academicJournals

8(4), pp. 106-114, 29 January, 2014
DOI: 10.5897/AJPP2013.3839
ISSN 1996-0816 © 2014 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

The effect of medium chain saturated fatty acid (monolaurin) on levels of the cytokines on experimental animal in *Entamoeba histolytica* and *Giardia lamblia* infection

Zeinab Hassanein Fahmy[1]*, Eman Aly[1], Ibrahim shalsh[1] and Amira H. Mohamed[2]

[1]Department of Parasitology and Electron Microscopy, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt.
[2]Department of Haematology, Theodore Bilharz Reasearch Institute, Imbaba, Giza, Egypt.

Accepted 30 January, 2014

The aim of this study was to demonstrate the effect of medium chain saturated fatty acid (monolaurin) on experimentally infected *Giardia lamblia* and *Entamoeba histolytica*, and measurement of IFN-γ, TNF-α, IL-4, IL-10, TGF-β levels of cytokines. To study the effect of monolaurin on the duodenal mucosa of the studied infected hamsters, a group of sixty golden Syrian hamsters were used, which were further divided into two subgroups: Subgroup I, in which hamsters were infected by oral administration of 10,000 *G. lamblia* cysts and Subgroup II, in which hamsters were infected by 10,000 *E. histotytica* cysts. Each subgroup was divided into (6) groups. Subgroup I included the group from (1 to 6), although subgroup II included from 7 to 12 groups. In *G. lamblia* infected subgroup I, best results were observed by the reduction in both vegetative and cystic forms, respectively shown in group (6) treated with combination of metronidazole and monolaurin post infection 94.68 and 96.55%, respectively. In the Subgroup II infected with *E. histolytica*, the high reduction in trophozoite and cystic forms in intestinal contents were in the group (12) which was treated with a combination of metronidazole and monolaurin post infection (90.12 and 92.56%, respectively). Cytokines levels IFN-γ, TNF-α, IL-4, IL-10 and TGF-β were measured in serum using sandwich enzyme-linked immunosorbent assay (ELISA). The best result was shown in the group (6) treated with a combination of metronidazole and mololaurin post infection 130,129, 35, 165 and 240 Pg/ml. Also histopathological examination gave the best healing in the groups (6) infected with *G. lamblia* than thos infected by *E. histolytica*.

Key words: *Giardia lamblia*, *Entamoeba histolytica,* lauric acid, monolaurin, IFN-γ, TNF-α, IL-4, IL-10, TGF-β, histopathological examination.

INTRODUCTION

Entamoeba histolytica (*E. histolytica*) and *Giardia lamblia* (*G. lamblia*) are common causes of diarrhea and malabsorption in humans (Rauch et al., 1990). The infection may produce severe acute diarrhea in children less than five years of age with chronic infections resulting in weight loss and growth retardation (Fraser, 1994; Fraser et al., 1997; Newman et al., 2001). A parasitological assessment of drinking water in Egypt demonstrated a high prevalence of *G. lamblia*, an intestinal parasite of humans and various animals (Stauffer et al., 2006). *E. histolytica* is a protozoan causing amebic dysentery. Invasive amoebiasis is manifested by amebic colitis which

*Corresponding author. E-mail: zein201244@yahoo.com

can be complicated by intestinal perforation and peritonitis or hepatic abscess which may be fatal (Petri et al., 1987). There are little data on the true prevalence and incidence of *E. histolytica* infection in Africa; however, Egypt has high rates of asymptomatic infection as detected by the stool examination (Stauffer et al., 2006).

Therapeutic strategy has included diverse pharmaceutical agents of traditional use such as metronidazole, quinacrine and furazolidone (Gardner and Hill, 2001; Harris et al., 2001). However, evidence points to an increasing frequency of cases refractory to treatment with these drugs (Mendelson, 1980; Brasseur and Favennec, 1995; Sarker et al., 2010). Of these, metronidazole and albendazole may be the most representatives of anti-*E. histolytica* and *G. lamblia* of traditionally and recently used. Lemee et al. (2000) pointed to an increased frequency of cases refractory to treatment with these drugs and emergence of the drug-resistance species. Metronidazole has proved to acquire potential carcinogenicity and mutagenic effect in rats (Hill, 2000).

Cytokines may have a role in *G. lamblia* and *E. histolytica* infection. Cytokines or Interleukins are secreted by lymphocytes, monocytes or macrophages. They act on other cells of the immune system to regulate their function. Interleukins cause inflammatory response in parasitic diseases. (Beutler and Cerami, 1989). Tumor necrosis factors (TNF), a mediator of inflammatory response also plays an important role in parasitic diseases (Beutler and Cerami, 1986). Furthermore, this cytokine promotes the proliferation and recruitment of monocytes and neutrophils to inflammatory sites being the dominant cytokine for *E. histolytica* and *G. lamblia* parasite (Singer and Solaymani, 2010).

Singer and Nash (2000) illustrated the importance of T-cells in the control of giardiasis. Neither Th1 nor Th2 cells were absolutely necessary for the clearance of Giardia infection. This suggests that in the absence of Th1 cells, Th2 cells are sufficient for clearance of the parasite, or that in the absence of Th2 cells, Th1 cells are sufficient. Alternatively, Th3 cells (mucosal T cells) may play the major role in *E. histolytica* and *G. lamblia* infection. However, in interferon-gamma deficient animals, parasite clearance was delayed when compared to controls, suggesting the Th1 response may be more substantial in controlling Giardia infections. T-cell cytokines may also induce the production and release of antigiardial defensins into the intestinal lumen (Singer and Nash, 2000).

