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RESEARCH ARTICLE

The effect of aqueous alcohol *Artemisia herba-alba* and *Thymus vulgaris* extract on the *Cryptosporidium parvum* in theWhite Mouse (BALB/C)

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Abstract

The present work aimed to evaluate the effect of aqueous alcohol *Artemisia herba-alba* and *thymus vulgaris* extract on the *cryptosporidium parvum* and there compared with spiromycine drug in the infected White Mouse (BALB/C)by using fifth groups (G1, G2, G3, G4 and G5) included 10 mice for each, the first four groups was experimentally infected with 106*Cryptosporidium parvum* oocysts. After infection of G1, G2 and G3 in mice were orally received aqueous alcohol *Artemisia herba-alba*, *thymus vulgaris* extract at dose of 1ml /100 g body weight and spiromycine drug at dose of 1ml /1000g body weight through17 days, G4 was infected non treated (control positive) while G5 was non-infected and non-treated (control negative).The results showed more clear effects in aqueous alcohol *Artemisia herba-alba* and *thymus vulgaris* extract treated groups and continued till reaching negligible degree or no oocysts detection at 10 and 14^{th}to17 days post treatment but spiromycine drug continues oocysts execration till end experimentwith significant difference ($p>0.05$).It was concluded administration *Artemisia herba-alba*or *thymus vulgaris*extract was very protecting susceptible host against Zoonotic protozoa such as *cryptosporidium parvum*.

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 diarrhea in immune- competent subjects but potentially life threatening in immune-compromised individuals, such as AIDS patients (5, 6).

Artemisia plant is a vigorous growing annual weedy herb, usually single-stemmed, reaching up to 2–3 m in height. The plant produces a beautiful portfolio of bioactive compounds including flavonoids, coumarins, steroids, phenolics, purines, lipids, aliphatic compounds, monoterpenoids, triterpenoids and sesquiterpenoids. Thus far, the most important of the sesquiterpenoids seems to be artemisinin, dihydroartemisinic acid, artemisinic acid and arteannuin B which are stored in the glandular trichomes found in the leaves and inflorescence (7). Artemisinin derivatives are also effective against some other pathogens including those responsible for cryptosporidiosis, amoebiasis, giardiasis, clonorchiasis and leishmaniasis (8, 9).

Thyme (*Thymus vulgaris*) is a perennial Labiatae of the Mediterranean region, which has been used for centuries as spice, home remedy, drug, perfume and insecticide. In Medicine, it is used as antispasmolytic, antibacterial, and antifungal, secrotolytic, expectorant, antiseptic, anthelmintic and antitussive as reported by other

authors (10, 11, 12, and 13).Known primary constituents of the thyme include essential oil (borneol, carvacrol, cymol, linalool, and thymol), tannin, flavonoids (apigenin and luteolin), saponins, and triterpenic acids. Essential oil and extracts from fresh leaves and flowers can be used as aromatic additives in foods, pharmaceuticals, and cosmetics,*T. vulgaris* has antispasmodic, diaphoretic, expectorant, and sedative properties. As a tinture, extract, or infusion, thyme is frequently used in throat and bronchial problems, including acute bronchitis, laryngitis, and whooping cough, and also for diarrhea, chronic gastritis, and loss of appetite (14).

Materials and methods:

Chemicals:

Used antibiotic (Spiramycine) to compare with extract plant in this experimental.

Preparation of the extract:

Dry plants were procured from local market in the Baghdad city. Plants were blended in an electrical blender (sharp,Japan) until obtained final powder , weight 150 gm of plant in flask and added 450 ml of alcohol solution 70% Ethanol, put the flask in freezer (-20°C) for (15) days after that put it in on a magnetic stirrer for 20 minutes, filter the extract by gauze then filter paper (240mm) in size then put in oven 37ċ for 3days to preparation of dose concentration (1gm dissolved in 10 ml distilled water to obtained 100 mg/ml dandelion extract(16).

***C. parvum* oocysts:**

Cryptosporidium parvum oocysts were collected from the feces of some naturally infected diarrheic calves of one to two months of age from veterinary field of Baghdad University. After preparing fecal smears, the modified acid fast staining method was used to detect oocysts. Positive samples were washed through a 100 mesh sieve with Tween water (0.1% Tween 80 in distilled water). This fecal suspension was centrifuged at 3000g for 10mins in a centrifuge tube, then the supernatant fluid was discarded, and the pellet was diluted in Tween Water. *Cryptosporidium parvum* oocysts from fecal suspension were extracted by using water-ether sedimentation procedure and it was then purified by discontinuous sucrose gradients. Then, the purified oocysts were preserved in 2.5% aqueous potassium dichromate solution and stored at 4°C. Application of these two stage techniques helped to achieve much more purified oocysts suspension (5, 6).

