

Efficacy of garlic and onion oils on murin experimental *Cryptosporidium parvum* infection

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Abstract

The present study was done to evaluate the therapeutic effect of garlic and onion oils against experimental cryptosporidiosis in mice, Forty BALB/c mice, 3-4 weeks age have been divided into 4 groups (10 mice each), every mouse of the G1, G2, G3 were infected with *C. parvum* at a dose of 10^3 oocyst /ml by stomach tube, G3 was positive control, while G4 was negative control. After confirmation of infection, each mouse of G1 and G2 received 0.2 ml of garlic oil and 1 ml/100g body weight of onion oil, orally by using stomach tube daily till the end of the experiment at day 15 post treatment. Therapeutic effect of garlic and onion oils were assessed by measuring oocyst number shedded in feces of infected groups and ileum histopathological examination at end of experiment. The results showed that garlic oil and onion oils were effective against experimental cryptosporidiosis, garlic oil showed a more potent than onion oil ($P \leq 0.05$). It was concluded that administration of garlic and onion oils was efficient in treating susceptible hosts against the zoonotic parasite *Cryptosporidium parvum* infection.

Keyword : *Cryptosporidium parvum*, mice, garlic, onion, stomach tube.

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دراسة التأثير العلاجي لزيوت البصل والثوم ضد الإصابة التجريبية بطفيلي الابواغ الخبيثة في

الفئران

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الخلاصة

أجريت الدراسة الحالية لتقييم التأثير العلاجي لزيوت الثوم والبصل ضد الإصابة التجريبية بطفيلي الابواغ الخبيثة في الفئران، جلبت أربعون فأرة BALB /c بعمر 3-4 أسابيع، وقد تم تقسيمها إلى 4 مجموعات (10 فئران لكل منها)، كل فار من مجاميع G1، G2، G3 أصيب بـ *C. parvum* بجرعة 10^3 كيس بيضة/ مل بواسطة أنبوب المعدة، وكانت مجموعة G3 سيطرة موجبة، في حين مجموعة G4 سيطرة سالبة. بعد التأكد من الإصابة، كل فأر من مجموعة G1 تلقى 0.2 مل من زيت الثوم و G2 تلقى 1 مل / 100 غم وزن الجسم من زيت البصل، فمويًا عن طريق استخدام أنبوب المعدة يوميًا حتى نهاية التجربة في اليوم 15 بعد العلاج. تم تقييم التأثير العلاجي لزيوت كل من الثوم والبصل عن طريق عد اكياس البيوض المطروحة في البراز للمجموعات المصابة وفحص التغيرات المرضية في انسجة اللفانفي في نهاية التجربة. وأظهرت النتائج أن زيوت الثوم والبصل كانت فعالة ضد الإصابة التجريبية بطفيلي الابواغ الخبيثة، وأظهر زيت الثوم فعالية أكثر من زيت البصل ($P \leq 0.05$) ومن ذلك نستنتج أن زيوت الثوم والبصل كان تتسم بفعالية علاجية للمظاهر المعرضة للإصابة ضد طفيلي الكريبتوسبورديوم الحيوانية المصدر.

الكلمات المفتاحية: الابواغ الخبيثة، الفئران، الثوم، البصل، أنبوب المعدة.

Introduction

Cryptosporidiosis is a protozoal disease caused by *Cryptosporidium* spp, which is an obligate protozoan parasite belongs to phylum Apicomplexa. It is one of the common water and

