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RESEARCH

First Report of the Anti-Parasitic Effect of a *Cannabis sativa full-spectrum* Extract on *Echinococcus granulosus sensu stricto*

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Abstract

Purpose Cystic echinococcosis is a parasitic zoonosis caused by the larval stage of *Echinococcus granulosus sensu lato*. Albendazole (ABZ) is the drug of choice, although its efficacy is variable. The present research aimed to assess the in vitro and in vivo efficacy of a *full-spectrum* extract of *Cannabis sativa* inflorescences against *E. granulosus sensu stricto* (*s.s.*).

Methods Protoscoleces and cysts were incubated in vitro with the *C. sativa* extract, achieving final CBD concentrations of 1, 5, 10, and 50 µg/ml. Viability was evaluated periodically. Structural and ultrastructural alterations were also recorded. For the clinical efficacy study, female CF-1 mice were infected. Six months later, mice were divided into groups ($n=10$): (a) water control; (b) ABZ; (c) *C. sativa* extract, and (d) ABZ + *C. sativa* extract. Treatments were administered every 24 h for 30 days. The efficacy of the treatments was evaluated according to the weight of the cysts collected and the ultrastructural alterations observed.

Results The *C. sativa* extract caused a significant decrease in the viability of protoscoleces and cysts in vitro. The greatest effect was observed with 50 µg/ml, which generated the reduction in protoscoleces viability to 0% between 6 and 24 h post-incubation (pi) and the collapse of 92 ± 13% of the cysts after 24 h pi. All the in vivo treatments reduced the weight of the cysts and caused ultrastructural alterations, especially the combination of ABZ + *C. sativa* extract.

Conclusion We demonstrated the in vitro and in vivo efficacy of a *full-spectrum* extract of *C. sativa* inflorescences against *E. granulosus s.s*.

Keywords Cystic echinococcosis · *Echinococcus granulosus sensu stricto* · *Cannabis sativa* · Plant extract · Phytotherapy.

Introduction

Cystic echinococcosis is a parasitic zoonosis that affects more than 1 million people worldwide, representing a significant concern in terms of social, economic, and public health aspects [1]. Despite being categorized as a neglected tropical disease by the World Health Organization (WHO) and the intention to eradicate this disease by 2050, cystic echinococcosis is still endemic in areas like South America, the Mediterranean, and the Middle East [2].

The causative agent of cystic echinococcosis is the larval stage of *Echinococcus granulosus sensu lato*. The life cycle of this parasite involves a definitive host (canids, mainly domestic dogs) and an intermediate host (farm and wild ungulates, like cattle, sheep, camels, pigs, and equines) [3]. Humans can also act as intermediate hosts by accidentally ingesting parasite eggs, either through direct contact with

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parasitized dogs or by the consumption of contaminated water and food. On a suitable intermediate host, the parasite migrates to different organs (predominantly the liver and lungs) and develops into spherical, unilocular, fluid-filled cysts.

There are currently four alternatives for the treatment of cystic echinococcosis in humans: surgery, PAIR (puncture-aspiration-injection-reaspiration), anti-infective benzimidazole drug treatment, and the "watch-and-wait" approach [2]. The availability of resources, the size, location, and stage of the cyst, as well as the presence of symptoms or complications, are the main factors considered when deciding between these options [4].

The development of benzimidazoles has led to an increase in the use of pharmacotherapy in the last decades [5]. Currently, albendazole is the drug of choice indicated for the treatment of human cystic echinococcosis. However, both the optimal dose and the duration of treatment remain under discussion [6]. Patients should be monitored for at least 5 years due to high rates of recurrence and the uncertainty of a complete cure after the pharmacological treatment [2]. In addition, albendazole exhibits limited absorption in the human intestine (low aqueous solubility) and may be accompanied by side effects such as alopecia, hepatotoxicity, gastrointestinal distress, vertigo, and leukopenia [7].

Based on the aforementioned facts, further research is necessary to improve the pharmacological treatment of cystic echinococcosis. In the past few years, there has been a growing interest in natural products that could serve as alternatives for synthetic substances in the management of diseases. Plants are a source of a wide variety of compounds, which can display several biological effects. A large number of natural products and their derivatives have been screened for anthelmintic properties [5]. Essential oils of *Zataria multiflora*, *Ferula asafetida*, *Foeniculum vulgare*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Origanum vulgare*, *Cinnamomum zeylanicum*, *Menta* spp., and *Stevia* spp. extracts have shown great potential in vitro against *E. granulosus*. Furthermore, *Stevia* spp. extracts have demonstrated to be effective in vivo [8–14]. Several compounds isolated from different plants have also shown positive in vitro and in vivo results, including thymol and carvacrol [8]. Recently, promising in vitro and in vivo outcomes have emerged from the use of isolated cannabidiol (CBD), a secondary metabolite of *Cannabis sativa*, on cysts and protoscoleces of *E. granulosus sensu stricto* (*s.s.*) [15].

Cannabis sativa L. (Cannabaceae) is an annual herbaceous plant that produces a great diversity of phytochemicals, such as cannabinoids, terpenoids, flavonoids, and other phenolic compounds, many of which are concentrated in the inflorescences of the plant [16]. All these phytomolecules can exert different —and potentially complementary— biological effects. Compounds can interact with each other, either preventing the degradation of a molecule, increasing its bioavailability, facilitating its binding to a receptor, limiting adverse effects, or activating multiple biological pathways [17, 18]. There is an increasing amount of evidence suggesting that these interactions between different components may have a significant impact on the bioactivity of *Cannabis* extracts [18].

C. sativa has been traditionally used for medicinal purposes by different cultures around the world. Its versatile applications have sparked scientific interest in recent years. In this context, previous research has demonstrated the neuroprotective, anticonvulsant, antioxidant, anti-inflammatory, analgesic, antitumor, antimicrobial, and antiparasitic effects of *C. sativa* and its components [19, 20]. Specifically, independent studies have found that *C. sativa* extracts are effective in vitro against *Leishmania major* and in vivo against *L. tropica* and *Trypanosoma evansi*. Similarly, CBD has demonstrated in vitro activity against *Giardia intestinalis*, and tetrahydrocannabinol (THC) has shown in vitro effectiveness against *Plasmodium falciparum* [21–25].