Experimental Infection:

Fifty susceptible Swiss albino mice (BALB/ C)aging 2-3 weeks. They were housed in clean wire mesh cages and offered dry feed and water. Mice were divided into 4 groups (G1, G2, G3, G4 and G5), groups 10 mice of each, each mouse of the first 3 groups was (experimentally infected with 10^6per mouse orally oocysts administered using the stomach tube. After confirmation of infection, each mouse of G1 and G2 daily received *Artemisia herba-alba*and *thymus vulgaris* respectively, orally by using stomach tube till the end of the experiment at a dose of 1 ml/100g body weight. The G3 mice was infected but treated with Spiromycine orally by gave 0.001 ml. The G4 mice was infected but non-treated (control positive) while the G5 mice was non-infected and non-treated (control negative) buffer saline administered.

Fecal Examination: From third days post infection, mouse fecal pellet were collected and fecal smears were examine daily for 17 days using with Modified Ziehl-Neelsen Stain (MZN) for detection of *Cryptosporidium*oocytes as the procedure described by Henriksen and Pohlens(17).Identified oocytes 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 microscopically fields by oil immersion lens according to method of Rasmussen et al.(18).

Histo-Pathological Examination: Immediately after scarification at the day 17 post treatment, the small intestine 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 (19).

Statistical Analysis

The Statistical Analysis System- SAS (20) was used to effect of factors (group and days) in parameters study.

Results

Cryptosporidium Parvum Oocysts Shedding:

Examination of fecal smears from all mice groups for *Cryptosporidium parvum* detection and oocysts count per 50 microscopically fields revealed that infected non-treated control mice (G4) shed more oocysts than those of infected treated groups with aqueous alcohol *Artemisia herba-alba, thymus vulgaris* extract and Spiromycine (G1, G2 and G3) throughout the experiment(17day of treatment) with significant difference($p>0.05$).There was gradually

reduction in oocytes shedding in treated mice groups (G1, G2 and G3); this reduction more clear inaqueous alcohol *Artemisia herba-alba* and *thymus vulgaris* extract treated groups and continued till reaching negligible degree or no oocysts detection at 10 and 14th to17 days post treatment but spiromycine drug continues oocysts execration till end experiment (fig. 1),while examination of fecal smears of non-infected non-treated mice (G5), revealed absence of any oocysts detected all over the experiment.

Histo-Pathological Examination:

Small intestine histo-pathological examination showed difference in the appearance of villi of the fifth mice groups (G1, G2, G3, G4 and G5), in this experiment through 17 days (Figs 2 a, b, c, d and e). The villi of infected non-treated mice (G4) had abnormal appearance with showing clear changes including atrophy, shortening, and villi fusion together leading to blunting with desquamation of epithelial lining layer in most villi, with infiltration of inflammatory cells in the lamina propria of intestinal mucosa (Fig. 2a).On the other hand showed normal and no changes in the villi or any atrophy of non-infected control mice (G5) were observed (Fig. 2b). While the treated groups (G1and G2) had appearance slight in the villi or no histopathological changes, but the spiromycine treated group (G3) showed histopathological changes and infiltration of inflammatory cells in the sub mucosa. The aqueous alcohol *Artemisia herba-alba* and *thymus vulgaris* extract treated groups (G1and G2) showed lower histopathological changes than spiromycine treated were observed (Fig. 2c, d and e).