The drugs of choice in amebiasis are nitroimidazoles (Freeman et al., 1997; Iamp et al., 1999). It is effective and available at low cost. Trophozites of *E. histolytica* are able to adapt to therapeutically relevant levels of the drug (Samarawickerma et al., 1997; Wassmann et al., 1999). Lauric acid is a naturally derived fatty acid belonging to the medium chain saturated fatty acids (MCSFAs) which is suggested to have antimicrobial and antiparasitic properties. MCSFAs are natural fats that are easily digested, quickly absorbed and readily utilized for energy production in mammals. Due to their relative ease of absorption by the body, these compounds are ideal for individuals with digestive tract problems such as diarrhea and malabsorption of long chain fatty acids containing foods (Petschow et al., 1998).

Lauric acid (dodecanoic acid, C: 12) is a medium chain saturated fatty acid which is reported to have anti-giardial effect with lethal dose 50 (LD_{50}) concentration comparable to that of metronidazole which is the drug of choice in treatment of giardiasis. Dodecanoic acid appears to induce *in vitro* trophozoite death by accumulating within the parasite cytoplasm resulting in rupture of cell membrane (Rayan et al., 2005). Lauric acid is transformed into a substance called "monolaurin" in the human body. Monolaurin is a glyceride ester derivative of lauric acid (Hegde, 2006). Also, it was reported that monolaurin can destroy various pathogenic bacteria and protozoa such as *G. lamblia.* This is why lauric acid is the fundamental building block of the most effective anti-pathogenic of all the medium chain saturated fatty acids (MCSFAs) (Rayan et al., 2005).

The present study was carried out to evaluate lauric acid, as a treatment of plant origin on *G. lamblia* or *E. histolytica* infection. This work also studies the histopathological changes occurring in the small intestine of infected animals and following treatment.

MATERIALS AND METHODS

G. lamblia and *E. histolytic*a cysts were obtained from diarrheic patients attending parasitology laboratory in outpatient clinic of Theodore Bilharz Reasearch Institute (TBRI). Each hamster was infected orally by 10,000 of either *G. lamblia* or *E. histolytic*a cysts. The excreta of hamsters were examined daily to evaluate the time of maximal cyst excretion. A group of sixty golden Syrian hamsters were used, which were further subdivided into two subgroups: Subgroup I: in which hamsters were infected by oral administration of 10,000 *G. lamblia* cysts and Subgroup II: in which hamsters were infected by 10,000 *E. histotytic*a cysts through an esophageal tube. Each subgroup divided into (6) group: Subgroup I included the group from (1 to 6) although subgroup II included 7 to 12 groups. Group (1) constituted infected animals added as control (5 animals), group (2) infected with Giardia cysts (5 animals) and treated with metronidazole in a dose of 120 µg/kg receiving twice daily for 5 successive days. Group (3) infected with Giardia cysts (5 animals) received dose of 500 mg/kg monolaurin for 7 consecutive days pre infection, group (4) infected with Giardia cysts (5 animals) received 500 mg/kg monolaurin for 7 consecutive days post infection. Group (5) infected with Giardia cysts (5 animals) received dose (2/3) 300 mg/kg monolaurin for 7 days consecutive days pre infection, then treated with metronidazole 1/3 dose twice daily for 5 successive days. Group (6) infected with Giardia cysts (5 animals) received a dose of 300 mg/kg monolaurin for 7 consecutive days post infection in a combination treated with metronidazole 1/3 dose twice daily for 5 successive days. Group (7) infected with *E. histolytic*a cysts. Group (8) infected with *E. histolytic*a cysts and treated with metronidazole120 µg/kg twice daily for 5 successive days. Group (9) infected with *E. histolytic*a cysts (5 animals) and treated dose 500 mg/kg monolaurin for 7 consecutive days pre infection. Group (10) infected with *E. histolytic*a cysts (5 animals) three consecutive days post infection. Group (11) infected with *E. histolytic*a cysts (5 animals) received a dose of 300 mg/kg monolaurin

for 7 consecutive days pre infection, then treated with metronidazole 1/3 dose twice daily for 5 successive days and group (12) infected with *E. histolytic*a cysts (5 animals) received a dose of 300 mg/kg monolaurin for 7 consecutive days post infection in combination with metronidazole 1/3 dose twice daily for 5 successive days. The number of trophozoites were investigated in the duodenal part of intestine of hamsters.

Drugs

*Metronidazole (flagyle) was supplied by Rhone Opulence Rorer Company, as suspension. The dose given to each hamster was 120 µg/kg twice daily for 5 successive days. The dose for hamsters was calculated according to the chart for drug doses in experimental animals Paget and Barnes (1964). *Monolaurin (lauric acid) was supplied by Manufactured for Ecological formulas CONCORD, CA 9456 (Rayan et al., 2005). The dose was 500 mg/kg given for 7days, pre and post infection.

