Original research

In Vitro Protoscolicidal Efficacy of Boswellia Resin Extract and Its Nanoemulsion Against Echinococcus granulosus

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Abstract

Cystic echinococcosis, caused by *Echinococcus granulosus*, remains a significant zoonotic disease with limited treatment options, necessitating the exploration of novel therapeutic agents. This study aimed to evaluate the in vitro protoscolicidal efficacy of Boswellia resin hydroalcoholic extract and its nanoemulsion formulation against *E. granulosus* protoscoleces. Protoscoleces were obtained from liver cysts of infected sheep at Birjand slaughterhouse and treated with serial dilutions (0.1%, 0.01%, 0.001%, and 0.0001%) of both formulations over varying exposure times (5 to 30 minutes). Viability was assessed using 0.1% eosin staining, and data were analyzed with SPSS22 software using the chi-square test. The hydroalcoholic extract exhibited protoscolicidal effects only at concentrations above 0.01%, achieving 100% mortality at

0.1% after 30 minutes, though effects at lower concentrations were not statistically significant compared to the control ($P > 0.05$). In contrast, the Boswellia nanoemulsion demonstrated significantly superior protoscolicidal efficacy, achieving 100% mortality at lower concentrations and shorter exposure times (e.g., 0.1% at 15 minutes and 0.01% at 20 minutes), with statistical significance confirmed at these levels ($P < 0.05$). These findings highlight the potential of Boswellia nanoemulsion as a promising natural agent for hydatid cyst treatment due to enhanced bioavailability and efficacy compared to the extract alone.

Keywords*: Echinococcus granulosus*, *Boswellia* resin, nanoemulsion, protoscolicidal, in vitro

1.Introduction:

Echinococcus granulosus is a parasitic cestode responsible for causing cystic echinococcosis (CE), commonly known as hydatid disease, which is a significant zoonotic infection worldwide. The parasite's life cycle involves canids, primarily domestic dogs, as definitive hosts, and ungulates like sheep as intermediate hosts. Humans become accidental intermediate hosts by ingesting E. granulosus eggs that are shed in the feces of infected dogs and subsequently contaminate food or water sources (1,2). The disease is globally prevalent, especially in regions where livestock farming and close contact with dogs are common, such as South America, Africa, the Middle East, and Central Asia. CE poses a substantial public health concern due to its chronic nature and potential to cause severe organ damage. Economically, it burdens the livestock industry by causing decreased productivity and condemnation of infected organs, leading to significant financial losses (3,4). Conventional treatments for CE, such as surgical excision and chemotherapy with benzimidazole drugs like albendazole and mebendazole, face significant challenges, including risks of protoscolex spillage leading to recurrence, severe immunological reactions, and toxic side effects like liver toxicity and bone marrow suppression, limiting their applicability. (5)(6). These limitations highlight the pressing necessity for alternative treatment options that are more efficient and result in fewer negative effects. Historically, medicinal plants have played a crucial role in treating parasitic infections due to their accessibility, cost-effectiveness, and lower toxicity (7). The investigation of natural products for antiparasitic treatment is, thus, a promising path to create safer and more efficient therapies.

The Boswellia species, commonly known as frankincense, are part of the Burseraceae family and originate from the arid and semi-arid regions of the Arabian Peninsula, India, and East Africa (8). The resin derived from Boswellia is rich in phytochemicals, notably boswellic acids, which are considered the primary active constituents responsible for its well-documented anti-inflammatory and immunomodulatory effects (9). Traditionally, Boswellia extracts have been employed for various medicinal purposes (10). While previous research has highlighted diverse pharmacological activities of Boswellia, including anticancer, antimicrobial, and antiviral properties (9,11), and some studies have suggested potential antiparasitic effects against other organisms (Reff), its specific efficacy against *E. granulosus* protoscoleces, particularly when formulated to enhance bioavailability, remains largely unexplored. This represents a significant gap this study aims to address.Nanoemulsions represent an innovative drug delivery platform, characterized by thermodynamically stable, colloidal dispersions of nanoscale droplets (typically 10-200 nm). Their unique physicochemical properties, including high surface area-to-volume ratio and enhanced stability, significantly improve the solubility and bioavailability of poorly water-soluble compounds (12). This nanotechnology offers distinct advantages over conventional formulations by facilitating better absorption, potentially enabling targeted delivery, and protecting active compounds from degradation, thereby maximizing therapeutic efficacy (13) (14). The application of nanoemulsion technology to enhance the delivery of plant-derived compounds like those found in Boswellia resin is particularly promising for improving treatment outcomes in parasitic diseases.

