated lymphocytes (astrik) (H&E. X 1000). Inset a semithin section in the same group showing villi fusion, blunting, erosion and adhesion (arrow), goblet cells can be seen (astrik) (T.B X1000). (C) From G IV after Ginger (20 mg/kg/day) showing few short columnar enterocytes covering areas of the villi (arrow), decrease in the inflammatory infiltration in the lamina propria (astrik) (H&E. X 100). Inset of a semithin section in the same group showing some cuboidal cells lining the villi (arrow), with moderate inflammatory cells (astrik) (T.B X1000). (D) From G VI after Cinnamon (20 mg/kg/day) showing normal tall, finger-like villi, most of them were covered with tall columnar enterocytes and goblet cells (arrow), but some villi have dislodged tips (arrow head) (H&E. X 100). Inset of a semithin section in the same group showing regular continuous brush border (arrow), other areas showed flat surface epithelial cell of the villi (arrow head) with mild inflammatory cells infiltrated the lamina propria (astrik) (T.B X1000)

An insignificant increase in the number of goblet cells in the infected non-treated group was noticed. A significant dose-dependant decrease in ginger-treated groups was observed (Table 2). The intestinal mucosa showed a significant decrease in villi height and an insignificant increase in crypt depth in the infected non-treated group. After treatment with ginger and cinnamon, the villus height increased and crypt hyperplasia was observed (Table 2).

Table 2: The number of goblet cells, villi height, crypt depth and villi/crypt ratio of control and treated groups

Group	the number of goblet cells Mean ± SD	Villi height (mm)	Crypt depth (mm)	Villi/Crypt ratio
Non-infected non-treated (G I)	8.22 ± 2.52	7.8±2.1	3.2±0.7	2.5±0.4
Infected non-treated group (G II)	8.55 ± 3.22	4.3±1.3*	4±0.9	1.1±0.2*
Ginger 10 mg (G III)	3.74 ± 1.50*	5.2±1.3*	4±0.7	1.3±0.3*
Ginger 20 mg (G IV)	2.93 ± 2.20*	5.2±1.2*	3.2±0.5	1.6±0.4*
Cinnamon 10 mg (G V)	10.81 ± 2.18	6.1±1.9	4.4±0.8*	1.4±0.2*
Cinnamon 20 mg (G VI)	5.36 ± 1.69	5.7±1.6	3.1±0.8	1.8±0.4*

P-value represents the relationships between GI and all other groups. / * Significant at ($P \leq 0.05$).

Scanning electron microscopic (SEM) examination

SEM of the infected non-treated group (GII) showed damage of the intestinal mucosa with short, swollen and fused microvilli. Linear circular imprints at the sites of previous trophozoite attachment were detected with multiple epithelial gaps. A large number of typical pear-shaped trophozoites with smooth intact ventral and dorsal surfaces were observed. Some of these trophozoites either fell into these gaps or attached *in-situ* with their convex surface projecting above the microvillus brush border, while others entangled in thick mucus sheets (Fig. 2, 3). After exposure to ginger and cinnamon 10 mg/kg/day(GIII and G V), there was a decrease in the epithelial gaps and an increase in the amount of mucus covering the intestine which denoted the beginning of intestinal healing while flat circular imprints were still found. Some swollen or shrunken trophozoites while others with some irregularities, erosion and peeling could be seen. After exposure to ginger and cinnamon 20 mg/kg/day (GIV and GVI), more progression of intestinal healing was observed, which was indicated by more closure of the epithelial gaps, the increase in the mucosal coverage and the marked decline in the number of trophozoites? Some of these trophozoites were completely misshaped and still attached in-situ showing irregular dorsal and ventral surfaces while other trophozoites after cinnamon treatment were swollen but still keeping their pear shape. All the previously observed changes were more obvious after ginger treatment (Fig. 2, 3).

