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RESEARCH ARTICLE

Antigiardial efficacy of portulaca oleracea extract *in vitro* and prevalence of infection in sheep in Anbar Province, Iraq

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Abstract

This study aimed to determine the incidence of giardiasis in sheep of Anbar Province by inspecting 660 faecal specimens. An examination using a flotation technique confirmed the presence of *Giardia lamblia* in 46.2 % of specimens. The gender had no significant effect on the infection percentage, as it was similar for males (46.9 %) and females (45.4 %). The highest infection rate was 61.7 % for animals aged 1 month to 2 years. During the summer, the infection rate was 61.8 %, whereas it was 27.8 % during the winter, which clearly depicts the seasonal variation in the parasite infestation. The current study systematically evaluated the impact of *Portulaca oleracea* against *G. lamblia*. An aqueous extract was prepared by macerating dried the plant material in distilled water, yielding a 15 mg/mL stock solution, which was serially diluted to concentrations of 1.5, 3.0, 4.5, 6.0 and 7.5 mg/mL, the extract exhibited a concentration-dependent inhibitory effect over 48 hrs, with a concentration of 7.5 mg/mL significantly reducing parasite viability. However, complete eradication was not achieved and requires statistical confirmation. As cytotoxicity assays on host cells were not conducted, the safety of the extract remains unverified. Giardiasis was found to be widespread among sheep in Anbar Province. In contrast, the extract demonstrated promising *in vitro* anti-Giardia activity. Further studies, particularly those examining cytotoxicity and in vivo evaluations, are necessary before recommending it as an alternative treatment.

Keywords: aqueous extract; giardiasis; Iraq; *Portulaca oleracea*; sheep

Introduction

Giardiasis is a disease caused by a flagellated protozoan parasite, a single-celled organism that reproduces in the small intestine, forming colonies and lead to infection (1). *Giardia lamblia* parasite is responsible for this condition and lives in the digestive tracts of humans and various animals, including mammals such as cats, rabbits, beavers, deer, cattle, sheep, birds and amphibians, it is one of the more widespread parasitic illnesses worldwide, particularly prevalent in tropical and subtropical regions (2-4). Infection occurs when the contaminated food or drinks are infected with the cyst stage. Parasite cysts can survive in cold water for weeks or even months, retaining their infectivity (5, 6). These cysts are found in wells, ponds, rivers, water networks, contaminated reservoirs, lakes, swimming pools, water parks and groundwater (7).

Cysts are transmitted from infected animals to healthy animals and humans. Upon entering the host's body, they disintegrate, releasing the active parasites (8). *G. lamblia* is a protozoan parasite that exists in two morphological stages: the active trophozoite and the infectious cyst (9). In domestic animals such as sheep, goats and cattle, giardiasis poses a substantial health risk. Infected animals, especially young or immunocompromised ones, may suffer from chronic or intermittent diarrhea, dehydration, growth retardation and impaired feed conversion efficiency (10). These consequences may result in significant economic losses due to inadequate weight gain, lower productivity and increased susceptibility to various illnesses (11). Furthermore, infected animals can act as asymptomatic carriers, contributing to environmental contamination and possibly aiding zoonotic transmission. Therefore, studying the prevalence and control of *Giardia* in livestock is essential for both animal health and public safety, particularly in endemic regions such as Anbar Province, Iraq (12).

Plant extracts provide safe and effective solutions for treating disorders, including parasitic infections. Unlike many chemical drugs, they contain active compounds that eliminate parasites without causing adverse effects or harm (13). The *Portulaca oleracea* plant, commonly called purslane, belongs to the Portulacaceae family. It is a perennial plant with flat or ascending stems, measuring 15-35 cm in height. *P. oleracea*, abundantly found in agricultural fields along stagnant irrigation canals and sandy or clay-rich soils, is a medicinal plant of high ethnopharmacological relevance. Several investigations have established its medicinal characteristics, including antioxidants, anti-inflammatory, antibacterial and antiparasitic activities (14). The plant contains numerous phytochemicals, including

flavonoids, alkaloids, tannins, glycosides, phenols and essential fatty acids such as alpha-linolenic and linoleic acids, as well as vitamins A, C and E (15). These chemicals are considered to contribute to its biological function, particularly pertinent to the current work, since aqueous and alcoholic extracts of *P. oleracea* have demonstrated strong *in vitro* activity against protozoan parasites, including *G. lamblia*, in experimental settings (16). These findings highlight the promise of *P. oleracea* as a natural antiparasitic, but additional toxicological and pharmacological research is needed before it can be clinically validated.

