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Research Article

An Epidemiological, Diagnostic and Therapeutic Study of *Giardia lamblia* in Anbar Province – Iraq

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ABSTRACT

This study was carried out to investigate the epidemiology of *Giardia lamblia* parasites in patients who visited some of the hospitals in Anbar province, which included (Fallujah Teaching Hospital, Ramadi Teaching Hospital, Ramadi Teaching Hospital for Women and Children and Hit Hospital) during by examining 864 stool samples in a direct examination method, The results revealed the infection rate was 41.7 % and the percentage of infection among males 47.8% is higher than that of females 35.4% with significant differences ($p \leq 0.05$). The age groups (1-9) years recorded the highest rates 55.4% and the lowest rate 13.6% in the age group (40-49) years. The highest rate of infection was 62.5% during the month of June, while the month of October was the lowest rate 5% and significant differences. The incidence rate in rural areas was 50.6% higher than in the urban areas 32.5%. The study also included the effect of *Teucrium polium* L. on the parasite in the culture media HSP-1, the concentrations of 0.5-3 mg / mL significantly affected Giardia, it was noted whenever the greater the concentration, the greater the effect during different treatment periods (1-4) days, as the highest concentration 3 mg/ml killed all *Giardia* parasites on the fourth day of treatment.

Keywords: epidemiological, therapeutic, *Giardia lamblia*, *Teucrium polium*, Anbar.

INTRODUCTION

Giardia lamblia is a parasite that causes Giardiasis, a common human-animal disease[1], affecting more than 200 million people in developing countries[2], spreading in tropical and subtropical regions[3], the incidence of parasitic infection ranges between 1-7% and may reach 30% in different countries of the world and the incidence of infection increases in densely populated and poor areas[4]. The parasite affects the duodenum, the upper part of the small intestine of humans and some animals such as cattle, sheep, goats, horses, Birds, dogs[5], the parasite has two phases, the Trophozoite and the Cyst phase, the infection occurs when the bags are devoured with contaminated food and drink[6]. The parasite causes many pathological symptoms, such as Steatorrhea, Flatulence, Anorexia, Nausea, Vomiting, and Abdominal cramp[7], this parasite can cause infection in both healthy and sick people and cause diarrhea in children and adults and may lead to acute and chronic infection[8]. Several studies have been carried out in several countries to investigate the prevalence of *Giardia lamblia* parasites, including Hanevik recorded a rate 4.6% in children under 2 years of age in Tanzania[9]. Osman *et al*., found rate 28.5% during the examination of 249 children in two schools in Lebanon[10], Zylberberg *et al*., reported a 0.11% injury rate in the United States[11]. In Iraq, Al-Fahdawi recorded a 25.7% infection rate during the examination of 2,140 stool samples in four hospitals in Anbar province[12], Mehdi recorded 13.8% of the parasitic infection in the city of Ramadi[13], while Al-Fatali reported a 13.2% infection in Diwaniyah province[14], In addition to the study of Sa' el, which recorded a rate of 12.9% in the city of Baghdad[15], and the study of Shenawah, which found 17.8% in the province of Karbala[16], in addition, the researchers AL-Mehna and AL-Hamidawi which recorded a rate of 14.8% in Najaf province[17], Abdul-Abbas *et al*., recorded 58.7% of the infection in the same province[18], while Atia in Alexandria city in Babylon province recorded 48%[19]. Many plants and medicinal herbs have been used in the treatment of diseases including parasitic diseases such as Giardiasis. They have been found to contain many effective compounds including, resins, glycosides, fats, flavones, carbohydrates, titans, volatile oils, alkaloids and others[20]. The Germander plant or *Teucrium polium* plant belongs to the Labiatae (Lamiaceae) family, which is under a small shrub ranging from 25-40 cm, long, with ascending stems covered by thick gray or white cypresses, blooming from mid-April to early July, growing Plant in the flatlands and hill slopes. It is an aromatic plant used in the treatment of ulcers, intestinal inflammation, wound healing, diabetes, gum treatment, and repairs, and also treat intestinal parasites and helps to get rid of intestinal disorders accompanied by diarrhea. The most important chemical compounds are the glycosides, alkaloids, turbines, flavones, and sterols such as Apigenin and Stigmasterol, volatile oils[21]. Of the studies carried out for the treatment of parasite *G. lamblia*, the study of Kubaisi *et al*., which used the water extract of Cucurbita sp. for the effect on the parasite in vivo[22], and also the study of Hamad using the water extracts of the fruits of chili, Capsicum frutescens and black pomegranate

