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Therapeutic Effect of Onion (*Allium cepa)* and Cinnamon (*Cinnamomum zeylanicum)* Oils on Cryptosporidiosis in Experimentally Infected Mice

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Abstract: In this study, we investigated the effect of administration of onion (*Allium cepa*) and cinnamon (*Cinnamomum zeylanicum*) oils on the development and progression of the experimental cryptosporidiosis in mice. Four groups of susceptible mice (G1, G2, G3 and G4); 10 mice each were used. Each mouse of the first 3 groups was experimentally infected with 10^6 *Cryptosporidium parvum* oocysts. After establishment of infection in mice of G1 and G2 were orally received onion and cinnamon oils, respectively daily till the end of the experiment at a dose of 1 ml/100g body weight. The G3 mice was infected but non-treated (control positive) while the G4 mice was non-infected and non-treated (control negative). Fecal smears from mice were examined daily for 17 days post-treatment and the assessment of results was by oocysts count and mucosal histology for experimentally infected mice. The results showed that the two oils were effective against experimental infection of mice with *Cryptosporidium parvum*. *A. cepa* oil showed more potent than *C. zeylanicum.* It was concluded that administration of onion or cinnamon oils was beneficial in protecting susceptible hosts against opportunistic zoonotic parasites such as *Cryptosporidium parvum*.

Key words: Onion (*Allium cepa*) · Cinnamon (*Cinnamomum zeylanicum*) · Cryptosporidiosis · Mice · Anti-protozoa

INTRODUCTION

Cryptosporidium parvum is one of the important species of obligate enteric protozoan parasites belongs to phylum Apicomplexa which infects the gastrointestinal epithelium of most mammalian hosts including human worldwide [1, 2].

Cryptosporidium parvum has become recognized as a cause of water and food born disease in humans and animals [3, 4]. Also the parasite is common etiologic agent associated with self-limited diarrhoea in immune-competent subjects but potentially life threatening in immune-compromised individuals, such as AIDS patients [5, 6].

Many drugs have been subjected to screen tests for their efficacy against *Cryptosporidium* infection in mammals [7]. The emergence of parasites resistant to current chemotherapies highlights the importance of plant essential oils as novel anti parasitic agents and the essential oils, or their active component, have activity against parasites reside in the intestine such as Cryptosporidium, Coccidian and nematodes [8].

Onions (*Allium cepa*) plant is used as traditional remedy in the treatment of various disorders so it has particular medicinal importance [9]. Several authors have reported pharmaceutical activity of extracts of *A. cepa* including anti-tumor, anti-diabetic, antioxidant, antimicrobial, anti-allergic and molluscicidal activity [10-12].

Moreover, the pharmaceutical investigation suggested that onions has an anti-parasitic activity for many helminthes and protozoa such as, *Trichinella spiralis* and *leishmania* [13-16].

Now Cinnamon (*Cinnamomum zeylanicum*) is grown in almost every tropical region of the world and the cinnamon aromatic oil has been in use for thousands of years and possesses abroad antimicrobial spectrum, being antibacterial, antifungal, antiviral and anti-

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parasitic [17-19]. Cinnamon also benefits the immune system and improves the health of colon and thereby reducing the risk to colon cancer [20], anti oxidant activity [21] and has anti- diabetic or high cholesterol control effect [22].

Pharmacological investigation suggested that cinnamon oil has anti-parasitic activity against the flagellates, *Trichomonas* and *Histomonas meleagridis*, in chicken [23] head lice [24]. Also, it possesses insecticidal properties against termites (*Coptotermes formosanus*) also in controlling house-dust mites and arthropod pests [25-27].

Light microscopy, with haematoxylin and eosin (H&E) staining, is often sufficient to suggest or confirm diagnosis small organisms such as *Cryptosporidium* spp. [28].

This study is one of the first aimed to evaluate the anti-parasitic (anti-protozoal) activity of *Allium cepa* and *Cinnamomum zeylanicum* oils against *Cryptosporidium parvum* infection in mice.

MATERIALS AND METHODS

Experimental Infection: 40 susceptible Swiss mice aging 2-3 weeks obtained from a colony maintained in National Research Center, Dokki, Egypt. They were housed in clean wire mesh cages and offered dry feed and water. Mice were divided into 4 groups (G1, G2, G3 and G4), 10 mice of each, each mouse of the first 3 groups was experimentally infected with 10^6 *Cryptosporidium parvum* oocysts originally obtained from naturally infected calves. After confirmation of infection, each mouse of G1 and G2 daily received *Allium cepa* and Cinnamon oils respectively, orally using stomach tube till the end of the experiment at a dose of 1 ml/100g body weight. The G3 mice was infected but non-treated (control positive) while the G4 mice was non-infected and non-treated (control negative).

