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Research Article

Experimental infection of *Sarcocystis moulei* in mice and treated by using the alkaloid extract of *Ruppia sp.* in Basrah governorate southern of Iraq

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Abstract

Sarcocystosis is caused by different *Sarcocystis* species. It's a protozoal infection with worldwide distribution in many species of animals. The current study aimed to transfer infection of *S.moulei* into the mice as well as morphological diagnosis to confirm this aquatic plant and treat the mice experimentally infected by *S.moulei*. The plants of the family Ruppiaceae are monocotyledons, containing only one genus, which has many species in the world., Plant samples of *Ruppia* sp. were isolated from one of the salt marshes(swamps) in a province in the area of five miles and were carried out for phenotypic and then molecular diagnosis for confirmation. The whole plant is approximately 30 cm (40-65) cm in length and less than 1 mm (0.9 mm) in stem diameter. The plant is monocious, and the flowers are bisexual and sessile in groups at the base of the leaves; the peduncles are more than 5 cm long and reach 22–23 cm in length, flexuous but not spirally coiled, and bearing 5–11 obliquely ovoid (2-2.5) mm in length. The leaves are distributed alternately across the stem. Experimental transfer of the infection was carried out to laboratory mice, and three concentrations were used to treat the infection (0.106, 0.156, and 0.206) g/kg of extract alkaloid of *Ruppia sp.*, showing the two concentrations (0.156 and 0.206 g/kg) complete efficacy and complete recovery compared to the other groups and other concentrations, as well as the group (infected and treated with albendazole 0.250 g/kg).

Keywords: *Sarcocystis moulei*, Aquatic plants, *Ruppia* sp., Mice, Basrah

Introduction

Sarcocystis species are coccidian intracellular protozoan parasites from the genus *Sarcocystis*, which belongs to the Apicomplexa phylum's family Sarcocystidae. Sarcocystosis is a zoonotic protozoan illness that affects a wide range of mammals, reptiles, and birds worldwide. There are around 200 genuine species of *Sarcocystis*, which range in pathogenicity to the host, ranging from virulent to severe disease. Many species are zoonotic (Dubey, 2015; Dubey et al., 2016).

The domestic sheep and goats destined for human consumption in Basrah governorate were infection with macroscopic and microscopic *Sarcocystis moulei* (Saood et al., 2022).

More than 65 percent of the world's population, particularly in underdeveloped nations, uses plants and algae as a therapy source for a variety of diseases, according to the World Health Organization (2001). Phytochemicals are as important as synthetic medicines since in some regions it is the only source of medicine. The extracts of plant and algae provide around 80% of the world's health needs (Cowan, 1999).

Phytochemicals play an important role in the pharmaceutical industry as raw materials or as particular drugs. Secondary metabolites obtained from plants are found to be an important source of various phytochemicals that could be used for the production of pharmaceuticals. In developing countries, populations still rely on traditional medicine derived from plants for their health care needs. Thus, the demand for herbal medicines is continuously increasing day by day in comparison to synthetic drugs, which are used in crude forms as decoctions, infusions, tinctures, and poultices.

The current study aimed to transfer experimental infection *S.moulei* to laboratory mice and use the alkaloid extract from aquatic plants, *Ruppia sp.* to treat.

Material and Methods

Sample collection of *Sarcocystis moulei*

A total of 50 esophagus from sheep and goats, aged 1-6 years were randomly inspected and collected during postmortem inspections of the slaughtered animals at the slaughterhouse of Basrah province, Iraq to search for infection by *Sarcocystis* Additionally, whole fresh esophageal samples were collected from animals slaughtered outside the abattoirs from butcher markets.

Samples were collected during the month of January 2021 All esophageal tissues were inspected for the detection of the macroscopic cysts of *Sarcocystis* before taking samples.

The macroscopic examination of the entire sample was performed in the lab to visualize macroscopic *Sarcocystis*. The size and dimensions of the macroscopic *Sarcocystis* were measured by a ruler (Zangana and Hussein, 2017, Dong et al., 2018).

The esophagus Samples were longitudinally sectioned in order to examine the internal and the external walls (Bittencourt et al., 2016). Several macroscopic cysts were selected and processed for transport of infection to the labrotary animals.

Samples collection of Aquatic plants *Ruppia* sp.

Ruppia sp. samples were collected by hand from swamps in the 5-mile area in Basrah city, southern Iraq. On December 29, 2020, they were placed in clean plastic bags. Then samples were brought to the laboratory in the Department of Biology, College of Education for Pure Sciences, University of Basrah. They were placed in large glass Petri dishes. The samples were washed several times with tap water to get rid of the impurities and mud stuck to them, then they were washed with distilled water It is located in salt ponds in Basra Governorate, southern Iraq several times to ensure that they were clean well (Weideman,1984).