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Figure 1: The Germander plant *Teucrium polium.*

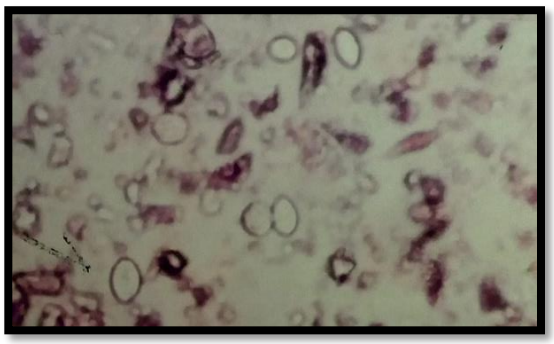

Figure 2: Cysts for *G. lamblia* parasite Dyed with iodine stain (10x).

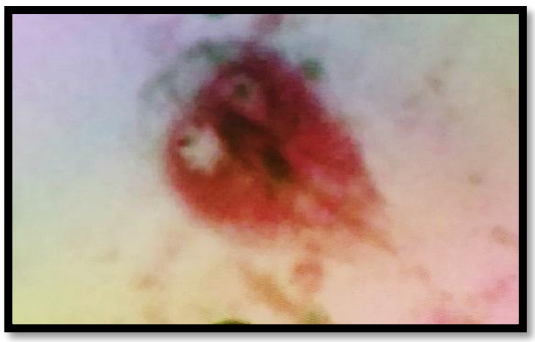

Figure 3: Trophozoite for *G. lamblia* parasite dyed with iodine stain (40x).

Table 1: Number and percentage of *G. lamblia* parasites infection by sex.

sex	Number of examined	Number of infections	%
male	441	211	47.8*
female	423	150	35.4
Total	864	361	41.7
Significant variations			Less than 0.001

* = significant

crusts Punica granatum to influence these parasites in the culture media[23], In addition to the study of al-Musawi *et al*.,[24] where they used raw powder for pomegranate peel to influence the parasite in experimental cats. The objective of this study was to investigate the epidemiology of *Giardia lamblia* in Anbar province and to determine the effect of the water extract of the *Teucrium polium* plant on the parasite in the culture media, This is the first study in the country in which this plant is used to treat this parasite to provide alternatives using plant extracts because they contain effective substances that help kill these parasites because they are less toxic than chemical drugs used to treat parasitic diseases.

MATERIALS AND METHODS

Collection and examination of stool samples

A total of 864 samples of feces were collected from patients who visited some of the hospitals in Anbar province, which included (Fallujah Teaching Hospital, Ramadi Teaching Hospital, Ramadi Teaching Hospital for Women and Children and Hit Hospital) during 2018 of both sexes and for age groups 1 to 59 years. The samples were placed in plastic containers containing 10% Natural salt with formalin for fixation, and the patient's information was taken including name, age, gender and accommodation area. The samples were examined in two ways:

Direct examination method

The sample was examined with the naked eye in addition to the microscopic examination according to the method of Price[25] by placing a drop of 0.9% normal saline solution on a clean glass slide and taking the mount of faces by the stick by different regions, especially the mucus and blood. The sample was mixed well and covered with the slide cover It was examined on the force 10 X and 40X.