Fecal Examination: From third days post infection, mouse fecal pellet were collected and fecal smears were examined daily for 17 days using with Modified Ziehl-Neelsen Stain (MZN) for detection of *Cryptosporidian* oocysts as the procedure described by Henriksen and Pohlens [29]. Identified oocysts of *Cryptosporidium parvum* were collected by Sheathers sugar solution then stored in 2.5 % potassium dichromate at 4°C. The oocysts were counted in G1, G2 and G3 in 50 randomly selected microscopical fields by oil immersion lens according to method of Rasmussen *et al*. [30].

Histo-Pathological Examination: Immediately after scarification at the day 17 post treatment, the ilea of mice of all groups were collected and fixed in 10% formalin, embedded in paraffin, sectioned at 5 microns and stained with haematxylin and eosin for histo-pathological examination [31].

RESULTS

Cryptosporidium Parvum Oocysts Shedding: Examination of fecal smears from all mice groups for *Cryptosporidium parvum* detection and oocysts count per 50 microscopical fields revealed that infected non-treated control mice (G3) shed more oocysts than those of infected treated groups with *Allium cepa* and Cinnamon oils (G1and G2) throughout the experiment (17day pot treatment). There was gradually reduction in oocysts shedding in both *Allium cepa* and Cinnamon oil treated mice (G1and G2); this reduction more clear in *Allium cepa* than Cinnamon oil treated group and continued till reaching negligible degree or no oocysts detection at 14 to17 days post treatment. Examination of fecal smears of non-infected non-treated mice (G4), revealed absence of any oocysts detected all over the experiment (Fig. 1).

Histo-Pathological Examination: Histo-pathological examination of ileum epithelia of the four mice groups (G1, G2, G3 and G4), shows sharp indicative clear difference in the appearance of villi at the termination of experiment (17dpi) (Figs 2 a, b, c and d).

The villi of non-infected mice (G4) had normal appearance without showing any atrophy or villi fusion and no inflammatory response in the lamina propria were observed (Fig. 2a). On the other hand the appearance of villi of infected non-treated control mice (G3) showed evident changes including shortening, atrophy and villi fusion together leading to blunting with desquamation of most villi, association with necrosis of some entrocytes, basophilic oval or round organism embedded in microvillous border of entrocytes, hyperplasia in goblet cells and the lamina propria showed congestion reticular cell hyperplasia with mixed inflammatory response (Fig. 2 b). The villi of infected treated groups (G1and G2) show slight or no pathological changes and regained their normal appearance resembling those in non infected control group and the *A. cepa* oil treated group (G1) showed lower histo-pathological changes than *C. zeylanicum* treated one (Figs. 2 c and d).

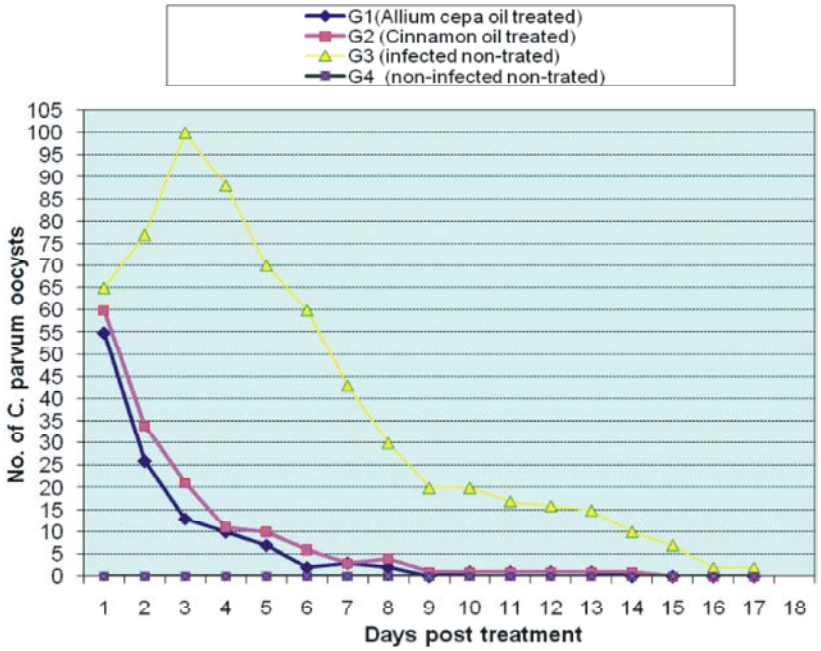
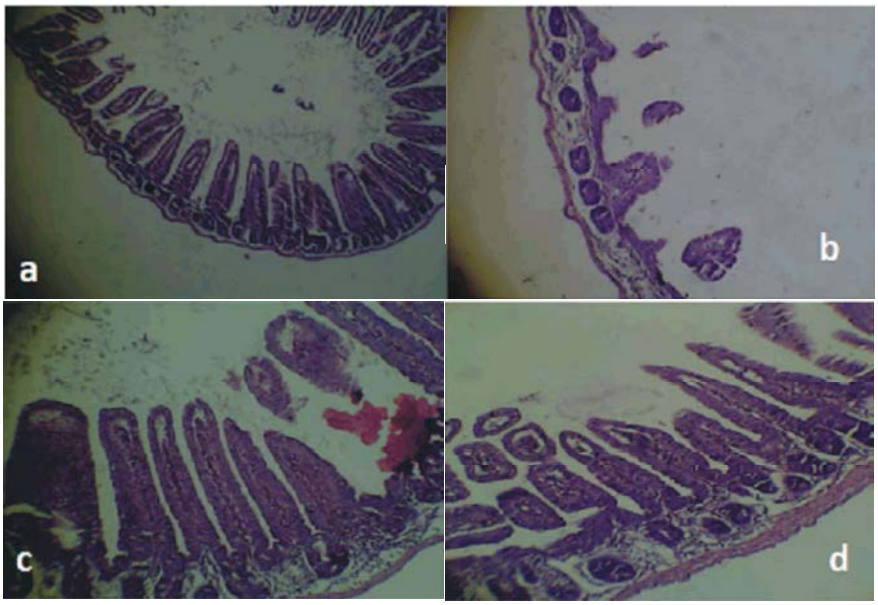


