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Original paper

A preliminary study on the therapeutic potential of pulsed electric fields (PEF) in hydatid cysts: *ex vivo* targeted detachment of the germinal layer in daughter cysts

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ABSTRACT. Cystic echinococcosis (CE), a globally parasitic disease is primarily caused by the larval stage of *Echinococcus granulosus*. This zoonotic infection carries significant medical, veterinary, and economic implications. In human the disease occurs by the ingestion of parasite eggs and can create in any organ especially liver and lungs. Current therapeutic methods have some limitations. Therefore, effect of pulsed electric fields (PEF) as a non-invasive method was utilized on daughter cysts as an important part of hydatid cyst. Daughter cysts was extracted from sheep liver hydatid cyst, divided into three groups. The first group was exposed to PEF at intensities of 70V/cm, 150 V/cm and 1300V/cm. The second group was treated with albendazole (100 µg/ml). The third group, was exposed with different intensities of PEF (as above) following incubation of daughter cysts with albendazole (100 µg/ml). Structural changes in the daughter cysts were analyzed using a stereomicroscope. The laminated layer remained undamaged in all three groups. In the first group (PEF), some degree of opacity and detachment of germinal layer was observed especially in higher pulse intensity (1300 V/cm). Albendazole treatment (second group) induced significant opacity. In the combined PEF and albendazole (third group), although germinal layer detachment was occurred in addition to opacity but the level of detachment was not similar to those that observed in the first group. The destructive effect of PEF on the germinal layer of daughter cysts can be considered as a promising result in the treatment of CE.

Keywords: pulsed electric fields, hydatid cysts, daughter cysts, albendazole

Introduction

Cystic echinococcosis (CE), a neglected tropical disease with global prevalence, is primarily caused by larval stage of *Echinococcus granulosus* [1,2]. This zoonotic infection carries significant medical, veterinary, and economic implications [3]. It is most common in rural areas of many countries where livestock farming is prevalent, and access to veterinary and public health services is limited [4]. In human infections, hydatid cysts have potential to form in virtually any organ of the body. However, CE primarily affects to liver, which is involved in about 75% of cases. It can also disseminate to other organs, including the lungs (5–15%), and less frequently to spleen, brain, heart, and kidneys, with involvement rates ranging from 10% to 20%. This pattern of distribution underscores the systemic nature of the disease and need to comprehensive clinical monitoring [5].

The clinical manifestations of CE depend on the location and size of the cysts and may take years to

appear, as the disease progression follows a silent clinical course. Liver involvement can lead to abdominal pain, hepatomegaly, hyperbilirubinemia, and weight loss, while lung infections may result in coughing, chest pain, and dyspnea [6,7]. Transmission occurs when animals ingest eggs through contaminated food, water or via direct contact with infected hosts. Carnivores such as dogs, wolves and foxes maintain the life cycle by excreting eggs in their feces after consuming infected intermediate hosts like sheep or rodents [8].

Preventive measures focus on deworming for high-risk dogs and vaccinating livestock, particularly sheep to disrupt the transmission cycle [9]. Hydatid cysts sometimes result in the development of secondary cysts within the primary structure. These can range from a solitary large cyst to multiple smaller and larger cysts [10]. Daughter cysts are structurally similar to the mother cyst, originating from the germinal layer and possessing the ability to produce protoscoleces. This formation occurs as a response to adverse conditions or complications within the mother cyst. The survival mechanism is triggered by factors such as injury, exposure to harmful substances like bile, immune pressure, or pathological changes such as infection or rupture. This process is considered a defensive strategy, enabling the parasite to maintain its life cycle despite challenges [10–12].

Treatment of hydatid cysts involves several approaches, each with specific applications and limitations [13]. Surgical intervention is commonly used for large or complicated cysts, with options including total cyst removal, partial cystectomy, or even partial hepatectomy. However, surgical procedures carry risks such as bleeding, infection, and potential damage to surrounding organs [14]. Pharmacological treatments, particularly albendazole and mebendazole, are effective in eliminating and preventing cyst growth. These drugs are often preferred for smaller cysts or when surgery is not feasible, but their effectiveness is reduced for larger cysts, and they can cause side effects [15,16]. Minimally invasive techniques, such as PAIR (puncture, aspiration, injection, and re-aspiration) and PAIRD (which includes intra cystic drainage), are commonly used for smaller cysts or when surgery is not feasible. While these methods are effective for treating smaller cysts, they may not be adequate for larger or more complex cases [17,18]. Endoscopic treatments, such as ERCP (endoscopic retrograde cholangiopancreatography), are particularly useful for cysts connected to the bile ducts, but their application is limited to these specific cases and may not be effective for larger or more intricate cysts [19].