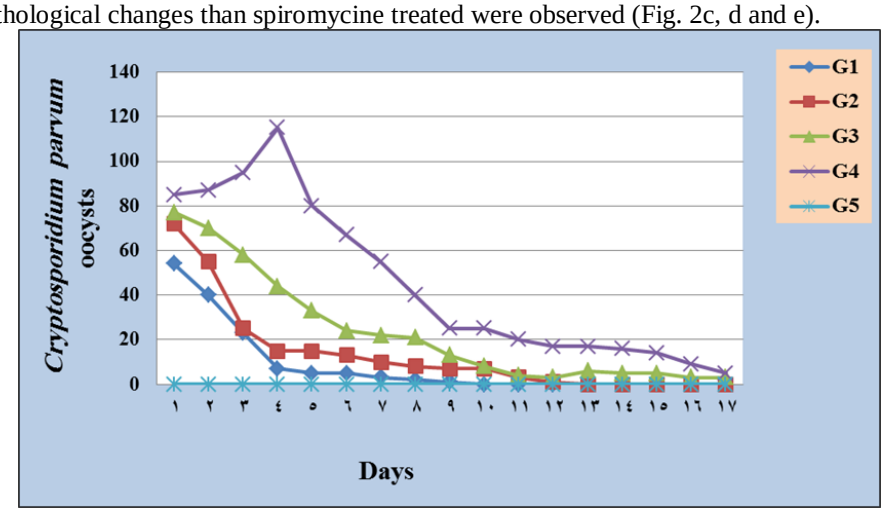

Fig1. Effect of group and day in *cryptosporidium parvum*oocyste shedding experimentally infected treated (G1) aqueous alcohol *Artemisia herba-alba*, (G2)aqueous alcohol *thymus vulgaris*extractand (G3)spiromycine(G4) infected non treated and (G5) non infected non treatedmice.

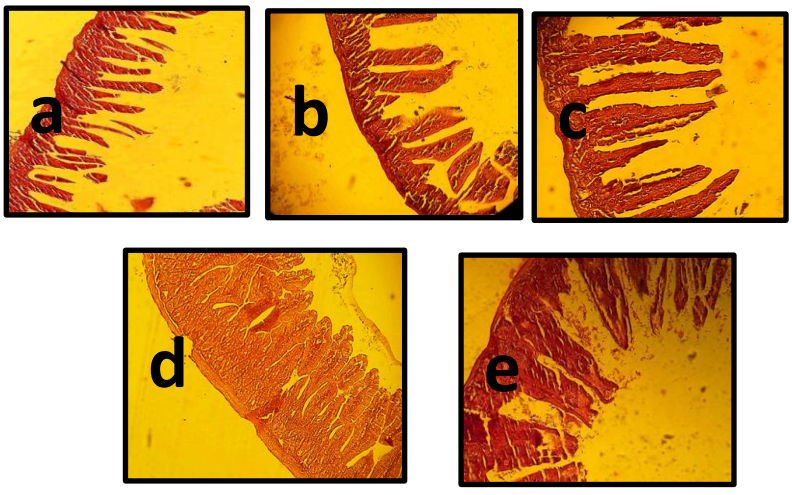

Fig 2.Histopathologyof mice intestinal epithelia stained with H&E (X100) *Cryptosporidium parvum* non infected (a), infected non-treated (b), infected treated with *Artemisia herba-alba*(c), infected treated *thymus vulgaris* with (d) and infected treated with Spiromycine (e).

Discussion

The results of this study revealed that both aqueous alcohol *Artemisia herba-alba* and *thymus vulgaris* extract was high effect on the density of *Cryptosporidium parvum* oocysts and show anti- protozoal activity on oocysts shedding in infected mice with *Cryptosporidium parvum*.

Aqueous alcohol of *Artemisia herba-alba* extract was effect in *cryptosporidium* infected mice at the 14 days due to the presence of active penetration process on the host cells due to it found some chemical compound as Artemisinin in *Artemisia herba-alba* which had offer protection against cellular damage due to influence calcium element confusion of parasite and it inhibited of penetration process of *cryptosporidium* into cells(21), the aqueous alcohol extract of *A. herba-alba* was active in *cryptosporidium* infected mice at the 14 days due to the presence of active Artemisinin compounds in *Artemisia herba-alba*. Shnawa (22) proved that the water extract of *Artemisia* sp. was active against the intestinal flagellate *Giardia lamblia* inside the body of rats. Al – Rubaie (23) showed that that water extract of *A. herba – alba*(20%) decreased the viability of protoscolices of *Echinococcus granulosus in vitro* and prevented the infection with hydatid cysts in mice bodies.