Purification, drying and preservation of *Ruppia* sp.

The method of (Giusti, 2005) was used to purify aquatic plant, which involved washing the plant with tap water several times to get rid of the mud, algae and materials attached to the aquatic plants, then washing with distilled water afterwards. Ultrasonicator Telesonic for three minutes. To get rid of small algae sticking to plant, as well as bacteria and fungi, if any, these sample were washed with distilled water 12 times (Weidman, 1984) and then placed on Wattman N0.1 filter papers to dry at laboratory temperature Biomass was dried using a (Topt 10 D freez dryer).

Preparation of alkaloid extract

The alkaloid extract was prepared according to the method of Al-Samarrai, (1983).

Identification of chemical compounds in *Ruppia* sp. extract using GC-Mass technology

Identification of chemical compounds with biological activity for alkaloid extracts of *Ruppia* sp. was done in the College of Education for Pure Sciences/Basrah in chemistry laboratories. At 700 Ev, an Agilent 7890A gas chromatography detector and a mass 5975C spectrometer with computer control were used. The capillary column was Hewlett Packard HP-5MSssilica, with a diameter of 250 m, a length of 30 m, and a thickness of 0.25m. The carrier gas was helium at a stable flow rate of 2 ml/min and an injection volume of 1 µl with an injector temperature of 250°C. 18 min was the scanning time. The initial oven temperature was 60°C for 1min. The National Institute of Standards and Technology (N.I.S.T) 2008 mass spectral library was used (Dallee et al., 2022).

Alkaloid detection

Alkaloids were detected according to the method (Harborne, 1984).

Albendazole drug

The effective concentration dose of albendazole determined was according to LD50 (Al-Waeli et al., 2015). A broad-spectrum anti-parasitic was produced by the Indian company Meshen (Derosa and Teggi, 1995).

Assignment of sub-lethal dose from *Ruppia* sp. to laboratory mice LD50 and effective dose

Selected the sub-lethal dose LD50 of plant extracts and albendazole was tested on male mice and then it was calculated according to the mentioned equationas to determine an effective dose. They were administered orally in a dosing tube attached to a 1 ml syringe. The animals were monitored for 72 hours. If there are cases of weakness, instability of gait, loss of balance, loss of appetite, or death during it during this period, the LD50 was calculated for the extract and albendazole based on the law formulated by Litchfield and Wilcoxon, (1949) according to Table 1 and the following equation, which shows the LD50 of the alkaloid extract of *Ruppia* sp. against mice (*Mus musculus* L.).

Infection transfer and treatment in laboratory mice

Preparation and breeding of laboratory mice

During the current study, male albino laboratory mice of the type *Mus musculus* L. (Balb / C strain) were brought from the Drug Control Center in Baghdad, and were raised in the animal house of the Department Biology-College of Education for Pure Sciences - University of Basrah under controlled conditions according to (Al-Maliki, 2000).

The mice were fed the ready-made, purchased ration from the Marine Sciences Center, University of Basrah.

Table 1. Shows LD50 of alkaloid extract of *Ruppia* sp. against mice (*Mus musculus* L.)

Groups	Concentration Mg/Kg	N	Dead animals	A	b	a X b
1	4000	4	2	-	-	-
2	7000	4	2	3000	2	6000
3	10000	4	4	3000	3	9000
						∑ab=15000

N : 4 for each dose .
Where :
a : is the value of difference between two successive doses and
b : is the mean mortality in mice for two successive doses

$$LD_{50} = \text{High dosage} - \frac{\sum ab}{N}$$

$$LD_{50} = 10000 - \frac{15000}{4} = 10000 - 3750 = 6250 \text{ mg/kg}$$

The effective dose = 6250 / 40 = 156.25 mg/kg
156.25 / 1000 = 0.156 g
0.156 + 0.05 = 0.206 g
0.156 – 0.05 = 0.106 g

Transfer method of infection to laboratory mice

The experiment was divided into four groups (A,B,C, and D). Each group contains 25 mice, uninfected (negative control), infected but not treated with aquatic plant extract *Ruppia* sp. or albendazole, infected and treated with albendazole (positive control 0.250g/K), infected and treated with aquatic plant extract *Ruppia* sp., respectively, as mentioned in Fig.1

Mice aged 8–10 weeks and weighing 23–27 grams were infected by feeding one cyst after a 12-hour fast. Where one cyst only was administered to each mouse in the infection groups (2,3 and 4), group 1 was uninfected (negative control).

The average bradyzoites number (102350) (Saood et al., 2022), was calculated by the following equation, taken from calculating the number of white blood cells for the four squares in a hematocytometer: x 200 divided by the number of squares (Kruse et al., 1973). The cyst dimensions ranged from 5 x 2 mm to 0.07g on average.