Due to the health and economic impacts of this disease on various livestock species, this research aimed to assess the incidence of the *G. lamblia* parasite in sheep and explore the *in vitro* effects of *P. oleracea* aqueous extract on this parasite. To our knowledge, this is the first study in Iraq to assess the antigiardial activity of *P. oleracea* extract in sheep. Although earlier Iraqi research has explored the plant's medicinal properties, such as its antibacterial and antioxidant effects (17, 18), its application against giardiasis in livestock remains unstudied, leaving a notable gap in regional knowledge.

Materials and Methods

Collection and analysis of fecal specimens

A total of 660 fecal specimens were collected from sheep of both sexes, aged between one month and eight years, in livestock fields in Anbar Province in 2024. Each specimen was placed in a sterile plastic container containing a mixture of normal saline and 10 % formalin for preservation; the samples were then transported to the Parasitology Laboratory for examination using the zinc sulphate flotation technique. For preparation, one gram of feces was mixed with 10 mL of warm distilled water in a test tube to form a fecal suspension (19). The suspension was centrifuged at 2000 rpm for one minute and the sediment was retained. This centrifugation process was repeated until a transparent layer formed on top of the sediment. After discarding the supernatant, 2 mL of zinc sulphate solution was added to break up the sediment. The test tube was then filled to the top and a coverslip was carefully placed on it. After a short settling time, the coverslip was transferred to a microscope slide, stained with Lugol's iodine and examined under a compound microscope at 10X and 40X magnifications (20).

Plant extraction

The *P. oleracea* plant (Fig. 1) used in this research was collected from the Al-Muhammadi region, which is located west of Al-Ramadi in Anbar Province, Iraq. The plant material was taxonomically verified and authenticated by specialists at the Herbarium of Al-Anbar University, where a voucher specimen was deposited under the accession number (AAUH-PO-2024-56), ensuring both traceability and future verification. Fresh aerial parts of Portulaca oleracea, including leaves and stems, were harvested, carefully washed and shade-dried at room temperature until their weight stabilized, following the method outlined in reference (21). Then, 40 g of the dried material was ground and soaked in 160 mL of distilled water for cold maceration, keeping a plant-to-solvent ratio of 1:4 w/v. The grinding process was performed in an ice bath to preserve thermolabile constituents. The mixture was then stirred continuously using an electromagnetic stirrer for 60 minutes and left at 4 °C for 24 hrs to enhance

Fig. 1. Habit of *Portulaca oleracea* plant.

extraction efficiency. After maceration, the extract was filtered through the Whatman No. 1 filter paper and the resulting aqueous extract was kept in sterile, sealed glass containers at -10 °C until required. Although the extraction yield was not calculated in this study, the process was standardized to ensure reproducibility and consistency. For the *in vitro* assay, a stock solution of 100 mg/mL was prepared by dissolving 2 g of crude extract in 20 mL of sterile distilled water; serial dilutions in sterile water produced working concentrations of 1.5, 3.5, 5.5 and 7.5 mg/mL.

Purification of the parasite's encysted stages

According to the method described by (22), a stool sample was prepared by diluting 1 gram of feces in 10 mL of purified water, followed by filtration through a metal sieve with a pore size of 90 -120 microns to remove coarse debris. Approximately 4-5 mL of the filtrate was transferred into a centrifuge tube and centrifuged at 1800 rpm for five minutes. The supernatant was discarded and the sediment was resuspended in 10 mL of purified water. This centrifugation step was repeated to purify the sample further. Subsequently, four mL of distilled water were added to the precipitate to suspend it. And it was then stored at 4 °C till needed. The 105 cells/mL concentration was obtained by counting the parasite's encysted stages using a hemocytometer.

Preparation of HSP-1 medium

Following the technique of (23). 1 g of phytoene, 0.15 g of L-cysteine hydrochloride, 0.05 g of glucose and 8.5 mL of Hanks' balanced salt solution were combined and dissolved to create the culture medium. The medium's pH was adjusted to 6.7 before autoclaving for 10 minutes at 121 °C and 15 psi to sanitize it. The medium was supplemented with human blood serum, 500 units/mL of penicillin and 50 units/mL of streptomycin. To cultivate the *G. lamblia* parasite, culture media (5 mL) were added to each tube after sterilization and storage at 4 °C.

Parasite growth in culture media

The culture medium HSP-1 for *G. lamblia* was made. The specimen was cultured by adding 1 mL of a suspension containing 10^5 encysted stages and 4 mL of human serum to the culture medium. The inoculation tubes were angled and incubated at 37 °C for three days. Using a 40x magnification, a small amount of culture was inspected on a slide on the third day of culture. Both encysted and active stages of the parasite were observed. After ten days, a second culture was performed, detecting 10^6 active stages within that period, which confirms the daily persistence of these phases. Later, three milliliters of the old

medium were removed and replaced with one milliliter of fresh growth medium (24).