Parasitological study

In the group infected with *G. lamblia* and *E. histolytica* one week following infection, stool analysis was performed for all (12) groups to verify infection. Analysis was repeated every other day till end of second week post-infection. Treatment to all groups were given one week following infection. One week later stool analysis was done by direct examination of fresh stool for trophozoites and merthiolate iodine formaldhyde concentration (MIFC) technique (Blagg et al., 1955). Hamsters infected with *G. lamblia* and *E. histolytica* were sacrificed ten days after treatment and small bowel was removed and the duodenal contents were analyzed and the number of vegetative forms was counted. In hamsters infected with *E. histolytica,* large intestine was excised and colonic contents were examined. In the group treated with monolaurin pre-infection, hamsters were administered monolaurin daily for one week pre infection and the hamsters were sacrificed after three weeks post infection.

Histopathological examination

After sacrifice of the animals, part of the small intestine were fixed by formaline then pieces of tissues were processed for paraffin embedding stained with hematoxylin-eosin and masson trichrome stain (Bancroft and Stevens, 1975).

Serum cytokine and chemokine measurement

Levels of the cytokines IFN-γ, TNF-α, IL-4, IL-10, TGF-β (R & D systems Inc., Minneapolis) were measured in serum using sandwich enzyme-linked immunosorbent assay (ELISA) (eBioscience). The results were expressed as pg/ml, based on standard curves (Baqai, 1996). In brief, ELISA plates were coated with 50 µl (lµg/ml) of capture antibody (IFN-γ, TNF-α, lL-4, IL-10, TGF-β) (Beckton Dickenson & Co.) and allowed to incubate at 4°C overnight. Plates were washed six times with Phosphate buffered saline (PBS)/Tween 20. Excess protein binding sites were blocked with 200 µl of skimmed milk. The wash step was repeated and 50 µl of serum samples were added and incubated 1 h in water bath at 37°C. The washing step was repeated and the biotin labeled anti-(IFN-γ, TNF-α, lL-4, IL-10, TGF-β) monoclonal detector antibody (lµg/ml) (Beckton & Dickenson & Co) was added to each well and the plates incubated at room temperature for an hour. After a further washing step 100 µl of avidin-alkaline phosphatase was added to each well and the plates incubated at room temperature for 30 min. The washing step was repeated and 100 µl of p-nitrophenyl phosphate (pNpp) (Sigma Aldnch) (lmg/ml) in 0.2 M tris buffer was added to each well to detect bound antibody. The re-action was visualized by the addition of 100 µl/well of p-nitrophenyl phosphate (pNpp) (Sigma) substrate solution for 30 min in the dark at room temperature. The reaction was stopped by adding 50 µl/well of 8 N H_2SO_4 and plates were read at 405 nm using ELISA microplate reader (Bio Rad).

Ethical considerations

The experimental animal studies were conducted in accordance with international valid guidelines and they were maintained under convenient conditions at the Schistosom Biological Supply Progrm (SBSP) animal house of Theodor Bilharz Research Institute (TBRI), Cairo, Egypt.

Statistical analysis

The statistical package for social sciences (SPSS) for Windows (version 11) computer program was used for statistical analysis. Means of different groups were compared using unpaired 2-tailed students t-test. Data were considered significant if “p” values were less than 0.05.

RESULTS

Parasitological parameters

Table 1 showed that the reduction in trophozoite forms of *G. lamblia* in intestinal contents was 67.0% in group (3) treated with monolaurin pre-infection and 87.34% in the group (4) treated with monolaurin post-infection compared to infected control group (1). The reduction in the cysts of *G. lamblia* treated with monolaurin pre and post infection (group 3 and 4) was 73.96 and 91.15%, respectively (Table 2). Tables 1 and 2 showed that there was a reduction in trophozoite and cystic forms of *G. lamblia* in intestine (92.15 and 93.23, respectively), when treated with metronidazole (group 2). The best results in a percentage reduction rate in both vegetative and cystic forms, respectively was shown in the group (6) treated with a combination of metronidazole and monolaurin post infection 94.68 and 96.55%, respectively (Tables 1 and 2). In Tables 3 and 4, in the group infected with *E. histolytica,* the high reduction in trophozoite and cystic forms in intestinal contents were in the in group (12) treated with a combination of metronidazole, and monolaurin post infection were 93.08 and 92.56%, respectively. The reduction in trophozoite forms of *E. histolytic* in the groups (9 and 10) treated with monolaurin pre and post infection were 75.32 and 88.15%, respectively (Table 3). The difference for both treated groups was statistically significant from respective untreated control hamsters at ($P < 0.001$). The reduction in number of the cysts of *E. histolytic* pre and post infection (group 9 and 10) were 76.66 and 89.59%, respectively (Table 4). When treated with metronidazole in group (8) there were reduction in trophozoite and cysts forms of *E. histolytic* in intestinal contents were 89.14 and 90.03%, respectively (Tables 3 and 4).

Table 1. Effect of monolaurin on vegetative forms (trophozoite) in the small intestine infected with *Giardia lamblia*.

Group	Vegetative forms in small intestine (trophozoite) mean±SE	% reduction
Control infected groups	39.50±0.65	0
Infected treated with Metronidazole	3.1±0.95*	92.15
Treated with monolaurin pre-infection .	13.0±0.20**	67.0
Treated with monolaurin post-infection .	5.1±0.23***	87.34
Treated with monolaurin pre-infection + metronidazole.	9.9±0.12****	74.93
Treated with metronidazole + monolaurin post-infection	2.1±0.11****	94.68

Date were as mean ± SE (mean ± standard devination). *Significant difference compared to the infected control group (P > 0.001).