This study represents a pioneering effort to evaluate the protoscolicidal efficacy of Boswellia resin extract in a nanoemulsion formulation against *E. granulosus* protoscoleces, an approach that has not been previously explored in the context of hydatid disease treatment. To our knowledge, this is the first investigation into the potential of a Boswellia nanoemulsion for hydatid disease treatment. We hypothesize that the nanoemulsion formulation of Boswellia resin extract will exhibit superior protoscolicidal efficacy against E. granulosus protoscoleces compared to the hydroalcoholic extract alone, due to enhanced solubility, bioavailability, and targeted delivery of active compounds like boswellic acids.

2. Materials & Method

2.1. Collection and Preparation of Protoscoleces

In this experimental in vitro study, protoscoleces of *E. granulosus* were obtained from liver hydatid cysts of infected sheep collected from the Birjand slaughterhouse. The hydatid cysts were identified and aseptically opened in the laboratory to aspirate protoscoleces under sterile conditions, followed by washing at least three times with sterile normal saline to remove debris. The viability of the protoscoleces was assessed using the 0.1% eosin staining method (15), counting at least 100 protoscoleces, and only samples with viability over 90% were used in the experiments (**Figure 1**).

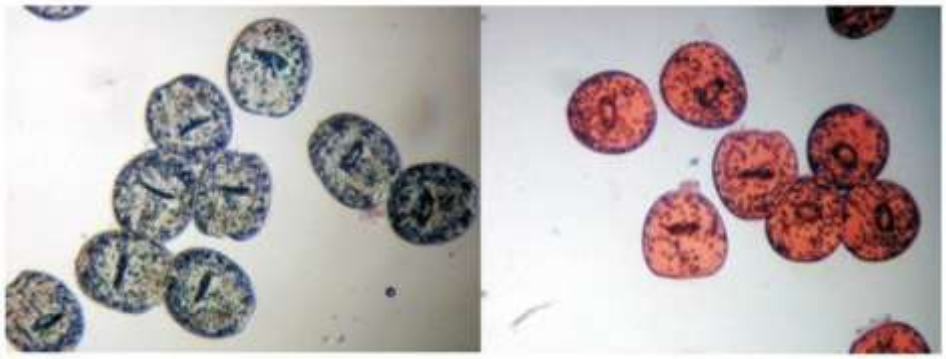

Figure 1. Microscopic evaluation of *E. granulosus* protoscoleces viability using eosin staining. Left panel: Viable protoscoleces excluding eosin stain, appearing unstained with intact morphological features. Right panel: Non-viable protoscoleces after treatment with Boswellia nanoemulsion, showing eosin uptake (red staining) indicating loss of membrane integrity and death (400× magnification).

2.2. Preparation of Boswellia Extract

High-quality yellow resin of *Boswellia* was purchased, and its identity was confirmed by morphological comparison with authenticated specimens at the Herbarium of the School of Pharmacy, Birjand University of Medical Sciences; impurities were removed before use. For the preparation of the hydroalcoholic extract, 300 grams of powdered *Boswellia* resin were mixed with 100 mL of 70% ethanol and distilled water, gently stirred to facilitate extraction, filtered to obtain the initial extract, and then the solvent was evaporated using a rotary evaporator at 80°C to concentrate the extract.

2.3. Preparation of Boswellia Nanoemulsion

The Boswellia nanoemulsion was prepared using the spontaneous emulsification method by testing various ratios of emulsifiers and the extract to achieve a stable nanoemulsion, employing co-solvents like propylene glycol and polyethylene glycol. The selection of specific surfactants (optimized empirically through ratio testing) and co-solvents like propylene glycol and polyethylene glycol was based on their established roles in reducing interfacial tension, enhancing the solubility of hydrophobic compounds like those in Boswellia resin, their common use in pharmaceutical formulations due to favorable safety profiles, and their ability to contribute to the formation of stable nano-scale emulsions. The aqueous phase was slowly added to the oil phase under constant stirring. The spontaneous emulsification method was chosen as an initial low-energy approach to form a coarse emulsion, followed by high-speed homogenization using an ultrasonicator for 20 minutes. This high-energy step is crucial for reducing droplet size to the nano-range (as confirmed by DLS), ensuring homogeneity, and enhancing the kinetic stability of the final nanoemulsion formulation, a common and effective strategy for preparing stable nanoemulsions. This process yielded a transparent, single-phase nanoemulsion. A nanoemulsion gel was formulated by hydrating carbomer polymer in distilled water, adjusting the pH to 6.8 using triethanolamine, and incorporating the nanoemulsion into the gel base until homogeneous.