Concentration Method

The fecal samples were examined with zinc sulfate, a zinc sulfate solution was prepared in 1 liter distilled water, and the stool was suspended by mixing 1 g of feces with 10 ml of warm water in test tubes. These tubes were placed in the centrifuge for one minute and at a speed of 2500 cycles / Minute, then the sediment was taken and the sedimentation process was repeated until a clear float was obtained over the precipitate, the suspended part was neglected, 2 milliliters of zinc sulfate solution was added, which dissolved the precipitate, the tube was filled to the top of the solution and covered with the slide cover, after that, another deposition was performed at the same velocity and the slide cover was transferred to a glass slide containing a drop of the iodine and examined with a microscope on force 10 X and 40 X, according to Hussein et al.,[26].

Preparation of the alcoholic extract of the plant

Table 2: Number and percentage of *G. lamblia* parasites infection by age.

Age	Number of examined	Number of infections	%
1-9	395	219	55.4**
10-19	177	85	48.0
29 -20	93	20	21.5
39-30	105	23	21.9
49-40	44	6	13.6
59-50	50	8	16.0
Total	864	361	41.7
Significant variations			Less than 0.001

** = high significant

Table 3: Number and percentage of *G. lamblia* parasites infections by month.

Month	Number of examined	Number of infections	%
January	56	16	28.5
February	48	16	33.3
March	32	11	34.3
April	54	23	42.5
May	96	38	39.5
June	208	130	62.5**
July	127	66	51.9
August	112	47	41.9
September	33	4	12.1
October	56	7	12.5
November	20	1	5.0*
December	22	2	9.0
Total	864	361	41.7
Significant variations			Less than 0.001

*= significant ** = high significant

The alcoholic extract was prepared from the Germander plant (Figure 1). Firstly, it was collected from an island area in Ramadi, which was diagnosed in Anbar University Herbarium. The plant parts were cleaned and dried and the extract was obtained according to the Harborne method[27], by weighing 25 g of dried plant parts and then dissolved in 350 ml of ethyl alcohol at 70% concentration by 10: 1 the mixture was placed in the magnetic stirrer for a homogeneous mix for 24 hours at room temperature. The solution was filtered into the Buchner Funnel and the leachate was collected and evaporated using a vacuum rotary evaporator at 60 ° C to remove the solvent, the solution was filtered with chlorophyll filter papers and was re-extracted into the evaporation device to get rid of the water and to obtain the concentrated extract. Then sterilize the extract by dissolving 1 g of it in 5 ml of Dimthyl Sluphoxide (DMSO), and then sterilized the mixture using the pasteurization process at 62 ° C for 10 minutes. Thus, the standard solution of the plant extract was obtained from which the concentrations used (0.5, 1.5, 2.5, 3) mg / ml were prepared in this study.

Purification of cysts for parasites

The cysts were purified according to Sheffield and Bjorvatan[28] by diluting the sample of 1 g in the 10 ml distilled water and the sample was filtered with a nylon cloth. The sample was filtered with a metal filter with holes 90-120mile micron, then took 4-5 ml of this sample and placed in the centrifuge at a speed of 1800 cycles/minutes for 5 minutes, the deposit was taken and 10 ml of distilled water was added and the process was repeated at the same speed, this process was repeated 3 times, then, the precipitate was suspended with the addition of 4 ml distilled water and preserved at 4 ° C until use. The parasite cysts were counted using a hemocytometer to obtain 10^5 cells/ml.