Table 2. Effect of monolaurin on the number of cysts excreted in stool infected with *Giardia lamblia*.

Group	Cysts/gm stool	% reduction
Control groups	7250.00±35.35	-
Infected treated with Metronidazole	490.2±25.22*	93.23
Treated with monolaurin pre infection	1887.5±21.34**	73.96
Treated with monolaurin post-infection	610.2±15.11***	91.15
Treated with monolaurin pre-infection + metronidazole	400.9±0.22****	94.48
Treated with metronidazole + monolaurin post-infection	250.0±0.012*****	96.55

Date were as mean±SE (mean ± standard deviation). *Significant difference compared to the infected control group (P > 0.001).

Table 3. Effect of monolaurin on vegetative forms (Trophozoite) in the small intestine infected with *E. histolytic.*

Group	Vegetative forms in small intestine (trophozoite) mean±SE	% reduction
Control infected groups	50.65±1.35	
Infected treated with Metronidazole	5.5±0.35*	89.14
Treated with monolaurin pre- infection	12.5±1.13**	75.32
Treated with monolaurin post- infection	6.0±2.11***	88.15
Treated with monolaurin pre-infection + metronidazole	5.0±0.02****	90.12
Treated with metronidazole + monolaurin post-infection	3.5±0.22*****	93.08

Date were as mean ± SE (mean ± standard devination). *Significant difference compared to the infected control group (P > 0.001).

Serum cytokine and chemokine measurement

Levels of the cytokines IFN-γ, TNF-α, IL-4, IL-10, TGF-β (R&D systems Inc., Minneapolis) were measured in serum using sandwich ELISA. There is a significant reduction observed in all groups, the greatest reduction in different cytokines was observed in group 3 and 6 while there is no significant reduction in the level of TGF-β (Table 5).

Table 4. Effect of monolaurin on the number of cysts excreted in stool infected with *E. histolytic.*

Group	Cysts/gm stool Mean± SE	% reduction
Control groups	6725.00±55.55	-
Infected treated with metronidazole	670.0±23.44*	90.03
Treated with monolaurin pre-infected	1569.2±11.24**	76.66
Treated with monolaurin post-infected	700.0±12.34***	89.59
Treated with monolaurin pre-infection + Metronidazole .	600.3±21.20****	91.07
Treated with metronidazole + monolaurin post-infection .	500.0±11.22*****	92.56

Date were as mean ± SE (mean ± standard devination). *Singnificant difference compared to the infected control group (P > 0.001)

Table 5. Effect of monolaurin on cytokines infected with *Giardia lamblia*.

Parameter	IFN – γ	TGF –b	IL – 4	IL – 10	IL – 6
	Pg/ml ± SEM				
Normal	166±5.11	256±29.2	17.9±1.7	99 ±12.4	140±5.1
Infected	612±19.6	131.2±31.1	69.6±12	510±29.1	587±94
Infected treated with Metronidazole	155.17±6.90	144.21±12.14	43.11±4.32	188.32±50.20	255.17±6.90
Treated with monolaurin pre- infection	311.23±2.72	219±33.7	27±1.3	107±20.2	164±32.6
Treated with monolaurin post-infection	267.81±5.32	142.62±23.90	61.31±14.11	121.22±12.13	171.81±4.32
Treated with monolaurin pre-infection + metronidazole	145.22±4.88	140.01±33.23	40.00±2.11	175.22±22.32	250.12±2.30
Treated with metronidazole + monolaurin post-infection	130.02±3.38	129.11±11.11	35.05±2.22	165.22±22.11	240.22±3.33

The Levels of the cytokines IFN- γ, TNF-α, IL-4, IL-10, TGF-β were measured in serum using sandwich ELISA.

In sub-groups infected with *E. histolytica*. There is a significant reduction observed in all groups, the greatest reduction in different cytokines was observed in group 9 and 12 while there is no significant reduction in the level of TGF-β (Table 6).

Histopathological examination

In the control infected non treated hamsters, atrophic degeneration of the intestinal villi was observed (Figure 1). Again, the group given monolaurin post-infection revealed partial villi atrophy (Figure 2), but exhibited complete healing in the group treated post-infection (Figure 3 and 4). While the group given combination of metronidazole and monolaurin post-infection with *E. histolytic* showed degeneration and healing in intestinal villi (Figure 5).

DISCUSSION

Millions of people are annually infected with *E. histolytica and G. lamblia,* making the diseases a major cause of morbidity worldwide (Teles et al., 2011). Metronidazole is known to be the drug of choice for treatment of trichomoniasis and giardiasis. However, some adverse reaction appears to be related to the high dosage and duration of treatment, resistance to the drug had led to the search for other suitable treatment (Martinez and Caumes, 2000). In human giardiasis, therapeutic failure that was recorded recently is up to 5 to 20% of cases (Fallah et al., 2007) occurs due to low compliance of drug therapy, frequent re-infection or emergence of parasite resistance to metronidazole and/or

