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Full Length Research Paper

Evaluation of the therapeutic effect of some natural plant extracts on experimental cryptosporidiosis

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Cryptosporidium parvum (C. parvum) **is a coccidian protozoon causing cryptosporidiosis, a parasitic disease of the mammalian intestinal tract. It is considered to be the most important waterborne diarrhoeic pathogen in developing countries. Infection causes drastic and retractable diarrhoea with dehydration. But unfortunately, this protozoon resists all levels of chlorination ever-known. The present work aims at evaluating the effect of both naural extracts: lauric acid (monolaurin or coconut), and ginger in *C. parvum* infected mice. Fifty laboratory bred albino mice were used in the experiment. Animals were divided into 5 groups: Group 1: infected treated with Lauric acid. Group 2: infected treated with ginger. Group 3: infected given combined half doses of both Lauric acid and ginger. Group 4: infected treated with Nitazoxanide antiparasitic drug (infected treated control). Group 5: infected untreated control. Drugs were given for seven consecutive days, followed by sacrifice of all mice. Stool samples were collected for oocysts count. In addition, histopathological examination of upper part of the small intestine was done, and the duodenal contents were examined for oocysts count. There was a significant reduction in *C. parvum* oocysts count following treatment. The highest percentage of reduction was noticed in the Nitazoxanide treated group (96.7%), followed by Lauric acid (92.5%), followed by the combined treated group (84.4%). At last, came the Ginger treated group (72.3%). Histopathological examination revealed complete healing of intestinal mucosa after nitazoxanide treatment, while partial healing of the lining intestinal epithelium was noticed after lauric acid, ginger and combined treatment. In conclusion, this study showed that Lauric acid (monolaurin) or coconut offers an alternative or concurrent therapy to the conventional anti- cryptosporidial drugs. The best cure rates were obtained following treatment with Nitazoxanide followed by Lauric acid.**

Keywords: *Cryptosporidium*, Mice, Lauric acid, Nitazoxanide, Coconut, Ginger.

INTRODUCTION

Cryptosporidium parvum is a coccidian intracellular protozoon causing an important waterborne disease in developping countries called cryptosporidiosis (Haque *et al*., 2003). The disease causes severe life-threatening diarrhea in immunocompromised hosts (Guk *et al*., 2005). Infection is caused by faeco-oral route. The pathology is induced by invasion of the apical tip of the ileum by sporozoites and merozoites. Drugs used in the treatment are Nitazoxanide (Nitazoxid) and Azithromycin (Zithromax). These drugs can alleviate diarrhea by attacking the metabolic processes of the *C. parvum* organisms. However, these drugs are ineffective against the infectious cysts, can produce side effects, even toxicity and are not always available in developing countries. Therefore, one of the greatest challenges for public, rural, and medical health professionals involved in the control of parasitic diseases is finding novel, safe and cost-effective drug alternatives (Rosenblatt, 1992).

Actually, some of the naturally derived fatty acids, known as the medium-chain, saturated, fatty acids (MCSFAs) (Lauric acid) possess powerful antimicrobial properties against yeast *(Candida albicans)*, bacteria including the pathogens *Vibrio cholera, Neisseria gonorrhea* (Sun *et al*., 2003) and also proved to decrease *Giardia duodenalis* trophozoites in vitro (Mostafa *et al*., 2005). It was found that *Zingiberofficinale Roscoe* or Ginger is one of the world's most widely used herbal anthelmintic extract. Its activity ranges against sheep gastrointestinal nematodes (Zafar *et al*., 2006), free-living

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protozoa *Tetrahymenapyriformis* (Elmer-Rico *et al*., 2004) and antischistosomal activities (Mostafa *et al*., 2011). The present work was done to evaluate the potential effect of medium-chain, saturated, fatty acids (MCSFAs) Lauric acid and ginger versus the commercially used (Nitazoxanide) in experimental animals infected with *Cryptosporidium parvum* oocysts.

MATERIAL AND METHODS

This study was conducted at the animal house and parasitology department, Theodor Bilharz Research institute (TBRI), Egypt. The TBRI approved the experimental study which was conducted according to International Valid Guidelines.