Prepare the HSP-1 culture media

The culture media was prepared according to the method of Meyer[29], by mixing and dissolving (1 g Phytone peptone, 0.15 g L-cysteine hydrochloride, 0.05 g Glucose, 8.5 ml Hanks balanced salt solution) pH of the media was adjusted to 6.7, and was sterilized by the device under the pressure of 15 pounds / Ing and temperature of 121 ° C for 10 minutes, and was added to the culture media (50 units / ml of streptomycin and 500 units / ml penicillin) and also the addition of human blood serum after the sterilization by filtration, was placed 5 ml of the culture media in each sterile tube and save at 4 ° C for transplantation of parasites *G. lamblia.*

Development of parasite in the culture media

The HPS-1 culture media was prepared for *G. lamblia* parasite, followed by the transplantation of the sample by taking 1 ml of suspension containing 10^5 cells from the cysts to the culture media, and adding 4 ml of the human serum, after that, the tubes were incubated 37 ° C in an oblique manner for 3 days. And after the third day of the transplant took a drop of the farm and placed on a glass slide and examined on 40X where it was observed the presence of the cysts and trophozoites of the parasite. A secondary transplant was performed after 10 days, and the number of trophozoites was evaluated in the first days (10^6) to maintain the survival of the trophozoites. Each day, 3 mL of the developing medium was taken and neglected and 1 mL of the new media was added.

Effect of Different Concentrations of the alcoholic extract on Vitality of Parasite

The culture media were examined and the active trophozoites of the parasite were measured (12 x 10^6) trophozoite/ml, the culture was developed in 6 groups of glass tubes, each group consists of 5 tubes containing 5 ml of culture media, 5 groups were treated with extracts (0.5, 1.5, 2.5, 3) mg/ml, respectively, while the sixth group was

Table 4: Number and percentage of *G. lamblia* parasites infection by Accommodation area.

Accommodation area	Number of examined	Number of infections	%
rural	440	223	50.6**
urban	424	138	32.5
Total	864	361	41.7
Significant variations			Less than 0.001

** = high significant

Table 5: Effect of alcoholic extract of the *Teucrium polium* plant on the vitality of *G. lamblia* parasites in culture media.

Exposure period / Concentration (mg / ml)	Killing rate % 1	2 days	3 days	4 days
control	0.0	0.0	0.0	0.0
0.5	52.2	74.1	85.0	90.5
1.5	72.4	82.2	92.3	100
2.5	88.0	94.7	100	100
3	100*	100	100	100
mean	78.1	87.7	94.3	97.6

* Concentration 3 mg/ml is most effective in killing the parasite after one day of treatment with plant extract

considered a control group (without treatment), the tubes were incubated at 37 ° C for 4 days. The parasite counts were calculated using the Haemocytometer slice and the percentages of the activity of Giardia parasite trophozoites were recorded daily according to the following equation:

% of live trophozoite

$$= \frac{\text{Number of live trophozoites} \times 100}{\text{Total number of trophozoite}}$$

% of dead trophozoite

$$= \frac{\text{Number of dead trophozoite} \times 100}{\text{Total number of trophozoite}}$$

Chemical detection of active compounds in plants[30]

Detection of glycosides

5 ml of Benedict reagent was added to 1 ml of extract in a test tube and within a water bath heated to 100°C. After 5 minutes, the tube was cooled, and the presence of a red deposit indicated the existence of glycosides.

Detection of flavonoids

A 10 ml solution of 50% ethanol was added to 10 ml of 50% potassium hydroxide and mixed in an equal amount; the presence of a yellow color indicated the existence of flavonoids.

Detection of phenols

1 ml of extract was added to 1 ml of 1% ferric chloride solution; the surfacing of a green or green-like shade indicated the presence of phenols.

Detection of alkaloids

10 grams of plant extract was boiled with 50 ml of distilled water containing 4% hydrochloric acid (HCL). The solution was cooled and filtered. Following this, 0.5 ml of leachate was tested in a watch glass with 0.5 ml of Meyer reagent; the emergence of a white deposition confirmed the presence of alkaloids.

Detection of terpenes

a mixture of the following chemical materials was prepared: 1 gram of plant extract was dissolved in 2 ml of chloroform, followed by a drop of anhydrous acetic acid and a drop of concentrated sulfuric acid. The presence of brown sediment indicated the existence of terpenes.