Table 6. Effect of monolaurin on cytokines infection with *E. histolytica.*

Parameter	IFN – γ	TGF –β	IL – 4	IL – 10	IL – 6
			Pg/ml±SEM		
Normal	166±5.11	256±29.2	17.9±1.7	99±12.4	140±5.1
Infected	612±19.6	131.2±31.1	69.6±12	510±29.1	587±94
Infected treated with metronidazole	170.22±5.50	150.21±11.24	43.11±4.32	192.22±40.50	255.17±6.90
Treated with monolaurin pre-infection	315.27±2.81	233.0±12.6	28.02±2.3	117.6±20.2	170±22.5
Treated with monolaurin post-infection	277.81±5.32	152.73±13.80	64.31±12.12	125.02±12.13	176.52±4.44
Treated with monolaurin pre-infection+metronidazole	168.11±4.4	148.22±22.3	40.22±2.12	169.33±34.22	245.13±5.72
Treated with metronidazole + Monol post-infection	150.01±4.4	135.73±13.70	30.21±13.14	150.13±22.11	234.22±6.22

The Levels of the cytokines IFN- γ, TNF-α, IL-4, IL-10, TGF-β were measured in serum using sandwich ELISA.

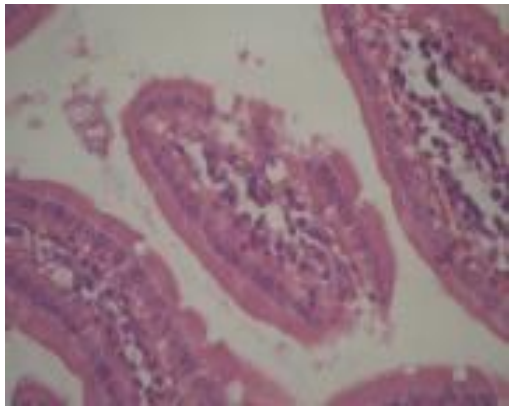

Figure 1. Infected control of *E. histotytic*a degeneration of the intestinal mucosa (40×).

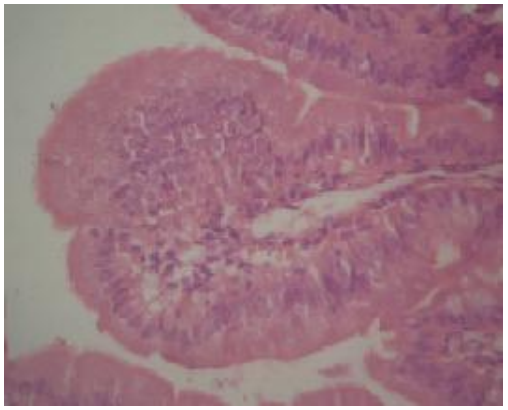

Figure 2. Healing of the intestinal villi after metronidazole treatment of *E. histotytic*a (Hx &E ×40).

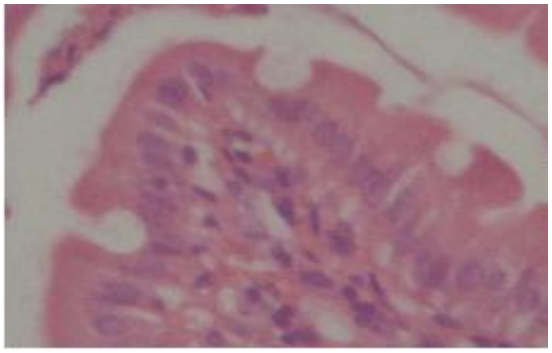

Figure 3. Treated with monolaurin post-infection with *E. histotytic* showing-degeneration and heal in intestinal villi (40×) E.

nitromidazole (Lemee et al., 2000).

In this study regarding the effect of metronidazole on *G. lamblia* infection, it was found that there was a high significant difference between control and all treated *G. lamblia* infected groups. Metronidazole administration resulted in a percentage reduction rate of 92.15 and 93.23% in both vegetative and cystic forms, respectively (Tables 1 and 2) group (2), compared to 67 and 73.96% when monolaurin was given pre-infection in group (3). The best results were in a percentage reduction rate in both vegetative and cystic forms obtained in group (6) which was treated with a combination of metronidazole and monolaurin post infection 94.68 and 96.55%, respectively. These results are in agreement with Amer et al. (2007) who made a trial to increase the effectiveness of metronidazole on *G. lamblia*. The study revealed a highest cure rate in vegetative forms (99.32%) and cystic forms (98.6%) in hamsters infected with *Giardia* and treated by a combination of metronidazole and lactospore. The authors added that, when metronidazole or lactospore were given separately, the reduction rates of trophozoites

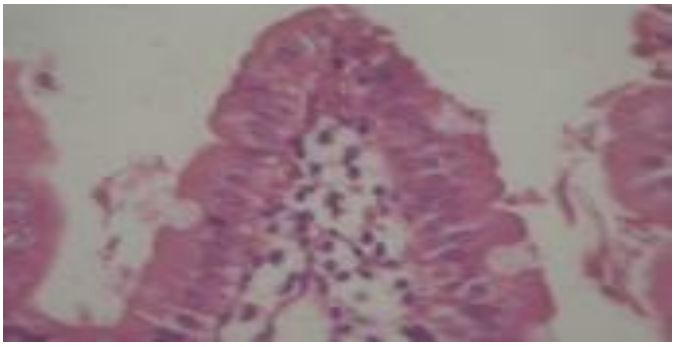

Figure 4. Treated with monolaurin Post-infection with *E. histotytic*a – reagenration and helling in instinal filli (40×) E.H.

