EVALUATION OF GIARDIA LAMBLIA TREATMENT IN EXPERIMENTALLY INFECTED HAMSTERS

By

SHIMAA MOHAMMED ABDEL AAL¹, ABDALLAH MICHEL BOGHDADI¹, NAGLAA FOUAD ABBAS IMAM¹, IBRAHIM RABIA ALY², And ABEER SAID ABDEL- GHANY AL-ANTABLY³

Department of Medical Parasitology¹, Faculty of Medicine, Cairo University, and Department of Medical Parasitology², Theodore Bilharz Research Institute, Imbaba, P.O. Box 30, Giza, Egypt (*Correspondence: asalantably@kasralainy.edu.eg, Orchid ID: 0000-0002-8381-7002)

Abstract

This study evaluated Curcumin Chitosan nanoparticles versus Nitazoxanide on G. lamblia infected hamsters. They were divided into six groups: G1 healthy, G2 infected, G3 infected treated with Nitazoxanide, G4 infected treated with Curcumin, G5 infected treated with Chitosan nanoparticles and G6 infected treated with Curcumin Chitosan nanoparticles. Treatment started 3 weeks post infection. Stool samples were daily collected and examined. The results showed reduction in cysts in Nitazoxanide, Curcumin, Chitosan nanoparticles and Curcumin Chitosan nanoparticles treated groups compared to control.

Key words: Giardiasis - Curcumin - Chitosan – Nano particles - Nitazoxanide.

Introduction

Giardia species are unicellular flagellates that infect the gut of many vertebrates (Thompson and Monis, 2012), by ingestion of cysts either by fecal oral route or indirectly by consumption of contaminated food or water (Gardner and Hill, 2001). Clinical manifestations varied between asymptomatic to severe symptomatic cases (Anuar et al, 2015), which was characterized by watery, sometimes foul-smelling diarrhea alternated with soft, greasy stools, fatigue, stomach cramps and bloating, gas, nausea, and weight loss (Lalle, 2010). Moreover, giardiasis and Helicobacter pylori co-infection is common in Egyptian school aged children and modulates gastrointestinal manifestations (El-Badry et al, 2017). The risky groups were children than adults, pregnant women and immune compromised people or without access to safe drinking water (Faubert, 2000).

Diagnosis of Giardia is based on detection of microscopic cyst in stool samples (Soares and Tasca, 2016) or duodenal sampling (Beal et al, 1970) and the update on the evaluation, diagnosis (Leung et al, 2019).

Medications included Metronidazole or Flagyl® (Abdel-Fattah and Nada, 2007), and Tinidazole or Tindamax® both have many side effects, but it can be given in a single dose (Vakkilainen et al, 2020). Also, Nitazoxanide (Alinia®) in a liquid form, nitazoxanide may be easier for children to swallow. Side effects may include nausea, gas, yellow eyes and brightly colored yellow urine (Matadamas-Martinez et al, 2020).

Chitosan is primarily chitin product widely distributed in nature, mainly as the structural component of the exoskeletons of crustaceans, insects, in marine diatoms & algae, as well as in some fungal cell walls (Tharanathan and Kittur, 2003). Commercial interest in chitosan and its derivatives rose from the fact that they combine several favorable biological characters, included biodegradability, biocompatibility and non-toxicity that made them valuable materials for therapeutic, biomedical and industrial applications (Raafat and Sahl, 2009). Meanwhile, curcumin have therapeutic properties of antioxidant, anti-inflammatory and remarkable safety for oral ingestion with poor aqueous solubility and rapid metabolism (Bavarsad et al, 2018), effectiveness in colon cancer (Baell and Walters, 2014), and Alzheimer’s disease (Bordonino et al, 2014) and others.

This study aimed to use curcumin chitosan nanoparticles in treating giardiasis experim-
mentally infected hamsters.

**Materials and Methods**

Experimental animals: A total of 54 parasite-free laboratory bred, male hamsters of 7 weeks old & weighed about 80-120gm were used. They were divided into six groups of nine hamsters each as G1: Healthy neither infected nor treated (negative control), G2: Infected but untreated (positive control), G3: Infected and Nitazoxanide (100mg/ kg/day). G4: Infected and Curcumin (20mg/kg/ day). G5: Infected and chitosan nanoparticle (100 μg/kg/day). G6: Infected and Curcumin Chitosan nanoparticle (100μg/kg/day). All drugs were given 7 days after ensuring infection.