Examine the infected mice to ensure that the infection occurred

After 24 hours of infection, five mice from each group were killed by inhaling chloroform for a few seconds and then dissected. The intestines were isolated from each mouse, placed in a dish, and frozen to be examined by the P.C.R. technique. Retry killing of mice to confirm infection 10 days after infection to confirm chronic infection.

Treating mice infected with *Ruppia* sp. alkaloids and Albendazole

After ten days of infection, three doses (0.106, 0.156, and 0.206) g/kg of extract alkaloid of *Ruppia* sp. were administered (first dose) to three subgroups (A, B, and C) from the main group (4), each group containing five replicates. As for group 3 (infected and treated with Albendazole 0.250g/kg) as shown in diagram (1), each of these weights was dissolved in 0.3 ml of physiological solution, and each concentration was given orally to each mouse and for all replicates in the experiment.

Mice aged 8–10 weeks and weighing (23–27g) were starved for 24 hours before the experiment began for each of the experimental mice. The dosing needle (stomach tube) was attached to a 1 ml syringe according to the method described by Sugane and Oshima (1982).

The second dose was given with the same concentrations and method of administration as the first dose and for all the sub groups mentioned in the treatment of the first dose, as well as group 3, after 12 days of giving the first dose of treatment.

After twenty-eight days of infection, five mice from each group (1,2,3,A,B, and C) were also killed to re-examine the intestines by P.C.R techniques to observe and determine the efficacy of the therapeutic alkaloid extracts in *Ruppia* sp. and compare them with the drug treatment and the other untreated groups (control groups).

Results

Identification of *Ruppia* sp.

This genus was classified according to the following taxa: Kingdom: Plantae; Clade: Monocots; Clade: Angrosperms; Clade: Tracheophytes; Order: Alismataley; Family: Ruppiaceae; Genus: *Ruppia* sp., which is characterized by some morphological characteristics represented by its presence in an environment consisting of small ponds in Basrah governorate in winter months, especially in December 2020. It was morphologically classified according to (Dandy, 1971; Dandy, 1985) as bearing the following features: The length of the plant is approximately 30cm (40-65) cm, with an internode 5-7cm in length and 0.9 cm in diameter.

The main branch is usually branched dichotomously. These aquatic plants are monoicous. The fruits are born on special branchless 22–23 cm in length with clusters of fruits between 6–11 (2.25-2.5) mm in length and (0.5–0.75) mm in diameter. While flowers are found as sessile clusters between branches (leaf branches) 5-10 mm in diameter Fig. 1 (A-D).

Transfer of infection and treatment to laboratory animals (mice)

Experimental Design

0 - day (Infection) — **100-mice**

1	2	3	4
Group 1: Uninfected N: 25 (Negative control)	Group 2: Infected but not treated with extract or Albendazole. N= 25	Group 3: Infected & treated with Albendazol ,N: 25 (Positive control)	Group 4: Infected & treated with alkaloid extract . N= 25

24 h. (Examination by P.C.R. Technique – Intestine) **(N=5 replicate)**
(Acute infection)

90-mice

Group 1: Uninfected (Negative control)	Group 2: Infected but not treated with extract or Albendazole.	Group 3: Infected & treated with Albendazol (Positive control)	Group 4: Infected & treated with alkaloid extract .

10-day (Examination by P.C.R. Technique – Intestine) **(N=5 replicate)**
(chronic infection) **80-mice**

Group 1: Uninfected N: 19 Negative control	Group 2: Infected but not treated with extract or Albendazole. N= 19	Group 3: Infected & treated with Albendazol ,N: 19 Positive control	Group 4: Infected & treated with alkaloid extract . N= 19

10- day (Treatment - The first dose)

Group 1: Uninfected Negative control	Group 2: Infected but not treated with extract or Albendazole.	Group 3: Infected & treated with Albendazol Positive control	Group 4: Infected & treated with alkaloid extract .

Group 3 splits into: Group A -N= 5 D=0.106g | -Group B -N= 5 -D=0.156 g | -Group C -N= 5 D=0.206 g

