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The in vivo effect of some medicinal plant extracts on *Cryptosporidium* parasite.

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ABSTRACT

Cryptosporidium is known as a parasite of humans especially children of both those who are immunodeficient and immunocompetent. The effect of prolonged diarrhea and dehydration can be dangerous, especially for young and immunodeficient persons. This study was designed to find out the watery and alcoholic effect of some medicinal plant extracts against the parasite. No significant differences between the watery and alcoholic extracts of the three medicinal plant used (*Corindrum sativum, Curcuma longa, Viscum album*) were noted. All the plants were dose related, *Curcuma longa* had the highest effect on *Cryptosporidium* oocysts shedding in laboratory infected Balb/c mice. With rate of 100% on the 7th day of treatment at 750 mg/kg and on the 5th day at 1000mg/kg in the watery extracts. And a rate of 100% on the 4th day at 1000mg/kg in alcoholic extracts, followed by *Viscum album* with rate of 48, 54% on the 7th day at 750, 1000mg/kg respectively for watery extract and 73, 76% on the 7th day at 750, 1000mg/kg respectively for alcoholic extract. The *Coriondrum sativum* had the lowest effect at all concentrations used in both watery and alcoholic extracts. No significant differences were seen with folic acid and potassium chloride adding to the watery and alcoholic plant extracts. Except with the antibiotic (azithromycin) in which its activity was increased to 100% on the 4th day of treatment whereas its effect was only 68% without them.

Introduction.

One of the most biologically intriguing, and clinically frustrating features of cryptosporidiosis is its resistance to antimicrobial drugs. Unlike many of its relative (*Toxoplasma, Eimeria,* and *Plasmodium*), there is no curative therapy for cryptosporidiosis, despite the *in vitro* and *in vivo* testing of many compounds. One possible explanation for this is that *Cryptosporidium* establishes a compartment within the host cell, which is morphologically different from the setting used by the related parasites. This unique parasitophorous vacuole may somehow shelter the parasite from antimicrobial drugs [1].

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Because the clinical course of cryptosporidiosis depends largely on the immune status of the host, treatment options vary accordingly. In immunocompetent adults and children, no specific therapy is indicated, since the disease is self-limiting. However, as in any diarrheal illness, hydration must be carefully monitored. In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below 200/mm^3, cryptosporidiosis can be life-threatening and must be treated aggressively. Initially the nutritional hydration, and electrolyte status of the patient should be assessed and corrected with intravenous hydration, if necessary, antimotility agents such as opiates and somatostatin analogues may also be used. Several antibiotics that have some efficacy against *Cryptosporidium* have been reported, spiramycin and dicalzuril sodium have produced partial responses against the parasite (partial decrease in diarrhea and

decreases in stool oocyst number) [2]. Paromomycin has been shown to decrease the intensity of infection and improve intestinal function and morphology, paromomycin is a poorly absorbed broad spectrum antibiotic similar to neomycin [3]. The *in vitro* activity of nitazoxanide alone and in combination with azithromycin and rifabutin was investigated, nitazoxanide had showed moderate anti cryptosporidial activity (>50%), a parasite reduction of 79.8-83.9% was observed when nitazoxanide was combined with azithromycin and rifabutin [4]. When azithromycin was used alone for cryptosporidial diarrhea treatment in AIDS patients it showed good reduction in symptoms but didn't eradicate the oocysts from the stool [5].

. An experiment on more than 100 drugs *in vitro* only 40 drugs showed some affection on the parasite, no one of the antiparasitic drugs used (guinive, chloroquine, Pyrimethamin, difluromethyl orinthine ,trimethoprim-sulfamethoxazol, diclazuril) had affected the parasite[6]. Fujikawa [7] believes that paromomycin sulfate is an effective drug against *Cryptosporidium* and because of difficulties for getting the drug it was replaced by clarithromycin.

.The infection may become chronic and life-threatening which can lead to death in some individuals specifically children, the elderly, and immnosuppressed patients. There is no completely satisfactory treatment for cryptosporidial enteritis has been successfully developed, and a wide variety of medications and components have been tested as possible treatments for the illness but there is currently no drug that can cure cryptosporidiosis therefore efforts are still needed to develop an effective drug [8, 9]. Our study is a trial for finding a treatment for the parasite.

Materials and methods.

Oocysts preparation and isolation.

. *Cryptosporidium* oocysts were obtained from human feces which were diagnosed by Modified Ziehl-Nelson (M.Z.N.) procedure[10].The positive stool samples were diluted with saline or D.W and sieved through stainless steel mesh (75 µm). The sieved stool was distributed in centrifuge tubes (0.5ml of diluted stool + 10ml of D.W) and centrifuged at 2500rpm for 10min. The deposit was washed again with D.W. the second deposit was used for sucrose flotation isolation procedures. The purified oocyst was incubated with antibiotics (penicillin 5000 Iu/ml, streptomycin 5 mg/ml and amphotericin-B 50 µg/ml) at 37C° for 12hrs to kill microbial contaminants. The number of the oocysts were counted using neubar slide chamber and the dimensions of the oocysts were measured by ocular micrometer and stage micrometer slide. The oocysts were stored in aqueous $K_2Cr_2O_7$ (2.5% wt/vol) at 4-8C° until used later [11].