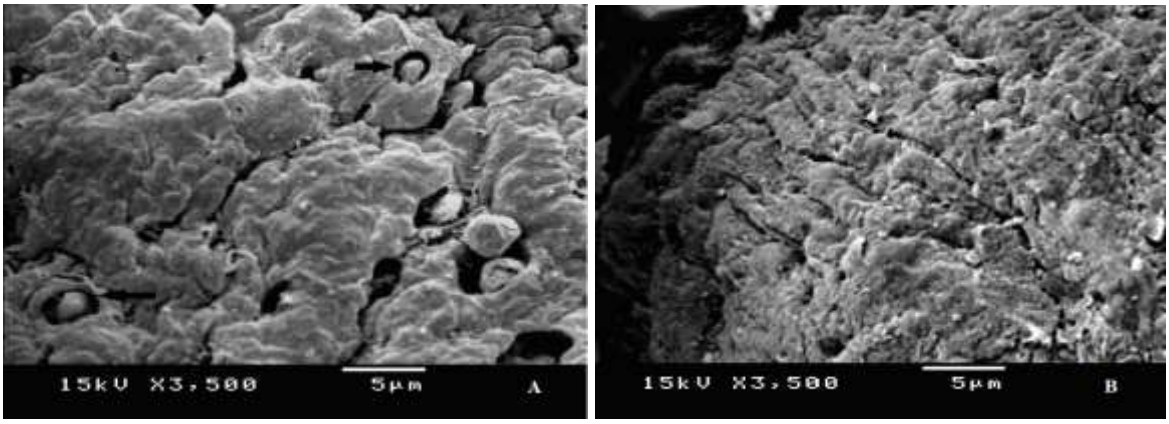

Fig. 2: SEM of intestinal mucosa (A) From GII (infected non-treated group) showing multiple epithelial gaps with many trophozoites falling into them (arrows). (B) From G IV (after Ginger 20 mg/kg/day) showing intestinal healing with closure of most of the gaps with no observed trophozoites

