

Effect of *Commiphora molmol* (Myrrh) Extract on Mice Infected by *Giardia lamblia*

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Abstract

Objective: To investigate the efficacy of (*Commiphora molmol* (Myrrh)) extract as alternative treatment in hamsters against giardiasis. This study was conducted to evaluate the effect of the new compound (*Commiphora molmol* (Myrrh)), as agent a natural product extracted from the roots of *Zingiber officinalis*, on *Giardia lamblia* infection as anti-protozoal and to study the parasitological and Histopathological impact of this treatment on the duodenal mucosa of infected hamsters. Fifty hamsters were divided into five groups: Group (A): normal control. Group (B): *Giardia lamblia* infected and untreated hamsters served as control. Group (C): infected with *Giardia lamblia* and treated with metronidazole in dose (120 µg/kg body weight twice daily for 7 successive days). Group (D) *Giardia lamblia* infected and orally treated with Myrrh (500 mg/Kg for 1 week). Group (E): *Giardia lamblia* infected and orally treated with combination 1/3 dose of (120 µg/kg body weight) metronidazole and Myrrh (250 mg/Kg) for 1 week). After 2 weeks of the treatment, hamsters were sacrificed. The highest percentages of reduction in the number of *Giardia lamblia* cysts and trophozoites were in the group receiving combined metronidazole and myrrh. Histopathological 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 and either myrrh plant treatment.

Keywords

Giardia lamblia, Metronidazole, *Commiphora molmol* (Myrrh) Extract, Infected Mice

1. Introduction

Giardia lamblia (syn. *G. intestinalis*, *G. duodenalis*) is an important zoonotic parasite that infects many mammals, including other animals and humans [1]

[2]. The flagellate protozoan *G. lamblia* infection in individuals can lead to abdominal cramps, acute or chronic diarrhea, and malabsorption [3] [4]. Infection is more common in children than in adults [5] [6].

Giardia is intestinal protozoan parasites. Infection with these entero-pathogens may cause diarrhea, dehydration, abdominal discomfort and weight loss. Symptoms can be present in the absence of any significant morphological injury to the intestinal mucosa, and infections may remain asymptomatic or become chronic for reasons that remain obscure. Despite the worldwide prevalence of these diseases, the pathophysiological mechanisms underlying intestinal disturbances in giardiasis remain incompletely understood [7].

In industrialized countries, Giardia causes outbreaks of diarrheal diseases in day-care centers and water-associated outbreaks [8].

Trophozoites inhabit the upper small intestine and are responsible for the symptoms of the disease, whereas cysts develop in the lower intestine and are excreted with the feces. This allows *Giardia* survival outside the intestine and transmission between susceptible hosts [7].

There are some agents to treat giardiasis such as metronidazole, tinidazole, furazolidone, paramomycin and nitazoxanide [9]. However, many problems are associated with the currently used chemotherapeutic agents including treatment failure, unpleasant side effects, activity against normal intestinal flora, possible carcinogenicity and parasite resistance [10].

Medicinal plants used in folk medicine contain a wide range of substances that can prevent and treat many diseases. The medical value of these plants is due to the presence of some bioactive constituents such as flavonoids, phytosterols, diterpenes, triterpenes, and polyphenolic compounds [11] [12] donated that *Commiphora molmol* (Myrrh) extract possesses antiulcer effects in rats and is used as a treatment of gastric ulcer.

The use of herbal medicine represents a long history of human interaction with the environment. According to WHO, more than 80% of the world's population depends upon traditional medicine for their primary healthcare needs. Medicinal plants have been used in healthcare, they contain a wide range of bioactive substance that can prevent and treat many diseases [13].

Commiphora molmol (Family *Burseraceae*) is small perennial tropical plant tree that grown in arid and semiarid regions in East Africa, Saudi Arabia and India [14]. Myrrh is a resinous exudate obtained from the stem of the plant trees. For many years, myrrh of *Commiphora molmol* is used for healing wound injuries [14]. The benefits of using myrrh in medicine have been proven in many scientific studies [15]. Previous studies showed that *Commiphora* species produced anti-inflammatory [16], analgesic [17], antiulcer [18], antioxidant [19] [20], and hypolipidemic [21] effects. *Commiphora* tree also induced antibacterial [12] [22] [23] and antischistosomal activities [24].

To avoid the resulting side effects and reduce the dose and long duration when treated with metronidazole. The use of combination of plant extract *Commiphora molmol* (Myrrh) and metronidazole hoping a good result as a line of

treatment of giardiasis.