Treatment experiments: For trials to treating *Cryptosporidium* parasite three medicinal plants were chosen which have been used for treating intestinal disorders and diarrhea, the plants was *Curcuma longa* rhizomes, *Coriandrum sativum* and *Viscum album* fruits. The plant parts were obtained from local markets in Kirkuk and the species were confirmed in traditional Plant Center (T.P.C.) in College of Science / Tikrit University. For extracting the plant component Prabhakar [12] procedures were used with some modifications.

Alcoholic plant extraction.

.Medicinal herbs were grounded by rotary grinder to yield a fine powder,10 gm of plant powder was weighted and placed in a maceration jar with 200ml of 95% ethanol mixed thoroughly.The jar was closed tightly to prevent evaporation of alcohol and was left in a dark place for 72hrs at room temperature, shacked tightly at least twice for 3 min each day.After 72hrs, the jar was opened, the extraction was filtered with gauze then with filter paper (Whatmann No. 1).The solvent was evaporated in water bath at 50-60C°. the dry extracts percentage yield were calculated and stored at 4C° until used, (table1), [12].

Watery plant extraction**.**

. 10gm of powdered plant were weighted, added to pyrex beaker containing 200ml D. W. . The mixtuer was heated for 2hr on a hot plate at 60C°. Heat source was removed and the beaker cooled at room temperature. Solution was filtered, dried, stored as in alcoholic extracts above[12]....

In vivo screening of anti-*Cryptosporidium* effects of plant extracts.

.Seventy seven groups (plant extract treating groups + control groups) of 3 Balb/c mice (total number of mice used were 231) 4-8 weeks old 11-16g weight were administered orally with 10^3 oocysts isolated from human feces. The mice feces were examined daily for oocysts recovery [13].The infection was recovered after 3-4 days post infection. The infected mice were treated with watery and alcoholic plant extracts at concentrations of 250,750,1000mg/kg twice daily each 12 hrs. for 7 days by using stomach tube. The oocysts number in feces of infected mice were counted daily by collecting and weighting 1gm feces and diluting it in to known volume of saline. A drop of this dilution (0.1ml) was smeared on a slide and stained as in [10] procedure. The number of the oocysts were counted in the 0.1ml of

the feces and the total number of the oocysts in 1gm of feces were calculated [14].

. The feces examination was continued for three days after disappearance of oocysts in the feces to confirm the disappearance of the oocysts. For control, two groups of mice were used, one treated with an antibiotic (azithromycin) 10mg/kg as a positive control and the other not treated with plant extracts or with the antibiotic as a negative control. The synergetic effect of folic acid 0.34mg/kg and potassium chloride 5mg/kg with the watery and alcoholic plant extracts and with the antibiotic were examined too by daily feces oocysts counting.

Toxicity testing of plant extracts**.**

.The toxic effect of plant extracts were studied by weighting the mice and weighting their livers and kidneys, noting the daily activity of the mice, and measuring the enzymes (GOT, GPT) and urea levels in the blood serum for liver and kidney dysfunction recovery. For this test mice which were used for treating experiments at highest concentration (1000 mg/kg), positive control and negative control were sacrificed. Their blood were collected using insulin needle 29 gauge by heart puncher. The blood was left to clot at 4C° then centrifuged for 10 min at 3000 rpm. The serum was genteelly separated from clotted blood for GOT, GPT, and urea measuring, the morphologies and weights of the liver and kidney were noted and recorded.

Serum GOT(Glutamic-oxalo acetic transaminase) and GPT (Glutamic-pyruvic transaminase) measuring: For GOT and GPT assay kit were used from Randox com.

Procedure: 0.1ml of the sera were pipette into test tubes (0.1ml D.W in Reagent blank tube), 0.5ml of phosphate buffer 100mmol/L, PH7.4 was added to each tube, mixed, incubated for exactly 30min at 37C°, 0.5ml of 2.4-dinitrophenylhydrazine 2.0mmol/L was added to each tube. Mixed, allowed to stand for exactly 20min at 20-25C°.0.5ml of sodium hydroxide 4.0mol/L was added. Mixed, the absorbance of samples was read at 546nm against the blank. GPT and GOT levels were obtained from standard absorbance tables in u/ml.

Serum urea measuring: Serum urea was measured using kit from biolabo reagents com.

Procedure: 1ml of working reagent (salicylate 31mmol/L, nitroprussiate 1.67mmol/L and urease ≥15Kul/L) were pipette into test tubes (Blank, standard, assay),5µl of demineralised water was added into blank tube,5µl of urea 40mg/dL (6.66mmol/L) into standard tube and 5µl of sera into assay tubes were added too.Mixed and waited for 4min at room temperature, 1ml of base solution sodium hypochlorite 7 mmol/L and sodium hydroxide 62mmol/L were added to each tube. Mixed, and let stand for 8min at room temperature. Absorbance were read at 600nm against blank.. Serum urea levels were calculated by this equation: Urea $= \frac{\text{Abs(Assay)}}{\text{Abs (Standard)}} \times$ standard concentration.