The aim of this study was to evaluate the in vitro and in vivo efficacy of a *full-spectrum* extract of *C. sativa* inflorescences against *E. granulosus s.s*. We first assessed its potential in vitro effect against protoscoleces and cysts, and subsequently evaluated the clinical efficacy of this extract in a murine model of cystic echinococcosis.

Materials and Methods

Plant Material

The plant material was supplied by the NGO CBG 2000 from Mar del Plata, Argentina, and characterized by the Plant Diversity Laboratory, belonging to the Marine and Coastal Research Institute (IIMyC) of the National University of Mar del Plata (UNMdP). The voucher number issued is, as recorded in the MDQ Herbarium Database: IMyCHer: MDQ:00630.

The total potency for this variety is 89 mg/g CBD (DW), 52 mg/g tetrahydrocannabinol (THC) (DW), and 0.1 mg/g cannabinol (CBN) (DW).

Preparation of the *Full-Spectrum* Extract of *C. Sativa* Inflorescences

The full-spectrum extract of *C. sativa* inflorescences was prepared from CBD-rich plants (chemotype III). Air-dried inflorescences (20 g) were finely chopped and extracted by maceration assisted by shaking. This process was repeated three times. The liquids obtained were combined

Table 1 Chemical characterization of the *full-spectrum* extract of *C. sativa* inflorescences

Polyphenols (mg/ml)	Flavonoids (mg/ml)	Anti-oxidant capacity (mg/ml)
14.0 ± 0.7	3.8 ± 0.2	104 ± 5.2

and evaporated using a rotary evaporator under vacuum at 50 °C. To achieve decarboxylation, the dry extract was kept under vacuum and taken to 100 °C for 45 min.

Chemical characterization of the *C. sativa* extract was performed by colorimetric methods (Table 1). Total polyphenol content, flavonoids, and antioxidant capacity were determined in quadruplicate [26]. The total content of phenolic compounds was determined by the Folin-Ciocalteu method with some modifications [27]; the calibration curve was constructed using different concentrations of a standard solution of gallic acid and the absorbance was measured at 760 nm. To determine the total content of flavonoids, the calibration curve was constructed using different concentrations of a standard solution of quercetin and the absorbance was measured at 420 nm [28]. The antioxidant capacity was determined by analyzing the decrease in the absorbance of the radical ABTS before and after reacting with the *C. sativa* extract [29]. The calibration curve was constructed using different concentrations of TROLOX as a standard compound and the absorbance was measured at 734 nm [29]. In all cases, the content of the different phytomolecules and the antioxidant capacity of the *C. sativa* extract were estimated from the linear regression previously obtained [26].

On the other hand, the concentration of CBD present in the *C. sativa* extract was determined by gas chromatography coupled to mass spectrometry (GC-MS), using analytical standards and the calibration curve method [20]. The CBD concentration obtained was 25.84 mg/ml (Fig. 1).

Chemicals

Standard solutions of CBD, THC, and CBN were purchased from Restek (Bellefonte, Pennsylvania, United States). Solvents were purchased from Sintorgan (Buenos Aires, Argentina) and used as received.

For in vitro studies, a stock solution was prepared by dissolving the *C. sativa* dry extract in dimethyl sulfoxide (DMSO). To achieve the final CBD concentrations to be tested in the culture tubes, aliquots from the stock solution were taken. Since the maximum volume of stock solution was used to reach the highest concentration tested (50 µg/ml), that same amount of DMSO was added to the control tube, which did not exceed 1%.

For in vivo studies, the dry extract was dissolved in sesame oil (Nutra Sem, Buenos Aires, Argentina). The suspension of albendazole (ABZ) (5.375 mg/ml) was prepared using pharmaceutical-grade ABZ (Parafarm, Buenos Aires,

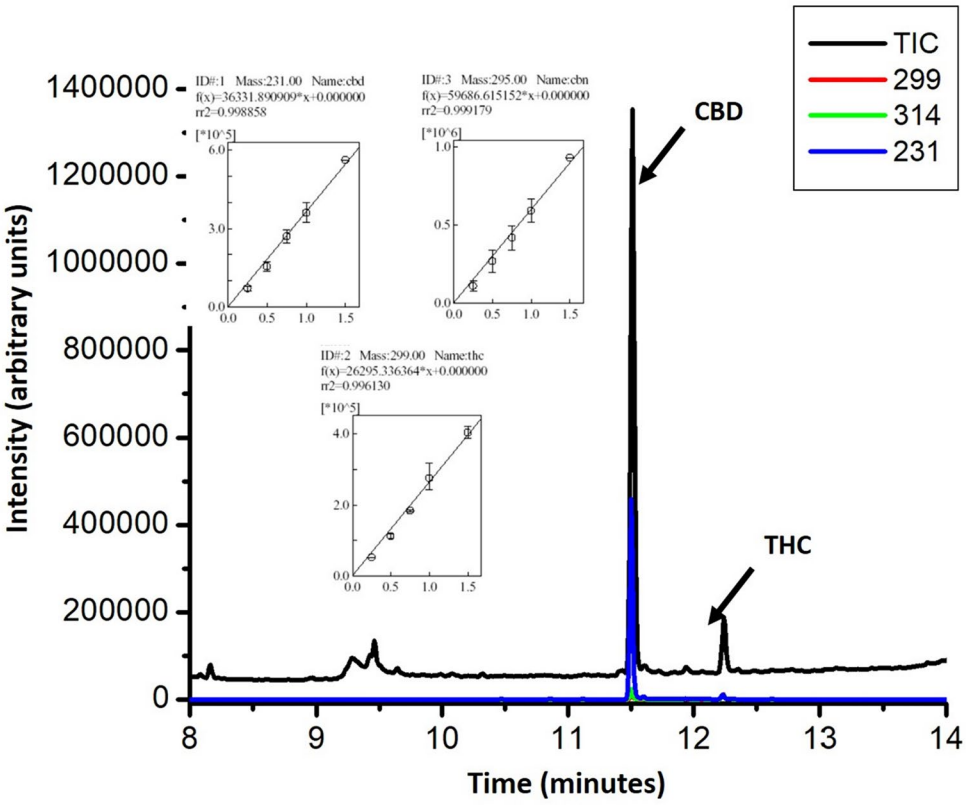

Fig. 1 Ion Chromatogram (TIC) for the *C. sativa* extract, as well as selected ions for Single Ion Monitoring (SIM) chromatogram and calibration curves for THC, CBD, and CBN. Note that the peak corresponding to CBN is not visible due to the substantially lower magnitude compared to CBD

Argentina). Pure ABZ was dispersed in distilled and deionized water (pH = 7.0), and then, this formulation was shaken overnight and finally sonicated for 1 h.