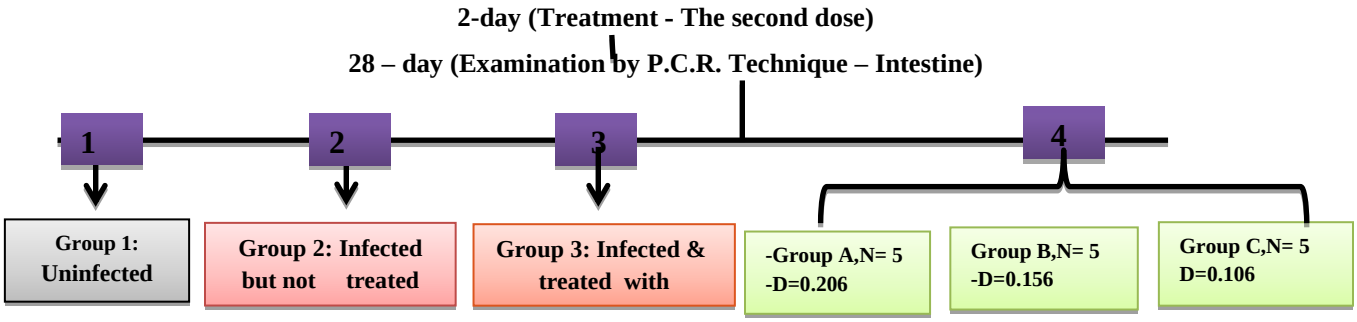

Diagram. 1 Experimental method of infection in laboratory animals (mice)

Chemical characterization of alkaloid extracts to *Ruppia* sp. by chromatography technique Gas Chromatography - Mass Spectrometry

Fig. 2 and Table 2 Showed five alkaloid compounds that have been diagnosed with Gas Chromatography-Mass Spectrometry technique in alkaloid extract of aquatic plant *Ruppia* sp.

***Sarcocystis moulei* infection transfer and treatment in mice**

Examine the infected mice to ensure that the infection occurred

The finding showed that killing after 24 h infection and retrying to confirm infection 10 days after transfer of infection showed a negative result in all replicates S1-S5 in the uninfected group, as shown in Fig.3, and positive results in all replicates S1-S5 in the infected group, as shown in Fig. 4

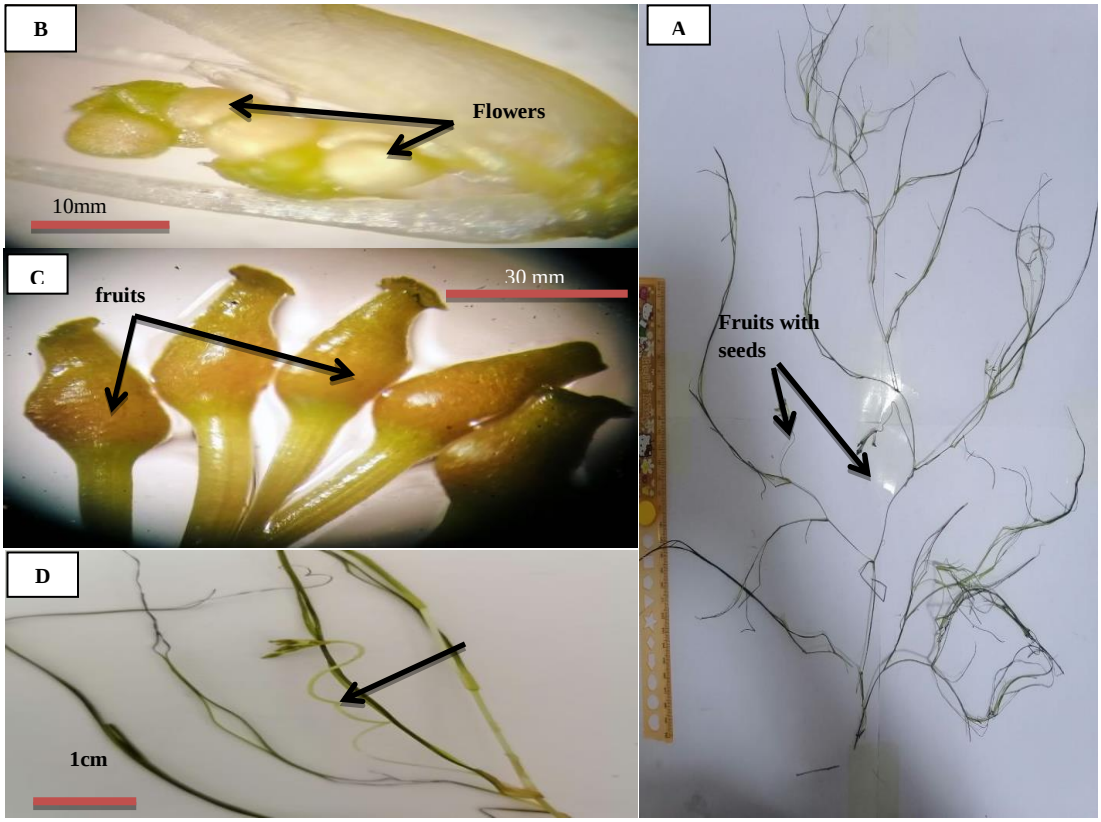

Fig. 1 Morphological characteristic of *Ruppia* sp. A: Whole plants, B: Clusters of flowers, C: fruits, D: Branchless bearing fruits (Arrow)

Table 2. Alkaloid compounds diagnosed by GC-Mass spectroscpy technique from *Ruppia* sp.