Effect of different concentrations of the aqueous plant extract on parasite survival

The number of actively staged parasites (12×10^6 trophozoites/mL), was determined by analyzing the culture medium. Six glass tubes were used to hold the culture; each set included five tubes, each containing 5 mL of culture. The extract concentrations administered to the five groups were 1.5, 3.5, 5.5 and 7.5 mg/mL; the sixth group served as the control, receiving no therapy. The tubes were incubated at 37 °C for four days. A hemocytometer was used to measure the number of parasites and the following equation records the daily proportion of active parasite stages.

$$\text{live trophozoite \%} = \frac{\text{live trophozoites number x100}}{\text{total number of trophozoites}}$$

$$\text{dead trophozoite \%} = \frac{\text{dead trophozoites number x100}}{\text{total number of trophozoites}}$$

Identification of active components in the plant (25)

Glycoside identification

One milligram of dried plant extract was combined with 10 mL of distilled water, filtered and then treated with a few drops of Fehling's reagent. Glycosides are present when a red hue appears.

Tannins identification

5 mL of the plant extract were mixed using several drops of lead acid (1 %); tannins are available when a white, gelatinous deposit forms.

Volatile oil identification

The presence of volatile oils is indicated by the formation of a grey hue when drops of the extract are applied to Paper filters until saturated and exposed to UV light.

Alkaloid Identification

10 g of the plant's extracts were heated with 50 mL of purified water, which had been acidified with a 4 % HCl and the mixture was then purified. Half a mL the mixture was examined with half a milliliter of Meyer's reagent in a watch glass; the appearance of white deposits verified the presence of alkaloids.

Phenol Identification

One milliliter of the dry plant extract was combined with 1 mL of a 1 % $FeCl_3$ solution. Phenols are indicated when a blue or olive hue appears.

Terpene Identification

The existence of terpenes is shown by forming a brown precipitate after one gram of the dried extract was diluted in 2 mL of chloroform and concentrated H2SO4 and anhydrous acetic acid were added.

Saponin Identification

When 5 mL of the plant extracts were combined with 3 mL of a 1 % mercuric chloride ($HgCl_2$) solution, a white precipitate formed, demonstrating the existence of Saponins.

Statistical analysis

SAS statistical software was used for all statistical analyses; the chi-square test assessed differences in giardiasis prevalence among categorical variables, One-way analysis of variance (ANOVA) was used to evaluate the antigiardial efficacy of *P. oleracea* aqueous extract at different concentrations, followed by the least significant difference (LSD) test to determine pairwise differences between treatment groups. A significance level of $p < 0.05$ was considered statistically significant for all tests (26).

Results and Discussion

Gastrointestinal parasites cause acute or chronic intestinal inflammation, reducing livestock productivity. Therefore, this research was conducted to determine the occurrence of giardiasis by inspecting 660 fecal specimens from sheep in the Anbar province. The overall *G. lamblia* infection rate was 46.2 %. The parasite was diagnosed based on the morphological characteristics of the cysts and trophozoites; the cysts appeared oval, containing four nuclei on one side (Fig. 2). The trophozoites seemed pear-shaped, with rounded anterior and pointed posterior ends. They included two oval nuclei, two suction discs and four pairs of flagella (Fig. 3).

There were no discernible variations ($p \leq 0.05$) in the impact of gender on the rate of infections, as the rates were 46.9 % for males and 45.4 % for females (Table 1). These results are higher than those reached by (27), who recorded an infection rate of 0.8 % with this parasite during their examination of 620 stool samples from sheep in Tibet, China and the study by (28),

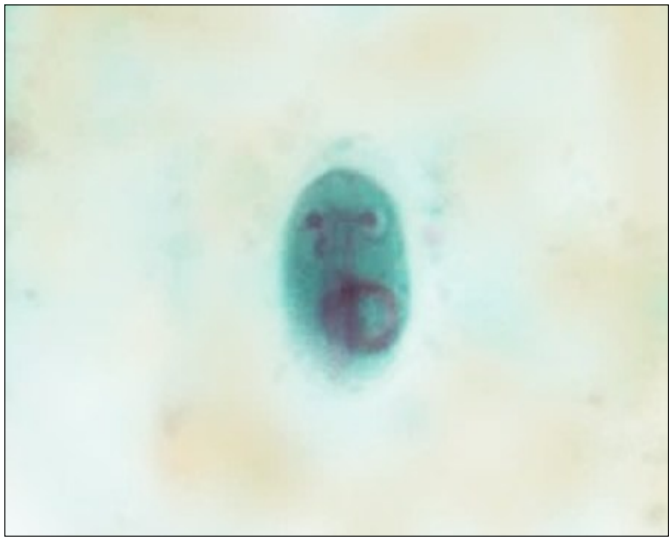

Fig. 2. Cyst of *G. lamblia* stained with local iodine (100x).

