



Iraqi Journal of Pharmacy

Journal homepage: <https://iphr.uomosul.edu.iq/>

Research Article:

Effect of *Boswellia sacra* Extracts on Preventing Secondary Hydatid Cysts in BALB/c Mice

Baraa Basil Ramadan¹  , Asmaa Abdulazeez Ali² , Luay Rashan³ ¹ Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Nineveh University, Mosul, Iraq.² Department of Biology, College of Education for Pure Science, Mosul University, Mosul, Iraq.³ Biodiversity Unit, Research Center, Dhofar University, Salalah, Oman.

Article Information

Article history:

Received on: 03 June 2026
 Revised on: 11 July 2026
 Accepted on: 19 July 2026
 Published on: 01 September 2026

Keywords:

Cystic Echinococcosis;
Boswellia sacra extract;
 Methanolic extract;
 Hepatomegaly;
 BALB/c mice

Abstract

Background: Cystic echinococcosis caused by *Echinococcus granulosus*, creates global health challenges due to costly and toxic chemotherapy. To the authors' knowledge, *Boswellia sacra* extracts have not previously been evaluated against secondary hydatid cysts in murine models. This study aimed to investigate the *in vivo* efficacy of various *Boswellia sacra* oleo-gum extracts against secondary hydatid cysts in BALB/c mouse models. **Methods:** BALB/c mice inoculated with *Echinococcus granulosus* protoscoleces were allocated into positive, negative, and vehicle controls, alongside treatment groups receiving ethanolic extract, methanolic extract, or crude oleo-gum resin. Efficacy was assessed via total cyst numbers, weight, diameter, and hepatosplenomegaly parameters. **Results:** *Boswellia sacra* extracts treatment induced a highly significant reduction ($P \leq 0.0001$) in recovered hydatid cysts compared to the positive control. However, ANOVA revealed no statistically significant differences ($P > 0.05$) in overall cyst weights and diameters. A reduction in hepatosplenomegaly was noted, though this systemic alleviation was not consistent across all treated groups. **Conclusion:** *Boswellia sacra* extracts potently suppress secondary hydatid cyst proliferation by significantly decreasing overall cyst counts. While the extracts did not significantly arrest the diameters or weight, and exerted only a partial reduction, in-consistent effect on related organomegaly, the reduced parasite burden underscores their potential as natural adjunctive agents.

2026 Iraqi Journal of Pharmacy. Published by University of Mosul, Iraq. This is an open access article licensed under CC BY: (<https://creativecommons.org/licenses/by/4.0>)

1. Introduction

Cystic Echinococcosis (CE) is a zoonotic parasitic disease that poses a considerable global health challenge and economic burden (1,2). This condition is caused by the larval stage of the tapeworm *Echinococcus granulosus*, is classified as a neglected Zoonotic parasitic disease. (3). The global economic impact of CE is estimated at nearly 3\$US billion annually, encompassing direct medical costs and substantial livestock industry losses, including liver condemnation and reduced animal productivity (4,5). This disease is highly endemic in rural communities,

particularly across the Middle East and North Africa, where transmission rates range between 3% and 7% (6,7). Like other tapeworms, *Echinococcus* depends on two distinct hosts to complete its life cycle. The adult worms develop and reproduce in the definitive hosts, while the larval stages require a separate intermediate host to continue their development (8,9). Current treatment of human CE includes surgical intervention, a "watchful waiting" approach, and chemotherapy, primarily using benzimidazoles such as Albendazole. (10,11). While surgery remains a definitive option, the use of effective protoscolicidal agents during or after intervention is critical to prevent cyst rupture and subsequent recurrence, which can trigger severe anaphylactic reactions or death (5,12,13). However, conventional chemical protoscolicidal agents (e.g., hypertonic saline, silver nitrate, and cetrime) are frequently associated with severe adverse effects, including liver necrosis, fibrosis, and biliary tract dysfunction.(3). Therefore, developing novel, effective, and safer alternative protoscolicidal agents is an urgent medical necessity (3).

*Corresponding author: Baraa Basil Ramadan, Department of Pharmaceutics and Industrial Pharmacy, College of Pharmacy, Nineveh University, Mosul, Iraq.

Email: baraa.basil@uoninevah.edu.iq

How to cite:

Ramadan, B., B., Ali, A., A., Rashan, L., (2026). Effect of *Boswellia sacra* Extracts on Preventing Secondary Hydatid Cysts in BALB/c Mice. Iraqi J. Pharm. 23(3), 130-139.

DOI: <https://doi.org/10.33899/iraqij.p.2026.172464.1235>

The genus *Boswellia* (Burseraceae) comprises approximately 25 discrete species, the most prominent of which are *Boswellia sacra*, *Boswellia serrata*, *Boswellia carterii* (13,14,15). *Boswellia* trees are known for producing an aromatic oleo-gum resin, commonly known as frankincense (Figure 1). *Boswellia sacra* frankincense has been used in traditional medicine, cultural, and religious ceremonies for thousands of years (16,17). The distinctive aroma of these resins has been valued for its therapeutic potential and has held cultural significance since the ancient Egyptian civilization. *Boswellia* spp. extracts have long been utilized in traditional medicine for a wide range of health conditions (7). Frankincense resin possesses a broad spectrum of pharmacological properties, including hypoglycemic, antioxidant, and gastroprotective activities, as well as therapeutic effects against gastric and colonic ulcers, Crohn's disease, osteoporosis, and cognitive impairment (18).



Figure 1. *Boswellia sacra* tree and its harvested oleo-gum resin

Extensive pharmacological investigations highlighted the therapeutic relevance of boswellic acids in the management of chronic inflammatory disorders such as ulcerative colitis, rheumatoid arthritis, Crohn's disease, and bronchial asthma. In addition, these compounds have demonstrated potential antidepressant and anxiolytic effects, along with supportive benefits in patients with brain tumors. (15,19). Among the bioactive constituents of frankincense, 11- keto- β - boswellic acid (KBA) and acetyl-11-keto- β - boswellic acid (AKBA) are considered the most potent non-steroidal anti-inflammatory agents and are largely responsible for its anti-inflammatory activity.(15,20).The anti-inflammatory mechanism of frankincense is primarily attributed to its ability to modulate cytokine production and suppress leukocyte infiltration, supporting its long-standing use in inflammatory diseases, including rheumatoid arthritis and Crohn's disease. (16). Furthermore, *Boswellia* spp. resins have demonstrated therapeutic utility in a variety of pathological conditions, including neurodegenerative disorders, malignant tumors, cardiovascular diseases, hepatic and biliary disorders, and inflammatory bowel disease. Their anticancer activity has been reported across several cancer models, including leukemia, hepatocellular carcinoma, melanoma, fibrosarcoma, colorectal, prostate, and meningeal tumors (16,21,22).