Animals

Fifty laboratory bred albino mice were provided by the Schistosome biological supply program (SBSP) in TBRI. Animals were divided into 5 groups (10 mice each).

Group 1: mice given Lauric acid (Monolaurin).

Group 2: mice given ginger (Ginger).

Group 3: mice given a combination of ginger and Lauric acid half the doses of each.

Group 4: infected treated control mice given Nitazoxanide .

Group 5: infected untreated control mice group.

Infection of animals

A) Isolation of *C. parvum* oocysts from diarrheic calves. The stool samples were collected in sterile clean stool cups and were repeatedly concentrated by sedimentation and centrifugation to obtain the oocysts (Arrowood and Donaldson, 1996).

B) Counting of oocyts: the infecting dose was calculated by taking the average of 3 counts, each of them done in 1ml of stool sediment by taking 1 µl from the stool sample and counting the oocysts in it.

C) Infection dose: a pilot study was performed to calculate the infecting dose, infecting each mouse with 1000 oocysts did not cause infection, increasing the dose to 3000 oocysts succeeded to cause the infection but further increase to 5000 oocysts caused death of mice.

D) Infection: mice were orally infected with *C. parvum* oocysts using tuberculin/esophageal tube.

Drug administration: *Drugs used:*

1) Monolaurin (Lauric acid) ® (300 mg) manufactured and provided by [ECOLOGICAL FORMULAS, CA 94518, CONCORD, USA].

2) Ginger (ginger) ® (400mg) was manufactured and provided by [Arab Company for pharmaceutical and Medical Plants MEPACO-MEDIFOOD, Enshas-Sharkeya, Egypt].

3) Nitazoxanide (Nitazoxid) ® (100mg),liquid form, was manufactured and provided by [Medizen Pharmaceutical industries for Utopia Pharmaceuticals].

Dose and administration: All drugs were given for seven consecutive days in a suspension form seven days post infection. Animals were divided into five groups:

Group 1: mice given Lauric acid in a dose of 65mg/kg body weight dissolved in 200 µl distilled water, i.e. 20µg/mouse/day.

Group 2: mice given ginger orally in a dose of 100mg/kg body weight dissolved in 200 µl distilled water i.e. 20 µg/mouse/day.

Group 3: mice given a combination of half the doses of ginger and Lauric acid.

Group 4: mice given Nitazoxid orally in a dose of 65 mg/mouse/day.

Group 5: infected control mice.

Parasitological examination: Four days post infection; stool samples were examined to detect *C. parvum* oocysts. Mice included in groups I, II, III, and IV were given the corresponding drug for seven consecutive days starting seven days post infection. Two weeks post treatment, specimens were examined microscopically, to count the number of *C. parvum* oocysts per high power field. Staining was done by Modified Zeihl Nielsen stain according to (Henricksen and Pohlenz, 1981).

Animal sacrifice: Animal sacrifice was done two weeks post treatment. It was performed by intraperitoneal anesthesia. The upper part of small intestine was removed and subjected to histopathological examination.

Histopathological examination: Histopathological examination was performed at the pathology department, faculty of medicine, Ain Shams University. Pieces of small bowels were removed after mice sacrifice. Three segments of one cm length each were excised and stained with haematoxylin-eosin, then submitted to histopathological examination.

Statistical analysis of data: The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Descriptive statistics: mean, standard deviation (± SD) and range for parametric numerical data. Analytical statistics: ANOVA test, Post Hoc Test, Paired t-test.

RESULTS

Parasitological results

A significant reduction in *C. parvum* oocysts count occurred in stools of all treated groups compared to infected control group (table 1, figure1)

Table1 Number of *Cryptosporidium* oocysts in stools and percentage reduction two weeks post treatment.