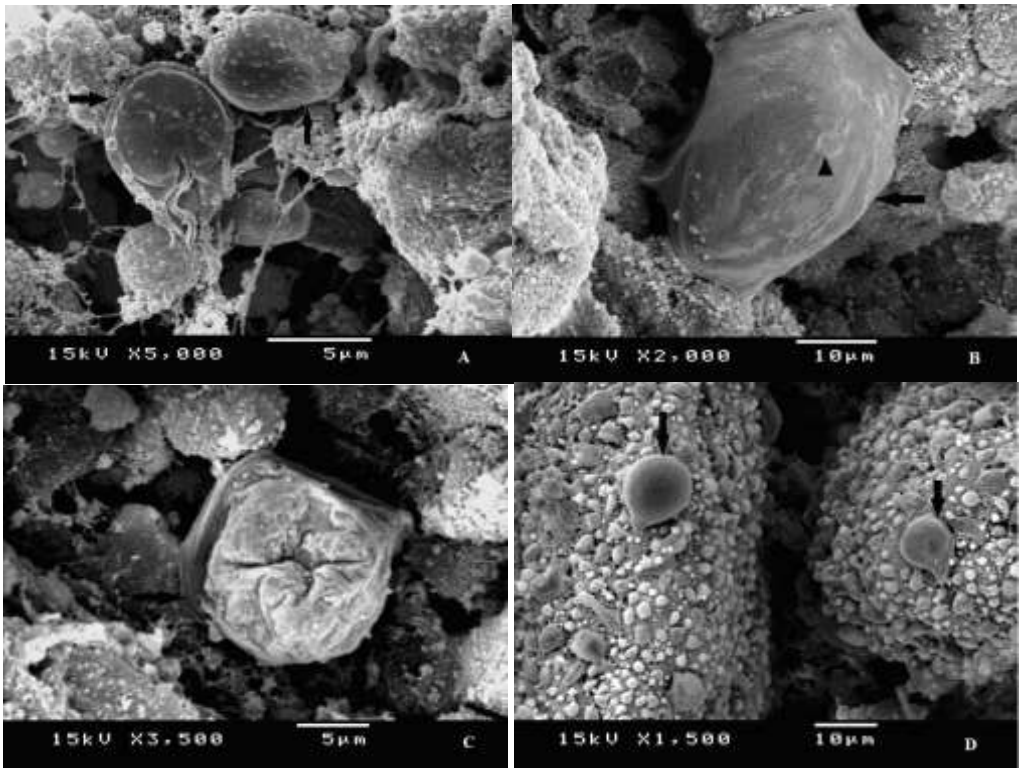

Fig. 3: SEM of *G. lamblia* trophozoite (A) From GII (infected non-treated) showing normal trophozoites with smooth intact ventral and dorsal surface (arrows). (B) From G III (after Ginger 10 mg/kg/day) showing swollen trophozoite, still attached in situ (arrow) with multiple erosions (arrow head). (C) From G III (after Ginger 20 mg/kg/day) showing irregularities of the trophozoite, with complete peeling of the outer surface (arrow). (D) From G VI (after Cinnamon 20 mg/kg/day) showing swollen pear-shaped trophozoites (arrows)

Transmission electron microscopic (TEM) examination

Ultra-structural examinations of the mucosa from GI (non-infected non-treated) showed that the intestinal villi were regular in height, diameters and spacing and covered with a single layer of columnar epithelial cells. The cytoplasm of enterocytes had low electron density, scattered vesicles with numerous electron-dense mitochondria, smooth endoplasmic reticulum and multivesicular bodies. The supranuclear cytoplasm contains short rough endoplasmic cisternae and scattered granules (Fig. 4-A).

In GII (infected non-treated), the microvilli were disoriented and disrupted, forming separate laminated vesicles with disorganized enterocytes. The apical cytoplasm was degenerated with supranuclear cytoplasmic vacuoles. Part of the cytoplasm bulged into the gut lumen and appeared as blebs. Disrupted nuclei with increased peripheral chromatin and mitochondrial cristiolysis were noticed (Fig. 4-B).

In G III and G IV (ginger-treated groups) the enterocytes showed occasional loss of basic morphology of intracellular organelles of columnar cells and polymorphism of nuclei with increased peripheral chromatin (Figs.4-C), while in GV (after treatment with cinnamon 10 mg/kg/day), some enterocytes showed changes similar to those in GII. On the other hand, TEM of GVI (after treatment with cinnamon 20 mg/kg/day) revealed no ultrastructural differences from GI (non-infected non-treated) (Fig.4-D).