To the best of our knowledge, the present study represents the first global investigation into the therapeutic efficacy of different frankincense (*Boswellia sacra*) extracts (methanolic extract, ethanolic extract, and raw oleo-gum resin) against secondary cystic echinococcosis in mice,

using Albendazole as the standard baseline drug. Treatment parameters were evaluated based on changes in cyst number, diameter, and weight, while the host inflammatory response was assessed through hepatosplenomegaly indices.

2. Materials and methods

2.1. Laboratory animals

Three- to four -week-old male BALB/c mice were used in the present study. All experimental procedures were carried out at the animal house of the College of Education for Women, University of Mosul, during the period from 1/11/2025 to 1/4/2026.

2.2. Cysts samples and protoscoleces isolation

Hydatid cysts were aseptically obtained from the livers of sheep naturally infected with *Echinococcus granulosus*, collected from local slaughterhouses. The external surfaces of the cysts were disinfected with 70% ethanol, after which cyst fluid containing protoscoleces was carefully aspirated using sterile syringes and transferred into sterile containers. The collected protoscoleces were washed repeatedly with sterile phosphate-buffered saline (PBS) to remove host tissue debris and cyst remnants. Protoscoleces viability was assessed using the eosin exclusion test according to Smyth and Barrett (23). Briefly, 20 μ l of a 0.1% eosin solution was added to a drop of the protoscoleces suspension on a slide and examined under a light microscope. Viable protoscoleces remained unstained and appeared bright green due to exclusion of the dye, whereas non-viable protoscoleces absorbed eosin and appeared red. Only preparations containing viable protoscoleces were used for experimental infection. To induce secondary hydatid infection, each mouse was intraperitoneally inoculated with 2000 viable protoscoleces suspended in sterile PBS under aseptic conditions. All injections were performed using sterile syringes, and the animals were subsequently observed throughout the experimental period to monitor their general health and infection progression.

2.3. Boswellia extracts

The crude oleo-gum resin of *Boswellia sacra* fluck (Bursraceae) was sustainably harvested from its natural habitats in Wadi Doka, located on the plateau region approximately 40 km north of Salalah, Dhofar Governorate, Sultanate of Oman, during 2023. This ecological region experiences a distinct desert climate characterized by sharp diurnal temperature variations and low annual rainfall (less than 100mm). The collected material-characterized locally by Dhofarian peoples as Grade Houjari-Najdi resin- was obtained utilizing time- honored traditional tapping techniques practiced by indigenous collectors. After clearing the resin from all woden debris and particles, the botanical integrity and identity of the specimen were formally authenticated through direct taxonomic comparison with preserved voucher samples deposited at the Herbarium of Nizwa University, Oman. The finalized, standardized formulations of the *Boswellia sacra* extracts utilized across the subsequent experimental arms were prepared and supplied in their optimal status by our research collaborator in the Sultanate of Oman (24).

To ensure therapeutic reproducibility and standardization, the *Boswellia sacra* oleo-gum resin was initially cooled down to -20°C to facilitate grinding. For organic extractions, 25 g of freshly ground resin was extracted separately with 100 ml of methanol (100%) and another 25 g with 100 ml of ethanol (100%) at laboratory temperature for 1-2 hours under continuous stirring. Following extraction, the mixtures were centrifuged at 5000 x g for 5 minutes; the resulting supernatants were collected and evaporated to dryness using a rotary evaporator to yield the finalized methanolic and ethanolic extracts. For the extraction of volatile constituents, 200 g of freshly ground oleo-gum resin was subjected to hydro distillation. The resin was placed in a round-bottom flask containing 500 ml of deionized water, fitted onto a heating mantle, and hydrodistilled using a steam jacket maintained at atmospheric pressure and a constant temperature of 100°C. The companion essential oil fraction was obtained after 2 hours of hydro distillation, subsequently dried over anhydrous sodium sulfate (Na₂SO₄), and stored in dark glass bottles at a controlled temperature until further analysis. Prior to initial host delivery in the *in vivo* bioassays, all working extract stock preparations were freshly reconstituted and diluted to the target experimental concentrations immediately before use (24,25).

Phytochemical fingerprinting and HPLC-MS/MS conditions the qualitative and quantitative phytochemical profiles of the prepared extracts were verified against established reference analytical criteria (24). Chromatographic separation and quantification of the hallmark pentacyclic triterpenic acids were performed on an Agilent 1260 Infinity system (Agilent, Santa Clara, CA, USA) coupled with an AB API 2000 triple quadrupole mass spectrometer (Applied Biosystem, Foster City, CA, USA) equipped with an electrospray ionization (ESI) source. Data acquisition and processing were managed via Analyst 1.6.1 software (AB Sciex, Framingham, MA, USA).

Separation was achieved using a reversed-phase HPLC column (Dr.Maisch Reprosil-Pur Basic- C18HD, 3 µm, 125 × 3 mm; Dr.Maisch GmbH, Ammerbruch, Germany) protected by a precolumn (Dr.Maisch Reprosil Universal Rp, 5µm, 10 × 4 mm). MS/MS analysis were executed in the negative atmospheric pressure electrospray ionization mode under multiple-reaction monitoring (MRM) detection. The ion source temperature was maintained at 300°C, the ion spray voltage was adjusted to -4250 V, and the collision energy was optimized at -40V with a dwell time of 500 ms. Quantifications of the analyte concentrations were conducted via the external calibration method based on the standard workflow pioneered by Schmiech et al. (26). Parallel to the triterpenoid evaluation, the volatile constituents of the essential oil fractions were mapped via gas chromatography-mass spectrometry (GC-MS) under the parameters outlined by Di Stefano et al (27).