2. Materials and Methods

2.1. Experimental Animals

The present study was conducted on fifty laboratory-bred male Syrian hamsters (*Mesocricetus auratus*) with a weight range of 100 - 110 gm. The animals were provided by Schistosome biological supply program (SBSP) in Theodor Bilharz Research Institute (TBRI). Throughout the study, the animals were kept on a standard diet containing 24% protein, 4% fat and about 4% - 5% fiber and water ad-libitum in the biological unit of TBRI under a temperature of 24°C.

Parasites and infection: *G. Lambia* cysts were obtained from diarrheic patients attending parasitology laboratory in outpatient clinic of TBRI. Stool containing *G. lambia* cysts were repeatedly washed with saline and sieved in order to get rid of debris and concentrate the cysts. Faecal sample was collected and clean, wide-mouthed covered containers and examined by direct smear [25].

The method was used Methylate-iodine-formaldehyde concentration Technique (MIFC [26]). Each hamster was infected orally by 10,000 cysts of *G. lambia*.

2.2. Drug Administration

1) Metronidazole (Flagyl) was supplied by Rhone Opulence Rorer Company, like suspension. The dose given orally to each hamster was 120 µg/kg twice daily for 7 successive days [27].

2) *Commiphora molmol* (myrrh) in a dose of 500 mg/Kg dissolved in dimethylsulphoxide 1% (DMSO), then dissolved in 99% distilled water. The suspension was orally given orally for 7 consecutive days to each hamster.

*The doses were calculated by extrapolation of human therapeutic doses to animal doses according to the table of [28].

Commiphora molmol Resin

Dried oleo-gum resin of *Commiphora molmol* (myrrh), Family Burseraceae, in the present study were purchased from Harraz Company for agriculture seeds and medicinal plants, Cairo, Egypt. Myrrh resin is present in the form of yellowish masses as illustrated in Figure 1.



Figure 1. *Commiphora molmol* (myrrh) resinous exudate.

Preparation of Plant Extract

Two hundred grams of myrrh resin powder were soaked in one liter of 90% ethanol and kept in a refrigerator with daily shaking for 3 days. The ethanol extract was filtrated using a double layer of musk into getting rid of debris. The liquid extract was concentrated under reduced pressure using Rotatory evaporator adjusted at 50°C and connected to a vacuum pump. The prepared ethanol extract was kept in refrigerator till further use. This procedure was described by Shalaby and Hamowieh (2010) [13].

2.3. Ethics

Anesthetic procedures complied with the ethical guidelines approved by the Ethical Committee of the Federal Legislation and National Institutes of Health Guidelines in USA were approved by the Medical Ethical Committee of Theodor Bilharz Research Institute (TBRI) in Egypt.

2.4. Experimental Design

All animals included in the present study were divided into 5 groups according to the drug they received.

- hamsters were divided into five groups:
 - Group (A): normal control.
 - Group (B): *Giardia lamblia* infected and untreated hamsters served as control.
 - Group (C): infected with *Giardia lamblia* and treated with metronidazole in dose (120 µg/kg body weight twice daily for 7 successive days).
 - Group (D): *Giardia lamblia* infected and orally treated with Myrrh (500 mg/Kg for 1 week).
 - Group (E): *Giardia lamblia* infected and orally treated with combination metronidazole 1/3 dose of (120 µg/kg body weight) and Myrrh (250 mg/Kg) for 1 week). After 2 weeks of the treatment, hamsters were sacrificed.

2.5. Histopathological Examination

- After scarification of hamsters, their small bowels were removed. Three segments of one cm length each were excised at a distance 5, 15 and 25 cm from the gastro duodenal junction.
- The excised segments were submitted to Histopathological examination as follows:
 - The excised segment was opened longitudinally, oriented on a filter paper and fixed in 4% formaldehyde. After fixation; the tissues were processed for paraffin embedding.
 - Histopathological sections of 4 mm thickness were stained with haematoxylin-eosin.
 - They were examined microscopically under low power (×200) and high power (×400) to detect Histopathological changes that occurred due to Giardiasis and to assess the degree of healing of intestinal mucosa after drug administration [29].

2.6. Statistical Analysis

- Data were coded and entered using the statistical packages SPSS version 7.5.
- Comparisons between groups were done using chi-square test for qualitative variables and analysis of variants (T-test) and multiple comparison post Hoc test for quantitative variables.
- P-values lower than 0.05 were considered as statistically significant.

3. Results

The present investigation of the *in vivo* effect of the receiving myrrh extracts of *Commiphora molmol* (myrrh) in a dose of 500 mg/Kg one week, on hamster infected with *G. lamblia* showed effective variable anti-giardial activity.