.Different statistical tests were used for analyzing the results according to the data : Analysis of variance for factorial experiment with two factors and (F) tests. χ^2(chi-square) test in style of independent and in style of homogeneous. Duncans multiple –range test style of comparison between the levels of the factors. The level of significance used was $P<0.05$.

Results.

In vivo anti- *Cryptosporidium* effects of watery plant extracts:

.For *in vivo* testing of plant extracts on *Cryptosporidium* oocyst production , watery plant extracts of three medical plants with three concentrations were used and the results(table 2) showed that watery extracts at 250 mg/kg was significantly effective for *Curcuma longa* and *Viscum album* comparing with the control but not significantly effective when compared with azithromycin at $\rho < 0.05$ level. All the extracts were dose related and their effects were higher at 750, 1000 mg/kg. The *Curcuma* was the most significantly effective agent than the other plants and its effect was equal to azithromycin at 750 mg/kg. The Viscum and *Coriandrum* were significantly effective comparing with control but not with the *Curcuma* and azithromycin. At concentration of 1000 mg/kg, the *C. longa* was the most effective agent and it was even significantly higher than azithromycin followed by *V. album* which had equal effect with azithromycin. The *Coriandrum* was significantly higher comparing with control but not with azithromycin.

..The day of treating was significantly effective in increasing the plants effect at all concentrations used. The most effective one was *Curcuma* which caused the disappearance of oocysts on the 7th day with rate of 100% at 750 mg/kg and on the 5th day with rate of 100% at 1000 mg/kg. While *Viscum* and *Coriandrum* were effective with rate of 54, 41% at 1000 mg/kg on the 7th day for each one respectively comparing with azithromycin and control.

In vivo anti- *Cryptosporidium* effect of alcoholic plant extracts :

. There was no significant differences between the alcoholic (table 3) and watery (table 6) extracts, except for *Viscum* at all concentrations especially on the 7th day of treating with rate of 50,73,76% for 250, 750, 1000

mg/kg respectively comparing with 26, 48, 54% for the same concentrations in the watery extracts. The *Curcuma* was significantly higher than the two other plants at 250 mg/ml comparing with the control but not with azithromycin, followed by *Viscum* comparing with the control. The *Coriandrum* was not significantly effective at 250 mg/kg.

.At 750 mg/kg the *Curcuma* effect was equal with the effect of azithromycin comparing the two with control, followed by *Viscum* and *Coriandrum* comparing with control but not with azithromycin. The *Curcuma* had the highest effect at 1000 mg/kg compared with the other plants and with the azithromycin and control, followed by *Viscum* compared with the azithromycin and control. The *Coriandrum* was not significantly effective compared with azithromycin but not with control. The increasing of treatment period was significantly effective on decreasing of the oocysts seen in stool, for all the plants and the antibiotic compared with the control. The most effective was the *Curcuma* which caused the disappearance of the oocysts with rate of 100% on the 4th day of treatment at 1000 mg/kg ,and a rate of 82% at 750 mg/kg on the 7th day followed by *Viscum* with rate of 73% at 750 mg/kg and 76% at 1000 mg/kg on the 7th day. And the lowest was for *Coriandrum* with rate of 21, 38% at 750, 1000 mg/kg on the 7th day of treatment .

In vivo anti- *Cryptosporidium* effect of watery and alcoholic plant extracts with folic acid:

The folic acid had no synergetic nor antagonistic effect with both the watery or alcoholic extracts (table 4, 5), the effect of both plant extracts or the antibiotic were significantly not effected with adding of folic acid.

In vivo anti-*Cryptosporidium* effect of watery and alcoholic plant extracts with potassium chloride:

. The effect of potassium chloride on watery and alcoholic plant extracts (table 6, 7) were not significantly different from that of watery or alcoholic extract alone. Except with *Curcuma* at 1000 mg/kg which led to significantly decreasing the potency of the plant, and had changed the day of disappearance of the oocysts from the 4th day in alcoholic plant extract alone to the 7th day with adding of potassium chloride.

In vivo anti-*Cryptosporidium* effect of watery and alcoholic plant extracts with folic acid and potassium chloride:

The effect of adding potassium chloride and folic acid together to the watery and alcoholic plant extracts (table8, 9) were not significantly effective in decreasing the stool oocyst numbers at all concentrations used and at all days of treatment, compared with the watery and alcoholic extracts alone. But it was significantly effective with the azithromycin, they increased its affectivity. On the 4th day the effect was increased to 100% with both the watery and alcoholic extracts.