2.4. Experiment design

A total of 77 male BALB/c mice were randomly assigned to 11 experimental groups (n=7 mice per group). All infected groups were inoculated intraperitoneally at 21 days of age with 2000 viable *Echinococcus granulosus* protoscoleces suspended in sterile PBS, following the same standardized infection protocol. The experimental groups comprised 6 frankincense- treated groups, two groups of standard drug, a vehicle control group, a positive control group, and a negative control group. Three frankincense preparations were designated using internal laboratory codes (Bs2, Bs4, Bs13) to facilitate sample tracking. The ethanolic extract, a

methanolic extract, and powdered frankincense oleo-gum resin. Preparations were solubilized in 10% DMSO. The animals were randomly assigned to 11 experimental groups as follows: an uninfected control group, consisting of uninfected, untreated mice maintained under standard experimental conditions; a vehicle control group, comprising uninfected mice orally administered 10% DMSO according to the same treatment schedule as the intervention groups; an infected untreated control group, consisting of infected mice that received no therapeutic intervention; a prophylactic albendazole group, in which infected mice received oral albendazole (150 mg/kg body weight) once daily for 10 consecutive days beginning immediately after infection; a therapeutic albendazole group, in which infected mice received oral albendazole (300 mg/kg body weight) beginning 4 months after infection according to an intermittent regimen consisting of one treatment day followed by a 3-day treatment-free interval, for a total of six doses over 24 days; a low-dose ethanolic extract group (Bs2-L), in which infected mice received oral *Boswellia sacra* ethanolic extract (200 mg/kg body weight) beginning 4 months after infection using the same intermittent dosing regimen; a high-dose ethanolic extract group (Bs2-H), treated with oral *Boswellia sacra* ethanolic extract (400 mg/kg body weight) according to the same schedule; a low-dose methanolic extract group (Bs4-L), in which infected mice received oral *Boswellia sacra* methanolic extract (200 mg/kg body weight) beginning 4 months after infection using the same intermittent regimen; a high-dose methanolic extract group (Bs4-H), treated with oral *Boswellia sacra* methanolic extract (400 mg/kg body weight) according to the same schedule; a low-dose crude extract group (Bs13-L), in which infected mice received oral *Boswellia sacra* crude extract (200 mg/kg body weight) beginning 4 months after infection using the same intermittent regimen; and a high-dose crude extract group (Bs13-H), treated with oral *Boswellia sacra* crude extract (400 mg/kg body weight) according to the same dosing schedule. All therapeutic and extract-treated groups received six oral doses administered over a 24-day period, with each treatment separated by a 3-day treatment-free interval.

2.5. Mice dissection

At the end of experimental period, mice were euthanized under deep anesthesia and examined for hydatid cyst development. Following a midline abdominal incision, internal organs including the liver, spleen, lungs, kidneys and peritoneal cavities were carefully inspected for cyst distribution. Hydatid cysts were gently excised from surrounding tissues and collected for analysis. The total number of cysts per mouse was determined by direct manual counting. Individual cysts were weighed using a precision analytical balance to determine cyst weight, and cyst diameter was measured using a digital caliper.

3. Statistical analysis

The data were analyzed statistically according to the Completely Random Design (CRD) method, with seven replications for the purpose of detecting the effect of each of the 11 Groups and overlapping between them. The differences between the means were compared according to Duncan's Multiple Range Test. The statistical Analysis System (SAS)V9 was adopted in the implementation of all statistical analysis (28).

4. Results

4.1. *Boswellia sacra* extracts

High-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) quantitative screening distinct variations in the concentrations of the eight monitored pentacyclic triterpenic acids across the evaluated *Boswellia sacra* fractions (Figure 2). The complete quantitative distribution ($\mu\text{g}/\text{mg}$, w/w) for each extract is systematically summarized in (Table 1). The characterization profile demonstrated that the organic solvent fractions exhibited a substantially higher yield of total boswellic acids, compared to the aqueous derivatives. Specifically, acetyl- β -boswellic acid (β -ABA) and acetyl-11-keto- β -boswellic acid (AKBA) emerged as the predominant biomarkers within the ethanolic and methanolic extracts.

4.1.1. Effect of Frankincense extracts on hydatid cyst number

The one-way ANOVA analysis summarized in (Table 2) revealed highly significant differences among the experimental groups ($P \leq 0.0001$) regarding the total number of recovered hydatid cysts six months post-infection. This statistical finding indicates a marked reduction in the number of cysts across all extract-treated mice compared with the positive control group (Figures S-10). These results are further supported by gross observations; Figure 3 and 5 demonstrate a clear, visual reduction in cyst numbers in treated mice relative to the untreated controls. Notably, no hydatid cysts were visibly detected in specific clearance groups, as illustrated in Figures 9 and 10. Furthermore, multiple post-hoc comparisons using Duncan's multiple range test (Table 3) confirmed that treatment with the *Boswellia sacra* ethanolic extract resulted in the most profound reduction. The lowest mean cyst count (0.571) was recorded in the group treated with a 200mg/kg dose, whereas the positive control group exhibited the highest mean number of cysts (28.143) at six months post-infection.

4.1.2. Effect of *Boswellia sacra* extracts on hydatid cyst weight and diameter

With respect to cyst weight and diameter, the overall variance evaluated via ANOVA (Table 2) showed no statistically significant differences ($P > 0.05$) among the experimental groups; indicating that the extracts did not induce a statistically consistent arrest of these morphological parameters. Nevertheless, a descriptive evaluation of the data (Table 3) suggested minor biological inclinations. For instance, the positive control group recorded the highest mean cyst weight (11.547 g), While a notable biological reduction to the lowest mean weight (0.026g) was descriptively observed in the group treated with methanolic extract at a dose of 400mg/kg, followed by intermediate values in mice treated with crude oleo-gum Bs13(400mg/kg) and ethanolic extract Bs2(200 mg/kg).

4.1.3. Analysis of cyst diameter

Similarly, regarding cyst diameter (Table 3), an exploratory look at the values indicated a downward inclination, where the descriptive mean diameter was lower in the ethanolic extract Bs2 (0.2719 mm), followed closely by the Albendazole-treated group (0.3020 mm), compared to the positive control group (2.540 mm). However, these observational differences must be interpreted with caution and strictly as exploratory trends due to the lack of overall statistical significance.

4.1.4. Effect of *Boswellia sacra* Extracts on Hepatosplenomegaly indices

The one-way ANOVA analysis summarized in (Table 4) revealed highly significant differences among the experimental groups ($P \leq 0.0001$) for hepatosplenomegaly index. This confirms that infection and subsequent treatment induced distinct systemic pathological alterations in organ weights. Further pos-hoc comparisons using Duncan's multiple range test (Table 5) demonstrated a complex, non-uniform physiological response across the treatment groups:

Heptomegaly Index: The positive control group exhibited the highest liver enlargement (139.46), reflecting severe infection-induced pathology, which was significantly reduced back to physiological control levels in the negative control group (39.06). Interestingly, the vehicle control (DMSO) also lowered this index (73.87). Among the treated groups, the *Boswellia sacra* methanolic extract at 200mg/kg exerted the most potent systemic alleviation, significantly reducing the liver index (83.43) compared to the positive control.

splenomegaly Index: contrary to the liver responses, the positive control group recorded (34.44). The administration of certain *Boswellia sacra* formulation, particularly the crude resin oleo-gum at 400mg/kg, resulted in the highest splenomegaly (47.13). Other formulations, including ethanolic and methanolic at both dose levels, showed intermediate values that did not statistically differ from either the positive control or the Albendazole-treated group. These findings indicate that while certain *Boswellia sacra* treatments such as the methanolic extract at 200mg/kg, effectively alleviated heptomegaly, other high-dose crude preparations induced a splenomegaly. This enlargement is likely attributed to immune system stimulation and immunomodulatory responses elicited by the active constituents of the crude oleo-gum, which may trigger cellular infiltration or compensatory metabolic stress in lymphoid tissues, thereby explaining the inconsistent systemic organomegaly responses observed in this study.