Detection of the saponins

3 ml of mercuric chloride solution (1%) was added to 5 ml of the extract; the appearance of a white sediment indicated the presence of the saponins.

Detection of tannins

A few drops of lead acetate solution (1%) were added to 5 ml of the plant extract; the emergence of white gelatin deposits confirmed the presence of tannins.

Detection of resins

50 ml of ethyl alcohol (95%) was added to 5 grams of plant extract and heated in a water bath. The solution was then filtered and combined with 100 ml of distilled water containing 4% of HCL. The appearance of turbidity indicated the presence of resins.

Detection of Volatile Oils

A few drops of plant extract were added to the filter paper to reduce saturation and exposure to ultraviolet radiation, the appearance of gray color indicates the presence of volatile oils.

statistical analysis

The statistical analysis was performed using the Chi-square and the least significant difference (L.S.D.), using the statistical program SAS[31].

RESULTS AND DISCUSSION

The results showed that the percentage of the infection of parasite *G. lamblia* reached 41.7%, by examining 864 stool samples for patients of some hospitals in Anbar province. The percentage of infection among males was 47.8%, higher than that of females (35.4%) (Table 1) and significant differences ($p0 \leq 0.05$), and these results are higher than the previous studies in the provinces of the country where Al-Fahdawi[12] found the rate of infection with this parasite reached 25.7% during its examination of 2140 stool samples of four hospitals in Anbar province , and the study of Mahdi[13], which recorded a rate of infection of 13.8% in the city of Ramadi, as well as the study of Alftli[14], which recorded a rate of infection rate 13.2% in Diwaniyah province, and also, Sa·el[15] pointed out that the incidence of parasitic infection 12.9 % in Baghdad city, While Mehaynah and Al-Hamidawi[17] records the

Table 6: Chemical reagents on active compounds in the extract of the *Teucrium polium* plant.

active compounds	Type of Reagents	Reagents Guide	Result Reagents
glycosides	Vhlink Reagent	red color	+
Flavonoids	Potassium hydroxide 50%	Yellow precipitate	+
Phenols	Ferric chloride 1%	Greenish green sediment	_
Alkaloids	Meyer Reagent	White deposit	+
Terpenes	Sulfuric acid concentrates with chloroform	Brown deposit	+
The Saponins	Mercuric chloride 1%	White precipitate	_
Tannins	Lead acetate 1%	White gelatin deposits	+
Resins	Acid 4% HCL	The turbidity	_
volatile oils	expose the filter paper containing the extract to the ultraviolet	gray color	+

(+) the presence of an effective compound (-) Lack of effective compound

infection rate was 14.8% during their examination of 3,383 stool samples for patients with diarrhea in Najaf province. The percentage was lower than that found by Abdul Abbas *et al*.,[18] where they recorded a rate of 58.7% in Najaf. But it is agree with Atia[19] where the rate of infection was 48% in Alexandria area in Babylon province. The high incidence of *G. lamlia* parasites is attributed to the lack of attention to cleanliness of food and drink, the non-sterilization of drinking water, the spread of insects, especially flies, which is one of the most important mechanical methods of parasite transmission, and the lack of health awareness and lack of attention to the rules of public health and hygiene plays a large role in the spread of infection. The difference in the incidence rates of the current study compared to the previous studies is due to several reasons, including different methods of diagnosis, different size of sample examined, and different areas of study. The results showed that male infection is higher than that of females and this is consistent with the study of Muhaynah and Al-Hamidawi[17] in Najaf province, due to the fact that the chances of exposure of males to parasites are more than females because of their habits to go out and play outside the house and also eat from unhealthy and unclean shops It is hawkers who do not care about the rules of public health and hygiene.