No	Compound name	M.W	Formula	Peak height	R.T	Area
1	(2-Aziridinylethyl)amine	86	C4H10N2	1095500	1.243	7.92
2	Pterin-6-carboxylic acid	207	C7H5N5O3	1896102	1.283	6.98
3	4-Fluorohistamine	129	C5H8FN3	5681531	1.375	3.08
4	3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole	223	C7H9N7O2	1443087	1.653	2.62
5	1,2,4-Triazole,4-[N-(2-hydroxyethyl)-N-nitro]amino	173	C4H7N5O3	3736410	1.857	0.89

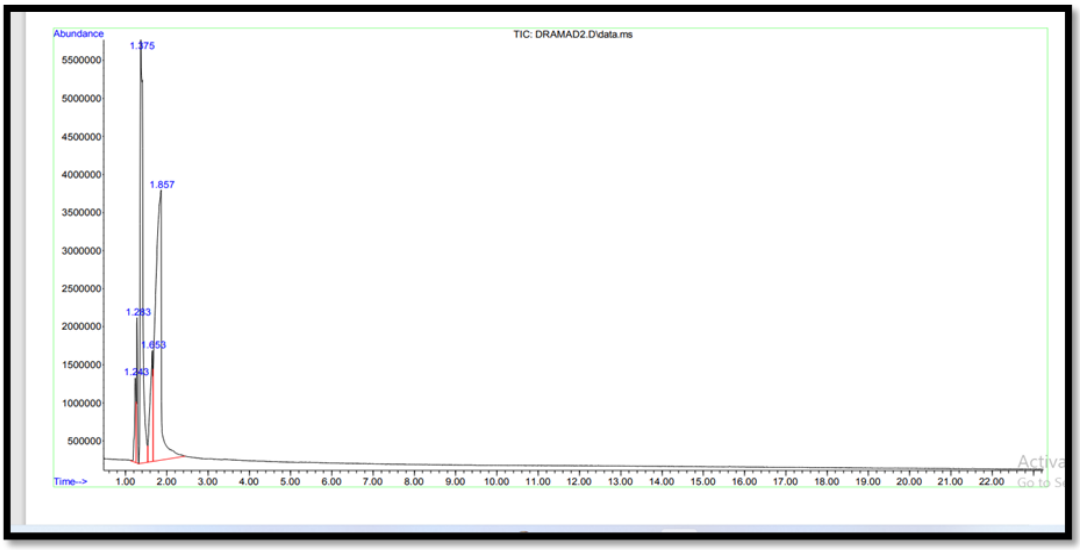

Fig. 2 GC-MS Chromatography of alkaloid extract of aquatic plants, *Ruppia* sp.

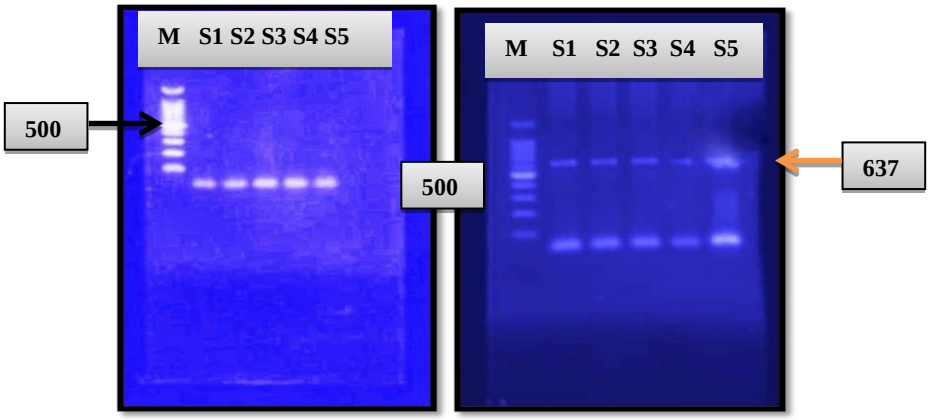

Fig. 3 Showed PCR products of the partial(18srRNA) gene in the intestines of mice. This figure revealed that all replicates of S1-S5 in group 1(uninfected) yielded negative results and this figure represented the finding after 24 h infection and retrying to confirm infection 10 days after transfer of infection

Fig. 4 Showed PCR products of the partial (18srRNA) gene in the intestines of infected mice were demonstrated by the *S.moulei* showed bands at 637 bp on 1% agarose gel electrophoresis, M 100 bp DNA ladder. This figure revealed that all replicates S1-S5 in group 2 (infected) yielded positive results and this figure represented the finding after 24 h infection and retrying to confirm infection 10 days after transfer of infection.