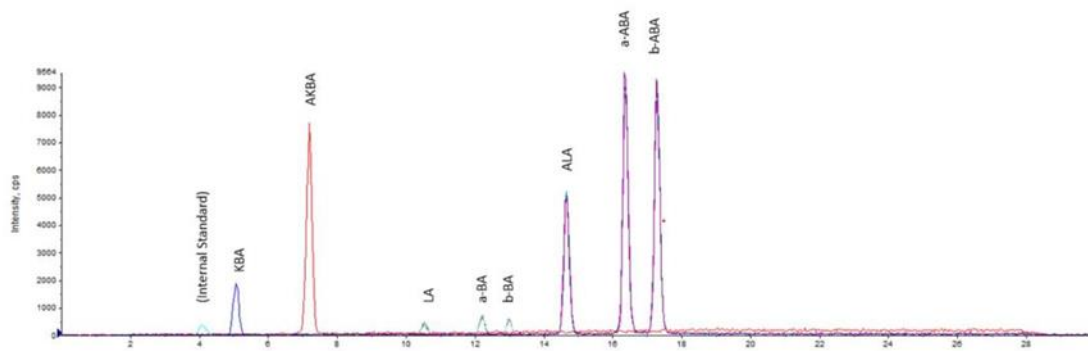


Figure 2. HPLC-MS/MS chromatogram showing the phytochemical fingerprint and separation of three major pentacyclic triterpenic acids from *Boswellia sacra* extracts. The identified biomarkers include: 11-keto- β -boswellic acid (KBA), acetyl-11-keto- β -boswellic acid (AKBA), lupeolic acid (LA), α -boswellic acid (α -BA), β -boswellic acid (β -BA), acetyl lupeolic acid (ALA), acetyl- α -boswellic acid (α -ABA), and acetyl- β -boswellic acid (β -ABA), along with internal standard. The y-axis represents ion intensity(cps) and the x-axis represents retention time (minutes)

Table 1. Phytochemical distribution of the eight primary pentacyclic triterpenic acids ($\mu\text{g}/\text{mg}$, w/w) across the evaluated *Boswellia sacra* extract fractions.

Conc. [$\mu\text{g}/\text{mg}$] (w/w)								
Sample	KBA	AKBA	LA	α -BA	β -BA	ALA	α -ABA	β -ABA
Ethanol extract	2.560	73.20	9.010	19.85	50.60	59.90	65.40	111.0
Methanol extract	2.550	58.50	9.600	18.50	43.85	46.60	52.80	92.25
Raw-gum resin powder	1.720	30.581	NT	6.459	16.561	NT	23.114	45.86
LOD (N32645)	0.0043	0.0039	0.0195	0.016	0.015	0.016	0.008	0.009
LOQ (DIN32645)	0.0260	0.0145	0.0719	0.060	0.058	0.06	0.0346	0.034

NT: not detected; Lod: limit of detection; LOQ: limit of quantitation

Table 2. Comparison of hydatid cyst numbers, diameters, and weights in experimentally infected mice using One-way ANOVA

Source of difference	Number of cysts				Weight of cysts (gm)			Diameter of cysts (mm)		
	DF	Squares	Mean Square	F value	Square	Mean square	F value	Square	Mean square	F value
Treatment	10	4710.93**	471.09**	75.99	821.58	82.158	1.40	37.1246	3.7124	1.60
Error	66	409.14**	6.199**		3867.09	58.592		153.377	2.323	

** $p < 0.0001$: highly significant differences in cyst numbers, $p > 0.05$: no significant differences in diameters and weights among groups.

Table 3. Comparison of hydatid cysts numbers, weights, and diameters by Duncan's multiple range test

Treatment	Number of cysts	Weight of cysts	Diameter of cysts
C+	28.143 a	11.547 a	2.546 a
C-	0.000 c	0.000 b	0.000 b
DMSO	0.000 c	0.000 b	0.000 b
albendazole150mg/kg	1.857 bc	0.287 b	0.302 b
albendazole300mg/kg	3.857 b	0.737 b	0.436 b
Crude oleo-gum200mg/kg	1.143 bc	0.303 b	0.381 b
Crude oleo-gum400mg/kg	0.714 c	0.030 b	0.036 b
Methanolic extract 200mg/kg	0.857 bc	0.439 b	0.892 ab
Methanolic extract 400mg/kg	0.857 bc	0.026 b	0.333 b
Ethanol extract 400mg/kg	1.857 bc	0.143 b	0.872 ab
Ethanol extract 200mg/kg	0.571 c	0.130 b	0.271 b

Means sharing the same letters are not significantly different, different letters indicate significant differences. ethanol extract; methanolic extract; crude oleo-gum.

Table 4. One-way ANOVA of hepatomegaly and splenomegaly in mice infected with secondary echinococcosis and treated with *Boswellia sacra* extracts

Source of difference	Hepatomegaly Factor				Splenomegaly factor		
	DF	Squares	Mean square	F value	Square	Mean square	F value
Treatment	10	48126.42**	4812.64**	6.39	6803.066**	680.30**	6.48
Error	66	49707.68**	753.146**		6929.396**	104.990**	

**P < 0.001 indicates highly significant differences. DF: degree of freedom.

Table 5. Comparison of mean hepatomegaly and splenomegaly indices among experimental groups using Duncan's multiple range test.

Treatment	Splenomegaly factor	Hepatomegaly factor
C+	34.442 b	138.46 a
C-	15.049 c	39.06 e
DMSO	20.291 c	73.87 d
Albendazole 150mg/kg	31.760 b	104.69 bcd
Albendazole 300mg/kg	38.882 ab	97.02 bcd
Crude oleo-gum 200mg/kg	43.342 ab	112.09 abc
Crude oleo-gum 400mg/kg	47.130 a	122.61 ab
Methanolic extract 200mg/kg	37.744 ab	84.43 cd
Methanolic extract 400mg/kg	40.707 ab	101.01 bcd
Ethanolic extract 400mg/kg	42.724 ab	108.08 abc
Ethanolic extract 200mg/kg	39.341 ab	94.85 bcd

Means sharing the same letters are not significantly different, different letters indicate significant differences.