Parasite Material, Protoscoleces Collection and Cyst Obtention

Liver and lung hydatid cysts were obtained from cattle slaughtered in an abattoir located in Buenos Aires, Argentina. Protoscoleces were removed aseptically [30].

The parasitic material was genotyped by sequencing a fragment of the gene that codes for mitochondrial cytochrome c oxidase subunit 1 (CO1), following established protocols [31].

To obtain murine cysts, female CF-1 mice (body weight 25 ± 5 g) were intraperitoneally inoculated with 1500 *E. granulosus s.s.* protoscoleces per animal suspended in 0.5 ml of medium 199 (Mediatech, USA). After 6 months, mice that developed experimental secondary cystic echinococcosis were euthanized. Necropsy was performed subsequently and hydatid cysts present in the peritoneal cavity were carefully removed [32].

In Vitro Assays

Protoscolecidal Activity

Approximately 3000 viable protoscoleces were placed per Leighton tube containing 6 ml of medium 199. *C. sativa* extract was added achieving final CBD concentrations of 1, 5, 10, and 50 µg/ml. Protoscoleces incubated in culture medium and culture medium + DMSO were used as controls. All culture tubes were kept at 37 °C under stable conditions of pH = 7-7.4 and 5% of CO_2, without changes in the medium during the entire experiment [33]. Cultures were performed in triplicate and the experiment was repeated 3 times.

Protoscoleces viability was evaluated regularly by the methylene blue exclusion test [34] for a maximum of 28 days. Culture tubes were examined by optical microscopy every day to record structural alterations. Ultrastructure studies were also carried out using scanning electron microscopy (SEM).

Cysticidal Activity

Ten murine cysts were placed per Leighton tube containing 6 ml of medium 199. *C. sativa* extract was added resulting in final CBD concentrations of 1, 5, 10, and 50 µg/ml. Cysts incubated in culture medium and culture medium + DMSO were used as controls. Throughout the experiment, all culture tubes were maintained at 37 °C under stable conditions of pH = 7-7.4 and 5% of CO_2, without changes in the medium [33]. Cultures were carried out in triplicate and the experiment was repeated 3 times.

A periodic follow-up of the cysts (for a maximum of 12 days) was performed using optical microscopy to register structural alterations. Loss of turgidity and collapse of the germinal layer were used as parameters of the cyst viability [35].

In Vivo Clinical Efficacy Study

In a preliminary study, the potential antiparasitic effect of sesame oil against *E. granulosus s.s.* was investigated, but no statistically significant differences were observed compared to the water control ($p > 0.5$; data not shown) (Fig. 2). Based on these findings and following the 3Rs principle to reduce the number of animals [36], only mice that received water were used as a negative control in this experiment.

Female CF-1 mice, weighing 25 ± 5 g, were infected via intraperitoneal injection with 1500 protoscoleces per animal, suspended in 0.5 ml of medium 199. Six months later, a total of 40 mice were randomly divided into the following experimental groups: (a) water control, animals that received water; (b) ABZ group, animals treated with ABZ suspension (25 mg/kg); (c) *C. sativa* extract group, animals treated with the extract (20 mg/kg of CBD); and (d) ABZ + *C. sativa* extract group, animals treated with ABZ suspension (25 mg/kg) + *C. sativa* extract (20 mg/kg of CBD). Treatments were administered intragastrically every 24 h for 30 days, based on [15].

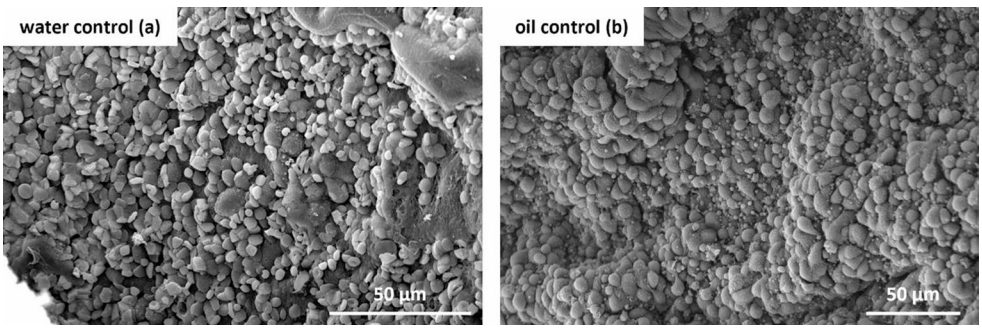

Fig. 2 Scanning electron microscopy of *E. granulosus s.s.* cysts recovered from mice that received water (**a**) or sesame oil (**b**). Both groups showed normal ultrastructure of the germinal layer of the cysts

At the end of the treatment period, all mice were euthanized and necropsy was carried out thereafter. Hydatid cysts were collected, situated in Petri dishes, and weighed on a digital scale with an accuracy of 0.01 g. Samples were taken for ultrastructural studies [35].

Electron Microscopy

Samples of protoscoleces taken from the in vitro assays, and samples of cysts taken from in vivo assays were processed for SEM following [37] with some modifications. The samples were fixed with a solution of 2.5% glutaraldehyde in 0.1% sodium cacodylate buffer for 48 h at 4 °C. Next, three washes in cacodylate buffer were made. The samples were dehydrated by sequential incubations in increasing concentrations of ethanol (50–100%) and then immersed in hexamethyldisilazane for 5 min, 1 h and then overnight. Finally, the samples were coated with gold (100-Å thickness) and examined using a "FESEM Zeiss Crossbeam 350 dual beam" scanning electron microscope operated at 15 kV.

Statistical Analysis

The statistical analyses and the respective Figures were performed in the R program [38].

To evaluate the effect of the *C. sativa* extract on protoscoleces and cysts of *E. granulosus s.s.*, a generalized linear model was applied. The 'emmeans' package [39] was employed to test the differences between pairs of conditions (Control, DMSO, 1, 5, 10, and 50 µg/ml). Differences between the weight of the cysts recovered from the different groups of the in vivo experiment were assessed by a Mann-Whitney test.

Ethical Statement and Experimental Animals

The Institutional Animal Care and Use Committee of the Faculty of Exact and Natural Sciences (National University of Mar del Plata, Argentina) approved the animal procedures and management protocols applied in this study (RD No. 40/2022). These procedures were performed in compliance with the revised version of The Guide for the Care and Use of Laboratory Animals [40].