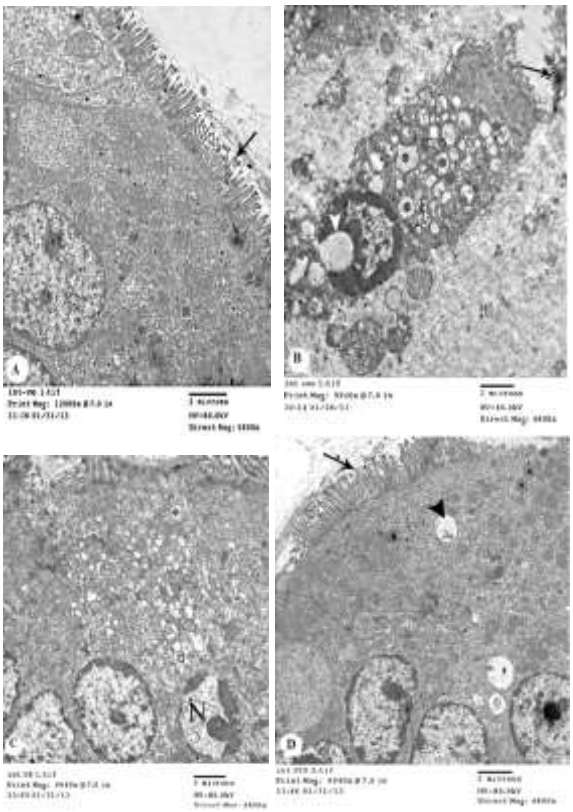

Fig. 4: TEM of small intestinal mucosa of control and treated rat groups. (A) From GI (non-infected non-treated) showing typical appearance of microvillus brush border (arrow) with numerous mitochondria (astrik) (X5800). (B) From GII (infected non-treated) showing the separate laminated vesicle (arrow), the disruption of nuclei (arrow head) with increased peripheral chromatin. Notice the mitochondrial cristiolysis and loss of mitochondrial dense matrix in the supranuclear region (astrik) (X 4800). (C) From GIV after (Ginger 20 mg/kg/day) showing enterocytes with membrane-bound cytoplasmic vacuoles (astrik). Notice the disruption and polymorphism of nuclei (N) with increased peripheral chromatin (X4800). (D) From G VI (Cinnamon 20mg/kg/day) showing the microvilli restored normal length, thickness, parallel orientation but irregular in height (arrow). The enterocytes regained normal mitochondrial (astrik) and nuclear chromatin density and electron-lucent Golgi vesicles reappeared near the lateral cell membranes. Notice the membrane-bound cytoplasmic vacuoles (arrow head) (X4800)

Discussion

The investigation of the in-vivo effect of carbon-dichloride (dichloromethane) extracts of ginger and cinnamon in a dose of 10 and 20 mg/kg/day on rats infected with *G. lamblia* showed variable but effective antigiardial activity. Antigiardial and antiprotozoal activities of *Zingiber* extracts were detected (25, 26, 27) while anti-protozoal activity of cinnamon was also proved (18, 19).

Significant dose-dependent reductions of both fecal cyst and intestinal trophozoites were detected with ginger and cinnamon. However, cinnamon showed more reduction of fecal cysts while ginger showed more reduction in the intestinal trophozoites. This may be due to the anti-oxidant action of cinnamon and ginger which help in the elimination of parasites (9, 16). We thought that cinnamon might have affected trophozoites attachment leading to their slipping and disintegration while with ginger many trophozoites were still attached in-situ and passed out after intestinal wash. The reduction of both fecal cyst and intestinal trophozoites was nearly similar to those in previously published reports (10, 21, 22, 28, 29, 30, 31).

Our histopathological results of the intestinal mucosa of the infected non-treated group are in line with several reports (10, 21, 22, 32). Many host factors as well as the interaction between trophozoites and the intestinal epithelium was responsible for the microvillus alterations and epithelial barrier dysfunction in giardiasis (33, 34).

The villi of intestinal mucosa in rats treated with ginger and cinnamon (10 mg/kg/day) showed some recovery while a pronounced improvement of pathological changes of villous architecture was observed with ginger and cinnamon 20 mg/kg/day. However, this improvement was more obvious in cinnamon than in ginger. These results were similar to other studies (10, 21, 22).

SEM examination of the duodenum and proximal jejunum of the infected non-treated group showed features of brush border injuries (22, 35, 36). All ultrasturactural mucosal changes observed in the infected non-treated group started to revert to normal in treated groups and the progression of intestinal healing increased when we doubled the dose of both extracts (22).

Regarding the electron microscopic changes of *G. lamblia* trophozoites observed in the intestine of the infected non-treated group, all the main distinguishing features of normal trophozoites were visible (10, 37). Our result after exposure to ginger and cinnamon extracts showed obvious structural changes in the ultrastructure of trophozoites (10, 22, 37). These changes can be explained by the fact some substances from the extracts interact with the Giardia membranes resulting in cell membrane discontinuity, cytoplasm leakage and parasite swelling which lead to loss of the osmoregularity and parasite death (22).

Transmission electron microscopic (TEM) examination of the intestinal mucosa in the infected non-treated group revealed damage of the brush border microvilli (38). Disrupted nuclei with increased peripheral chromatin and mitochondrial cristiolysis were noticed which denoted loss of functional efficiency (39). While in treated groups showed normalization of the microvilli and enterocytes, which was dose dependent in both extracts and more obvious with cinnamon.