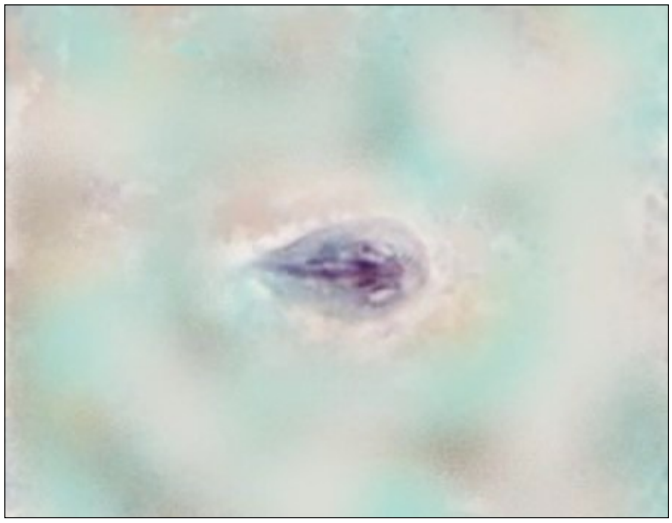

Fig. 3. Trophozoite of *G. lamblia* stained with local iodine (100x).

who recorded an infection rate of 21.8 % with the parasite during the examination of 325 stool samples from sheep. Additionally, this is lower than the rate mentioned by (29) in Henan Province, central China, where an infection rate of 6.6 % was recorded. And less than the study by (30) in the central regions of Iran, which recorded a rate of 19.8 %. This is also lower than the study by (31), who recorded an infection rate of 91.6 % in Wasit Governorate.

The elevated prevalence of giardiasis in sheep is attributed to the negligence of livestock breeders in maintaining the hygiene of ruminant enclosures, as well as the abundance of water sources that serve as potential vectors for transmission. Additional contributing factors include the widespread presence of agricultural lands and fields in the Anbar region, the large number of domestic animals and the proliferation of insects, particularly flies, which are recognized as potential vectors of this parasite (32).

Several factors may account for the variation between the findings of the present study and those of previous local and international studies, including differences in diagnostic techniques, geographical locations of the studies, the number of examined samples and climatic conditions (33). The findings indicated that the infection percentage was similar in males and females, which aligns with the previous study in Babylon province (34). This is explained by the reality that male and female sheep in breeding pens are subjected to identical conditions and contaminated resources, as the lack of distinction between males and females contributes to the animals' susceptibility to infection (35). The intestinal protozoa in sheep typically cause diarrhea when the appropriate conditions within the intestinal cavity exist, promoting the multiplication of parasites and the spread of infection (36).

The findings demonstrated a substantial relationship between sheep age and *Giardia* infections, the highest infection rate was observed in young sheep between one month and two years of age (61.7 %), while the rate decreased significantly in older sheep (6-8 years) to 20.6 % (Table 2), which aligns with the research conducted by (37) in Algeria, the increased infection rate in young sheep results from their weaker immune systems and continuous exposure to environmental sources of infection. Furthermore, infected animals frequently shed parasite cysts, contaminating their environment and facilitating the horizontal spread of the infection within the pen. Also, mothers play a pivotal role as a potential source of infection, transmitting the parasite to their offspring through direct contact or the surrounding environment (38) and reinforcing the importance of focusing on young age groups in preventive control programs by improving rearing management and hygiene, treating carriers and developing appropriate vaccination strategies.