Unnecessary animal suffering was prevented throughout the study. The animals were kept under controlled environmental conditions of temperature (22 ± 1 °C), relative humidity (60 ± 10%), and photoperiod (12 h light/dark cycle). Food and water were provided *ad libitum*.

At the end of the experiment, all mice remained alive. To obtain the murine cysts, they were euthanized by cervical dislocation after anesthesia (100 mg/kg ketamine + 10 mg/kg xylazine). Necropsy was carried out thereafter and hydatid cysts present in the peritoneal cavity were carefully removed.

Results

In Vitro Incubation of Protoscoleces with the *Full-Spectrum* Extract of *C. Sativa* Inflorescences

Protoscoleces viability after incubation with different concentrations of the *C. sativa* extract is shown in Fig. 3. The control group remained vital throughout the experiment. Statistically, each concentration tested had a different effect from the others ($p < 0.05$) and from the control ($p < 0.0001$). The concentration of 50 µg/ml led to a 50% decrease in protoscoleces viability after 1–2 h of treatment and the viability dropped to 0% within 6 to 24 h post-incubation (Fig. 3b).

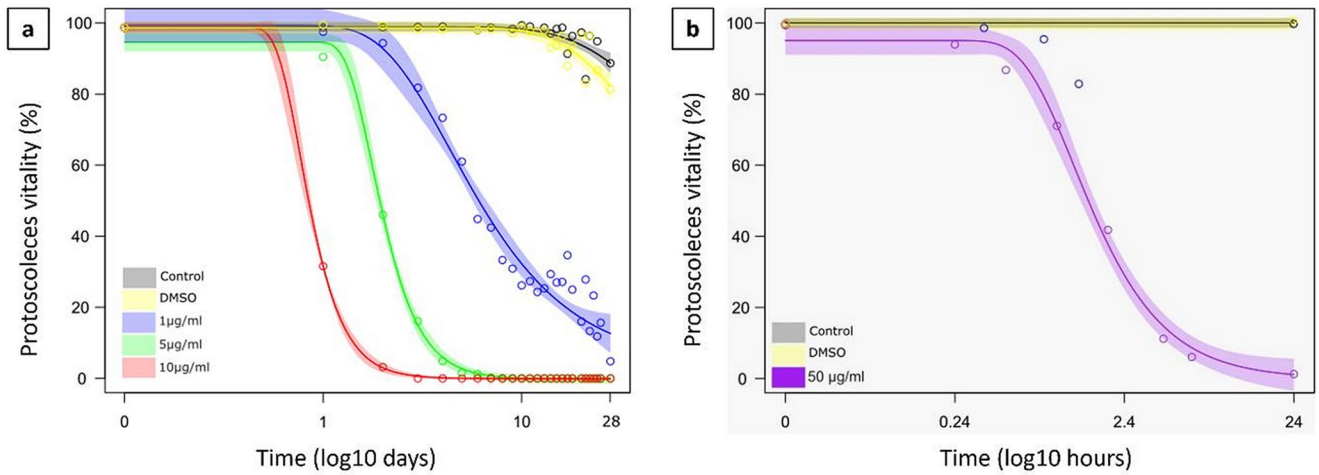

Fig. 3 Viability of *E. granulosus s.s.* protoscoleces after the in vitro exposure to different concentrations of the *full-spectrum* extract of *C. sativa* inflorescences

Concentrations of 10 and 5 µg/ml caused the reduction in viability to 0% on days 3 and 6, respectively (Fig. 3a). The concentration of 1 µg/ml reduced the viability to approximately 5% towards the end of the experiment (28 days) (Fig. 3a).

The effects of the *C. sativa* extract on protoscoleces viability were consistent with the outcomes from both structural (Fig. 4) and ultrastructural (Fig. 5) studies. No morphological changes were observed in the control group (Figs. 4b and 5b). In contrast, the *C. sativa* extract, at all concentrations tested, caused alterations from day 1, such as soma contraction (Figs. 4d- h and 5c-d), presence of blebs (Fig. 4c-e) and tegumental damage (Fig. 5f). In addition, the protoscoleces incubated with the highest concentrations (10 and 50 µg/ml) exhibited severe alterations, such as hook loss (Fig. 4g and h) and distortion of their normal morphology (Fig. 5g and h).

In Vitro Incubation of Cysts with the *Full-Spectrum* Extract of *C. Sativa* Inflorescences

Figure 6 shows the effect of different concentrations of the *C. sativa* extract on cysts. Control cysts remained turgid with no observable collapse of the germinal layer throughout the experiment (Fig. 7b). All concentrations tested had a marked effect, which differed statistically from the control ($p<0.05$). The concentration of 50 µg/ml caused the collapse of 92 ± 13% of the cysts after 24 h post-incubation (Fig. 6b). Both 10 and 5 µg/ml treatments resulted in the complete collapse (100%) of the cysts between 1 and 3 days post-incubation (Fig. 6a). With the concentration of 1 µg/ml, 80% of the cysts collapsed towards the end of the experiment (12 days) (Fig. 6a).

The effects of the *C. sativa* extract on cyst viability aligned with the outcomes from structural studies (Fig. 7). All concentrations tested caused structural alterations from the first minutes post-incubation, such as loss of turgidity (Fig. 7c), initiation of germinal layer detachment (Fig. 7d), and complete detachment of the germinal layer (Fig. 7e and f).

Clinical Efficacy Study

No behavioral or appearance changes were observed in animals throughout and after the *C. sativa* extract administration. Food and water intake remained unaltered. Table 2 shows the cyst weights (median and interquartile range) obtained after the treatment for the different experimental groups involved in the study.

The weight of the cysts recovered from treated mice was lower compared to the control group, especially those obtained after the treatment with ABZ ($p=0.09$) and the treatment with ABZ + *C. sativa* extract ($p=0.06$).

The SEM analysis of cysts recovered from the control group revealed their typical ultrastructure (Fig. 8a and b). In contrast, ultrastructural damage was detected in cysts recovered from all treated mice. The treatment with ABZ caused a reduction in the number of cells in the germinal layer (Fig. 8c); while the *C. sativa* extract induced morphological alterations in cells, as well as a reduction in cell number (Fig. 8d). The ultrastructural changes observed in the cysts recovered from mice treated with both ABZ + *C. sativa* extract were more pronounced compared to those caused by monotherapy (Fig. 8e).