Cinnamon extracts in this study especially in a dose of 20 mg/kg/day were more effective than ginger not only in decreasing fecal cyst count but also in improving the histopathological and electron microscopic changes of intestinal mucosa. However, ginger was more effective in decreasing and harming intestinal trophozoites. This is because cinnamon is an immune stimulant containing eugenol, which has local antiseptic and antiphagocytic properties (15, 40). This herb improved the appearance of the villi of the small intestine where the parasites colonized (19). Moreover, both herbs have an antioxidant activity (9, 16); both contained flavonoids, which protect against cellular damage (19).

An insignificant rise in the number of goblet cells in the infected non-treated group was observed while treated groups showed a dose-dependent decrease. Intestinal parasites including Giardia cause major changes in the goblet cells and mucins of the small intestine with evidence suggesting that mucus may play a part in either the clearance or invasion of *Giardia* (41, 42).

It is worthy to mention that no rats died after administration of ginger and cinnamon throughout the experiment, which at least proves the safety of these herbs at the given doses. Ginger rhizome is edible; therefore, it is safe for humans (43-45) while no mortalities were reported in mice treated with daily cinnamon oils (19).

Conclusion

The present study proved the effectiveness of ginger and cinnamon dichloro-methane extracts as promising natural therapeutic agents against *G. lamblia*. Further investigations will be necessary to identify and isolate the active compound(s) and performing toxicity test for its safety.

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References

1. Tian H, Chen B, Wen J. Giardiasis, drug resistance and target discovery. Infect Disorders Drug Targets. 2010; 10:259-302.
2. Mineno T, Avery MA. Giardiasis: Recent progress in chemotherapy and drug development. Curr Pharmaceut Design. 2003; 9(11):841-55.