2.4. Characterization of Nanoemulsion

Characterization of the nanoemulsion involved particle size analysis using Dynamic Light Scattering (DLS), which confirmed an optimal nanoscale dimension of approximately 33.7 nm, morphological examination using Transmission Electron Microscopy (TEM) to observe nano-sized particles, and Fourier Transform Infrared Spectroscopy (FTIR) analysis to identify functional groups and confirm the chemical integrity of the extract and nanoemulsion. These characterizations confirmed the successful formation and initial physicochemical properties of the nanoemulsion prior to its use in the protoscolicidal assays.

2.5. Preparation of Test Solutions

Test solutions were prepared by making a 0.1% (w/v) stock solution of the Boswellia extract with the addition of Tween 20 to enhance solubility, and serial dilutions were performed to obtain concentrations of 0.1%, 0.01%, 0.001%, and 0.0001% for both the extract and nanoemulsion formulations.

2.6. In Vitro Protoscolicidal Assay

In the in vitro protoscolicidal assay, protoscoleces were treated with different concentrations of the hydroalcoholic extract and nanoemulsion of *Boswellia*, while control groups included a positive control using 1% silver nitrate solution and a negative control using sterile normal saline. Equal volumes of protoscolex suspension and test solutions were mixed and incubated for various time intervals (5, 10, 15, 20, 25, and 30 minutes) in 48-well plates, with each concentration and time point tested in triplicate to ensure accuracy (20,21).

2.7. Assessment of Protoscolicidal Activity

Protoscolicidal activity was assessed by viability testing post-incubation, where samples were stained with 0.1% eosin solution and observed under a light microscope; dead protoscoleces absorbed the stain (appearing red), while live ones excluded it, and at least 100 protoscoleces were counted per sample to determine percent mortality (22,23).

2.8. Data Analysis

Data analysis was performed using SPSS software version 22. The chi-square test was employed to compare mortality rates (proportions of dead protoscoleces) between different treatment groups (extract vs. nanoemulsion vs. controls) and concentrations at each specific time point. Assumptions for the chi-square test, including categorical data type (live/dead), independence of observations, and expected cell frequencies, were verified prior to analysis. P-values less than 0.05 were considered statistically significant, indicating a significant difference in mortality proportions compared to the negative control group under the specified conditions.

3. Results:

The particle size analysis of the Boswellia nanoemulsion was performed using Dynamic Light Scattering (DLS), yielding an average particle size of approximately 35.07 nanometers (**Figure 2a**). Additionally, the zeta potential measurement showed that the nanoemulsion particles had a surface charge of -3.68 mV (**Figure 2b**).