Fig. 1: *Cryptosporidium parvum* oocysts shedding from experimentally infected treated (G1& G2), infected non-treated (G3) and non infected (G4) mice



Figs. 2: Histo-pathology of mice ileum epithelia stained with H&E (X 100). *Cryptosporidium parvum* non infected (a), infected non-treated (b), infected treated with Onion oil (c) and infected treated with Cinnamon oil (d)

DISCUSSION

The present results demonstrated that both of onion (*A. cepa*) and cinnamon (*C. zeylanicum*) oils show anti-protozoal activity in a murine model of *Cryptosporidium parvum* infection. Both oils had effect on oocysts shedding, although there was no complete elimination of the parasite these may be due to the anti oxidant action of *A. cepa* and *C. zeylanicum* which help elimination of parasite [10, 21].

A. cepa oil showed more effectiveness than *C. zeylanicum* on decline number of oocysts and histo-pathological changes these may be due to penetration of some chemical compound as thiosulfinates and

isothiocyanate found in *A. cepa* [32]. Moreover the anti-parasitic properties and the medicinal effect of *A. cepa* oil might be attributed to the presence flavonoids and sulphoid compound which had offer protection against cellular damage [33]. It offers direct chemo protective roles in animal cells and helps reduce oxidative stress and may also initiate the animal cells to produce their own chemical oxidative defense mechanisms [9, 34].

C. zeylanicum oil was reported to have an immune stimulant [20]. The oil was containing eugenol which is a member of the phenylpropanoids having local antiseptic and antiphagocytic properties [35]. Some plant oils have immunomodulatory effects that are useful for treating infectious diseases, particularly in cases where the oil has no direct adverse effect on the host. El-Meleigy *et al.* [12] indicated that, cinnamon oil was the most effective oil against bacteria and the effect cinnamon oil may be attributed to their anti-inflammatory effects. The antiparasitic effect of the cinnamon may be due to its phenolic compounds which were deemed responsible for its anti-protozoal effect [23].

In the present study and from the obtained data, that both of onion and cinnamon oils had induced a significant reduction in oocysts count of *Cryptosporidium parvum* starting from the 3rd day post-treatment till scarification of mice on day 17th post treatment. As infection with *Cryptosporidium* was highly related with the state of immunity of the host and was self-limiting in stimulating the immune system of the body rendering the intestinal cells less susceptible to infection with *Cryptosporidium* and consequently, leading to a sharp reduction in oocysts count. Furthermore, these herbal oils proved to have improved the appearance of villi of ileum, where the parasite colonized. The villi in treated mice retained their normal appearance, while, those in non-treated mice suffered from apparent shortening, atrophy and desquamation of most of villi. These results agreed with Harp *et al*. [36] who they indicated that plant oils might compete for or block receptor sites on the surface of ileum, thus, leading to reduction in *Cryptosporidium parvum* colonization. This study is evaluates the effectiveness of onion *(Allium cepaa)* and cinnamon *(Cinnamomum zeylanicum*) oils against experimentally *Cryptosporidium parvum* in mice as first time in Egypt and it point out to that administration of these oils proved too had an efficient therapeutic effect on the opportunistic zoonotic *C. parvu*m and such result could be adapted in similar infections in animal or even man.

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