Figure 5. Treated by combination of metronidazole and monolaurin for week post infection with *E. histotytic*a showing complet healing in intestine.

were 92.22 and 63.4%, respectively and of cystic forms were 93.8 and 79.5%, respectively. Another study reported 96.4 to 99.14% cure from Giardia cysts and 86.5 to 78.1% reduction in the vegetative forms in hamsters treated with metronidazole. Moreover, it was found that a cure rate over 90% in cystic forms *Giardia* when treated with metronidazole in other reports (Fawzy et al., 2003).

Monolaurin, the monoglyceride of lauric acid is the most powerful antiviral, antibacterial and antifungal fatty acid found in coconut oil. It has the greatest overall antimicrobial effect. It also had antiprasitic effect on blastocysts *in vitro* (Hassan et al., 2010). Monolaurin which is a natural compound derived from coconut was evaluated for its antigiardial effects on giardia as stated by Rayan et al. (2005).

In the present study in subgroup infected by *G. lamblia,* the best results in a percentage reduction rate in both vegetative and cystic forms was respectively observed in group (6) which was treated with combination of metronidazole and monolaurin post infection 94.68 and 96.55%, respectively. In the group (4) receiving mono-laurin post infection, the number of trophozoites and cysts forms of *G. lamblia* in intestinal contents were 87.34 and 91.15%, respectively compared to infected control group. This agree with Helmy (2010) who evaluated the effect of monolaurin, against *G. lamblia* in infected hamsters which the highest percentages of reduction in the number of *Giardia* cysts and trophozoites were in the group that received combined treatment (98.83 to 96.95%) followed by the group that received the metronidazole as treatment (93.77 to 95.5%) and the lowest percentage of reduction in the group that received the lauric acid as treatment (82.03 to 78.76%).

Similarly, in study of Hassan et al. (2010) reported the administration of monolaurin (lauric acid) was effective in the group infected with blastocystis. Dodecanoic acid (monolaurin ML) at 500 and 700 µg/ml induced highly significant reduction of concentration of blastocystis cells in culture after 2 h incubation ($p < 0.01$). Higher concentration (1000 µg/ml) caused rapid death of the parasite with viable cells were detected after 30 min incubation. These results agree with those found by Rayan et al. (2005) who confirmed that the dodecanoic acid has an anti-giardia effect with an LD_{50} concentration comparable to that of metronidazole.

Our result agrees with Fahmy et al. (2008) whose results showed that combined treatment of metronidazole together with arthemer and rosemary gave best results in highest percent reduction of cyst count in stool analysis and vegetative forms in small intestine caused by *G. lamblia*. Mixed treatment of metronidazole plus artemisia and rosemary showed complete regeneration of intestinal cells and sings of recovery was noticed.

El-Shennawy et al. (2009) studied the effect of pomegranate on the intestinal *G. lamblia* and concluded that the highest trophozoite reduction 98.7% was obtained in the group receiving metronidazole with pomegtanate (leaves). By histology, healing of mucosal ulcerations, preserved villi and reduced chronic inflammatory infiltrate of the lamina propria were detected with combined therapy.

Role of cytokines in *G. lamblia* infection is not clear. *G. lamblia* normally does not penetrate the epithelial barrier, therefore the spontaneous elimination of the parasite depends largely on immune mechanism. Cytokines or other inflammatory mediators may play a role in *G. lamblia* infection. IL4 appears to have some relationship in patients with *G. lamblia* infection as reported previously (Baqai, 1996).

Chronic diarrhoea and malabsorption produces mucosal inflammation associated with T cell activation and cytokine release (Farthing, 1993). Cytokines were not altered after infection of colonic cell with *G. lamblia* (Jung et al., 1995). As *G. lamblia* is a non invasive parasite, TNF alpha did not appear to have any role in giardiasis. This is in contrast with patients suffering from amoebiasis where TNF alpha was found because *E. histolytica* is an invasive parasite (Wang et al., 1992). TNF appears to act synergistically with other cytokines (Neta et al., 1988) and may be of therapeutic benefit in *G. lamblia* infection (Belosevic and Daniels, 1992).

In this study, the Levels of the cytokines IFN-γ, TNF-α,

IL-4, IL-10, TGF-β were measured in serum using sandwich ELISA. The best result was shown in group (6) which was treated with a combination of metronidazole and mololaurin post infection 130,129, 35, 165 and 240 Pg/ml in the subgroup I, infected with *G. lamblia*. In the present study, there is no significant reduction observed in all groups, the greatest reduction in different cytokines was observed in group 3, 6, 9 and 12, while there is no significant reduction in the level of TGF-β.

Our results agreed with Huma and Rakhshanda (2000) whose results indicate that IL-4 being an inflammatory regulator appears to have some relationship with it, probably because *G. lamblia* is a non invasive parasite giardiasis, while TNF alpha was not detected in patients. In our study, histopathological examination revealed complete healing of intestinal mucosa and regeneration and healing in intestinal villi after treatment with combination of metronidazole and monolaurin post-infection in group infected with *G. lamblia*, while partial healing of the lining epithelium of the intestine was noticed after treatment with monolaurin post infection or metronidazole treatment. This agrees with Helmy (2010) who showed that histopathological examination and electron microscopic examination revealed complete healing of intestinal mucosa after the combined treatment, while partial healing of the lining epithelium of the intestine was noticed after metronidazole or lauric acid treatment. Amoebiasis is a significant cause of morbidity worldwide and is the third leading cause of death from parasitic diseases. Although, metronidazole is the drug of choice for treatment of amoebiasis and has been used in clinical practice for many years, inappropriate usage could lead to drug resistance (Bansal et al., 2006). Drug resistance in *E. histolytica* is uncommon but differences in drug susceptibility between different isolates have been reported and resistance of metronidazole may be develop in future (Bansal et al., 2006).