3. Lars-Eckmann A. Mucosal defences against *Giardia*. Parasite Immunology. 2003; 25:259-270.
4. Troeger H, Epple H, Schneider T, Wahnschaffe U, Ullrich R, Burchard G, Jelinek T, Zeitz M, Fromm M, Schulzke J. Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. Gut. 2007; 56(3):328-335.
5. Wright JM, Dunn LA, Upcroft P, Upcroft JA. Efficacy of antigiardial drugs. Expert Opin Durg Saf. 2003; 2(6):529-41.
6. Hoste H, Torres-Acosta JF, Alonso-Diaz MA., Brunet S, Sandoval-Castro C, Adote SH. Identification and validation of bioactive plants for the control of gastrointestinal nematodes in small ruminants. Proc. of 5th International Workshop: Novel Approaches to the Control of Helminth Parasites of Livestock. Tropical Biomedicine. 2008; 25(1 Supplement):56-72.
7. Nandi S, Saleh-e-In M, Rahim M, Bhuiyan NH, Sultana N, Ahsan A, Ahmed S, Siraj S, Rahman Z, Kumar Roy S. Quality composition and biological significance of the bangladeshi and china ginger (*zingiber officinale*). JMBFS. 2013; 2(5):2283-2290.
8. Jeena K, Liju VB, Kuttan R. Antioxidant, anti-inflammatory and Antinociceptive activities of essential oil from ginger. Indian J Physiol Pharmacol. 2013; 57(1):51–62.
9. Sadhana S, Gupta AK. Evaluation of Phenolics Content, Flavonoids and Antioxidant activity of *Curcuma amada* (Mango Ginger) and *Zingiber officinale* (Ginger). Research and Reviews. J Chem. 2013; 2(1):32-35.
10. Abdalla SF, Ramadan NI, Mohamed MAA, El-Deeb HK, Al-Khadrawy FM, Badawy AF. A study on the effect of *Myrtus Communis* and *Olibanum* on *Giardia lamblia* infection in Egypt. P U J. 2011; 4(1):89-100.
11. Sanderson L, Bartlett A, Whitfield PJ. *In-vitro* and *in-vivo* study on the bioactivity of a *ginger (Zingiber officinale)* extract towards adult *Schistosomes* and their egg production. J Helminthol. 2002; 76(3):241-7.
12. El-Melegy MA, El-Saify GH, Hassab-El-Nabi SE. Evaluation of therapeutic effect of ginger compared to flubendazole on experimental trichinelosis in mice. Egyp J Med Sci. 2006; 27(2):25-48.
13. Merawin LT, Arifah AK, Sani RA, Somchit MN, Zuraini A, Ganabadi S, Zakaria ZA. Screening of microfilaricidal effects of plant extracts against *Dirofilaria immitis*. Res Vet Sci. 2010; 88(1):142-7.
14. Moazeni M, Nazer A. *In-vitro* lethal effect of *Zingiber officinale* on protoscolices of hydatid cyst from sheep liver. Microbiology Research. 2011; 2(25):91-94. DOI: 10.4081/mr.2011.e25.
15. Wondrak GT, Villeneuve NF, amore SD, Bause AS, Jiang T, Zhang DD. The Cinnamon-derived dietary factor cinnamic aldehyde activates dependent antioxidant response in human epithelial colon cells. Molecules. 2010; 15(5):3338-3355.
16. Wei A, Shibamoto T. Antioxidant/Lipoxygenase inhibitory activities and chemical compositions of selected essential oils. J Agric Food Chem. 2010; 8:(12):7218-7225.
17. Chaudhary SS, Imtiyaz S, Tariq M. Cinnamon: A Common Medicinal Spice. Am J Pharm Tech Res. 2013; 3(2):2249-3387
18. Zenner L, Callait MP, Granier C, Chauve C. *In vitro* effect of essential oils from *Cinnamomum aromaticum, Citrus limon* and *Allium sativum* on two intestinal flagellates of poultry, *Tetratrichomonas gallinarum* and *Histomonas meleagridis*. Parasite-Journal de la societe Francaise de Parasitologie. 2003; 10(2):153-157.
19. Abu El Ezz NM, Khalil FA, Shaapan RM. Therapeutic effect of onion (*Allium cepa*) and Cinnamon (*C. zeylanicum*) oils on cryptosporidiosis in experimentally infected mice. Global Veterinaria. 2011; 7(2):179-183.
20. El-Menshawi B. The Use of Biotechnological Methods for Drug Discovery from Egyptian Plants. Technical Report Phase I Cairo Acad Sci Res & Technol. 2003; 361-366.
21. Abdel-Fattah NS, Nada OH. Effect of Propolis versus metronidazol and their combined use in treatment of acute experimental giardiasis. J Egypt Soc Parasitol. 2007; 37(2):691-710.
22. Fathy FM. Effect of mirazid (*Commiphora molmol*) on experimental giardiasis. J Egypt Soc Parasitol. 2011; 41(1):55-177.
23. Drury RAB, Wallington EA. Carleton histological technique. 5th ed. Oxford University press. New York: Toronto; 1980.
24. Hummadi LA. Adverse effects of Soriatane on rats enterocytes, light microscopy and ultra-

structural studies. J Cytol Histol. 2012; 3(1):133. Doi:10.4172/2157-7099.1000133.