While groups B (0.156 g/kg) and C (0.206 g/kg) recovered completely and gave a negative result in all replicates for groups B and C see Fig. 8, 9. This result was identical to that of group 3 (infected and treated with albendazole at 0.250 g/kg) as shown in Fig. 10

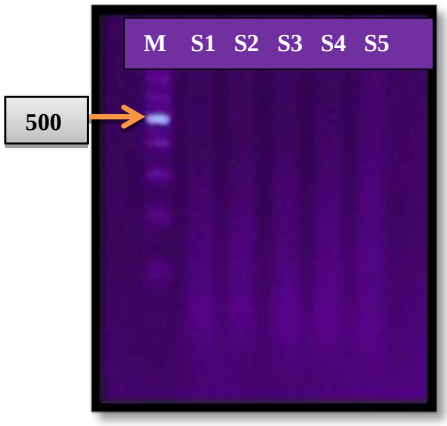

Fig. 5 PCR products of the *S.moulei* partial 18SrRNA gene showed bands at 637 bp on 1% agarose gel electrophoresis. A 100 bp DNA ladder, M. This figure revealed that all replicate S1, S2, S3, S4, and S5 in group 1(uninfected mice) yielded negative results

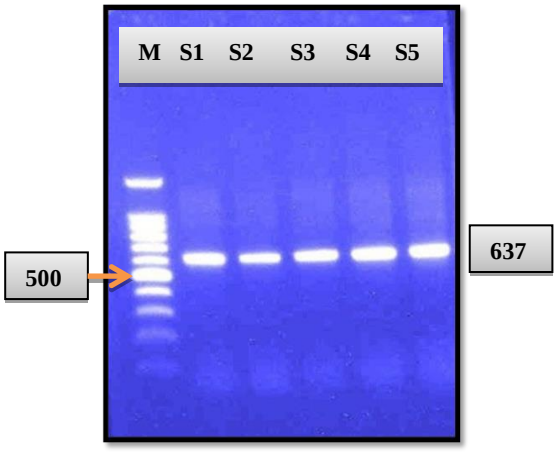

Fig. 6 Showed PCR products of the *S.moulei* partial 18srRNA gene showed bands at 637 bp on 1% agarose gel electrophoresis. A 100 bp DNA ladder, M This figure revealed that all replicates S1, S2, S3, S4, and S5 in group 2 (infected mice but not treated with an alkaloid extract of *Ruppia* sp. or albendazole) yielded positive results.

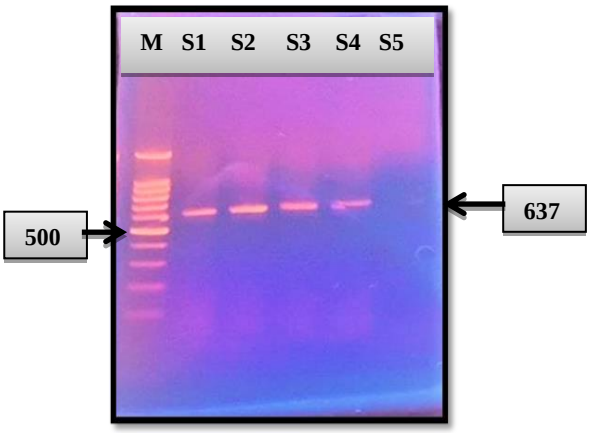

Fig. 7 Shows the effect of the alkaloid extract of *Ruppia* sp. and albendazole on the intestines of infected mice was demonstrated. PCR products of the *S.moulei* partial 18SrRNA gene showed bands at 637 bp on 1% agarose gel electrophoresis. A 100 bp DNA ladder, M In group A (0.106g), four replicates (S1, S2, S3, S4) were positive and one was negative to the other replicates (S5) (+4 with -1).

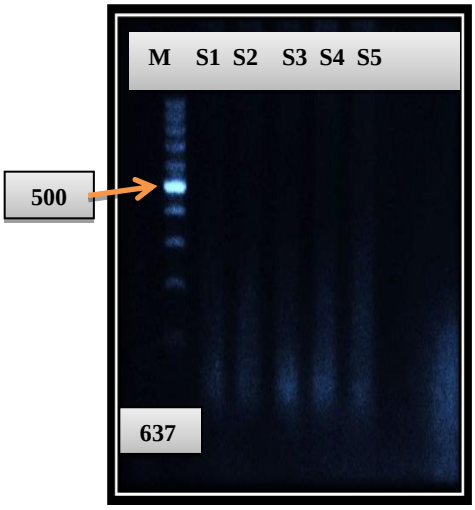

Fig. 8 Shows the effect of the alkaloid extract of *Ruppia* sp. and albendazole on the intestines of infected mice was demonstrated. PCR products of the *S. moulei* partial 18S rRNA gene showed bands at 637 bp on 1% agarose gel electrophoresis. A 100 bp DNA ladder, M This figure revealed that all rereplicate1, S2, S3, S4, and S5 in group B (0.156g) yielded negative results.