The toxic effect of the plant extracts:

. For determining the toxicity of the plant extracts the daily activity, animal weight, liver and kidney morphology and weight, and serum GPT, GOT and urea were observed in the treated group .The activity, feeding and weight 11-16 g of the mice under treatment were not affected comparing with control nor any mortality were seen among them. No morphological changes were seen on the livers and kidneys of the treated mice comparing with the control mice (fig.2).The weights of the livers and kidneys were not affected by plant extracts and it was 1.1-1.3 g for livers and 0.2-0.4 g for the two kidneys in both treated and control groups. The serum GPT, GOT, and urea measuring (table10) showed no significant differences between the GPT, GOT and urea levels in both treated and untreated mice compared with un infected control mice.

Discussion.

In vivo anti *Cryptosporidium* effect of watery plant extracts:

.The effect of the plant extracts on the number of oocysts produced by infected mice and the day of disappearance of this oocysts was depended on the dose and the days of treatment. This may because the amount of the effective component in low concentrations may be not enough to effect the parasite, the doses of treatment must be continued for several days in order their active component to act properly. Perrucci [15] found significant affectivity of *Mangiferin* at 250 mg/kg only after the end of treatment (10 days).

.*C. longa* was the most effective plant extracts on the parasite especially at 1000 mg/kg which cause the disappearance of the oocysts on the 5th day of treatment exceeding the effect of the azithromycin which is now used for treating the parasite. This was similar to Kadappu [5] results who improved that azithromycin treated mice had become asymptomatic after 7days of treatment, but stool samples was positive for *Cryptosporidium* even after 7days of therapy. Curcumin, a natural polyphenolic compound, has been found to be active against a variety of diseases including anti carcinogenic, antimicrobial and antiprotozoal effect [16,17].

. Curcumin inhibits cell proliferation and induces cell cycle changes in colon adinocarcinoma cell lines by a prostaglandin-independent pathway and may inhibit or stop the parasite proliferation and development, several

studies had confirmed the effect of *C. longa*. A study of chicks infected with the *Eimera maxima* demonstrated that diets supplemented with 1% turmeric resulted in a reduction in small intestinal lesion and improved weight gain [18]. Among two drugs and two plant extracts, used by Sinch [19] *C. longa* showed maximum vermifuge activity at the concentration of 50 µg/ml on *Pheretima pothuma* model. The curative effect of oil extract of *C. longa* on *Schistosoma mansoni* infected mice was recorded by El-Ansary [20], they showed that the *C. longa* was normalized the concentration of protein, glucose, AMP-deaminase and adenosine deaminase which were changed by the worm infection, moreover it was more potent in reducing egg count.

.The effect of *C. sativum* and *V. album* were not high and maximum effect of them was 40,54% at 1000mg/kg on the 7th day of treating respectively, this may because their active components are normal substances like proteins, fatty acida, carbohydrates, and minerals [21].This result is identical to several other results in trials for treating the parasite with different plant extracts each with some effect but any was not curative. The effect of *Punica granitium* was 53% at 1000 mg/kg, *Thymus vulgaris* was 50% at 1000 mg/kg [22]. Al-Alousi [13] had used five medicinal plants at 500mg/kg which their effects were ranged from 34.5% to 70.9% and not completely effected the parasite. Perucci [15] had showed reduction rate of 80% for *Mangiferin* at 1000 mg/kg. Not identical results was found by Al-Abaasi [23] who showed high effect of *Viscum album* at 1000 mg/kg on cutaneous leishmaniasis, Uma [16] showed high effect of *C. sativum* against infectious bacteria causing diarrhea at 4mg/ml, but Qadir [24] showed no significant effect of aqueous and alcoholic extracts of *C. sativum* at both high and low doses he used (0.45, 0.9 g/kg) on *Haemonchus contortus* nematode in infected sheeps. Same results were observed by Eguale [25] who detected no significant effect of C. sativum on the same warm for both watery and alcoholic extracts on 7 and 14 days post treatment.

In vivo anti *Cryptosporidium* effect of alcoholic plant extracts:

No significant differences has been noted between the watery and alcoholic plant extracts and the results of alcoholic extracts on oocysts shedding in the laboratory infected mice were similar to that of watery extracts. This may because very closely related compounds has been extracts by the two solvents or due to extraction method which include heating in watery extracts and only maceration in alcoholic extracts which may have lead to extract the same active components for both solvents, this result was identical to results showed by Sinch, Eguale [19, 25] whom showed no significant differences between the watery and alcoholic extracts of *C. sativum* and *C. long*. Although the alcoholic extracts showed no significant difference with watery extracts but data showed that the alcoholic extracts were more effective with the *V. album* especially on the 7th day with rate of 50,73,76% for 250,750, 1000 mg/kg respectively, comparing with 26, 48, 54% for the same concentrations respectively in watery extracts.This agree with Al-Abbasi [23] who extracted 11 compounds in alcoholic extracts of *V. album* versus 9 compounds in watery extracts.