Parasite: Fresh stool samples positive only for *G. lamblia* cysts were obtained from diarrheic patients attended Parasitology Department’s Diagnostic and Research Unit. Positivity was approved by stained Modified Ziehl-N-Neelsen smear and formalin-ethyl acetate concentration examinations (Garcia, 2007).

Preparation of infective inoculum: Positive samples were pooled together, emulsified in normal saline, centrifuged at 2000rpm for 10 min. and sediment cysts were counted by hemocytometer. About 1000cysts/milliliter were prepared. All groups except G1 were orally infected with 1ml cysts by an esophageal tube. Weekly post-infection, hamsters’ fecal samples were collected and examined for giardiasis infections (Dyab et al, 2016). After approving giardiasis infection, drugs were given in a single daily dose for seven consecutive days.

Chitosan nanoparticles preparation (Fernandez-Urrusuna et al, 1999): A total of 500 mg of Chitosan (medium molecular weight and 85%deacetylgyte, Sigma, St. Louis, USA) was dissolved in 50ml of 1% Acetic acid and stirred at 1000rpm for 25min at room temperature until the solution became clear.

Curcumin loaded nanoparticles (Mofazzal et al, 2015) were prepared by adding chitosan to TPP solution with 500mg curcumin and chitosan (3mg/ml).

Nitazoxanide (Nanazoxid): 500mg tablets were smashed, weighed, dissolved in distilled water and given to hamsters using esophageal tube in a dose of 100mg/kg bodyweight for 7 days (Abd El-Aziz et al, 2014).

**Curcumin longa:** Curcumin powder was suspended in water 1:10 and given orally in a single dose of 20mg/kg/day dose for 7 days (Bharti et al, 2003).

Treatment efficacy was done by cysts in feces (absent, or viability & count), by using 0.1% eosin vital stain 15 min. after exposure, cysts without absorbed dye were potentially viable (Fathy, 2011).

Chemical examinations: Seven days post-treatment the ALT, AST, serum urea, serum creatinine, WBCs, RBCs, and platelet count were measured (Bartels et al, 1972).

Ethical approval: The work was approved by the Institutional Animal Care and Use Committee (IACUC), Cairo University.

Statistical analyses: Data were collected, tabulated and analyzed by using SPSS software version 20. Percentages were used to express the rates. Chi square test compared the differences among groups of variables. P-value at < 0.05 was significance.

**Results**

The results were given in tables (1 & 20 and figure (1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cysts/gm on 1st day</th>
<th>Cysts/gm on 2nd day</th>
<th>Cysts/gm on 3rd day</th>
<th>Cysts/gm on 4th day</th>
<th>Cysts/gm on 5th day</th>
<th>Cysts/gm on 6th day</th>
<th>Cysts/gm on 7th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>10500±386</td>
<td>11200±589.9</td>
<td>12033±612.1</td>
<td>11566±621.8</td>
<td>12816±865.8</td>
<td>12666±875.6</td>
<td>12333±843</td>
</tr>
<tr>
<td>G3</td>
<td>4993±276.6 (52.4%)</td>
<td>4833±196 (56.8%)</td>
<td>4450±225.8 (63%)</td>
<td>3133±709 (72.9%)</td>
<td>2083±649.4 (83.7%)</td>
<td>1266±332.7 (90%)</td>
<td>650±137.8</td>
</tr>
<tr>
<td>G4</td>
<td>9916±285.8 (5.6%)</td>
<td>10266±240.3 (8.3%)</td>
<td>1141±241.7 (7.4%)</td>
<td>1033±493.6 (10.7%)</td>
<td>1140±752.4 (11%)</td>
<td>1130±608.3 (10.8%)</td>
<td>1100±380.8 (10.8%)</td>
</tr>
<tr>
<td>G5</td>
<td>8266±605.5 (21.3%)</td>
<td>7963±606.7 (28.9%)</td>
<td>7165±249.6 (40.5%)</td>
<td>6558±3466 (43%)</td>
<td>6116±440.1 (52.2%)</td>
<td>5833±3168 (53.9%)</td>
<td>5641±305.6 (54.3%)</td>
</tr>
<tr>
<td>G6</td>
<td>8416±7308.3 (19.6%)</td>
<td>8020±1023.1 (28.4%)</td>
<td>7817±1037.7 (35%)</td>
<td>7538±1028.9 (34.8%)</td>
<td>8533±1080.1 (33.4%)</td>
<td>8436±1064.2 (33.4%)</td>
<td>8301±1043.4 (32.7%)</td>
</tr>
</tbody>
</table>
After 7 days, cysts in G3, G4 & G6 showed significant difference (p <0.001) compared to G2, but G4 not significant (P>0.05). Compared to G3, cysts in G4, G5 & G6 showed a high significant difference (P <0.001). Compared with G4 & G5, cysts in G3, G4 & G6 showed a high significant difference (P <0.001), but G2 not significant (P >0.05). Viability was in G2, G3, G4, G5, & G6 (Tab. 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Viability 7th day</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2 (positive control)</td>
<td>95.7±2.7</td>
<td></td>
</tr>
<tr>
<td>G3 (NTZ)</td>
<td>29.5±3.3</td>
<td>69.2%</td>
</tr>
<tr>
<td>G4 (Curcumin)</td>
<td>93.8±1.6</td>
<td>1.9%</td>
</tr>
<tr>
<td>G5 (Chitosan nanoparticles)</td>
<td>40±9.5</td>
<td>58.2%</td>
</tr>
<tr>
<td>G6 (Curcumin Chitosan nanoparticles)</td>
<td>62.5±8.2</td>
<td>34.7%</td>
</tr>
</tbody>
</table>