Discussion

Due to the challenges in achieving successful pharmacological treatment and the disadvantages of the compounds currently used, the development of an effective therapy for cystic echinococcosis is crucial [5].

C. sativa is particularly interesting due to its great pharmacological potential. Throughout history, different cultures have utilized this plant for medicinal purposes, but its main psychoactive component, tetrahydrocannabinol (THC), led to its classification as an illegal substance for a long time [20]. However, recent studies suggest that CBD, another cannabinoid, may have the potential to counteract the negative effects of THC, especially when administered in higher proportions compared to THC [41]. This has prompted numerous investigations to explore the therapeutic applications of *C. sativa*, revealing its anti-parasitic properties against various groups of organisms, including *Leishmania major*, *L. tropica*, *Trypanosoma evansi*, *Giardia intestinalis*, and *Plasmodium falciparum* [21–25].

The *C. sativa* extract tested in this study showed a significant in vitro protoscolicidal effect against *E. granulosus s.s.* after short exposure times. This aligns with the structural and ultrastructural alterations observed, such as soma contraction, presence of blebs and tegumental damage, coupled with loss of hooks and distortion of their normal morphology at the highest concentrations (10 and 50 µg/ml).

The *C. sativa* extract also exhibited a significant in vitro cysticidal effect against *E. granulosus s.s*. Consistent with previous studies [10–14], it caused a rapid detachment of the germinal layer and collapse of the cysts.

The findings of this study are consistent with previously obtained results from our group, which demonstrated the in vitro activity of other natural products against protoscoleces and cysts of *E. granulosus*. The efficacy of the essential oils of rosemary, mint, thyme, oregano, cinnamon, and stevia extracts has been examined in previous studies

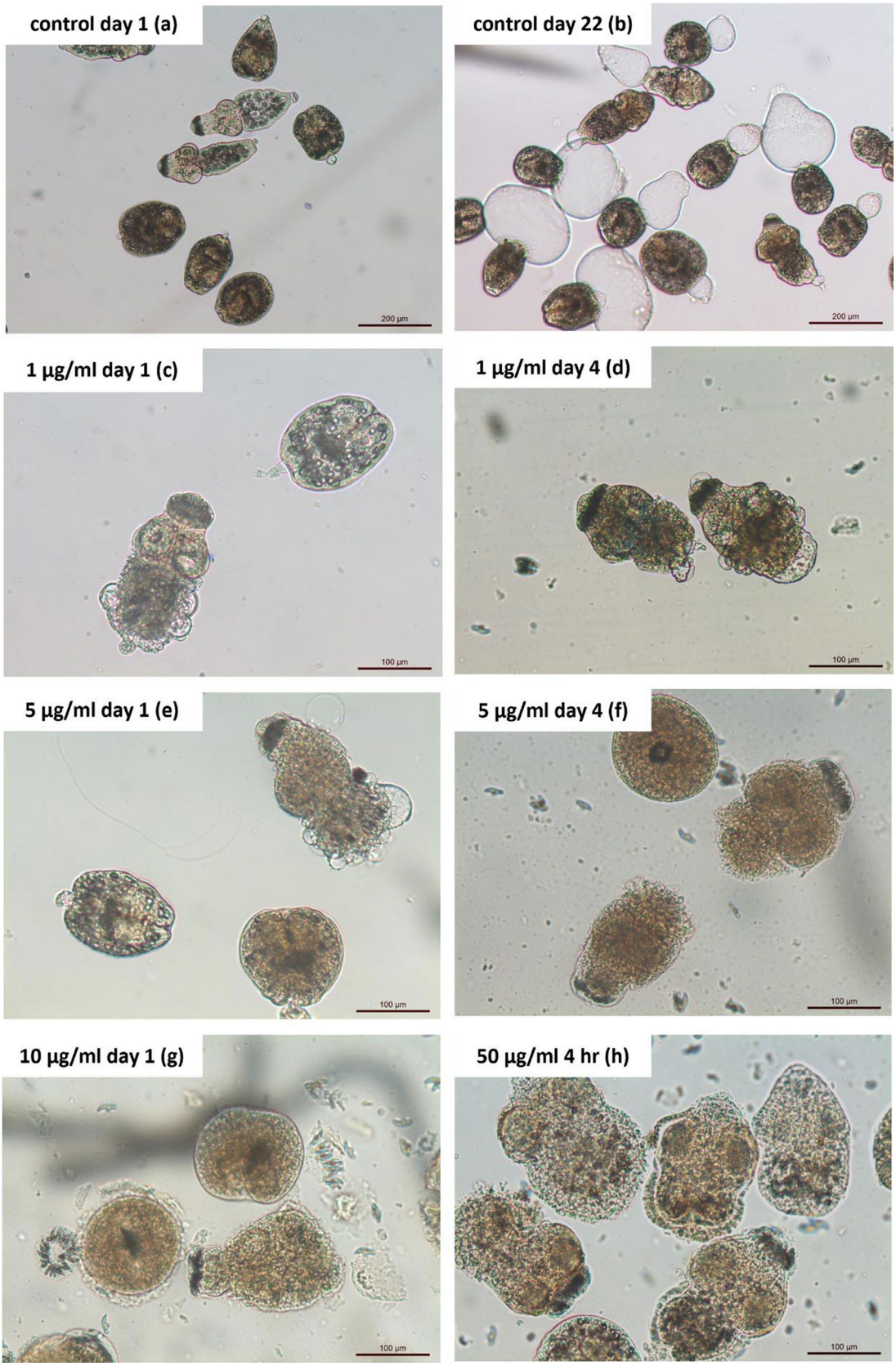

Fig. 4 Light microscopy of *E. granulosus s.s.* protoscoleces incubated in vitro with different concentrations of the *full-spectrum* extract of *C. sativa* inflorescences. Observe the normal structure of protoscoleces (**a** and **b**). Note the presence of structural alterations: presence of blebs (**c**, **d**, and **e**), tegumental damage (**f**), hook loss and distortion of their normal morphology (**g** and **h**)