food borne diseases in human and mammals and found worldwide (1). It highly infects the epithelia of the intestine and cause acute short-term infection. It spreads by the fecal–oral route and often through contaminated water, Transmitted in hard environment by oocysts which not killed by conventional disinfectants and chloridination (2). These oocysts when ingested they will excyst in the small intestine and invade its epithelial tissue (3). *Cryptosporidium* infection is characterized by water diarrhea and massive dehydration, electrolyte imbalances, malnutrition, wasting and weight loss (4). The increased resistance of the parasites to the present chemical drugs highlights the importance of oils of plants as alternative anti parasitic drugs (5). Onions (*Allium cepa*) plant is effective in the treatment of many diseases so it has particular medicinal importance (6). The onion pharmaceutical activity include anti-diabetic (7), antioxidant (8), antimicrobial, anti-allergic (9) and anti-parasitic activity for many helminths and protozoa such as, *Trichinella spiralis* (10) and *leishmania* (11). garlic (*Allium sativum*) has been used as food and medicine in many civilizations for hundred and thousands of years, It has been recognized not only as a spice but also as a substance which make a control on microorganisms (12). Garlic has been shown to have many beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, hypoglycemic (13, 14) and antitumor activities (15,16). At recent days garlic has been used widely to treat intestinal parasites (17, 18). Because of the growing need to find effective drugs to eliminate cryptosporidiosis, we design the current study to investigate the antiprotozoal efficacy of Onion and Garlic oils as medicinal plant oils in mice experimentally infected with *C. parvum*.

Material and Method

- **Oils preparation:** In this experiment, animals were administrated Garlic and onion as crude oils. The crude extract was prepared as follows: Fresh garlic bulbs and onion were separated, peeled and washed with distilled water. After drying, about 500 g bulbs of garlic and 500 g of onion were crushed separately in a blender until a uniform consistency was achieved for each one. Use soxhlet system in extraction, 500 ml of 70% ethanol at 65°C for 5 hours, filtered through Whitman No1 filter paper, concentrated in rotary evaporator vacuum, this concentrate will be diluted by sun flower oil to reach 50 ml market and stored at 25 C until use.
- **Oocyst preparation:** Feecal samples were collected from calves aged 1-2 months have a *C. parvum* positive infection (confirmed by cold Modified Ziehl-Neelsen Stain of fecal smears). These samples have been brought to the lab and washed by 10 ml normal saline and strained with tea strainer, the resulting suspension was centrifuged at 3000g/ 10 minutes the supernatant was discard (the washing step was repeated twice), the sediment resuspended with Sheather's solution and let stand for 10 minutes, the supernatant was collected and washed with normal saline (this step was twice repeated), the resulting deposit stored in 2.5% potassium dichromate 1:1 at 4°C till use. just before infection the stored oocysts solution was washed through centrifugation with normal saline 3 times to clear potassium dichromate, using hemocytometer the oocysts have been counted with a WBC count equation to calculate the required concentration for the infection (10^3 oocysts/ ml) which was prepared by dilution of the oocysts suspension in the adequate amount of distilled water (19).
- **Experimental study:** Forty BALB/c mice of 3-4 weeks age were obtained from the National Center for Control and Pharmaceutical Research/ Iraq to the animal house of Veterinary Medicine College, they have been divided in 4 groups (10 mice each) by wire mesh cages and fed dry standard pellets and water, bedding was changed everyday. The mice were allowed to adapt to the laboratory environment for one week before the experiment (20). Every mouse of the G1, G2, G3 were infected with *C.parvum* (10^3 oocyst/ ml) by stomach tube, G3 was positive control, while G4 was negative control. After confirmation of infection (through shedding of oocysts in G1, G2, G3 mice feces), daily each mouse of G1 and G2 received 0.2 ml garlic oil

(19) and 1 ml/100g body weight onion oil (21), respectively orally by using stomach tube till the ending of the experiment at day 15 post treatment. Efficacy of onion and garlic oils were assessed by daily measuring of oocysts shedding count in faeces till the end of the experiment (19, 20, 21) and ileum histological changes of all groups (22).

- **Fecal Examination:** The stool samples of all groups were collected daily to make smears stained with cold Modified Ziehl-Neelsen Stain and examined by microscope to count *C.parvum* oocysts in 50 randomly selected fields under the oil immersion lens, and mean of the counts were considered (20).
- **Histo-Pathological Examination:** The scarification of mice of all groups was achieved 15 days post treatment using over dose of ether. Small intestines were collected and fixed in 10% formalin, embedded in paraffin sections then stained with hematoxylin and eosin stain (H&E). Then intestinal sections were examined to study histopathological changes (23).
- **Statistical Analysis:** The Statistical Analysis System- SAS (2010) (24) was used to study effect of factors (group and days) as parameters of study. Lest significant difference-LSd test was used to significant compare between oocysts shedding means in this study.