Animal groups	No. of *Crypt.* Oocysts/HPF 2 weeks post treatment(Mean± SD)	Percentage of reduction in number of *Crypt.*Oocysts
Group **1**(Infected treated with Lauric acid	6.8±0.7	92.5%*
Group **2** (Infected treated with ginger	25±4	72.3%*
Group **3** (Infected treated with combination of Lauric acid and ginger)	13±2	84.4%*
Group **4** (Infected treated with nitazoxanide	3±1	96.7%*
Group **5** (infected control)	91±11	_

*High significance of P-value < 0.001

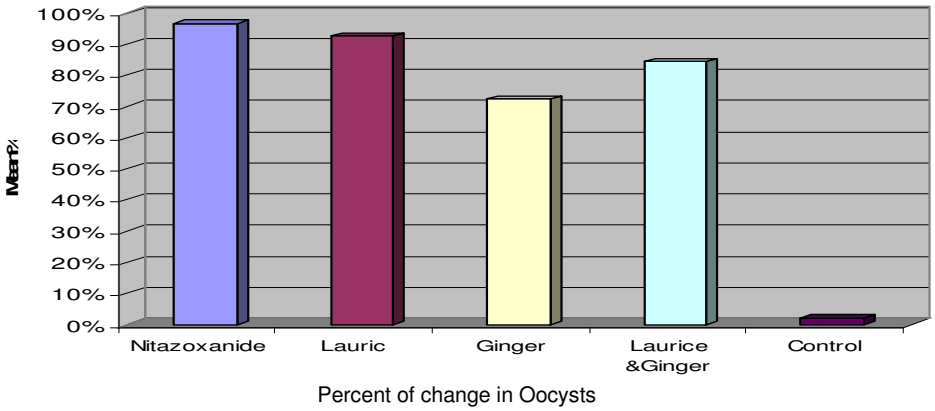

Figure 1 Comparison between different groups as regards percent reduction in *Cryptosporidium* oocysts post treatment.

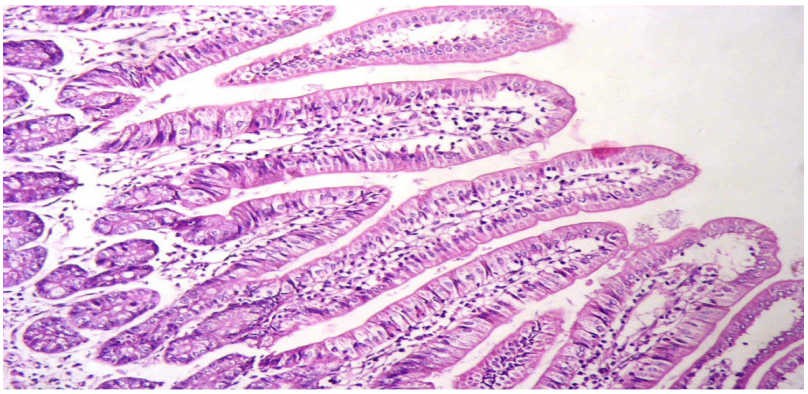

Figure 1 Complete healing of the intestinal mucosa in group (**4**) after treatment with Nitazoxanide (Nitazoxid) showing marked decrease of inflammatory infiltrate in lamina propria (H& E stain x 200).

Treatment with Nitazoxanide, resulted in the highest reduction rate (96.7%), followed by Lauric acid (92.5%), followed by the combined treated group given half the doses of lauric acid and ginger (84.4%), then the ginger treated group (72.3%) came at last.

Histopathological results

Histopathological examination revealed complete healing of intestinal mucosa after the nitazoxanide treatment (figure1), while partial healing of the lining epithelium of

Figure 2 Partial healing of the intestinal villi in group (**1**) after Lauric acid treatment with focal areas of degeneration (black arrows) still present with inflammatory infiltrate (H&E stain x 200).

the intestine was noticed after lauric acid (figure2), ginger and combined treatment as compared with the infected control group.