There were Significant dose-dependent reductions Percentage of reduction cysts/gm. stools 99.78% in faecal cyst and intestinal trophozoite counts 81.33% was detected in group treated with myrrh, the higher number of *Giardia* trophozoites and percentage of reduction in the contents of the small intestine of infected hamsters received a combination of metronidazole and myrrh was 91.77%.

Number of *Giardia* cysts and the percentage of reduction in the stool samples of infected hamsters two weeks after received a combination of metronidazole and myrrh 99.92% (Table 1).

Histopathological examination of duodenal showing in group A (normal control) (Figure 2).

Group B showing section of small intestine with ulceration of the lining intestinal epithelium, with *Giardia* trophozoites in the intestinal lumen (Figure 3).

Partial healing of the intestinal villi in hamsters of group C after treated myrrh with the presence of few *Giardia* trophozoites in the intestinal lumen (Figure 4).

Group E showing sections of small intestines following treatment with metronidazole and myrrh with no *Giardia* trophozoites in the lumen (Figure 5).

Table 1. Effect of Metronidazole 1/3 dose of (120 µg/kg body weight) versus or combined 1/2 dose of myrrh (250 mg for 7 days, 2 weeks post-treatment, the number of *Giardia lamblia* cysts excreted/gm stool and the number of vegetative forms (Trophozoites) in small intestine were estimated.

Animal group	Trophozoites in small intestine Mean ± SD	% of reduction	Cysts/gram stool Mean ± SD	% of reduction
Group (A) normal hamster	0		0	
Group (B) infected control	45 ± 21.41		5724 ± 355	
Group (C) infected receiving Metronidazole	21.5 ± 6.22**	52.22	185 ± 16***	96.77
Group (D) infected receiving myrrh	8.4 ± 0.71***	81.33	12.4 ± 0.2***	99.78
Group (E) infected receiving combination Metronidazole and myrrh	3.7 ± 0.25***	91.77	4.2 ± 0.5***	99.92

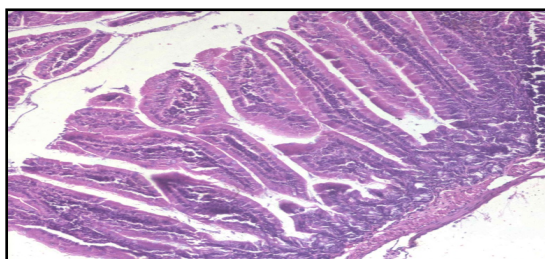


Figure 2. Group A (normal control) (Hx & E stain $\times 200$).

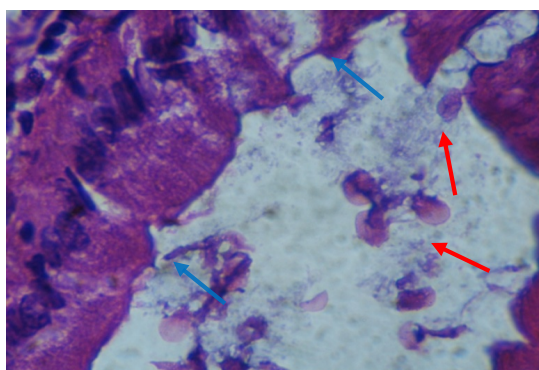


Figure 3. Section of small intestine in group B (infected control hamster) showing ulceration of the lining intestinal epithelium (blue arrow) with *Giardia* trophozoites (red arrow) in the intestinal lumen (Hx & E stain $\times 1000$).

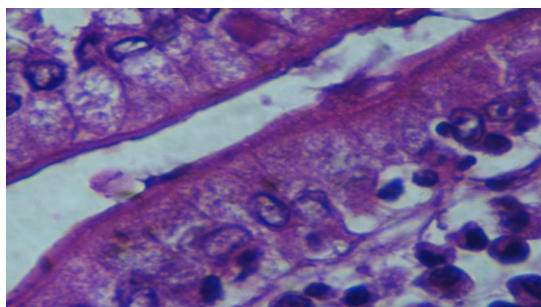


Figure 4. Partial healing of the intestinal villi in hamsters of group c after treated myrrh with the presence of few *Giardia* trophozoites (blue arrow) in the intestinal lumen (Hx & E stain $\times 1000$).

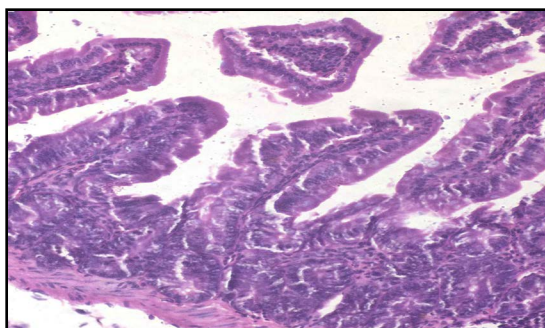


Figure 5. Sections of small intestines of hamsters in group E following treatment with metronidazole and myrrh with no *Giardia* trophozoites in the lumen (Hx & E stain $\times 400$).