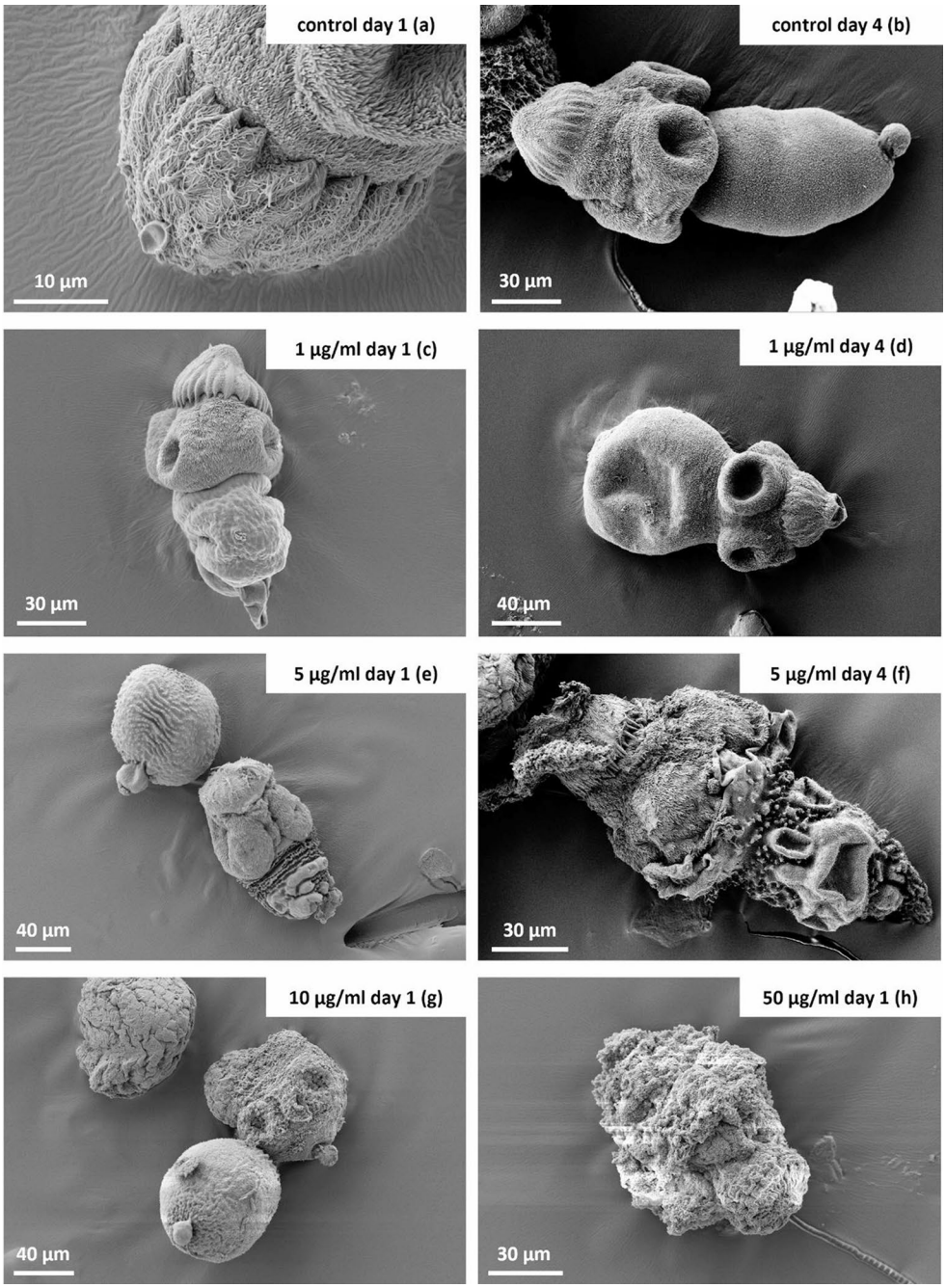

Fig. 5 Scanning electron microscopy of *E. granulosus s.s.* protoscoleces incubated in vitro with different concentrations of the *full-spectrum* extract of *C. sativa* inflorescences. Observe the normal ultrastructure of protoscoleces (**a** and **b**). Note the presence of ultrastructural alterations: soma contraction (**c** and **d**), presence of blebs and tegumental damage (**e** and **f**), hook loss and distortion of their normal morphology (**g** and **h**)

[9–14]. However, unlike the rapid effect observed with the *C. sativa* extract, none of the above-mentioned natural products caused a decline in viability of 50% or more before 7 days of treatment at the same concentrations. The *C. sativa* extract tested in this study demonstrated in vitro efficacy within a couple of days, even hours, showing to be more effective in shorter periods, making it clearly superior.

The clinical efficacy study revealed that all treatments reduced the weight of the cysts. Particularly, the outcomes from the treatment with ABZ and the coadministration of ABZ + *C. sativa* extract were statistically different from the control group. However, the combination of ABZ and the *C. sativa* extract produced the greatest effect on cysts of *E. granulosus s.s.*, even greater than the effect of monotherapy with ABZ. These results were accompanied by ultrastructural changes in the germinal layer, including a reduction in the number of cells and morphological alterations of their structure. Similar outcomes have been seen in *E. granulosus* cysts recovered from mice treated with other natural products, such as stevia extracts [13, 14].

The dose of CBD administered in this study (20 mg/kg) has been previously tested in other investigations and

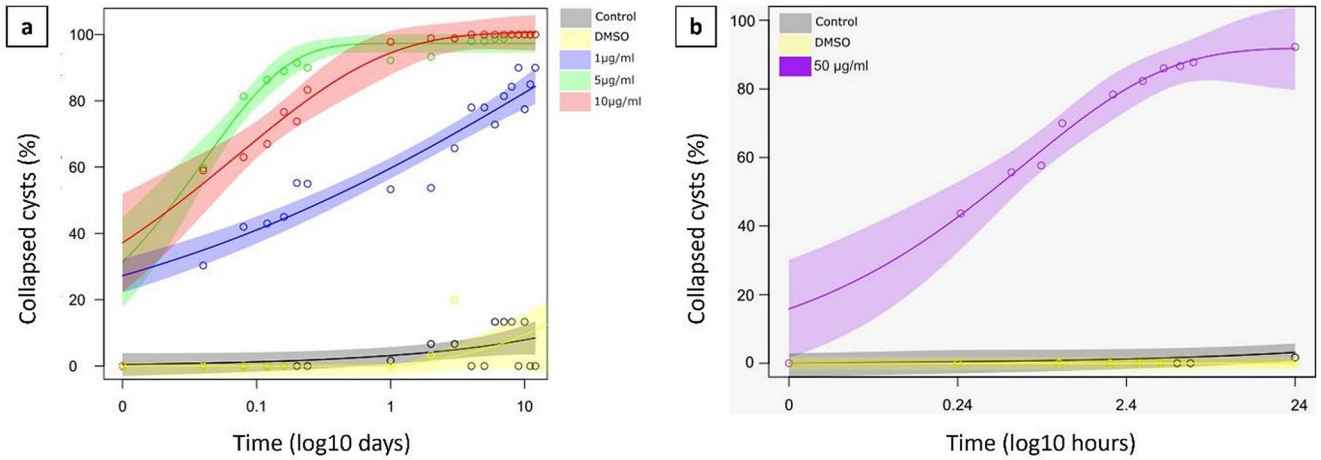

Fig. 6 Collapse of the germinal layer of *E. granulosus s.s.* cysts after the in vitro exposure to different concentrations of the *full-spectrum* extract of *C. sativa* inflorescences