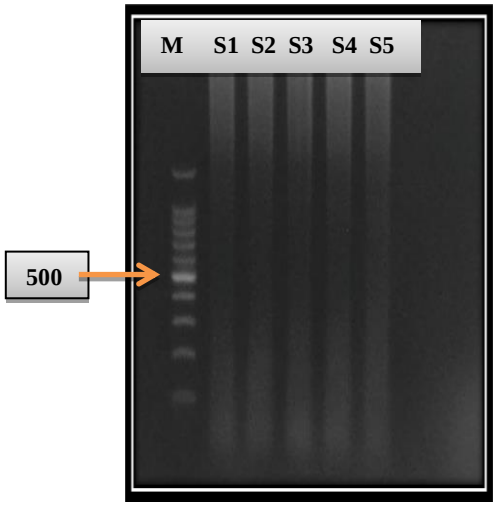

Fig. 9 Shows the effect of the alkaloid extract of *Ruppia* sp. and albendazole on the intestines of infected mice was demonstrated. PCR products of the *S.moulei* partial 18srRNA gene showed bands at 637 bp on 1% agarose gel electrophoresis. A 100 bp DNA ladder, M This figure revealed that all replicate S1, S2, S3, S4, and S5 in group C (0.206g) yielded negative results.

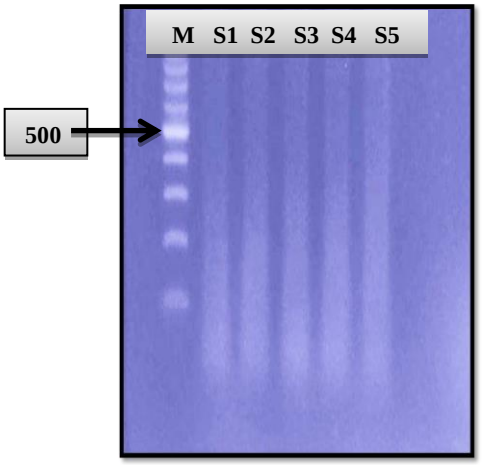

Fig. 10 Shows the effect of the alkaloid extract of *Ruppia* sp. and albendazole on the intestines of infected mice was demonstrated. PCR products of the *S.moulei* partial 18srRNA gene showed bands at 637 bp on 1% agarose gel electrophoresis. A 100 bp DNA ladder, M This figure revealed that all replicate S1, S2, S3, S4, and S5 in group 3 (infected and treated with albendazole at 0.250 g/kg) yielded negative results.

Discussion

The most common protozoan illness in a variety of ruminant farm animals, such as cattle, sheep, and goats, is sarcocystosis. species of *Sarcocystis*. It is a parasite with a large geographic range and strong zoonotic potential (Castro-Forero et al., 2020).

This is the first study of its kind in Iraq and the world using alkaloid extracts of *Ruppia* sp. against *S.moulei*. There were no prior studies on this type of aquatic plant in Iraq, and the reason for choosing this aquatic plant in the current study is due to the lack of studies on it, as we mentioned earlier, and because of its high distribution in the Iraqi environment and in the province of Basrah in particular, which gives a large live mass without the need to culture it in the laboratory.

In the current study, the muscles of the esophagus were examined. Previous studies indicated that this organ is the most common site for *Sarcocystis* infection, so this agrees with several studies (Latif et al., 1999; Barham et al., 2005; Minuzzi et al., 2019; Gjerde et al., 2020).

***Ruppia* sp.**

The reason for choosing these plants in the current study is the lack of studies on them, as we mentioned earlier, and their wide spread growth and high distribution in the environment, which give a large live mass without the need to culture them in the laboratory. This study is considered the first to identify and confirm the aquatic plant *Ruppia* sp. in Iraq (morphology study).

Identification of active chemical compounds (alkaloids) by the gas chromatography technique GC-mass

The present study is considered the first study in Iraq about the analysis of components of *Ruppia* sp. by Gas Chromatography-mass spectrometry. The results of the current study showed the presence of 5 peaks through technical analysis GC Mass of the aquatic plant extracted *Ruppia* sp. contains many of the active chemical alkaloid compounds, and some of these compounds occupy a larger area than the rest of the compounds during the analysis process. From among the total area of the diagnosed compounds, Manilal et al., (2010) mentioned that the compound that gives the largest percentage of the total area of compounds diagnosed by GC mass may be due to its biological activity.