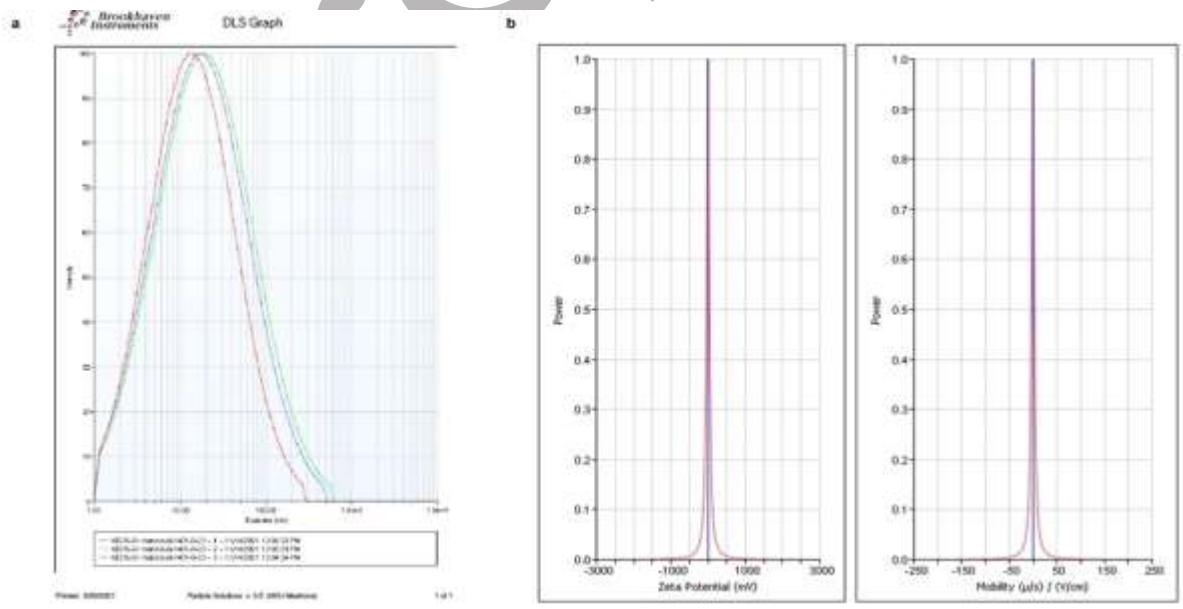

Figure 2. Characterization of Boswellia resin nanoemulsion. (a) Dynamic Light Scattering (DLS) analysis showing particle size distribution. (b) Zeta potential measurements indicating the surface charge and mobility of the nanoemulsion particles.

The morphological examination of the Boswellia nanoemulsion was conducted using Transmission Electron Microscopy (TEM) to visualize the nanoparticles and confirm their morphology and size distribution. TEM imaging revealed that the nanoemulsion particles were spherical and uniformly distributed, consistent with the nanoscale size determined by Dynamic Light Scattering (DLS) analysis (approximately 35.07 nanometers). The representative TEM images showcased well-dispersed particles with consistent spherical shapes, indicating successful formulation of the nanoemulsion and homogeneity of the particle size (**Figure 3**).

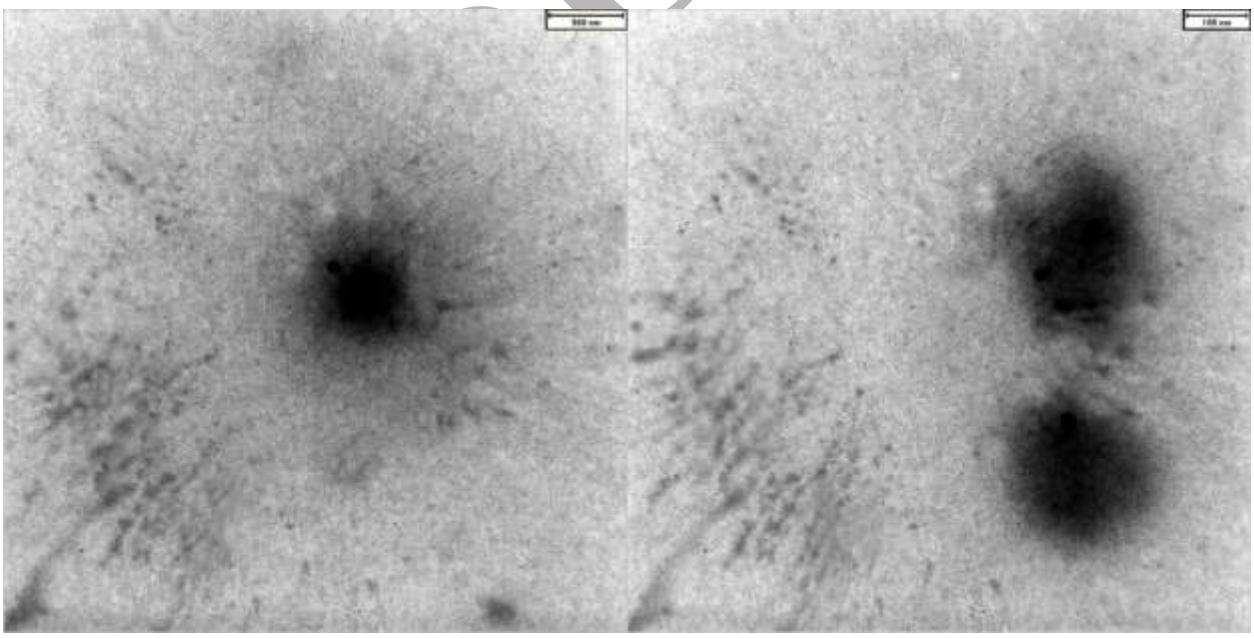

Figure 3. Transmission electron microscopy (TEM) images of Boswellia resin nanoemulsion showing spherical nanoparticles. TEM micrograph at 900 nm and 100 nm scale bar demonstrating

multiple uniform spherical particles, confirming the successful formulation of the nanoemulsion with consistent morphology.