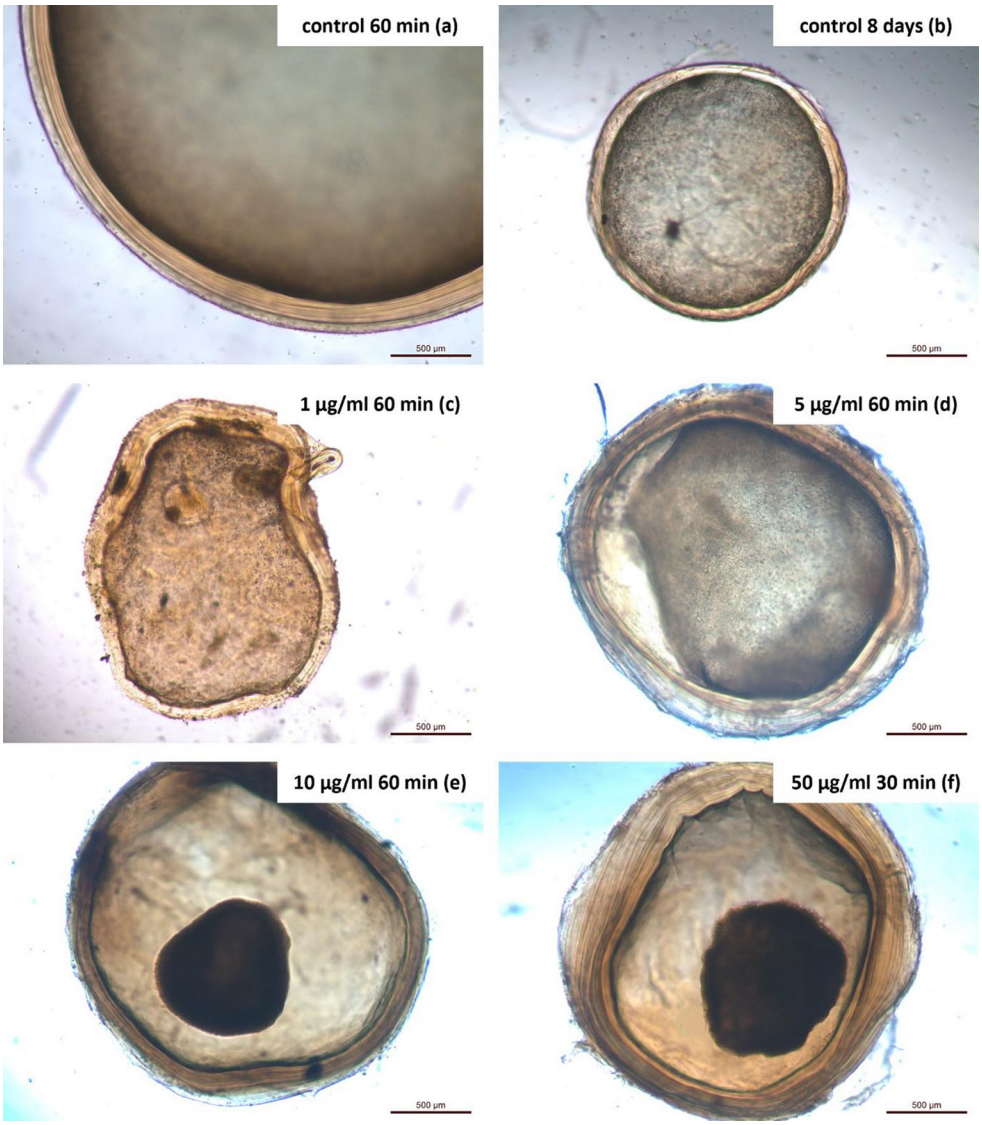

Fig. 7 Light microscopy of *E. granulosus s.s.* cysts incubated in vitro with different concentrations of the *full-spectrum* extract of *C. sativa* inflorescences. Observe the normal structure of cysts (**a** and **b**). Note the presence of structural alterations: loss of turgidity (**c**), initiation of germinal layer detachment (**d**), and complete detachment of the germinal layer (**e** and **f**)

Table 2 Clinical efficacy study. Median weight (g) and interquartile range (IQR) of the *E. granulosus s.s.* Cysts recovered from experimentally infected mice, untreated and treated groups

Group	Median weight of cysts (g)	Interquartile range (IQR)
Control	2.16	1.24
ABZ	1.50*	0.96
C. sativa extract	1.84	0.66
ABZ + *C. sativa* extract	0.73**	1.70

* Statistically significant differences with the control group ($p = 0.09$).

** Statistically significant differences with the control group ($p = 0.06$)

is considered safe. A CBD-rich *Cannabis* extract, when administered orally to mice, has only produced adverse effects at doses 30 times higher [42].

Drug development research often focuses on the study of individual molecules, but plant extracts represent a complex mixture of phytomolecules that possess multiple biological effects [18]. Recently, we demonstrated the activity of CBD against cysts and protoscoleces of *E. granulosus* [15]. Nevertheless, several studies have shown that *full-spectrum* extracts of *C. sativa* are markedly more effective and potent at lower dosages compared to isolated compounds. This allows the achievement of equivalent clinical benefits using a lower concentration of cannabinoids, resulting in a significant reduction of side effects such as increased hepatic enzymes commonly associated with high-dose CBD consumption [43].