A novel approach, nanosecond pulsed electric fields (nsPEF), has been applied to treat solid tumors by effectively disrupting the integrity and permeability of cell membranes [20,21]. In recent years, minimally invasive techniques such as ultrasound waves have been explored to target hydatid cyst and protoscoleces damaging them and inhibiting their growth [22,23]. Incorporating these advancements, this study explores the potential of PEF as a non-thermal, nonchemical ablation therapy for the eradication of daughter cysts, serving as an important part and a model for mother hydatid cyst, and offering a promising alternative to radical surgical interventions.

Materials and Methods

Collection of hydatid daughter cysts

The hydatid daughter cysts were carefully extracted from sheep liver under sterile conditions. Following separation, the cysts were removed from the hydatid liver cysts and placed in phosphate-buffered saline (PBS) to prevent disruption. Each study group consisted of five daughter cysts, each measuring between 1 and 1.5 cm. The cysts were maintained under controlled environmental conditions for subsequent treatment and analysis.

Preparation of albendazole solution

Albendazole was dissolved in phosphate-buffered saline (PBS) to achieve a concentration of 100 µg/ml and thoroughly mixed to prevent precipitation.

Grouping of experimental daughter cysts

Involved three experimental groups was designed to evaluate the effects of PEF, albendazole and combination of both. The first group (PEF) was consisted of five daughter cysts exposed to varying levels of PEF including 70V/cm (number of pulses: 4000, frequency: 5KH), 150 V/cm (number of pulses: 4000, frequency: 5KH) and 1300V/cm (number of pulses: 8, frequency: 1KH) (24–26). The second group (albendazole), was consisted of five daughter cysts incubated with albendazole (100 µg/ml) for one hour without exposure to PEF to evaluate its effect of albendazole on the cysts. In the third group (PEF with albendazole) was consisted of