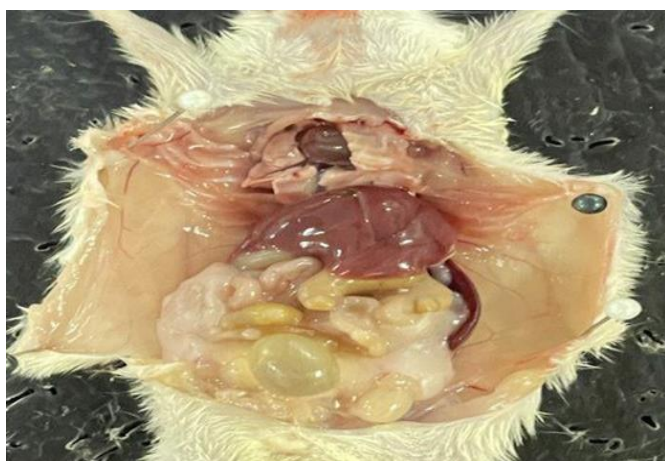
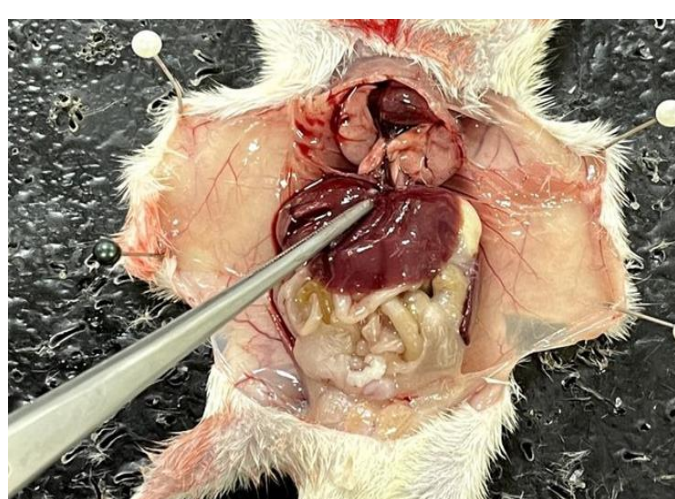
**Figure 3.** Mouse infected with protoscoleces and treated with Albendazole ABZ (300mg/kg) for six months, revealing residual hydatid cysts**Figure 5.** Mouse experimentally infected with protoscoleces and treated with ethanolic extract Bs2(200mg/kg) for six months, demonstrating a reduction in hydatid cysts**Figure 4.** Mouse experimentally infected with protoscoleces and treated with ethanolic extract Bs2 (400mg/kg) for six months, demonstrating a reduction in hydatid cysts**Figure 6.** Mouse infected with protoscoleces (positive control group) after six months of infection, showing growing cysts



Figure 7. Mouse infected with protoscoleces (positive control group) after six months of infection, indicating growing cysts

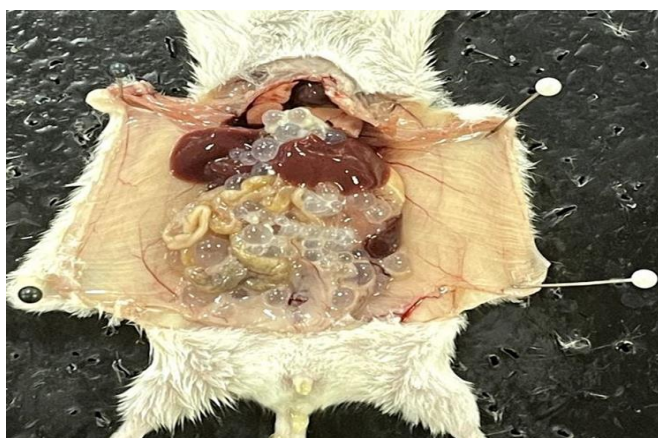


Figure 8. Mouse infected with protoscoleces (positive control group) after six months of infection, showing secondary cysts

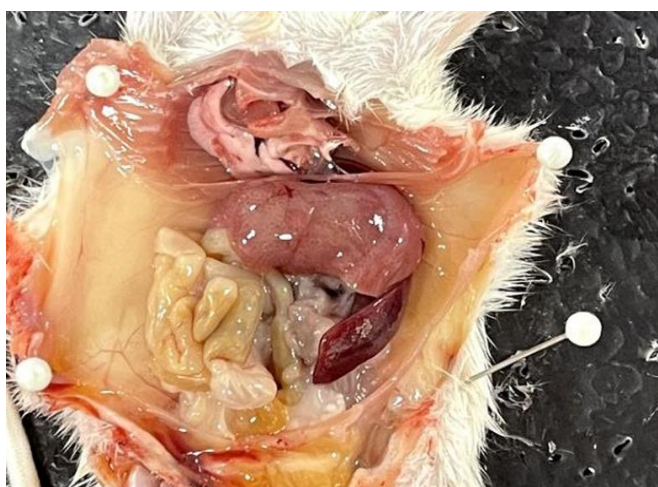


Figure 9. Mouse infected with protoscoleces and treated with crude oleo-gumBs13 (200mg/kg) for six months, showing no detectable cysts



Figure 10. Mouse experimentally infected with protoscoleces and treated with ethanolic extract Bs2 (200 mg/kg) after six months, showing no detectable cysts

5. Discussion

Treatment of hydatid cysts is often costly and complicated, typically requiring extensive surgery combined with prolonged pharmacological therapy to prevent relapses and recurrence. While surgery, particularly radical procedures, is considered the primary treatment for cystic echinococcosis, it often requires follow-up with scolicidal agents to eliminate remaining protoscoleces and reduce recurrence (5). Herbal medicines are increasingly recognized as a valuable platform for developing novel, effective scolicidal agents with minimal adverse effects (29,30). An ideal protoscolicidal agent for clinical use should exhibit high efficacy at low concentrations, act within a short exposure time, maintain stability within hydatid cyst fluid, and be accessible and affordable. (30). Frankincense extracts, derived from various *Boswellia* species, have long been utilized globally in traditional medicine for treating a wide range of inflammatory conditions. (31,15).