In subgroup II, infected with *E. histolytica*, results showed that the high reduction in trophozoite and cystic forms in intestinal contents in group (12) treated with combination of metronidazole and monolaurin post infection were 93.08 and 92.56%, respectively. Then, the group (10) treated with monolaurin post-infection gave the best result in percent of reduction of cyst count in stool analysis and vegetative form 88.15 and 89.59% when infected with *E. histolytica*.

In our study, in the reduction in cytokines when infected *with E. histolytica*, the best result was observed in group (12) which was treated with combination of metronidazole and mololaurin post infection; the level of different cytokines was 150,135, 30, 150 and 234 Pg/ml (IFN-γ, IL-4, IL-6, IL-10). In the group (8) which treated with metronidazole, the reduction in cytokines levels show in group (2) which was 170, 150, 43.1, 192 and 255 Pg/ml (IFN-γ, IL-4, IL-6, IL-10).

Histopathological examination revealed complete healing in the group treated with combination of metronidazole and monolaurin post-infection. While the group given the treatment post-infection with *E. histolytic* showed degeneration and healing in intestinal villi. Our work showed promising results with monolaurin, which can be used as a complementary food product in combination with metronidazole in treatment for *G. lamblia* or *E. histolytica* infection.

Conclusion

In *G. lamblia* infected subgroup I, best results were observed by the reduction in both vegetative and cystic forms, respectively shown in group (6) treated with combination of metronidazole and monolaurin post infection 94.68 and 96.55%, respectively. In the subgroup II infected with *E. histolytica*, the high reduction in trophozoite and cystic forms in intestinal contents were in group (12) which was treated with a combination of metronidazole and monolaurin post infection (90.12 and 92.56%, respectively). Cytokines levels IFN-γ, TNF-α, IL-4, IL-10, TGF-β were measured in serum using sandwich ELISA. The best result was shown in the group (6) treated with a combination of metronidazole and monolaurin post infection 130,129, 35, 165 and 240 Pg/ml. Also histopathological examination gave best healing in groups (6) infected with *G. lamblia* than those infected by *E. histolytica*.

REFERENCES

Amer N, Mahmoud S, Helmy A, Hammam O (2007). The role of Probiotcs in controlling Giardia intestinal is in experimental animals. The New Egyptian J. Med. 37:13-23.

Bancroft, Stevens, 1975: Histopathological stains and their diagnostic uses. Churchill Livingstone. Edunberg, London and New York. PP.7,H,25,46.

Bansal D, Sehgal R, Chawla Y, Malla N, Mahajan RC (2006). Multidrug resistance in amoebiasis patient. Indian J. Med. Res.124:189-194.

Baqai R (1996). Role of cytokines in parasitic disease. J. Pak. Med. Assoc. 46:48-49.

Belosevic M, Daniels CW (1992). Phagocytosis of *Giardia lamblia* tropliozoites by cytokine activated niacrophage. Clin. Exp. Immunol. 87:304-9.

Beutler B, Cerami A (1986). Cachectin and Tumor Necrosis Factor as the sides of the same biological coin. Nature 320:584-88.

Beutler BA, Cerami A (1989). The biology of Cachectin, TNF and primary Ann. Rev. Immunol. 7:625-55.

Blagg W, Mansour NS, Khalof GI (1955). A new concentration technique for the demonstration of protozoa and helminthic egg in faeces. Am. J. Trop. Med. Hyg. 14:23.

Brasseur P, Favennec L (1995). Two cases of giardiasis unsuccessfully treated by albendazole. *Parasite* 2:422.

El-Shennawy AM, Ali E, El- Komy W, Fahmy ZH A. El- EAwakel (2009). Evaluation of Ponytail Antiparasitic Activity of Pomegranate Juice, Peels and Leaves Against *Giardia lamblia* . J. Schistosomiasis and infect and endem.Dis. (31):61-68.

Fahmy ZH, Mohamed AH ,Nosseir MM (2008). Ulrastructural and histopathological changes in *Giardia lamblia* –infected hamsters following administration of two natural compounds and / or metronidazole. The New Egy. J. Med. 38:53-61.

Fallah M, Rabiee S, Moshtaghi AK (2007). Comparsion between efficancy of a single dose of tinidzole with a 7 days standard dose course of Metronidazole in giardiasis . Pak. J. Med. Sc. 23:43- 46.

Farthing MJG (1993). Pathogenesis of giardiasis. Trans. R. Soc. Trop.