Among the most notable compounds diagnosed by GC mass is the (2-aziridinylethyl) amine, which is in the amine functional group and is a type of secondary metabolite of plants. Apart from being a part of cell processes such as the division of cells and the formation of nucleic acids and proteins, amine has also functioned as a component of chemical and physical defenses in dealing with herbivorous and pathogenic attacks (Bouchereau et al., 2000). In the references, (2-aziridinylethyl) amine was found on the seed of *Persea americana* (Avocado) (Maduka et al., 2020).

On the other hand, the results of the current study also showed that pterin-6-carboxylic acid was the compound with the second-highest. Dallee et al. (2022) mention that it is one of the alkaloid compounds of anti-parasite, anti-psychotic

and mood stabilizer, and this result agrees with the current study, which showed a clear effect on the disappearance of white streaks in groups infected with *S. moulei* treated with aquatic plant alkaloid extract *Ruppia* sp., and it made a full recovery, as did the result of the examination by P.C.R technique.

The other compound that appeared in the diagnosis is the compound 4-fluorohistamine. This compound is one of the compounds of unknown biological activity until this moment, and this result is in agreement with Dallee et al. (2022) who identified the alkaloid chemical compounds in the leaves of *P. aoleracea*.

While other compounds detected by GC-mass include 1,2,4-triazole and 4-[N-(2-hydroxyethyl)-N-nitro]amino], the compound's pharmacological actions include biological activities such as anti-microbial activity (Kadhim et al., 2016) and appear to be anti-parasite active against *S.moulei* in our study.

Measurement the effect of alkaloid extracts of *Ruppia* sp. and Albendazole on mice infected with *S.moulei* by using the P.C.R technique

The current study reported the first infection of laboratory mice with *S.moulei* in Iraq, where this type was isolated and diagnosed in sheep and goats in the governorate of Basrah. Three concentrations of the extract of *Ruppia* sp. were used in the present study, and two of them had activity against the *S.moulei* cyst. The results found that the two concentrations (0.156 and 0.206) mg/kg of the alkaloid extract of the aquatic plant *Ruppia* sp. affected the efficacy of bradyzoites inside the bodies of mice after being treated with these extracts. Thus, when examined by PCR, this effect was reflected on the mice of infection severity, which is consistent with what Tabari et al. (2017) stated that alkaloid extract of *Piganum harmala* is a potent natural anti-trichomonal agent, effective against *T.gallinae* with full recovery of the infected birds when administered at a lower dose. In addition to full recovery in the groups of infected mice and the disappearance of the changes that were seen with the naked eye and clearly appeared on the skeletal muscles of the back and thighs as well as on the peritoneum membrane surrounding the internal organs of the body in the form of white threads, the whitish intramuscular streaks were first seen on the 28 day after the infection, and this is in agrees with Armando Ruiz and Frenkel (1976), who mentioned that cysts were first seen in skeletal muscle 28 days after infection, This agrees with Armando Ruiz and Frenkel (1976), who mentioned whitish intramuscular streaks 5 mm long were observed in a house mouse (*Mus musculus*) trapped alive on September 7, 1973 in Desamparados, a suburb of San Jose, Costa Rica. Microscopic examination revealed cysts containing a large number of small, sausage-shaped bradyzoites.

The reason for this positive effect and the appearance of a complete recovery is due to the presence of one of the alkaloid compounds that are normally present in *R.maritima*, namely pterin-6-carboxylic acid, which acts as a good anti-parasitic to eliminate or inhibit *S.moulei*, in addition to the other compounds identified in the extract. This result is in agreement with the findings of both Hussein et al. (2016) and Al-Dallee et al. (2022), they diagnosed the same alkaloid compound (Pterin-6-carboxylic acid) and proved its effectiveness as an antiparasitic.

It is difficult to speculate on the mechanism by which these bioactive compounds act as parasitecidal agents. In this regard, Sepulveda-Boza and Cassels (1996) suggested that many bioactive chemical compounds exhibit their parasitecidal activity by virtue of their interference with the redox balance of the parasites, acting either on the respiratory chain or the cellular defenses against oxidative stress. It is also known that some bioactive compounds act by binding to the DNA of the parasite. For example, dihydroorotate dehydrogenase (DHOD), the fourth enzyme in the de novo pyrimidine biosynthetic pathway, is essential to parasites, including the electron acceptor capacity and cellular localization (Nara et al., 2000).

It is reported that this study is the first to document an anti-parasite effect of aquatic plant alkaloid extract *Ruppia* sp. against *S.moulei* in mice in Iraq.

Conclusion

The result concludes that this is the first study to aim to transfer infection of *S.moulei* into the mice as well as a morphological diagnosis to confirm this aquatic plant and alkaloid extract to treat the mice experimentally infected by *S.moulei*.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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