The effect of folic acid and potassium chloride on the plant extractes:

.Folic acid, a water-soluble vitamin of the B-complex group when supplemented with the plant extracts had no significant effect on oocysts shedding per days of treatment. Same result was indicated by Khanna [26] who showed very little synergistic effect of folic acid when used with two medicinal plant extracts (*Ocimum sanctum*, *Commiphora mukul*) on lipid peroxidation in experimentally-induced hyperlipidemia mice. No significant effect was noted with using of potassium chloride with all plant extracts and azithromycin except with *C. longa* which decreased its effect, this may because of that the potassium chloride is interfere with the active components of the plants leading to reduction in its activity.

.The using of potassium chloride with folic acid again had no significant effect with all plant extracts but they increased the azithromycin activity, this agree with that found by Al-Jarjary [27] in which she referred to the synergetic effect of vitamin E with azithromycin and spriamycin.

The toxic effect of the plant extracts.

.The watery and alcoholic plant extracts had no toxic effect, the activity and weight of the treated mice were not affected comparing with control,this in agreement with Certad and Theodos[14, 28] whom not had recorded significant body weight change in mice infected with *Cryptosporidium*.

The weight or morphologic changes were not seen in the livers and kidneys of the treated mice, nor the serum GPT, GOT and urea were effected at the concentrations of the extracts which were used.

.For the *Coriandrum* and *Curcuma* no toxic effect had been recorded by the previous studies [29, 30] but in *Viscum album* little toxicity was recorded in very high concentrations Al-Abbasi [23] had recorded 3000 mg/kg

as LD50 and Nawaygerae [31] had recorded 1525 mg/kg as LD50.

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Table (1) Plant extracts weights yield with percentages.

Plant used	yield in gm. In alcoholic	Percentage %	yield in gm. In watery	Percentage %
Curcuma longa	0.63	6.3	1.56	15.6
Viscum album	0.51	5.1	0.58	5.8
Coriandrum sativum	0.88	8.8	1.22	12.2

Table(2):The means of oocyst numbers after different periods from giving the watery plant extracts.

Watery Plant Extract	Con. mg/kg	means of oocyst numbers / days													
		1	%	2	%	3	%	4	%	5	%	6	%	7	%
Coriandrum sativum	250	5795	0	6493	0	6887	19	6170	30	6231	21	5901	0	5008	4
	750	5038	7	6560	0	5695	34	5310	40	4889	38	4049	20	3870	26
	1000	5860	0	6792	0	5330	38	3124	65	3344	57	3243	36	3090	41
Curcuma longa	250	5511	0	5659	8	5608	33	4002	55	4010	50	4100	19	4002	23
	750	5589	0	4766	23	4020	53	3101	65	1899	76	339	93	0	100
	1000	5048	8	3023	51	2150	75	233	97	0	100	0	100	0	100
Viscum album	250	5229	4	6002	2	6206	28	5980	32	4019	49	4020	20	3872	26
	750	5112	6	5030	18	4900	43	4545	48	3341	57	2831	43	2693	48
	1000	5010	7	4925	20	4858	43	4005	54	3304	58	2360	53	2388	54
azithro mycin	10	4562	16	3931	36	2374	72	3095	65	2779	65	2182	57	1988	62
Control without treatment	.....	5444		6154		8587		8791		7850		5029		5222	

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Table (3):The means of oocyst numbers after different periods from giving the alcoholic plant extracts.

Control without treatment	Azithromycin	*Viscum album*			*Curcuma longa*			*Coriandrum sativum*			Alcoholic plant extract	Means of oocyst numbers / days
.....	10	1000	750	250	1000	750	250	1000	750	250	Con. mg/kg	
5444	4562	5140	5119	5409	3872	5006	5305	5010	5090	5109	1	
	16	6	6	0.7	29	8	3	8	7	6	%	
6154	3931	4050	6132	6201	2841	4049	5341	6211	6090	6274	2	
	36	3	0	0	54	34	13	0	1	0	%	
8587	2374	4705	5011	5801	158	4018	4505	4809	5120	7030	3	
	72	69	42	32	98	53	48	44	40	18	%	
8791	3095	3893	4095	5950	0	3180	4270	5015	5093	6400	4	
	65	56	53	32	100	64	51	43	47	27	%	
7850	2779	2496	2873	4070	0	3009	4200	3800	4172	5806	5	
	65	68	63	48	100	62	47	52	47	26	%	
5029	2182	1504	1900	2581	0	1216	2663	3425	3010	5011	6	
	57	71	62	49	100	76	47	32	40	0.4	%	
5222	1988	1233	1395	2623	0	961	2571	3261	4121	5003	7	
	62	76	73	50	100	82	51	38	21	4	%	

Table (4):The means of oocyst numbers after different periods from giving the watery plant extracts with folic acid.