It is also reported that the efficacy of *Thymus vulgaris* was (19.1%), which is also reported to be parasite killer. Its chemical contents include beta caryophyllena,(24).The effect of plant extracts on the shedding of oocysts in experimental mice, decrease the shedding of *Cryptosporidium* oocysts in mice by using 250 mg / kg body weight, route it was shown that the efficacy of *Peganum harmala* followed by *Artemesia herba-alba*, *Ricinuscommunis* and lowest was *Thymus vulgaris* (25).While the Spiromycine drug continued shedding oocysts after treatment because of a reverse after the treatment (26). And this is agreed with the Gross *et al*. (27) that referred to resistance some of the parasites at the inefficiency of the dose used where active again for the infection to occur later.

In the present study and from the obtained data, that of *A. herba-alba*, *Thymus vulgaris* and spiromycine had induced a significant reduction in oocytes count of *Cryptosporidium parvum* starting from the 10 day post-treatment till scarification of mice on day 17thpost 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 oocytes count. Furthermore, these aqueous alcohol herbal extract proved to have improved the appearance of villi of small intestine because the Artemisia have antioxidant properties act as radical scavenger, inhabit lipid peroxidation and therefore protect the body from several diseases attributed to the reaction of free radicals, therapeutic effect of Artemisia are attributed to phenolic antioxidant such as flavonoids, tanins, coumarins, xanthens and antioxidant micronutrients eg. (cu, zn and mn). The antioxidant activity of flavonoids is efficient in trapping superoxide anion, hydroxyl, peroxyl and alcohxyl radicals, Artemisia has been used as acytoprotective agent for gastric ulcer, and that believed that dehydroleucodine is the active principal of this plant, increase gastric glycoproteins synthesis and prevent lesion of the gastric mucosa induce by ethanol and other necrotizing agents (28).

It is also reported that the efficacy of *Thymus vulgaris* which is also reported to be parasite killer. Its chemical contents include beta caryophyllena (24).While, spiromycine drug no proved to have improved of villi and found infiltration of inflammatory cells in the sub mucosa due to it not observed the phenomenon of self-limiting due to host immunity as well as Auto-infection, which plays an important role in the continues of infected and increase the number of oocysts in feces (26). 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*. (29) who they indicated that plant extract might compete for or block receptor sites on the surface of small intestine, thus, leading to reduction in *Cryptosporidium parvum* colonization.

This study is evaluates the effectiveness of aqueous alcohol *Artemisia herba-alba* and *Thymus vulgaris* extract against the parasite *cryptosporidium parvum* in mice it usefulness therapeutic effect on the opportunistic zoonotic *C. parvum* and such result could be adapted in similar infections in animal or even man.

References

1- Xiao, L.;Fayer,R.;Ryan,U.and Upton, S.J. (2004)*Cryptosporidium* taxonomy: recent advances and implications for public health. ClinMicrobiol Rev., 17: 72-97.
2- Caccia, S.M.(2005). Molecular epidemiology of human cryptosporidiosis. Parassitologia., 47: 185-192.
3- Del Coco, V.F.; Córdoba, M.A. and Basualdo,J.A.(2009). Cryptosporidiosis: an emerging zoonosis Rev. Argent Microbiol., 41: 185-196.
4- O'Donoghue, P.J.(1995). *Cryptosporidium* and cryptosporidiosis in man and animals. In J. Parasitol., 25: 139-195.
5- Pönka, A.;Kotilainen,H. ;Rimhanen-Finne,R.; Hokkanen, P.; Hänninen,M.L.;Kaarna,A.; MeriT.andKuusi,M. (2009). A food-borne outbreak due to *Cryptosporidium parvum* in Helsinki. Euro Surveill., 16: 19269.