When comparing the outcomes of this study with those reported for isolated CBD [15], the *full-spectrum* extract of *C. sativa* inflorescences exhibited a comparable or even greater in vitro efficacy on protoscoleces and cysts of *E. granulosus s.s*. The extract showed a more pronounced effect on protoscoleces at concentrations of 50 and 10 µg/ml. Protoscoleces viability dropped to 0% within 6 to 24 h after the incubation with 50 µg/ml of CBD present in the *C. sativa* extract, whereas it took 48 h for the same concentration of isolated CBD to achieve the same result. Similarly, after 24 h of incubation with 10 µg/ml of CBD present in the *C. sativa* extract, protoscoleces viability decreased to 30%, while isolated CBD only caused a slight decrease to 85%. On the other hand, the *C. sativa* extract displayed greater activity on cysts at all concentrations tested. After 24 h of incubation with 50 µg/ml of CBD present in the *C. sativa* extract, 92% of the cysts collapsed, compared to only 50% when incubated with the same concentration of isolated CBD. Even at 10 µg/ml, the extract caused 99% of cysts to collapse after 24 h, while isolated CBD only led to a collapse of 7%.

Other authors have also observed significant differences in the efficacy of a *full-spectrum* extract of *C. sativa* and its components. A study conducted on cell cultures and animal models of breast cancer has revealed that an extract of *C. sativa* inflorescences has a more potent antitumor effect than pure THC [44]. Moreover, it was reported that *C. sativa* extracts are 4 times more potent in managing episodes of epilepsy compared to the administration of pure CBD [45].

This phenomenon could be explained by a complementary interplay between the various phytochemicals found in a *full-spectrum* extract. Several authors documented a synergistic interaction within the different constituents of *C. sativa* [18]. This plant produces a vast array of secondary metabolites, including over 100 different cannabinoids,

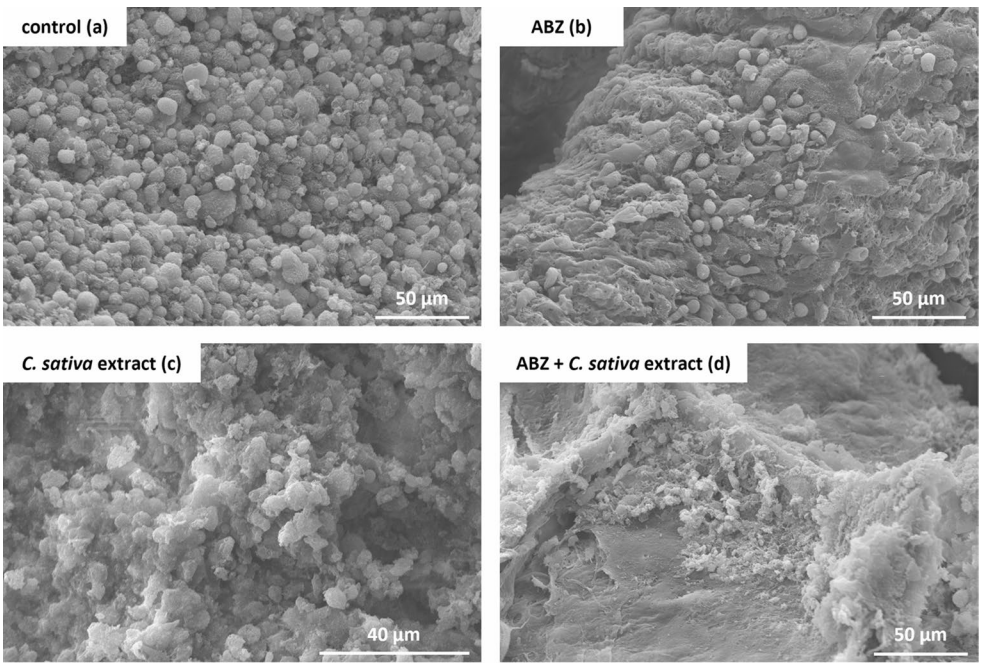

Fig. 8 Scanning electron microscopy of *E. granulosus s.s.* cysts recovered from mice after the clinical efficacy study. Observe the normal ultrastructure of the germinal layer (**a**), the effect of ABZ causing a reduction in the number of cells (**b**), the effect of the *full-spectrum* extract of *C. sativa* inflorescences causing morphological alterations and a reduction in cell number (**c**), and a more pronounced effect when treated with ABZ + the *full-spectrum* extract of *C. sativa* inflorescences (**d**)

as well as terpenoids, flavonoids, and other phenolic compounds [16].

Cannabinoids, like CBD and THC, have a broad range of therapeutic applications [46]. The exposure of *Caenorhabditis elegans* (Nematoda) to cannabinoids causes alterations in their behavior, specifically affecting their feeding, locomotion, and nociception, key processes in the biology of these organisms [47].

Terpenoids are among the most numerous groups of compounds found in *C. sativa*. Not only can they exert biological effects per se, but they can also modulate the effects of cannabinoids [46, 48]. Studies have shown that terpenoids cause damage to the tegument of *Schistosoma mansoni* (Platyhelminthes) [49–51]. Due to their lipophilic nature, they can penetrate biological membranes and produce intracellular disruption. Ultimately, this leads to the death of the parasite [51].

On the other hand, flavonoids possess a variety of biological effects similar to cannabinoids and terpenoids [46]. These compounds bind to membrane proteins, which can cause major structural changes that alter the permeability and substance exchange [52].

When administered as a *full-spectrum* extract, the combination of all these phytomolecules may enhance the therapeutic effect of *C. sativa* by acting in multiple ways [18, 53]. In general, the complexity and multi-component nature of plant extracts present significant challenges to their standardization. However, in the case of *Cannabis sativa*, combining genetic uniformity (via clonal propagation), environmental standardization (via indoor cultivation), and chemical fingerprinting of key constituents allows the reliable production of reproducible and efficacious cannabis-derived extracts for experimental or therapeutic purposes [54].

Conclusion

This research reports for the first time the in vitro efficacy of a *full-spectrum* extract of *C. sativa* inflorescences against protoscoleces and cysts of *E. granulosus s.s*. Additionally, the clinical efficacy of this extract against established cysts in mice was demonstrated. The results obtained in this study are highly promising in the search for novel, safer, and more effective alternatives for the treatment of human cystic echinococcosis. Based on these findings, we plan to conduct a dose titration study to achieve precise dosing of the *C. sativa extract* on the murine model of cystic echinococcosis.

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Data Availability The data that support the findings of this study are available from the corresponding author upon request.

Declarations

Competing Interests The authors declare no competing interests.

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