FTIR spectroscopic analysis was conducted to investigate the chemical composition and molecular interactions in both the hydroalcoholic extract and the nanoemulsion formulations of Boswellia. The FTIR spectra of both formulations exhibited characteristic absorption bands at 3440 cm^{-1}, corresponding to O–H stretching vibrations of hydroxyl groups such as phenols and alcohols; 2929 cm^{-1}, attributed to C–H stretching vibrations of aliphatic hydrocarbons; 1713 cm^{-1}, assigned to C=O stretching vibrations indicative of carbonyl groups like ketones, aldehydes, carboxylic acids, or esters; 1456–1378 cm^{-1}, due to C–H bending vibrations confirming the presence of methyl and methylene groups; and 1242 cm^{-1}, associated with C–O stretching vibrations of ethers, esters, or carboxylic acids (**Figure 4a**).In the nanoemulsion spectra, additional peaks were observed at 3500 cm^{-1}, related to amide groups, proteins, enzymes, and phenolic O–H groups; 1380 cm^{-1}, representing the NO$_2$ band of nitro compounds; and 1045 cm^{-1}, associated with C–F bonds in aliphatic fluoro compounds (**Figure 4b**). The presence of these signature peaks in both the extract and the nanoemulsion formulations confirms the successful incorporation of the Boswellia extract components into the nanoemulsion while maintaining their chemical integrity. The protoscolicidal activity of both the Boswellia hydroalcoholic extract and its nanoemulsion formulation was evaluated against *Echinococcus granulosus* protoscoleces at varying concentrations (0.1%, 0.01%, 0.001%, 0.0001%) and time intervals (5, 10, 15, 20, 25, 30 minutes) (**Figure 5**). The hydroalcoholic extract exhibited significant protoscolicidal effects only at concentrations higher than 0.01%, with mortality rates increasing over time and with higher concentrations. Specifically, at a concentration of 0.1%, the extract achieved 100% mortality of protoscoleces after 30 minutes of exposure, identifying it as the minimum effective concentration for significant activity. Statistical comparisons with the negative control (sterile normal saline) showed that the extract's effects at lower concentrations (0.001% and 0.0001%) were not statistically significant **($P > 0.05$)** across the tested time points.

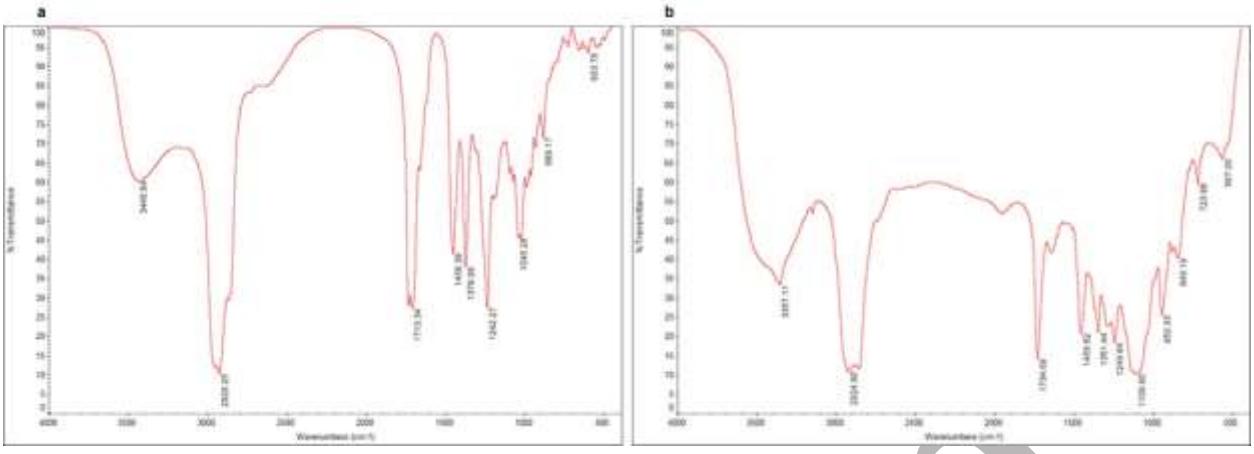

Figure 4. FTIR spectra of Boswellia formulations. (a) FTIR spectrum of Boswellia hydroalcoholic (b) FTIR spectrum of Boswellia nanoemulsion gel