The results showed a high infection rate in the age groups (1-9) years and (10-19 years), which amounted to (55.4, 48), respectively (Table 2). The statistical analysis showed significant differences ($p0 \leq 0.05$), and this is consistent with the study Fahdawi[12] in Anbar province, this is due to the fact that this parasite is one of the most prominent parasites that affect small age groups due to lack of health awareness and lack of attention to the rules of public health and also the transition of parasites among them during play and exchange of food as well as the decline of natural immunity. Table (3) shows that the highest rate of infection was 62.5% during the month of June, while the lowest rate was 5% during the month of November and showed through the statistical analysis there were significant differences ($p0 \leq 0.05$) in the incidence rates for the months of the year, this is in accordance with the results of Al-Fahdawi[12] in Anbar province, as well as Al-Muhaynah and Al-Hamidawi[17] in Najaf. The reason is the increase in drinking water in the summer months and therefore the possibility of drinking water contaminated with cysts and the proliferation and spread of insects Especially for flies and mosquitoes, as well as large quantities of cysts with their feces, as well as favorable environmental conditions for parasite growth during the summer months, thus increasing the chances of exposure to parasites and high infection rates[33].

The results of the study showed that the incidence of infection in rural areas is 50.6% higher than in the urban area, 32.5%, with significant differences (Table 4). This corresponds to the results of the AL-Moussawi[34] in Babylon province. This is due to the lack of interest in public health rules and conditions, and rely on unhealthy sources of drinking water and non-sterilization and drinking directly from rivers and waterways and also eat vegetables and fruits from the farm without washing or sterilization, and raising animals near or inside the house, which are considered Carrier animals of the disease such as dogs, sheep and goats, and the use of animal manure and fertilizer reinforce the spread of infection among the populations of these areas[35].

The alcoholic extract of the plant showed a significant impact on the vitality of *G. lamblia* parasites in the culture media, where the concentration of 3 mg/ml killed all parasites in the rate of 100% after one day of treatment, while concentrations (0.5, 1.5, 2.5) mg/ml lead to an inhibition effect in parasite vitality at rate (52.2, 72.4 and 88.0%) respectively, and after 2 days the parasite killing rate increased to 74.1, 82.2, 94.7% respectively. However, after 3 days of treatment, the killing rate for the above concentrations was 85.0, 92.3% and 100% respectively. The highest parasite killing rate was observed after 4 days of treatment, while the lowest concentration of 0.5 mg / mL resulted in a killing rate of 90.5%, While the other concentrations had a complete inhibitory effect on parasite growth at 100% (Table 5). This is consistent with the Hamad study[23] using the extract of chili and black pomegranate crops on the parasite in vivo. The inhibitory effect on the vitality of the parasite because the plant extract contains many active compounds, including glycosides, alkaloids, turbines, flavones, volatile oils, which affect the internal enzymes of the parasite and lead to the disruption of enzyme production centers, and prevents the passage of ions through the cell membrane and affects the biological activities of the parasite by disrupting acetylcholinesterase, which controls all the

physiological functions of the parasite, leading to parasite death[36].

Table (6) shows the chemical reagents used on the extract of the *Teucrium polium* plant

to determine the effective compounds in it. The results showed that the plant contains many active compounds, namely glycosides, alkaloids, tannins, flavones, turbines and volatile oils that have a key role in the vitality of the parasite and eliminate it, Mustafa[37] mentioned that many plants and medicinal herbs contain effective substances and compounds that have the effect of antibiotics bacteria, fungi, and parasites.

CONCLUSIONS

The results showed a high incidence of *G. lamblia* parasites in the population of Anbar province by 41.7%. It was noted that the high incidence of infection in males as well as in small ages, and the high rate of infection in the summer months compared to months of winter. The results showed the effect alcoholic extract of the *Teucrium polium* plant significantly in the vitality of *G. lamblia* parasites, and a higher concentration of 3 mg / mL killed all parasites on the fourth day of treatment.

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