Frankincense consists of a complex mixture of over 200 chemical constituents, primarily comprising resin, essential oil, and gum. The essential oil fraction is predominantly composed of monoterpenes, diterpenes, sesquiterpenes, and phenolic compounds, specifically the diterpene alcohol serranol. The gum fraction contains pentose and hexose sugars, along with oxidizing and digestive enzymes. The resin of *Boswellia* species possesses potent bioactive compounds, chiefly boswellic acids, which are classified as pentacyclic triterpene acids (32,33,15). The anti-inflammatory effects of the resin are primarily mediated through the modulation of inflammatory mediators, such as cytokines, and the regulation of signaling pathways involved in the inflammatory response (34).

Our findings demonstrated a highly significant decrease ($P \leq 0.0001$) in the total number of hydatid cysts was observed in *Boswellia sacra* extract (BSE) treated mice relative to the control group, while the overall variance ANOVA in cyst diameter and weight did not reach strict statistical significance ($P > 0.05$), Duncan's test revealed a descriptive downward reduction in these parameters. Taken together, these findings strongly suggest the BSE primarily interferes with the establishment and early development of secondary hydatid cysts, leading to fewer successful cysts, rather than inducing a complete, uniform

regression of pre-existing, well-established cyst mass. In parallel, a highly significant alteration ($P \leq 0.0001$) in hepatosplenomegaly parameters among the experimental groups. Treatment with specific BSE led to a prominent attenuation of splenomegaly, with the methanolic formulation effectively lowering the spleen index compared to the positive control.

Conversely, an elevation in the liver index was observed in certain high-dose crude resin groups, suggesting an immunomodulatory response and immune system stimulation associated with leukocyte infiltration rather than classic hepatic damage. The differential impact on cyst number versus cyst size may be explained by the stage dependent susceptibility of *Echinococcus granulosus* to bioactive compounds. Boswellic acids, particularly acetyl-11-keto- β -boswellic acid (AKBA), which was present at higher concentrations in the ethanolic extract (Bs2), are known to exert stronger effects during early inflammatory and proliferative phases of parasite development. Once cysts reach an advanced stage, structural barriers and reduced metabolic activity may limit the penetration and efficacy of these compounds, accounting for the lack of significant changes in cyst weight and diameter. Collectively, these findings indicate that frankincense extracts exert a predominantly preventive and anti-establishment effect against secondary cystic echinococcosis, mediated through modulation of host inflammatory responses and inhibition of early cyst formation, rather than through direct cytoreductive effects on mature hydatid cysts.

The significant effects observed in this study may be partly attributed to the potent antioxidant and free radical scavenging activities of *Boswellia sacra* (Omani frankincense) extracts, which are known to be rich in bioactive components. Similar antioxidant activities have been previously reported for *Boswellia serrata*, which contain high phenolic and flavonoid contents [35,36]. Gupta et al. (2021) demonstrated that 3-O-acetyl-11keto- β -boswellic acid (KBA) induced intracellular oxidative stress, evidenced by the production of reactive oxygen species and nitric oxide in a concentration-dependent manner. Furthermore, the antimalarial effect of KBA was confirmed in *Plasmodium nigeriensis* and *Plasmodium yoeli* infected Swiss albino rats, where KBA dose-dependently reduced blood parasitemia, accompanied by an increase in hemoglobin levels [37].

Frankincense extracts have been shown to inhibit the growth of clinically isolated *Porphyromonas gingivalis* at concentrations ranging from 500–10000 $\mu\text{g/mL}$. Previous studies have demonstrated the antibacterial activity of frankincense extracts against various pathogenic bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, and *Klebsiella pneumoniae* [38,39,40]. Attallah et al. (2021) reported that frankincense extract suppressed the growth of *Porphyromonas gingivalis*, an effect attributed to the wide range of bioactive constituents, such as trans-nerolidyl formate and ursenoic acid 3-oxomethyl ester, present in the extract. Khalifa et al. (2023) demonstrated that the methanolic extract of *Boswellia sacra* resin exerts a protective effect on the tests of rats. The authors suggested that the extract's antioxidant properties, including increased superoxide dismutase and catalase activity, may shield testicular tissue from toxic agents known to induce damage via free radical formation, such as cyclophosphamide [41].

Frankincense (*Boswellia* spp.) has been widely reported to exhibit pronounced anti-inflammatory and analgesic properties in both *in vitro* and *in vivo* settings, primarily

through the suppression of key pro-inflammatory mediators, including cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α). In addition, ethanolic extracts of frankincense resin and its volatile oil have demonstrated immunomodulatory effects, particularly through the regulation of lymphocyte proliferation [33]. These mechanisms may, at least in part, account for the observed therapeutic effects reported in the present study.

6. Conclusion

The findings of this study demonstrated that frankincense extracts exhibit notable anti-hydatid activity, suggesting their potential role in the control of *Echinococcus granulosus* infection across a range of concentrations. *In vivo* results further revealed a significant reduction in the total hydatid cyst number, alongside a prominent attenuation of systemic splenomegaly. Conversely, an elevation in the liver index was observed in specific high-dose crude resin groups, which is strongly suspected to be associated with immune system stimulation and an immunomodulatory response rather than hepatotoxicity. While descriptive reduction was noted in cyst weight and diameter, the overall variance did not show consistent regression. Collectively, these findings demonstrate the potential of frankincense extracts as a promising alternative or complementary approach to conventional Albendazole therapy, primarily by interfering with the establishment of early cyst abundance.

Acknowledgements: We extend our thanks and appreciation to Prof. Dr. Luay Rashan, Director of Research Center, Dhofar University, Salalah/Oman for providing us with frankincense extracts. Our thanks to the College of Education for Pure Science at the University of Mosul for providing the laboratories where the study was conducted.

CrediT Authorship Contribution Statement: All authors contributed equally to the conceptualization, methodology, and design of the study. The acquisition, analysis, and interpretation of data, as well as the drafting and critical revision of the manuscript for important intellectual content, were shared collaboratively and equally among all authors. All Contributors have reviewed and approved the final version to be published.

Consent to participate: The corresponding author affirms on behalf of all contributors that each author has made substantial intellectual contributions to this study and has reviewed, ratified, and endorsed the final manuscript for submission.

Consent for Publication: All authors have read the final manuscript and hereby grant their collective and unconditional consent for its publication in the Iraqi Journal of Pharmacy.

Ethics Approval: Institutional guidelines for the care and use of animals in scientific experiments were followed. All procedures in this study were approved standards that were carried out by the ethics committee of College of Veterinary Medicine/ University of Mosul in ethical approval code: UM.VET.2023.157, in 13/11/2025.

Funding: This research did not receive any specific grant or external financial support from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest: The authors declare that they have no known competing Financial Interests or personal relationships that could have appeared to influence or bias the work reported in this paper.

Data Availability: All relevant data, Figures, and statistical outcomes supporting the findings of this study are fully processed and presented within the main text of this article.