Viability was in all infected treated G3, G5 & G6 with high significant difference (p <0.001) compared to G2, but G4 not significant (P >0.05) compared to G2. Viability in G2, G4, & G6 showed a high significant difference (p <0.001), but G5 not significant (p>0.05) compared to G3. All blood parameters except WBCS showed no significant difference in all groups (p >0.05) compared to G1, but WBCS in G2, G3, G4, G5 & G6 showed a high significant difference (P <0.001) compared to G1. All chemical blood parameters showed no significant difference (P>0.05) in all except G4 compared to G1, and in G4 showed a high significant difference (P <0.001) compared to G1.

Discussion


Many drugs were used as anti-giardiasis, but with adverse activities (Fallah et al, 2007), due to the development of drugs resistance (Harris et al, 2000; Vivancos et al, 2018). Many studies investigated the invitro antigiardial properties of other commonly used medicinal plants (Anquez-Trauxler, 2011; Sadjadi et al, 2006; Saffarharandi et al, 2006; Shahabi et al, 2008).

Carter et al. (2018) in the United Kingdom stated that with the nitroimidazoles growing number of refractory, clinicians were increasingly falling back on second-line and less well-known drugs to treat giardiasis. Dafni and Böck (2019) stated that all the Biblical Medicinal Plants were known as such in Ancient Egypt and/or Mesopotamia also. Examination of the list showed that all these plants were in continuous medicinal use in the Middle East down the generations, as well as being used in the Holy Land today. Precisely in King Solomon’s words, "That which has been is what will be, that which is done is what will be done. And there is nothing new under the sun" (Ecclesiastes 1:9).

In the present work, the four drugs used, showed different degrees in decreasing number of G. lamblia cysts and viability. But, none of them gave a complete eradication of cysts. This agreed with Nabarro et al. (2018) who reported that none of the anti-giardial treatments gave 100% parasitological cure rates, particularly failure of
treatment occurred in patients with hypogammaglobulinaemia or human immunodeficiency virus (HIV), or be due to nitroimidazole-resistant organisms.

In the present study NTZ proved to be the most effective drug against giardiasis as it gave (94.7%) reduction in cysts number and (69.2%) in cysts viability compared to negative control with highly significant difference. This agreed with Müller et al. (2006), who found that NTZ induced (95%) of the *Giardia* trophozoites, and Navarrete-Vázquez et al. (2015) *in-vitro* found that NTZ gave marked efficacy against *G. intestinalis*. Also, Abdel-Hafeez et al. (2016) who evaluated *Zingiber officinale* extract compared to NTZ in (PBS) as controls gave (92.93%) decrease in giardiasis.

Moreover, clinically Romero Cabello et al. (1997) in Mexico and Abaza et al. (1998) in Egypt evaluated NTZ in treating giardiasis infections, reported cure rate of 71% & 94% respectively.