Results

Shedding of *C. parvum* oocysts from infected mice groups G1, G2, G3 was begun at 5th day post infection (99, 102, 99 oocyst/ day respectively). The oocysts count means of G1 (garlic oil treated) and G2 (onion oil treated) were reduced gradually from 2nd day after treatment to the end of experiment at the day 15th post treatment, this reduction was more clear in G1 than G2. The oocysts count mean in G3 (infected not treated) was high significantly ($P \leq 0.05$) along the experiment compared with treated groups G1, G2. The G4 (not infected not treated) shed no oocysts (Fig. 1). Histopathological changes in G3 (positive control) show severe destruction of intestinal tissue, the villi show atrophy, shortening, fusion, desquamation of epithelial lining layer and infiltration of inflammatory cells in lamina propria, while there were no pathological changes observed in G4 (negative control) their ileum had a normal tissue appearance. The villi of G1 and G2 (infected treated groups) show no clear pathological changes and recover their natural appearance resembling those in G4 control group, and this was clear in garlic oil treated group (G1) (Fig. 2).

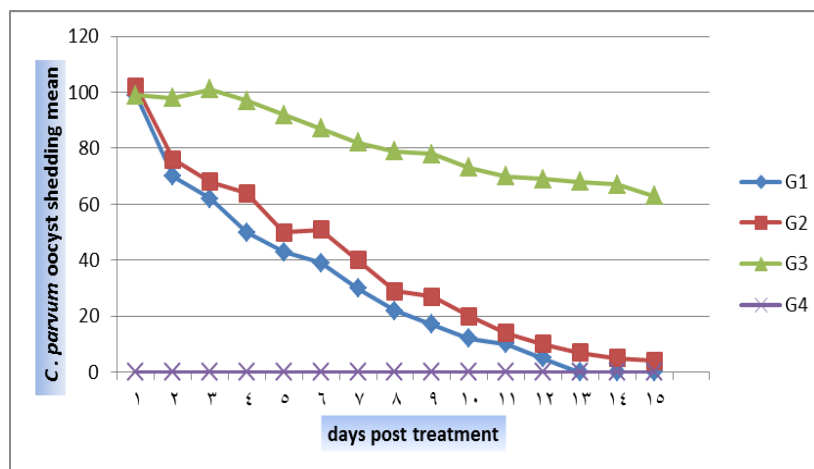


Fig. (1) *C. parvum* oocysts shedding means (oocyst/ day) for all groups G1, G2, G3, G4