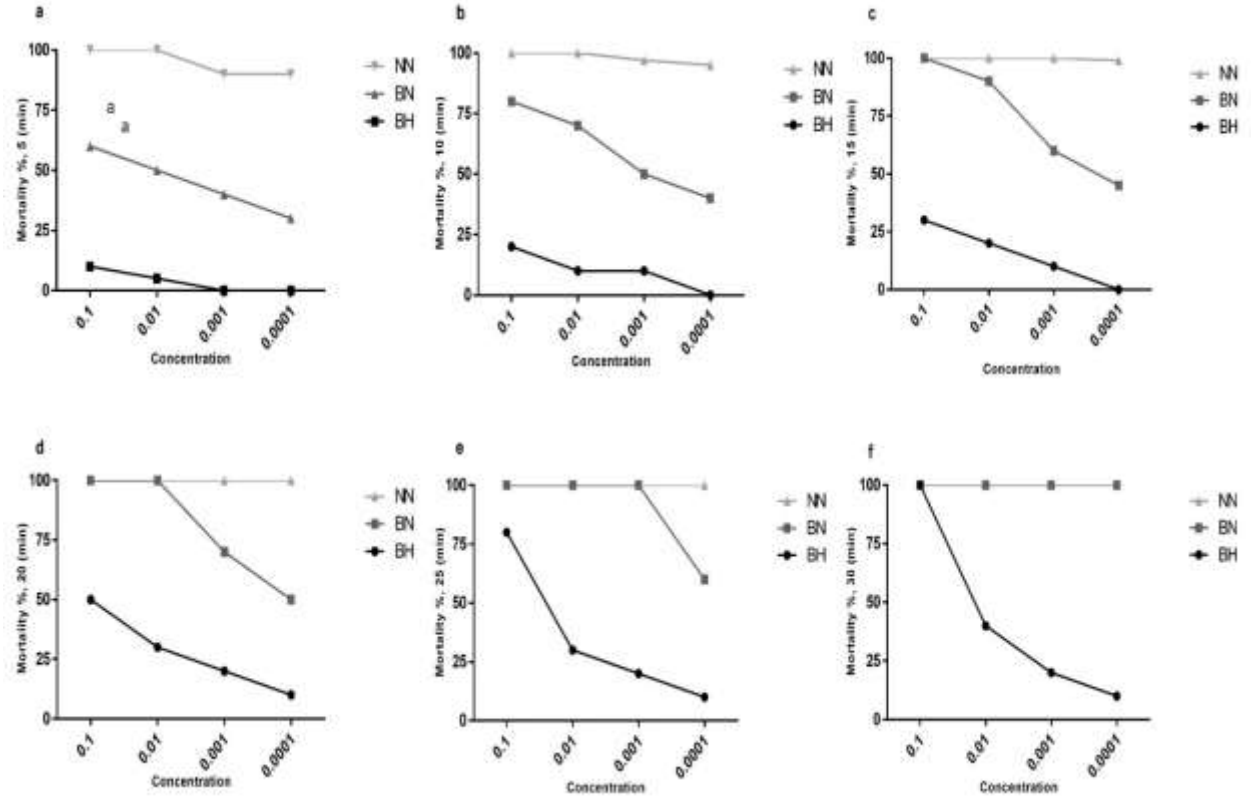

Figure 5. The mortality rates of protoscoleces were assessed after exposure to silver nitrate (SN), Boswellia nanoemulsion (BN), and Boswellia hydroalcoholic extract (BH) at concentrations

ranging from 0.1% to 0.0001% for (a) 5 minutes, (b) 10 minutes, (c) 15 minutes, (d) 20 minutes, (e) 25 minutes, and (f) 30 minutes.