In the present study, curcumin was the least effective drug against *Giardia* cysts, with 10.8% reduction in number and 1.9% reduction in viability. Chan et al. (2005) reported that curcumin gave non-significant reduction in cysts number due to its anti-inflammatory activity, which exacerbation infection. Also, Said et al. (2012) reported a low reduction rate (13.1%) of *G. lambia* cysts. But, Dyab et al. (2016) *in vitro* evaluated the anti-giardiasis efficacy of dichloromethane extracts of *C. longa* versus MTZ, and found that the dichloromethane extract gave a lethal effect up to (85%) on *Giardia* cysts. However, the lethal effect varied from one study to another as to the solvent used and extraction system of medicinal plants, part used and their essential oils (Rios and Recio, 2005; Amaral et al, 2006). Pérez-Arriaga et al. (2006) reported that curcumin caused cytotoxic effect, which inhibited *Giardia* growth, adherence capacity, and motility.

With chitosan nanoparticles, reduction in number of cysts and viability were 52.3%, & 58.2% respectively (Li et al, 2013). Besides, chitosan modulated the immune response by increasing cellular and humoral immunity (Choi et al, 2016). The chitosan nanoparticles, with its biological properties, minimized toxicity in crossing the body physiological barrier access to specific target tissues (Jahangiri and Barghi, 2018).

This agreed with Yarahmadi et al. (2016) who reported that chitosan affected giardiasis cysts viability causing complete mortality. Also, Said et al. (2012) reported that the anti-parasitic activity of Chitosan and Chitosan nanoparticles, which reduced cysts’ number (44.2%, & 68.2%) respectively.

The present data disagreed with Chabra et al. (2019) who *in vitro* found that the mortality rate of *Giardia* cysts treated with fungal chitosan, and chitosan nanoparticles was 78 & 87 %, respectively. The chitosan have the potential development advantage of the anti-giardiasis (Chabra et al, 2019).

In the present, curcumin chitosan nanoparticles reduced the cysts number and viability (32.7%, & 34.7%) respectively. The significant reduction in cysts number by curcumin chitosan nanoparticles may be due to the fact that it increased the bioavailability to target tissues. Said et al. (2012) in experimentally giardiasis infected rats found that combination curcumin and chitosan nanoparticles caused (81.3%) reduction in the *G. lambia* cysts. Besides, chitosan nanoparticles successfully treated *Leishmania infantum* (Kayser, 2001, *Plasmodium falciparum* (Föger et al, 2006), and *Cryptosporidium parvum* (Pujals et al, 2008). Akhtar et al. (2012) found that curcumin chitosan nanoparticles developed activity against *Plasmodium yoelii*.

In the present study, there was a significant increase in the leukocyte counts in all hamsters groups. This agreed with Al-Haboobi et al. (2013) who found increase in WBCs in children infected with different intestinal protozoa, especially among children infected with active giardiasis.

In the present study, there was significant increase in liver enzymes, urea and creatinine. This agreed with Dundar and Yilmazlar.
(2015) who reported hepatotoxic and hepato-renal syndrome due to natural bi-product like Curcumin. But, Osawa (2007), Wang et al. (2012) and Trujillo et al. (2013) reported that Curcumin was protective to the liver and the kidney especially renal injury.

**Conclusion**

The outcome data showed that NTZ gave the highest reduction rate. Chitosan nanoparticles were better than curcumin chitosan nanoparticles in reduction of cysts count and viability. Curcumin was the least one, and increased liver and kidney functions. Chitosan nanoparticle is a promising safe and nontoxic anti-Giardia.

**Conflict of Interest:** The authors declare that neither have interests or received fund.

**Authors' contributions:** All authors contributed to the study concept and design, material preparation, data collection, analysis, and discussion, and final approval by Abdel Aal, SM, Boghdadi, AM, Imam, NFA, Aly, IR, and Al-Antably, ASA. The draft manuscript was written by Abdel Aal, SM, Imam, NFA, and Al-Antably, ASA.

**References**


Carter, ER, Nabarro, LE, Hedley, L, Chiodi-
Fathy, FM, 2011: Effect of Mirazid (Comniphora molmol) on experimental giardiasis. J. Egypt-


Explanation of figures
Fig. 1: Mean cysts count in infected groups over 7 days P.T.
Fig. 2: Giardia lamblia cysts in fresh stool sample.