Watery Plant extract +folic acid 0.34 mg/kg	Means of oocyst numbers / days
Con. mg/kg	
1	
%	
2	
%	
3	
%	
4	
%	
5	
%	
6	
%	
7	
%	

Control without treatment	Azithromycin	*Viscum album*			*Curcuma longa*			*Coriandrum sativum*			Means of oocyst numbers / days
.....	10	1000	750	250	1000	750	250	1000	750	250	
5444	4371	4410	4849	4985	4233	4335	4560	5011	5010	5343	
	20	18	11	8	22	20	16	8	8	2	
6154	3583	5015	5232	5270	2662	4903	5136	5318	5509	5765	
	42	19	15	14	57	34	17	15	10	6	
8587	3121	5131	5461	5946	1172	4255	4892	5196	5240	5991	
	64	40	36	31	86	50	43	39	39	30	
8791	2665	5668	5932	6006	705	3325	4539	5022	5116	5752	
	70	36	33	32	92	62	48	43	42	35	
7850	2892	5188	5359	5520	311	2121	4322	4348	4476	4578	
	63	34	32	30	96	73	45	45	43	42	
5029	1924	3050	3108	3693	0	1464	2796	3158	3335	3695	
	62	40	38	29	100	71	44	37	34	27	
5222	1948	2405	3062	3389	0	31	2844	3115	3151	3844	
	63	54	41	35	100	99	46	40	40	26	

Table (5):The means of oocyst numbers after different period from giving the alcoholic plant extracts with folic acid.

Coriandrm sativum			Alcoholic Plant extract +folic acid 0.34 mg/kg	Means of oocysts numbers / days
1000	750	250	Con.mg/kg	
5250	5245	5419	1	
4	4	0	%	
5133	5293	5255	2	
17	14	15	%	
5111	5675	5863	3	
40	34	31	%	
5323	5509	5617	4	
40	38	36	%	
4467	4670	4699	5	
43	41	40	%	
3196	3261	3581	6	
36	36	29	%	
3148	3214	3503	7	
40	38	32	%	

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Control without treatment	Azithrom-ycin	*Viscum album*			*Curcuma longa*		
		1000	750	250	1000	750	250
…….	10	1000	750	250	1000	750	250
5444	4371	4562	4727	4799	3551	4551	4733
	20	16	13	11	35	16	13
6154	3583	4070	4401	4661	2878	4264	4892
	42	34	28	24	53	31	21
8587	3121	5234	5260	5312	1175	5010	5335
	64	39	39	38	86	42	38
8791	2665	4651	4909	4930	98	3792	5294
	70	47	44	44	99	57	40
7850	2892	3963	4216	4329	0	2421	4567
	63	50	46	44	100	69	42
5029	1924	2887	3254	3535	0	1017	3001
	62	43	35	30	100	79	41
5222	1948	1422	2992	3511	0	953	2678
	63	72	43	33	100	81	49

Table (6) : The means of oocyst numbers after different period from giving the watery plant extracts with potassium chloride.

Curcuma longa			*Coriandrum sativum*			Watery plant extract+p-otassium chloride 5 mg/kg	Means of oocyst numbers / days											
1000	750	250	1000	750	250	Con. mg/k-g	1		2		3		4		5		6	
4115	4431	4966	5360	5593	5651	1		%		%		%		%		%		%
24	19	9	2	0	0	%	4092	4290	4550	5960	6151	6544	2		%		%		%		%		%	
34	30	26	3	0	0	%	3106	3602	5803	5363	6261	6443	3		%		%		%		%		%	
64	58	32	38	27	25	%	1901	3125	5221	5294	5469	5991	4		%		%		%		%		%	
78	64	41	40	38	32	%	1497	2290	4604	4430	4878	6232	5		%		%		%		%		%	
81	71	41	44	38	21	%	316	1421	3112	3581	4372	4339	6		%		%		%		%		%	
94	72	38	31	16	14	%	0	1119	3110	4203	4291	4872	7		%		%		%		%		%	
100	79	40	16	15	7	%																		

Control without treatment	Azithromycin	*Viscum album*		
		1000	750	250
…….	10	1000	750	250
5444	4972	4900	5122	5663
	9	10	6	0
6154	3677	4560	5232	5982
	40	26	15	3
8587	3104	5501	5914	5916
	64	36	31	31
8791	2842	4905	5445	5675
	68	44	38	35
7850	2525	4197	5201	5116
	68	47	34	35
5029	2466	2908	3459	3513
	51	42	31	30
5222	2431	2725	3457	3513
	54	48	34	30

Table (7):The means of oocyst numbers after different period from giving the alcoholic plant extracts with potassium chloride.