Med. Hyg. 87:17-21.
Fawzy SM, Soheir SM, Amer NM, Shalaby MA (2003). Effect of four antihelminthic drugs on experimental giardiasis. Kasr El Aini Med. J. 9:57-72.
Fraser D, Dagan R, Naggan L, Greene V, El- On J, Abu- Rbiah Y, Deckelbaum RJ (1997). Natural history of *Giardia lamblia* and Cryptosporidium infections in a cohort of Israeli Bedouin infants : a study of a population in transition. Am. J. Trop. Med. Hyg. 57:544-549.
Freeman CD, Klutman NE, Lamp KC (1997). Metronidazole. A therapeutic review and update. Drugs 54:679-708.
Gardner TB, Hill DR (2001). Treatment of giardiasis. Clin. Microbial. Rev. 14(1):114-128.
Harris JC, Plummer S, Lloyd D (2001). Antigiardial drugs. *Appl. Microbiol. Biotechnol.* 57:614-9.
Hassan SI, Moussa HEM, Fahmy ZH, MohamedAH (2010). In Vitro Effect of Medium – Chain Saturated Fatty Acid (Dodecanoic Acid) on *Blastocytsis spp*. Int J. infect. Dis. (14):584.
Hegde B (2006). Coconut oil –ideal fat next to mother's milk (Scanning coconut's horoscope). JLACM. 7:16-19.
Helmy SH (2010). Evaluation of the effect of medium chain saturated fatty acid (lauric acid) on Giardiasis in hamsters. A thesis MS D . Faculty of Medicine Cairo University.
Huma Q, Rakhshanda B (2000). Role of Cytokines in Giardiasis. JPMA 50:113.
Jung HC, Eckmann L, Yang SK, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF (1995). A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J. Clin. Invest. 95:55-65.
Lamp KC, Freeman CD, Klutman NE, Lacy MK (1999). Pharmacokinetics and pharmacodynamics of the nitroimidazole antimicrobials. Clin. Pharmacokinet. 36:353-373.
Lemée V, Zaharia I, Nevez G, Rabodonirina M, Brasseur P, Ballet JJ, Favennec L (2000). Metronidazole and albendazole susceptibility of 11 clinical isolates of Giardia duodenalis from France. J. Antimicrob. Chemother. 46(5):819-821.
Martinez V, Caumes E (2000). Metronidazole. Ann. Dermat. Venerol. 128:903- 909.
Mendelson RM (1980): The treatment of giardiasis. Trans. Roy. Soc. Trop. Med. Hyg. 74:438- 9.
Neta R, Oppenheem JJ, Douches SD (1988). Interdependence of radioprotective hum an recombinant interleukin, Tumor Necrosis Factor and in urine recombinant granulocyte macrophage and colony stimulating factor. J. Immunol.140:108-111.
Newman RS, Murray B, Lussier C (2001). Confrontation with aggressive peers at school students' reluctance to seek help from the teacher. J. Educ. Psychol. 93:398–410.
Paget GE, Barnes JM (1964). Evaluation of results: Qualitative application in different species. In: Laurence DR, Backarach AL (eds.), Evaluation of drug activities. Pharmacometrics. Academic press, London and New York. 1:160-167.
Petri W, Smith R, Schlesinger P, Murfhy C, Radvin J (1987). Isolation of the galactosebinding Lectin that mediates in vitro adherence of *E. histolytica*. J. Clin. Invest. 80:1238-1244.
Petschow BW, Batema RP, Talbott RD, Ford LL (1998). Impact of medium-chain monoglycerides on intestinal colonization by Vibrio cholera or enterotoxigenic *Escherichia coli*. J. Microbiol. 47:383-389.
Rauch AM, Van R, Bartlett AV, Pickering LK (1990). Longitudinal study of *Giardia lamblia* infection in a day care center population. Periatr. Infect. Dis. J. 9:186-189.
Rayan P, Stenzel D, McDonnell PA (2005). The effect of saturated fatty acids on *Giardia* duodenalis trophozoites *in vitro*. Parasitol. Res. 10:1432-1435.
Samarawickrema NA, Brown DM, Upcroft JA, Thammapalerd N, Upcroft P (1997). Involvement of superoxide dismutase and pyruvate:ferredoxin oxidoreductase in mechanisms of metronidazole resistance in *Entamoeba histolytica*. J. Antimicrob. Chemother. 40:833-840.
Sarker SK, Mondal D, Siddique MA (2010). Study of sensitivity pattern of metronidazole on axenic culture of *E.histolytica* in vitro. *J Dhaka*
Singer SM, Solaymani-Mohammadi S (2010). *Giardia duodenalis:* the double-edged sword of immune responses in giardiasis. Exp. Parasitol. 126:292–297.
Singer SM, Nash TE (2000). T-cell-dependent control of acute *Giardia lamblia* infections in mice. Infect. Immun. 68:170-175.
Stauffer W, Abd-Alla M, Ravdin JI (2006). Prevalence and incidence of *Entamoeba histolytica* infection in South Africa and Egypt. Arch. Med. Res. 37(2):266-9.
Teles NS, Fechine FV, Viana FA, Viana IO,Nascimento DF, Leite AL, Bezerra FA, Moraes MO,Moraes ME (2011). Evaluation of the therapeutic efficacy of Mentha crispa in the treatment of giardiasis. Contemp Clin. Trials 32(6):809-13.
Wang W, Keller K, Chadie K (1992). Modulation of Tumor Necrosis factor production by macrophage in *Entainoeba histolytica* infection. Infect. Immunol. 60:3169-74.
Wassmann C, Hellberg A, Tannich E, Bruchhaus I (1999). Metronidazole resistance in the protozoan parasite *Entamoeba histolytica* is associated with increased expression of iron-containing superoxide dismutase and peroxiredoxin and decreased expression of ferredoxin 1 and flavin reductase. J. Biol. Chem. 37:26051-26056.