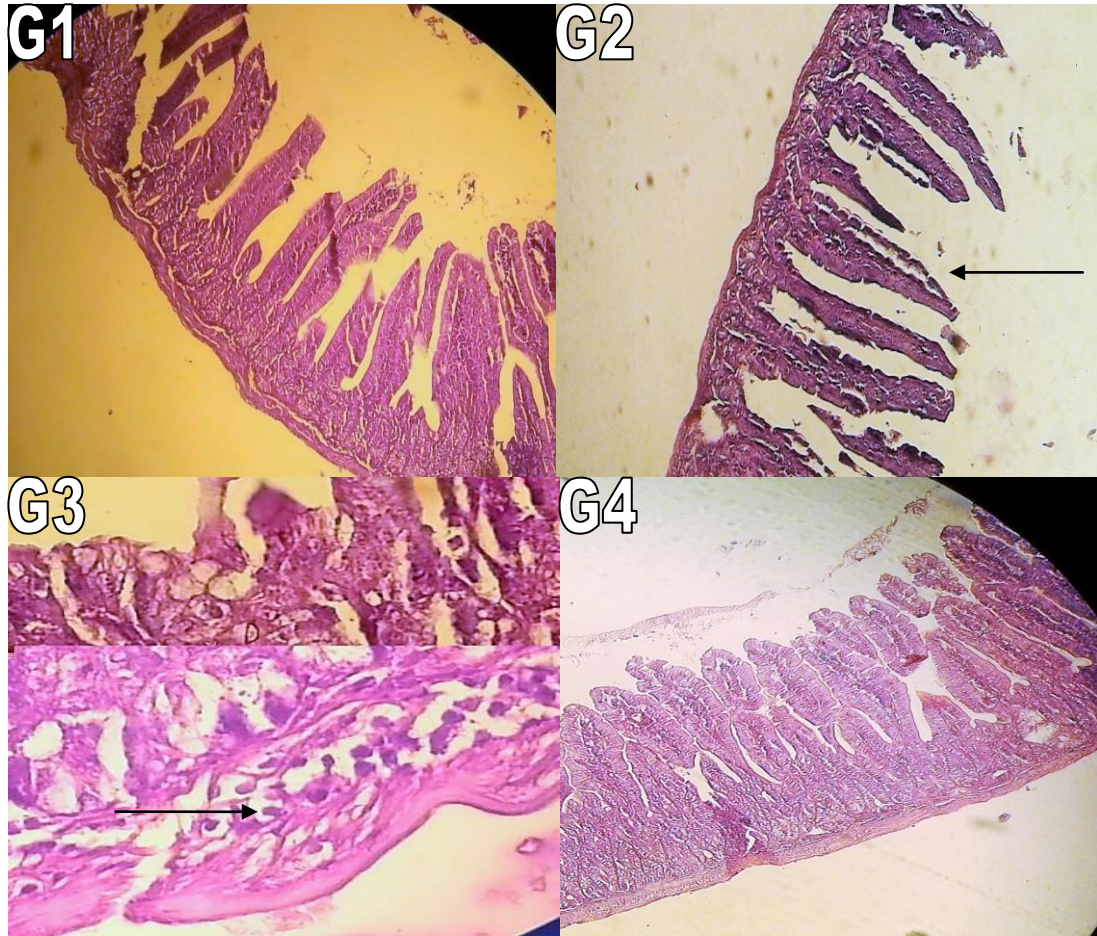


Fig. (2) Pathological changes of all study groups using haematoxylin and eosin stain X10:G1 (Garlic oil group) no clear pathological changes, G2 (Onion oil group) desquamation of epithelial lining of villi (arrow), G3 (Positive control) X40 villus atrophy (white arrow) and cellular infiltration of polymorphonuclear leukocytes in lamina propria (black arrow), G4 (Negative control) show normal architecture of intestinal tissue.

Discussion

The works to find plants have anti-parasitic activity has received a considerable attention in nowadays because of the increasing resistance of parasites to chemical drugs (25), thus this study was aimed to assess the antiparasitic efficacy of garlic and onion oils against experimental *C.parvum* infection in mice. The present results showed that both oils have antiprotozoal effect against cryptosporidiosis, they eliminate oocyst shedding, histopathologic changes in infected treated groups and have high therapeutic efficacy, that was agreed to (26) who noticed that when garlic was used for the treatment of HIV patients with cryptosporidial chronic diarrhea, complete healing occurred in some patients and partial healing occurred in the others, so agreed with (22) who demonstrated that onion oil show anti- protozoal activity in a murine model of *C. parvum* infection. In the current study the obtained data showed that both garlic and onion oils had induced reduction in oocysts count mean starting from the 2nd day post-treatment till scarification of mice at day 15th post-treatment, this agreed with (19, 27, 28). The antiprotozoal effect of garlic is belonged to allicin and some another compounds which act as antimicrobial, antioxidative and work on enhancement of phagocytosis and increase the natural killer cells activity which stimulate the immune system function, and boost the body's defense mechanism during treatment with garlic

(12). (20) reported that *A. sativum* hold up the normal physiological functions of the parasite like mobility, food absorption, and reproduction. The anti-parasitic effect of *A. cepa* oil might be attributed to the presence of antioxidants such as sulphoids and flavonoids which protect against cellular damage (29). It gives direct chemoprotective effect in body cells and reduce oxidative stress and may also help the body cells to produce their own oxidative chemical defense mechanisms (30). Based on our data, it can be concluded that garlic and onion oils have perfect effect as a promising therapeutic agent against Cryptosporidiosis and therefore validates the traditional use of them in parasitic infections. It is recommended that further studies be carried out on the uses of garlic and onion oils as a complementary medicine in the management of cryptosporidiosis.

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