Viscum album			*Curcuma longa*			*Coriandrum sativum*			Alcoholic Plant extract+ Potassium chloride 5mg/kg	Con. mg/kg	Means of oocyst numbers/ days													
750	250	1000	1000	750	250	1000	750	250			1		2		3		4		5		6		7	
5013	5020	3951	3970	4151	5009	5660	5929		%	0	%	0	%	5	%	30	%	26	%	24	%	43	%	54	%	66
8	7	27	27	24	8	0	0																	
5592	5722	3106	4205	4290	5107	5602	5870																	
9	7	50	32	30	17	9	5																	
4800	5677	3101	4120	5018	5339	5700	5991																	
44	34	64	52	42	38	34	30																	
4451	4960	2662	4055	5016	5239	5510	5665																	
49	44	70	54	43	40	37	36																	
3096	4102	2001	3163	4072	4993	5122	5029																	
61	48	75	53	48	36	35	36																	
2075	3449	900	2802	3115	3223	3531	3704																	
59	32	82	51	38	36	30	26																	
2203	3331	0	1755	3027	3260	3452	3777																	
58	36	100	66	42	38	34	28																	

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Control without treatment	Azithromycin												
.....	10	1000	4977	9	4876	21	4530	47	4317	51	3152	60	2069	59	1659	68
5444	4972		9		3677		3104	64	2842	68	2525	68	2466	51	2431	54
6154	3677		40		3104		2842	68	2525	68	2466	51	2431	54		
8587	3104		64		2842		2525	68	2466	51	2431	54				
8791	2842		68		2525		2466	51	2431	54						
7850	2525		68		2466		2431	54								
5029	2466		51		2431											
5222	2431		54													

Table (8) :The means of oocyst numbers after different period from giving the watery plant extracts with folic acid and potassium chloride.

Viscum album			*Curcuma longa*			*Coriandrum sativum*			Watery Plant extract+Folic acid 0.34mg/kg + Potassium-m chloride 5mg/kg	Means of oocyst numbers/ days													
1000	750	250	1000	750	250	1000	750	250	Con. mg/kg	1	%	2	%	3	%	4	%	5	%	6	%	7	%
4860	5102	5210	2204	3173	4117	5000	5201	5211															
11	6	5	59	42	24	8	5	5															
4639	4717	5100	1962	3204	4603	5215	5236	5722															
25	23	17	69	48	25	15	15	7															
4345	4595	4920	1067	2224	4962	5620	5870	5922															
49	46	43	88	74	42	35	32	31															
4109	4351	4781	182	2215	4201	4306	4405	4794															
53	51	46	98	88	52	51	48	45															
2855	3605	3825	0	871	2580	4391	4561	4887															
64	54	51	100	98	67	44	42	38															
2822	2991	3199	0	0	1898	3588	3911	3980															
44	41	36	100	100	62	29	22	21															
973	1142	2360	0	0	1802	3127	3373	3562															
81	78	54	100	100	65	40	35	32															

Table (9):The means of oocyst numbers after different period from giving the..alcoholic plant extracts with folic acid and potassium chloride.

Azithromycin	*Viscum album*			*Curcuma longa*			*Coriandrum sativum*			Alcoholic Plant extract+Folic acid 0.34mg/kg +Potassium chloride 5mg/kg	Means of oocyst.numbers/ days													
10	1000	750	250	1000	750	250	1000	750	250	Con. mg/kg	1	%	2	%	3	%	4	%	5	%	6	%	7	%
2302	4390	4505	4662	2932	4105	4420	4870	4801	5216															
58	19	17	15	46	25	19	11	12	4															
1114	4014	4761	5139	2701	4002	4117	4738	5519	5605															
82	35	23	16	59	35	33	23	10	9															
989	3209	3895	4670	1478	3421	3449	4481	5501	5555															
89	63	55	46	82	60	59	48	36	35															
0	3408	3523	4506	204	2901	3264	4452	4522	5499															
100	61	60	48	97	67	62	49	49	37															
0	1714	3048	4371	0	1659	3302	4107	4509	5185															
100	78	61	44	100	78	59	48	42	34															
0	982	1804	3235	0	984	2896	3819	3959	4306															
100	80	64	36	100	80	42	24	21	14															
0	0	1035	2186	0	0	2248	2723	2603	3817															
100	100	80	58	100	100	57	48	50	26															

Control without treatment	**……..**	**5444**	**6154**	**8587**	**8791**	**7850**	**5029**	**5222**				

Table (10) The toxic effect of plant extracts on the livers and kidneys function in the mice under treating.

Plant type	Extract	GPT Unit/ml	GOT Unit/ml	Urea Mg/dl
Curcuma longa	Alcoholic Watery	28.6 ±2.3 28.8±2.9	36.5±1.7 36.5±1.9	3.5±0.05 3.4±0.024
Coriandrum sativum	Alcoholic Watery	29.0±2 29.0±3	36.5±1.2 36.8±0.8	3.5±0.03 3.3±0.094
Viscum album	Alcoholic Watery	28.3±2 28.8±2	36.6±0.8 36.9±2.6	3.5±0.03 3.4±0.31
Control without plant extracts	-----------	29.2±1.3	36.5±2.5	3.4±0.095
Control without plant extracts without parasite	-----------	29.1±2.2	37.0±0.9	3.4±0.11