25. Sawangjaroen N, Subhadhirasakul S, Phongpaichit S, Siripanth C, Jamjaroen K, Sawangjaroen K. The *in-vitro* anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in Southern Thailand. Parasitol Res. 2005; 95(1):17-21.
26. Sohni YR, Kaimal P, Bhatt RM. The anti-amoebic effect of a crude drug formulation of herbal extracts against *E. histolytica in-vitro* and *in-vivo*. J Ethenopharmacol. 1995; 45(1):43-52.
27. Choi KM, Gang J, Yun J. Anti-*Toxoplasma gondii* RH strain activity of herbal extracts used in traditional medicine. Int J Antimicrob Agents. 2008; 32:360-2. Doi.org/10.1016/j.ijantimicag.2008.04.012.
28. Ortega E, Ward H, Keusch G, Pereira M. Growth inhibition of the intestinal parasite *Giardia lamblia* by a dietary lectin is associated with arrest of the cell cycle. J Clin Inves. 1994; 94(6):2283-88.
29. Tripathi DM, Gupta N, Lakshmi V. Antigiardial and immunostimulatory effect of Piper *longum* on giardiasis due to *Giardia lamblia*. Phytother Res. 1999; 13(7):561-65.
30. Jiménez-Arellanes A, Luna-Herrera J, Ruiz-Nicolás R, Cornejo-Garrido J, Tapia A, Yépez-Mulia L. Antiprotozoal and antimycobacterial activities of *Persea Americana* seeds. Complementary and Alternative Medicine. 2013; 13:109-118.
31. Elhadi IM, Koko WS, Dahab MM, El Imam YM, Abdu El Mageed MA. Antigiardial Activity of some *Cucurbita* Species and *Lagenaria siceraria*. Journal of Forest Products & Industries. 2013; 2(4):43-47.
32. Buret AG. Immunopathology of giardiasis: The role of lymphocytes in intestinal epithelial injury and malfunction. Mem Inst Oswaldo Cruz. 2005; 100(1):185-90.
33. Scott KGE, Logan MR, Klammer GM, Teoh DA, Buret AG. Jejunal brush border microvillous alteration in *Giardia muris* infected mice: Role of T-lymphocyte and interleukin-6. Infec Immun. 2000; 68(6):3412-3418.
34. Chin AC, Teoh DA, Scott K GE. Strain dependent induction of enterocyte apoptosis by *Giardia lamblia* disrupts epithelial barrier function in a caspase 3 dependent manner. Infect Immun. 2002; 70 (7): 3673-3680. Doi: 10.1128/IAI.70.7.3673-3680.
35. Khanna R, Joshi K, Kum-Kum A, Malik AK, Vinayak VK. An ultrastructural analysis of changes in the surface architecture of intestinal mucosa following *Giardia lamblia* infection in mice. J Gastroenterol. 1990; 25(5):649-658.
36. Buret A, Gall DG, Olson ME. Effect of murine giardiasis on growth, intestinal morphology and saccharidase activity. J Parasitol. 1990; 76:403-9.
37. Ponce-Macotela M, Rufi no-González Y, González-Maciel A, Reynoso-Robles R, Martínez-Gordillo MN. Oregano (Lippia spp.) kills *Giardia intestinalis* trophozoites *in vitro*: antigiardiasic activity and ultrastructural damage. Parasitol Res. 2006; 98:557-560.
38. Cheville NF. Ultrastructural pathology: The comparative cellular basis of disease. 2nd ed. Wiley-Blackwell. A John Wiley of Sons: Inc USA; 2009.
39. Robbins C. Pathological Basis of Disease. 5th ed. International edition: WB; 1995.
40. Mallavarapu RG, Ramesh S, Chandrasekhara RS, Rajeswara R, Kaul PN, Bhattacharya AK. Investigation of the essential oil of cinnamon leaf grown at Bangalore and Hyderabad. Flavour and Fragrance J. 1995; 10:233-286.
41. Kim YS, Ho SB. Intestinal goblet cells and mucins in health and disease: Recent Insights and Progress. Curr Gastroenterol Rep. 2010; 12(5):319–330. Doi: 10.1007/s11894-010-0131-2.
42. Venkatesa P, Finch RG, Wakelin D. A. comparison of mucosal inflammatory responses to *Giardia muris* in resistant B10 and susceptible BALB/c mice. Parasite Immunology. 1997; 9:137-143.
43. Abdul Rahuman A, Gopalakrishnan G, Venkatesan P, Geetha K, Bagavan A. Mosquito larvicidal activity of isolated compounds from the rhizome of *Zingiber officinale*. Phytother Res. 2008; 22(8):1035-9. Doi: 10.1002/ptr.2423.
44. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of *Z. officinale* (*Zingiber officinale Roscoe*): a review of recent research. Food Chem Toxicol. 2008; 46:409-420.
45. Rong X, Peng G, Suzuki T. A. 35-day gavage safety assessment of ginger in rats. Regul Toxicol Pharmacol. 2009; 54:118-23.