7. References

- Widdicombe J, Basáñez MG, Entezami M, Jackson D, Larrieu E, Prada JM. The economic evaluation of Cystic echinococcosis control strategies focused on zoonotic hosts: A scoping review. *PLOS Neglected Tropical Diseases* 2022;16(7):e0010568.
- Shakir MM, Hamad SS. Scolicidal Potential of *Tribulus terrestris* L. Aqueous Fruit Extract: An In Vitro Study on *Echinococcus granulosus* Protoscolices. *International Journal of Environmental Sciences* 2025;14(1):634-647.
- Niazi M, Saki M, Sepahvand M, Jahanbakhsh S, Khatami M, Beyranvand M. In vitro and ex vivo scolicidal effects of *Olea europaea* L. to inactivate the protoscolices during hydatid cyst surgery. *Annals of Medicine and Surgery* 2019;42:7-10.
- Houshmand E, Kamalifar HS, Elmi H. In vitro scolicidal effect of ginger (*Zingiber officinale* Roscoe) ethanolic extract against protoscolices of hydatid cyst. *Journal of Hermed Pharmacology* 2019;8(2):103-107.
- World Health Organization. Echinococcosis: Fact sheet [Internet]. Geneva: World Health Organization; 2018 Feb [cited 2026 Apr 7]. Available from: <https://www.who.int/news-room/fact-sheets/detail/echinococcosis>.
- Ceballos L, Elissondo C, Sanchez Bruni S, Denegri G, Lanusse C, Alvarez L. Combined flubendazole-nitazoxanide treatment of cystic echinococcosis: Pharmacokinetic and efficacy assessment in mice. *Acta Tropica* 2015;148:89-96.
- Rahdar M, Raffei A, Valipour-Nouroozi R. The combination of cytokines and albendazole therapy for prophylaxis and treatment of experimental/hydatid cyst. *Acta Tropica* 2020;201:105206.
- Mouahid G, Mintsu Nguema R, Al Mashikhi KM, Al Yafae SA, Idris MA, Moné H. Host-parasite life-histories of the diurnal vs. nocturnal chronotypes of *Schistosoma mansoni*: adaptive significance. *Trop Med Int Health* 2019;24(6):692-700.
- Aziz H, Seda P, Aswani Y, Gosse MD, Krishnakumari AJ, Pawlik TM. Cystic echinococcosis of the liver. *Journal of Gastrointestinal Surgery* 2025;29(3):101974.
- Akhan O. Percutaneous treatment of liver hydatid cysts: to PAIR or not to PAIR. *Current Opinion in Infectious Diseases* 2023;36(5):308-317.
- Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Tropica* 2010;114(1):1-16.
- Mahmoudvand H, Kheirandish F, Dezaki ES, Shamsaddini S, Harandi MF. Chemical composition, efficacy and safety of *Pistacia vera* (var. Fandoghi) to inactivate protoscolices during hydatid cyst surgery. *Biomedicine & Pharmacotherapy* 2016;82:393-398.
- Woolley CL, Suhail MM, Smith BL, Boren KE, Taylor LC, Schreuder MF, et al. Chemical differentiation of *Boswellia sacra* and *Boswellia carterii* essential oils by gas chromatography and chiral gas chromatography-mass spectrometry. *Journal of Chromatography A* 2012;1261:158-163.
- De Rapper S, Van Vuuren SF, Kamatou GPP, Viljoen AM, Dagne E. The additive and synergistic antimicrobial effects of select frankincense and myrrh oils—a combination from the pharaonic pharmacopoeia. *Letters in Applied Microbiology* 2012;54(4):352-358.
- Iram F, Khan SA, Husain A. Phytochemistry and potential therapeutic actions of Boswellic acids: A mini-review. *Asian Pacific Journal of Tropical Biomedicine* 2017;7(6):513-523.
- Frank MB, Yang Q, Osban J, Azzarello JT, Saban MR, Saban R, et al. Frankincense oil derived from *Boswellia carteri* induces tumor cell specific cytotoxicity. *BMC Complementary and Alternative Medicine* 2009;9(1):6.
- Johnson S, DeCarlo A, Satyal P, Dosoky NS, Sorensen A, Setzer WN. The chemical composition of *Boswellia occulta* oleogum resin essential oils. *Natural Product Communications* 2019;14(7):1934578X19866307.
- Namjou A, Rouhi-Broujeni H. Antihyperglycemic, antihyperlipidemic and wound healing of *Boswellia serrata* on experimentally induced diabetic rats. *Abanico Veterinario* 2021;10(1):35.
- Zhang Y, Ning Z, Lu C, Zhao S, Wang J, Liu B, et al. Triterpenoid resinous metabolites from the genus *Boswellia*: pharmacological activities and potential species-identifying properties. *Chemistry Central Journal* 2013;7(1):153.
- Du Z, Liu Z, Ning Z, Liu Y, Song Z, Wang C, et al. Prospects of boswellic acids as potential pharmaceuticals. *Planta Medica* 2015;81(04):259-271.
- Al-Yasiry ARM, Kiczorowska B. Frankincense-therapeutic properties. *Postępy Higieny i Medycyny Doswiadczalnej* 2016;70:1380-1391.
- Alotaibi SH. Biophysical properties and finger print of *Boswellia* Sp. Burseraceae. *Saudi Journal of Biological Sciences* 2019;26(7):1450-1457.
- Smyth JD, Barrett NJ. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1980;74(5):649-652.
- Rashan L, Hasson SS, Simmet T, Al Bulushi M, Al-Jabri AA, inventors; Boswellic Acid Extraction. South African patent ZA2019/02740. 2020 Jan 29. <https://patents.google.com/patent/ZA201902740B/de>
- Interdonato L, Impellizzeri D, D'Amico R, Siracusa R, Fusco R, Abdelhameed AS, Fritsch T, Rashan LJ, Cuzzocrea S, Calabrese V, Cordaro M, Di Paola R. Autophagy machinery plays an essential role in traumatic brain injury-induced apoptosis and its related behavioral abnormalities in mice: focus on *Boswellia sacra* gum resin. *Frontiers in Physiology* 2024;14:1320960.