6- Almeida, A.; Moreira,M.J.;SoaresMdle,S.; Delgado,L.;Figueiredo, J.; Silva, E.; Castro,A. and Cosa, J.M. (2010). Presence of *Cryptosporidium* Spp. and *Giardia duodenalis* in drinking water samples in the north of Portugal. Korean J. Parasitol. 48: 43-48.
7- Ferreira, J. F. S.and Janick, J. (1995). Production and detection of artemisinin from Artemisia annua. ActaHorticulturae, 390, 41–49.
8- Ma, Y.; Lu, D.; Lu, X.; Liao, L.and Hu, X. (2004). Activity of dihydro artemisinin against *Leishmania donovani* both in vitro and vivo. Chinese Medical Journal, 117, 1271–1273.
9- Yang, D. M. and Liew, F. Y. (1993). Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis. Parasitology, 106, 7–11.
10- Broucke, C.V.D.; Lemli, J. and Lamy, J.(1983). Spasmolytic activity of the flavonoids from *Thymus vulgaris*, Pharmaceutischweekblad scientific edition, Plantesmedicinalis et Phytotherapie, 16, 4, 310-317
11- Bouchberg, G.M., and Allegrini, J. (1976). ParfumiAromi, Saponi, Cosmet.. Aerosol, Essenze, 58, 10, 527.
12- Broucke, C.V.D. (1983). The Therapeutic of Thymus species. Fitoterapia, 4, 171-174.
13- Özgüven, M. and Aksu, F.(1987). Antibacterial activities of essential oils from MajoranahortensisMoench, Saturejamontana L. and *Thymusvulgaris* L., Journal of ANKEM 1, No: 3, 270-275.
14- Leung, A.Y. and Foster, S. (1996). Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. 2nd ed. New York, USA: John Wiley & Sons.
15- Griffiths, G.;Trueman,L.; Crowther,T.; Thomas, B. and Smith,B. (2002). Onions: a global benefit to health Phytother Res., 16: 603-615.
16- Ahmed, I.; Aqil, F. and Owais, M.(2006).Modern phytomedicine. Wiley. VHS Weinheim, Germany. ISBN: 3-527-31530-6.
17- Henriksen, S.A. and Pohlenz, J.F. (1981). Staining of *Cryptosporidia* by a modified Ziehl-Neelsen technique. Acta Vet Scand., 22: 594-596.
18- Rasmussen, K.R.; Healey,M.C; Cheng, L. and Yang,S.(1995). Effects of dehydroepiandrosterone in immunosuppressed adult mice infected with *Cryptosporidium parvum*. J. Parasitol., 81: 429-433.
19- Bancroft, I.D. and Stevens,A.(1990). Theory and practiceof histological techniques. Churchill Livingston, Edinburgh, UK. 3rd pp.: 255.
20- SAS. (2010). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
21- Brisibe, E.A.; Umoren, U.E.;Owal, P.U. and Brisibe,F.(2008).Dietary inclusion of dried *Artemisia* annua leaves for management of coccidiosis and growth enhancement in chickens. African J. Biotochnology, 7(22):4083-4092.
22- Shnawa, B.H. (1995). Biological and immunological studies on *Giardialamblia*(Stiles, 1915). Ph. D. Thesis, Coll. Sci., Univ. Basrah: pp: 102.
23- Al-Rubaie, S.S.M. (1999). Effect of some plant extracts in attenuation ofprotoscolecies of Echinococcusgranulosus (in vitro) and (in vivo) in white mouse (Musmusculus). M.Sc. thesis, Coll. Sci., Univ. Baghdad:95 pp.(In Arabic).
24- Maryam, F. ;Javid, S. and Fatemah, G. (2010) . Effects of two anticoccidial drugs,Monensin, Toltrazuril and the mixture of them on *Cryptosporidiumparvum* Invitro . Jundishapur J Microbiol., 4(2): 71-4.
25- Al-Dulaimi, F. H. A.; Al-Hamairy, A. K. A. and Mughier , A. H.(2013). Prevalence Of*Cryptosporidium* Sp. and Treatment By Using Some Plants Extracts In Al-Hilla City\Babylon Province. Journal of Babylon University/Pure and Applied Sciences/ No.(4)/ Vol.(21). Pp. 1211-1220.
26- Al-Azzawi, M. H. K. (2003). An epidemiological study of cryptosprdiosis isolation and diagnosis of oocysts antigen and use of some medical plant extracts for treatment. Ph. D. A thesis, University Baghdad, Veterinary Medicine College, department of parasitology.
27- Gross, T.L.; Wheat, J.; Bartlett, M. and O'connor, K.W. (1986). AIDS and multiple system involvement with *Cryptosporidium*. Am. J. Gastroentero. 81(6): 456-457.
28- Derakhshanfar, A. ;Oloumi, M.M.; Kabootari, J. and Arab, A.Y. (2006). Histopathological and biochemical study on the effect of *Artemisia sieberia* extract on experimental skin wound healing in rats. Iranian, J. Vet. Surgery, 1(1): 36-42.
29- Harp, J.A.; Jardon, P.; Atwill, E.R. ;Zylstra,M.;Checel,S.; Goff,J.P. and De Simone,C. (1996). Field testing of prophylactic measures against *Cryptosporidium parvum* infection in calves in a California dairy herd. Am. J. Vet. Res., 57: 1586-1588.