In contrast, the Boswellia nanoemulsion demonstrated markedly enhanced protoscolicidal activity, achieving higher mortality rates at lower concentrations and significantly shorter exposure times compared to the extract alone. Remarkably, the nanoemulsion achieved 100% mortality at a concentration of 0.1% within just 15 minutes ($P < 0.05$) and at a lower concentration of 0.01% within 20 minutes ($P < 0.05$). These mortality rates were statistically significant compared to the negative control group at these time points and concentrations. Control experiments validated these findings, where the positive control (1% silver nitrate solution) demonstrated rapid and complete protoscolicidal activity as expected, confirming the assay's validity, while the negative control showed negligible mortality.

4. Discussion

The present study investigated the protoscolicidal effects of hydroalcoholic extract of Boswellia (frankincense) and its nanoemulsion formulation against *E. granulosus* protoscoleces in vitro. The findings demonstrated that the nanoemulsion form of Boswellia exhibited enhanced protoscolicidal activity compared to the hydroalcoholic extract alone. Specifically, the nanoemulsion achieved 100% mortality of protoscoleces at a concentration of 0.1% within 15 minutes and at 0.01% within 20 minutes **($P < 0.05$)**. In contrast, the hydroalcoholic extract required a higher concentration (0.1%) and longer exposure time (30 minutes) to achieve similar efficacy **($P > 0.05$)**. These results suggest that the nanoemulsion formulation significantly improves the delivery and bioavailability of the active compounds in Boswellia, particularly boswellic acids, which are known for their anti-inflammatory and antimicrobial properties (10). The nanoscale size of the emulsion particles (~35 nanometers) likely contributes to increased surface area and enhanced interaction with the protoscoleces, leading to more effective penetration and disruption of the

parasite's cellular structures (16,17). The enhanced efficacy of the *Boswellia* resin nanoemulsion can be attributed to several potential mechanisms related to its physicochemical properties. Nanoemulsions are known to increase the solubility and bioavailability of hydrophobic compounds like boswellic acids, the primary active constituents of *Boswellia* resin (12). The nanoscale droplets (~33.7 nm) provide a larger surface area for interaction with protoscoleces, facilitating more efficient delivery and absorption of the active compounds (18).Furthermore, the small droplet size allows for enhanced permeation through biological membranes, potentially leading to greater penetration into the parasite's tegument and intracellular spaces. The surfactants used in the nanoemulsion may also disrupt the membrane integrity of protoscoleces, contributing to increased mortality. Additionally, nanoemulsions can protect the active compounds from degradation, maintaining their stability and prolonging their activity during the treatment period (25).Previous studies have highlighted the antimicrobial and antiparasitic properties of Boswellia. For instance, Al-Harrasi and Al-Saidi (8) reported the presence of bioactive triterpenoids in Boswellia species, which exhibit significant antimicrobial activity. Moreover, Mohammadi et al. (25) demonstrated the antifungal effects of Boswellia essential oil against clinical isolates of *Candida albicans*, indicating its potential in combating fungal infections.The enhanced efficacy of the nanoemulsion observed in this study aligns with other research emphasizing the benefits of nanoparticle formulations in drug delivery. Nanoemulsions can improve the solubility of hydrophobic compounds, protect active ingredients from degradation, and facilitate targeted delivery to pathogens(25). This is particularly relevant for hydrophobic compounds like boswellic acids, where conventional formulations may have limited efficacy due to poor solubility and bioavailability.The results of the FTIR spectroscopic analysis showed characteristic peaks at ~3400 cm□¹ (O–H stretching), ~1730 cm□¹ (C=O stretching), and ~2920 cm□¹ (C–H stretching), similar to those observed in the pure extract. The absence of significant peak shifts or new peaks suggests that the chemical structure of the extract was preserved during formulation.The preservation of characteristic absorption bands corresponding to key functional groups—such as the O–H stretching vibrations at 3440 cm□¹, C–H stretching at 2929 cm□¹, C=O stretching at 1713 cm□¹, C–H bending at 1456–1378 cm□¹, and C–O stretching at 1242 cm□¹—in both the extract and nanoemulsion spectra indicates that