4. Discussion

The main goal of the present study is to evaluate the (*Commiphora molmol* (Myrrh) anti-protozoal, which is used to treat gastrointestinal disease and its effect on *G. lamblia*. A traditional herbal medicinal product, containing myrrh, chamomile flower, and coffee charcoal, has been used in Germany for the relief of gastrointestinal complaints for decades. [4] [30] had been identified numerous waterborne outbreaks of diarrhoeal disease. Metronidazole, albendazole and mebendazole are used as drug for the treatment of giardiasis, but the high dosage and duration of treatment show some adverse reactions appear to be related to, including mutagenesis and carcinogenesis [31]. There is an increasing need for the evaluation of new and effective alternative therapies against *G. lamblia* without side effects. In addition, the parasite's resistance to drugs is a growing concern [32]. The treatment of gastrointestinal disorders used Plants, such as diarrhoea and dysentery, augmented the possibility of new alternative therapies [33].

Commiphora molmol (myrrh) (CME) extract exhibited a significant protective effect on gastric mucosa against aspirin—induced ulcer the protective effect of CME was evident by decreased volume and total acidity of gastric juice and length of ulcer [12].

The most commonly used solvents for investigation of antimicrobial activity from plant materials are organic solvents (methanol and ethanol) and water [34] [35], in this investigation, the methanol extract of pomegranate peel was tested for anti-Giardia activity and it demonstrated positive results.

Our study investigation of the *in vivo* effect of the receiving myrrh extracts of *Commiphora molmol* (myrrh) in a dose of 500 mg/Kg for one week, on mice infected with *G. lamblia* showed effective variable anti-giardial activity.

In our study, a higher significant dependent reductions percentage of reduction cysts/gm stools 99.78% in faecal cyst and intestinal trophozoite counts 81.33% was detected with myrrh.

These results go in harmony with Mahmoud *et al.* (2014) [36] who found that in the treatment with combination of metronidazole and myrrh, the high number of Giardia trophozoites and percentage of reduction in the contents of the small intestine of infected hamsters two weeks after received a combination of metronidazole and myrrh was 91.77. This result goes with the previous finding of [2] [37] [38].

This study found in the mean number of Giardia cysts and the percentage of reduction in the stool samples of infected hamsters two weeks after received a combination of metronidazole and myrrh was 99.92% (Table 1). This result agreed with Amer *et al.* (2007) [39]. On the other hand, Dyab *et al.* 2016 [40] estimated that, *in vivo* ginger (more effective) and curcumin extracts significantly treated infected mice, and this was evidenced by the faecal cyst and intestinal trophozoite counts reduction, in addition to evident improvement of intestinal mucosal damages induced by Giardia infection. *Z. officinale* and *C. longa* ex-

tracts may represent effective and natural therapeutic alternatives with low side effects and without drug resistance on the treatment of giardiasis.

Histopathologic examination of duodenal: in the control animals group A (**Figure 2**).

Group B showing ulceration of the lining intestinal epithelium with *Giardia* trophozoites in the intestinal lumen show in group B (infected hamster) (**Figure 3**).

Partial healing of the intestinal villi in hamsters of group c after treated myrrh with the presence of few *Giardia* trophozoites in the intestinal lumen, however with combined treatment, sections of small intestines of hamsters in group E following treatment with metronidazole and myrrh with no *Giardia* trophozoites in the lumen (**Figure 5**).

These results agree with Fahmy *et al.*, 2008 and El-Shennawy *et al.*, 2009) [41] [42] who resulted that in combination treatment, show complete repair of the intestinal cell projection as well as healing of mucosa and submucosa. Meanwhile, partial healing of destructed intestinal cell projection was observed in group receiving metronidazole alone.

Mokrzycka *et al.* (2010) [43], resulted that the different morphological and immunohistochemical (lack of immune-reactivity for iNOS) results in the duodenum of children with giardiasis can elucidate a variety of clinical symptoms from asymptomatic to severe infection.

5. Conclusion

The combined group (*Commiphora molmol* Myrrh and Metronidazole) was found to decrease trophozoite and cyst than Metronidazole. *Commiphora molmol* (Myrrh) had a promising effect on *G. lamblia* infection and was superior than treated with Metronidazole alone.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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