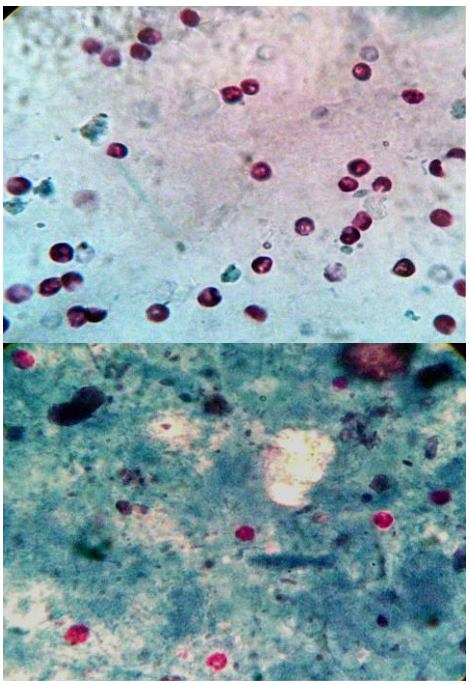
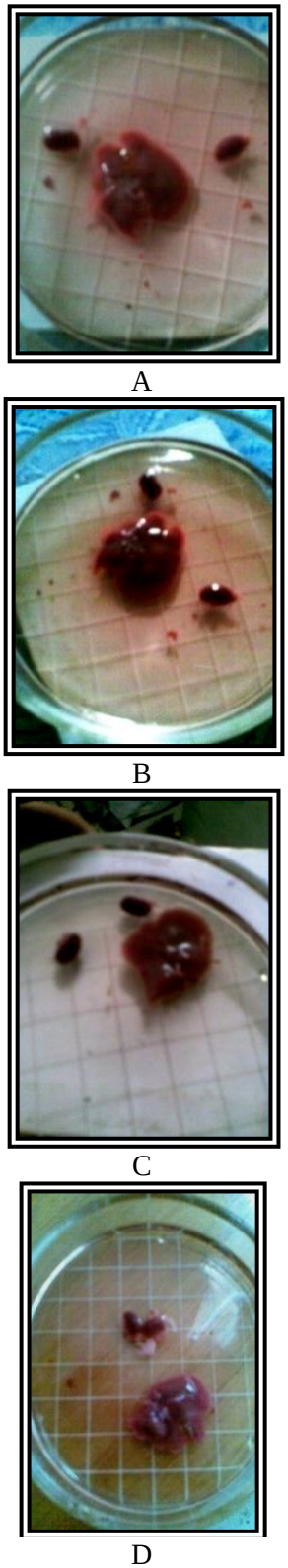


Fig.(1) Red or pink *Cryptosporidium* oocysts in human isolates stained by M.Z.N.1000x.



A
B
C
D

Fig. (2) Normal liver and kidney appearance of mice treated with the plants extracts: A (un treated) B, C, D (treated)

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تاثير بعض مستخلصات النباتات الطبية على طفيلي داء البويغات الخبيئة في الحي.

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الخلاصة .

يعرف داء البويغات الخبيئة بانه طفيلي يصيب الانسان و خاصة الاطفال من كلا الفئتين ذوي المناعة الطبيعية و ذوي المناعة القليلة . تأثير الاسهال المتسبب عن الطفيلي و فقدان السوائل الجسمية اثناء الاصابة قد يكون خطيرا جدا خاصة في الفئات العمرية الصغيرة و الاشخاص ذوي المناعة القليلة . صممت هذه الدراسة كمحاولة لأيجاد علاج للداء باستخدام المستخلصات الكحولية و المائية لبعض النباتات الطبية . لم يكن هناك فروق معنوية بين المستخلصات الكحولية والمائية للنباتات الثلاثة المستخدمة (الكركم ، الكزبرة ، الدبق), وكل المستخلصات المستخدمة كانت ذو علاقة طردية مع التركيز حيث ازدادت تاثيراتها بزيادة التراكيز . كان لنبات الكركم التاثير الاقوى لتقليل معدل الاكياس المطروحة في براز الفئران المعالجة نوع Balb/c, بمعدل تاثير 100٪ في اليوم السابع من العلاج عند تركيز 750 ملغم/كغم و اليوم الرابع عند تركيز 1000 ملغم/كلغم في المستخلص المائي و معدل 100٪ في اليوم الرابع من العلاج عند تركيز 1000 ملغم/كغم مع المستخلص الكحولي. الدبق كانت في المرتبة الثانية بمعدل تاثير 54,48٪ في اليوم السابع عند تركيز 750, 1000 ملغم/كلغم على التوالي للمستخلص المائي, ومعدل تاثير 76,73 ٪ في اليوم السابع لنفس التركيزين السابقين على التوالي للمستخلص الكحولي. نبات الكزبرة كانت لها التاثير الاقل عند كل التراكيز المستخدمة ومع كلا المستخلصين الكحولي والمائي. لم يلاحظ فروق معنوية عند اضافة حامض الفوليك او كلوريد البوتاسيوم معا او بصورة منفصلة الى المستخلصات النباتية ماعدا عند اضافتهما معا الى المضاد الحيوي ازثرومايسين حيث سببا زيادة في تاثيره الى نسبة 100٪ في اليوم الرابع من العلاج بعد ان كان نسبة تاثيره 68٪ فقط دون المادتين .