the fundamental molecular structures responsible for the extract's therapeutic properties remain intact. The additional peaks observed in the nanoemulsion spectra, including the amide groups and phenolic O–H at 3500 cm□¹, NO□ bands at 1380 cm□¹, and C–F bonds at 1045 cm□¹, suggest successful interaction and stabilization of the extract within the nanoemulsion matrix. This is a critical finding, as maintaining the structural integrity of the extract's functional groups is essential for preserving its biological activity and therapeutic efficacy. Therefore, the nanoemulsion formulated in this study is validated as an effective delivery system for the Boswellia extract, potentially enhancing its stability, bioavailability, and overall efficacy in pharmaceutical applications.Comparative studies with other plant extracts and nanoformulations further support the potential of using nanoemulsions for antiparasitic purposes. For example, Mahmoudvand et al. (20) found that plant extracts formulated in nanoparticle forms exhibited greater protoscolicidal activity against *E. granulosus* compared to their crude extracts. Similarly, research on other nanoparticles, such as zinc oxide nanoparticles synthesized with plant extracts, showed significant antiparasitic effects.The use of positive (1% silver nitrate) and negative (sterile normal saline) controls in this study validated the experimental conditions and ensured that the observed protoscolicidal effects were attributable to the Boswellia formulations. The negative control exhibited no significant protoscolicidal activity, confirming that the mortality rates observed were due to the treatments applied. Statistical analysis reinforced the significance of the findings, with mortality rates showing a clear dependency on both concentration and exposure time. The nanoemulsion's superior performance suggests that it could be a promising candidate for developing new protoscolicidal agents with potential applications in the treatment of hydatid cyst disease.Despite the promising in vitro results, several limitations must be acknowledged. Firstly, the study's in vitro design cannot fully replicate the complex biological environment of a host. Factors such as host immune responses, drug metabolism, and interactions with host tissues are absent in this model but would significantly influence efficacy and safety in vivo. Secondly, potential variability in protoscolex sensitivity due to genetic differences between *E. granulosus* strains or variations in developmental stages was not assessed and could impact the generalizability of these findings. Thirdly, and critically for therapeutic potential, this study did not evaluate the cytotoxicity of the Boswellia extract or

nanoemulsion against host cells. Assessing potential toxicity to relevant cells (e.g., hepatocytes, fibroblasts, immune cells) is essential before considering in vivo applications. Finally, the long-term stability of the prepared nanoemulsion under different storage conditions was not investigated, which is crucial for practical formulation development. Building upon these promising preliminary findings, future research should focus on addressing the identified limitations. Crucially, comprehensive in vitro cytotoxicity studies are required to determine the selectivity index of the Boswellia nanoemulsion (i.e., toxicity to parasites vs. host cells). Subsequent in vivo studies in appropriate animal models of cystic echinococcosis are essential to evaluate the nanoemulsion's efficacy, pharmacokinetics (absorption, distribution, metabolism, excretion), and safety profile, including potential organ toxicity, following relevant administration routes. Further research could also explore the precise molecular mechanisms underlying the enhanced protoscolicidal activity, potentially involving investigations into membrane disruption, metabolic interference, or apoptosis induction in the protoscoleces. Optimizing the nanoemulsion formulation for stability and potential targeted delivery could also enhance its therapeutic prospects.

In conclusion, the nanoemulsion formulation of Boswellia hydroalcoholic extract significantly enhances its protoscolicidal activity against *E. granulosus* protoscoleces in vitro. This enhancement can be attributed to the improved solubility and bioavailability of active compounds in the nanoemulsion. The results support the potential use of Boswellia nanoemulsion as an effective and natural protoscolicidal agent, which could contribute to safer and more efficient treatments for hydatid cyst disease. Further research, including in vivo studies and clinical trials, is warranted to fully realize its therapeutic potential.

Abbreviations

The following abbreviations are used in this manuscript:

CE: Cystic echinococcosis

Acknowledgments

The authors of this article sincerely thank the esteemed colleagues who assisted us in sample collection at the parasitology laboratory in university of medical sciences, Birjand, Iran.(code: 456990)

Data Availability:

The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval:

This study was approved under the ethical approval code IR.BUMS.REC.1402.077.

Conflict of interest

The authors have no competing interests to declare that are relevant to the content of this article.

Authors Contribution

Study concept and design:**TN** and **ATK**. Analysis and interpretation of data:**SMM**. Drafting of the manuscript: **ATK**. Critical revision of the manuscript for important intellectual content:**AM**. Statistical analysis: **AM**.

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