26. Schmiech M, Lang SJ, Werner K, Rashan LJ, Syrovets T, Simmet T. Comparative analysis of pentacyclic triterpenic acid compositions in oleogum resins of different *Boswellia* species and their in vitro cytotoxicity against treatment-resistant human breast cancer cells. *Molecules* 2019;24(11):2153.
27. Di Stefano V, Schillaci D, Cusimano MG, Rishan M, Rashan L. In vitro antimicrobial activity of frankincense oils from *Boswellia sacra* grown in different locations of the Dhofar region (Oman). *Antibiotics* 2020;9(4):195.
28. Midway S, Robertson M, Flinn S, Kaller M. Comparing multiple comparisons: practical guidance for choosing the best multiple comparisons test. *PeerJ*. 2020;8:e10387.
29. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms* 2021;9(10):2041.
30. Yaghoobi, H., Ghanbari, S., Hataminejad, M., & Azizi, H. Effect of Alhagi maurorum hydroalcoholic extract on preventing secondary hydatid cysts in BALB/c mice. *Jundishapur Journal of Natural Pharmaceutical Products* 2023;18(3):e135321.
31. Padhi M, Mahapatra S. *Boswellia serrata*: a review of its traditional uses, phytochemistry and pharmacology. *International Review of Biophysical Chemistry* 2013;4:74-83.
32. Shah BA, Qazi GN, Taneja SC. Boswellic acids: a group of medicinally important compounds. *Natural Product Reports* 2009;26(1):72-89.
33. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M. Frankincense (乳香 Rǔ Xiāng; *Boswellia* species): from the selection of traditional applications to the novel phytotherapy for the prevention and treatment of serious diseases. *Journal of Traditional and Complementary Medicine* 2013;3(4):221-226.
34. Aldahlawi AM, Alzahrani AT, Elshal MF. Evaluation of immunomodulatory effects of *Boswellia sacra* essential oil on T-cells and dendritic cells. *BMC Complementary Medicine and Therapies* 2020;20(1):352.
35. Beghelli D, Isani G, Roncada P, Andreani G, Bistoni O, Bertocchi M, et al. Antioxidant and ex vivo immune system regulatory properties of *Boswellia serrata* extracts. *Oxidative Medicine and Cellular Longevity* 2017;2017:7468064.
36. Singh HP, Yadav IK, Chandra D, Jain DA. In vitro antioxidant and free radical scavenging activity of different extracts of *Boerhavia diffusa* and *Boswellia serrata*. *International Journal of Pharma Sciences and Research* 2012;3(11):503-511.
37. Gupta M, Kumar S, Kumar R, Kumar A, Verma R, Darokar MP, et al. Inhibition of heme detoxification pathway in malaria parasite by 3-hydroxy-11-keto- β -boswellic acid isolated from *Boswellia serrata*. *Biomedicine & Pharmacotherapy* 2021;144:112302.
38. Di Stefano V, Schillaci D, Cusimano MG, Rishan M, Rashan L. In vitro antimicrobial activity of frankincense oils from *Boswellia sacra* grown in different locations of the Dhofar region (Oman). *Antibiotics* 2020;9(4):195.
39. Abers M, Schroeder S, Goelz L, Sulser A, St. Rose T, Puchalski K, et al. Antimicrobial activity of the volatile substances from essential oils. *BMC Complementary Medicine and Therapies* 2021;21(1):124.
40. Attallah NG, Negm WA, Elekhawwy E, Altwaijry N, Elmongy EI, El-Masry TA, et al. Antibacterial activity of *Boswellia sacra* Flueck. Oleoresin extract against *Porphyromonas gingivalis* periodontal pathogen. *Antibiotics* 2021;10(7):859.
41. Khalifa SA, Kotb SM, El-Seedi SH, Nahar L, Sarker SD, Guo Z, et al. Frankincense of *Boswellia sacra*: Traditional and modern applied uses, pharmacological activities, and clinical trials. *Industrial Crops and Products* 2023;203:117106.

تأثير مستخلصات نبات اللبؤذ المقدس (*Boswellia sacra*) على الوقاية من الأكياس المائية الثانوية في فئران BALB/c

الخلاصة

المقدمة: يُشكل داء المشوكات الكيسية، الناجم عن طفيل المشوكة الحبيبية (*Echinococcus granulosus*)، تحديًا صحيًا عالميًا نظرًا لتكلفة العلاج الكيميائي العالية وسميته. وحسب علم الباحثين، لم يسبق تقييم مستخلصات نبات اللبؤذ المقدس (*Boswellia sacra*) ضد الأكياس المائية الثانوية في نماذج الفئران. **الهدف:** هدفت هذه الدراسة إلى التحقق من فعالية مستخلصات صمغ اللبؤذ المقدس (*Boswellia sacra*) المختلفة في الجسم الحي ضد الأكياس المائية الثانوية في نماذج فئران BALB/c. **طرق العمل:** تم تقسيم فئران BALB/c المحقونة برؤوس المشوكة الحبيبية (*Echinococcus granulosus*) إلى ثلاث مجموعات: مجموعة ضابطة (إيجابية)، ومجموعة ضابطة (سلبية)، ومجموعة ضابطة (مجموعة التحكم)، بالإضافة إلى مجموعات علاجية تلقت مستخلصًا إيثانوليًا، ومستخلصًا ميثانوليًا، ورائحة صمغ خام. تم تقييم الفعالية من خلال قياس العدد الإجمالي للأكياس، ووزنها، وقطرها، ومؤشرات تضخم الكبد والطحال. **النتائج:** أدى العلاج بمستخلصات نبات اللبؤذ المقدس (*Boswellia sacra*) إلى انخفاض كبير إحصائيًا ($P \leq 0.0001$) في عدد الأكياس المائية المستعادة مقارنةً بالمجموعة الضابطة الإيجابية. مع ذلك، لم يُظهر تحليل التباين (ANOVA) أي فروق ذات دلالة إحصائية ($P > 0.05$) في الوزن الإجمالي للأكياس وأقطارها. لوحظ انخفاض في تضخم الكبد والطحال، على الرغم من أن هذا التحسن العام لم يكن متسقًا بين جميع المجموعات المُعالَجة. **الاستنتاج:** تُنبئ مستخلصات نبات اللبؤذ المقدس (*Boswellia sacra*) بشكل فعال تكاثر الأكياس المائية الثانوية عن طريق خفض العدد الإجمالي للأكياس بشكل ملحوظ. في حين أن المستخلصات لم تُوقف بشكل ملحوظ نمو الأقطار أو الوزن، ولم تُحدث سوى انخفاض جزئي، وتأثير غير متسق على تضخم الأعضاء المُصاحبة، فإن انخفاض عبء الطفيليات يُؤكد إمكانية استخدامها كعوامل مساعدة طبية.

الكلمات المفتاحية: داء المشوكات الكيسية؛ مستخلص نبات اللبؤذ المقدس (*Boswellia sacra*)؛ مستخلص ميثانولي؛ تضخم الكبد؛ فئران BALB/c