Table 1. Percentages of giardiasis infections by gender

gender	No. of samples examined	No. of samples Infected	%	p-value
males	341	160	46.9*	
females	319	145	45.4	
Total	660	305	46.2	
Significant differences			Less than 0.001	0.001 >

*=Significant difference

Table 2. Percentages of giardiasis infections by age

Age (years)	No. of samples examined	No. of samples infected	%	p-value
1Month-2	340	210	**61.7	
5-3	223	75	33.6	
8-6	97	20	20.6	
Total	660	305	46.2	
Significant differences			Less than 0.001	0.001 >

**= Highly significant differences

An apparent seasonal variation in the percentage of infections was noted, with summer recording the highest incidence rate of 61.8 %. In comparison, winter recorded the lowest rate of 27.8 % (Table 3). These results are consistent with those found (39). This variation happens because hot temperatures and humidity in summer support the survival and spread of Giardia cysts. More animals active near water sources also heighten the risk of contamination. Additionally, the high presence of disease-carrying insects, such as fleas and flies, aids in the mechanical transmission of cysts, helping to perpetuate and disseminate giardiasis during warmer seasons. In contrast, winter's colder and drier conditions usually lower cyst viability and decrease the chances of transmission (40). Supporting this, (41) highlighted that environmental factors, such as temperature and humidity, play a key role in *Giardia* cyst survival and infectivity, with warmer and more humid conditions allowing them to survive longer.

The results showed that *P. oleracea* plant aqueous extracts effectively inhibited *G. lamblia* parasites in the culture medium, with the effect increasing as the extract concentration rose. Concentrations of 1.5, 3.5 and 5.5 mg/mL significantly reduced parasite vitality by 48.5 %, 68.8 % and 78 %, respectively and the highest concentration of 7.5 mg/mL eliminated all the parasites after two days of treatment. After four days of treatment, an increase in the percentage of dead parasites was observed, reaching 70.2 %, 80.6 % and 92.8 %, respectively. A significant rise in parasite death was seen after the sixth day of treatment, reaching 83 %, 93.3 % and 100 %, respectively. After eight days of treatment, a 90 % fatality rate was achieved with the lowest dose of 1.5 mg/mL. In contrast, the remaining concentrations completely inhibited the growth of parasites, killing them 100 % of the time (Table 4). This finding is consistent with the research of (42), which utilized *Terminalia ferdinandiana* to inhibit *Giardia in vitro* and the study, which employed the alcoholic extract of the *Teucrium polium* plant, also affecting the parasite *in vitro* (32).

The aqueous extract of *P. oleracea* exerts a substantial effect on the viability of the *Giardia* parasite, attributable to its rich composition of beneficial compounds and active chemicals, including flavonoids, alkaloids, tannins, phenols and glycosides. These constituents inhibit enzyme activity and induce cell death

Table 3. Percentages of giardiasis infections by seasons

Season	No. of samples examined	No. of samples infected	%	p-value
Winter	187	52	*27.8	
Spring	150	66	44.0	
Summer	181	112	**61.8	
Autumn	142	75	52.8	
Total	660	305	46.2	
Significant differences			Less than 0.001	0.001 >

*= significant difference **= highly significant difference

Table 4. Killing rates of *G. lamblia* parasites using *P. oleracea* aqueous extract concentrations

Concentration mg/mL	% parasites killed			
	dyes2	dyes 4	6 dyes	dyes8
Control	0.0	0.0	0.0	0.0
1.5	48.5	70.2	83.0	90.0
3.5	68.8	80.6	93.3	100
5.5	78.0	92.8	100	100
7.5	*100	100	100	100

* 7.5 mg/mL concentration killed the parasite after two days

by disrupting the flow of ions across cellular membranes. They also alter the activity of the enzyme acetylcholine, which governs essential cellular functions, ultimately leading to the parasite's death (43).

The extract's phytochemical analysis revealed numerous bioactive compounds known to inhibit the growth of bacteria and parasites. Studies have shown that some medicinal plants contain phytochemicals that may be more effective than conventional antimicrobial drugs in destroying bacteria, fungi and protozoan parasites (44).

Conclusion

The present research demonstrated a substantial occurrence of Giardiasis infections in sheep in Anbar province, with the highest infection rates observed in young animals and during the summer season, indicating potential age- and climate-related risk factors. No statistically significant difference in infection rates was noted between sexes. The findings demonstrated the effectiveness of the *P. oleracea* plant's aqueous extract in suppressing the viability of *G. lamblia* parasites, the impact increased with more significant concentrations over the various treatment durations, which offers interesting potential for natural remedies as an effective alternative to chemical treatments and their harmful effects on human, animal and environmental health. However, this data alone does not establish the extract's suitability for treatment. Additional research is necessary to evaluate the compound's effectiveness and safety, including in vivo studies, thorough dose-response assessments and detailed toxicity and safety tests to confirm the *in vitro* findings and assess their practical relevance.

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Authors' contributions

SSS designed the study, supervised the parasitological diagnosis and reviewed the manuscript. AAZ performed microbiological analyses and participated in manuscript writing. YIS contributed to the data interpretation and helped in statistical evaluation. AJD was responsible for chemical sample preparation and laboratory support. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: None

Ethical issues: None

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