DISCUSSION

In this study, Lauric acid, a natural compound derived from coconut, was evaluated for its anti-cryptosporidial effect compared to the usual Nitazoxanide anti-cryptosporidial drug. It was found that Nitazoxanide was highly efective against *C. parvum* infection in mice. The percentage reduction in the number of oocysts/HPF was 96.7%. Previously, Gargala et al (2013), tested the effect of Nitazoxanide and three halogeno-thiazolides, RM-4850, RM-4865, and RM-5038 against *C. parvum* in experimentally infected immunosuppressed gerbils. They found that RM-5038 is more effective than nitazoxanide**.** Rossignol and El Gohary (2006), studied the effect of Nitazoxanide in diarrhea and enteritis caused by *Cryptosporidium* species. The authors noted that a 3-day course of Nitazoxanide is effective in treating diarrhea and enteritis caused by *Cryptosporidium* in immunocompetent patients**.**

In the present study, Lauric acid was evaluated for the treatment of *C. parvum* infection in mice. It showed a 92.5% percentage reduction in the number of *C. parvum* oocysts/HP. Previously, Christov et al (2004) performed a study on the effects of medium-chain saturated FA on protozoa in vitro. They found that these fatty acids can decrease protozoal numbers**.** Another study by Faciola et al (2012) was done to evaluate Lauric acid (LA) as a practical luminal protozoa suppressing agent. It showed high anti-protozoal activity reducing protozoa by 90% **.** Rayan *et al.* (2005) studied the effect of (Lauric acid) on *Giardia* trophozoites *in vitro* and reported that it induces *in vitro* reduction in the number of trophozoites.

Regarding the effect of ginger on experimental *C. parvum* infection, the present study showed a 72.3% percent reduction in the number of *Cryptosporidium parvum* oocysts/HPF. This goes with the previous assumption of Abo- Esa (2008). The author stated that the herbal control with ginger was safe and effective to treat the ectoparasitic protozoa *Trichodina* and *Epistylis spp***.** Mostafa et al (2011), postulated that in schistosomes, ginger proved to have antischistosomal activities. This provided a basis for subsequent experimental and clinical trials.

In this study, combined treatment with half doses of Lauric acid and ginger, caused an 84.4% percent reduction in the number of *C. parvum* oocysts/HPF. Histopathological examination of the infected control group revealed profound effect on the structure of the intestinal mucosa in comparison with the non-infected control group. This effect was in the form of villous shortening and atrophy, decrease in the ratio of villous height to crypt length, goblet cell depletion, mucosal ulceration and infiltration of lamina propria with inflammatory cells mainly lymphocytes and eosinophils with diffuse loss of the brush border of microvillous surface area. Again, *C. parvum oocysts* were detected in the intestinal lumen.These findings were in accordance with Randhawa *et al.* (1994). The authors revealed variable histopathological changes, ranging from partial to complete villous atrophy and inflammatory infiltrate attributed to *cryptosporidium* infection. Similar histopathological findings were reported by Eckmann and Gillin (2001).

The present work provides the first evaluation of combined administration of ginger and Lauric acid in the treatment of cryptosporidiosis; it also reports the first evidence for the *in vivo* effectiveness of Lauric acid as a line of treatment of cryptosporidiosis in mice.

CONCLUSION

Wide spread use of drugs to treat and control infecting organisms has almost led to the development of drug resistance. Moreover, chemotherapeutic intervention

presently offers a limited range of drugs. However, these drugs are ineffective against the infectious cysts, can produce toxic side effects, are expensive, with limited availability in developing countries.. Therefore, one of the greatest challenges for public, rural, and medical health professionals involved in the control of parasitic diseases is finding novel, safe and cost-effective drug alternatives.

The current efforts nowadays aim to improve the chemotherapy of infections including assays of natural products from different origins, which are more readily accepted and free of toxicity.

Therefore, the present study was carried out to evaluate the effect of Lauric acid, a natural product extracted from coconut oil and ginger against *C. parvum* infection. It was found that Lauric acid (monolaurin) offers an alternative or concurrent therapy with conventional antimicrobials for the treatment of cryprosporidiosis. The best cure rates recorded in this study were obtained with Nitazoxanide, followed by Lauric acid. This study could be of use in endemic areas were cryptosporidiosis could be a common devastating health problem.

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