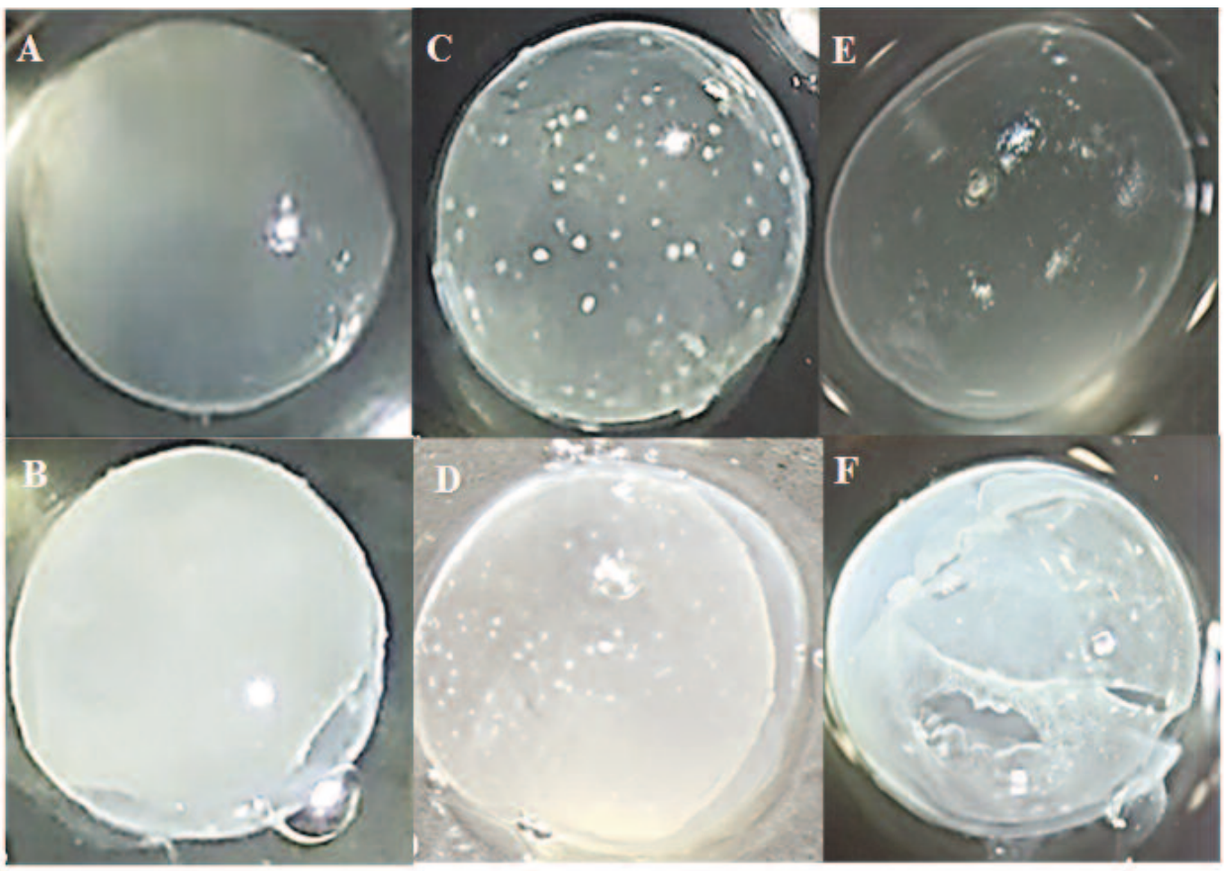

Figure 1. Exposure of daughter cysts to PEF at 70, 150, and 1300 V/cm. A. healthy cyst, B. cyst after 70V/cm exposure, C. healthy cyst, D. cyst after 150 V/cm exposure, E. healthy cyst, F. cyst after 1300 V/cm exposure

five daughter cysts incubated in albendazole (100 µg/ml) for one hour and subsequently exposed to PEF at electric field strengths of 70, 150, and 1300 V/cm as above. This experimental design aimed to evaluate how the initial incubation period influences both the therapeutic efficacy and structural integrity of the cysts.

Therapeutic assessment and data collection

The effects of PEF and albendazole on daughter cysts across the first, second, and third groups was evaluated. The analysis was focused on structural integrity, the detachment of the germinal layer from the laminated layer, and any structural changes observed using a stereomicroscope.

Results

Based on stereomicroscopic analysis, it appears that the laminated layer of daughter cysts was not damaged in any of the three groups. In first group, some degree of opacity and partial detachment of germinal layer was observed in three out of five cysts after one hour of exposure to PEF intensities of 70 and 150 V/cm (Fig. 1). At a higher pulse intensity of 1300 V/cm, slight opacity, rupture and complete detachment was observed in germinal layer of all examined daughter cysts (Fig. 1).

In second group, one-hour daughter cysts exposure to albendazole resulted in significant opacity in the daughter cysts, suggesting substantial structural changes (Fig. 2).

In third group, one hour after incubation of daughter cysts in albendazole and one hour after exposure to PEF at intensity of 70, 150 and 1300 V/cm, although germinal layer detachment was also occurred in addition to opacity but the level of detachment was not similar to those that observed in the first group (Fig. 3).

Discussion

The clinical management of hydatid cysts includes surgery, percutaneous intervention, pharmacological treatment, and an „expectant and observation" approach for inactive cysts [24]. However, evidence supporting pharmacological treatment is limited due to challenges such as small

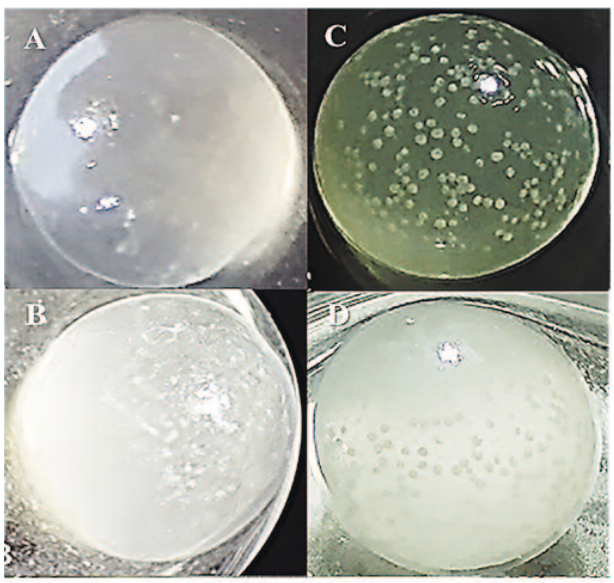

Figure 2. Structural changes in hydatid daughter cysts after one hour of exposure to albendazole (100 µg/ml). A and C. healthy cysts, B and D. cyst after one-hour exposure with albendazole

patient groups, intermittent treatment methods, and the chronic nature of CE, which requires long-term monitoring [15]. Therefore, the limitations, definitive treatment for hydatid cysts remains cystectomy, which is accompanied by an extended course of chemotherapy with albendazole and/or mebendazole for up to two years' post-surgery. In cases where surgery is not feasible, chemotherapy alone serves as an alternative treatment strategy. Standard chemotherapy protocols for hydatid cyst treatment typically involve administering albendazole in two daily doses (10–15 mg/kg/day) or mebendazole in three daily doses (40–50 mg/kg/day). The treatment duration usually spans over six months and may extend to several years in challenging cases. This prolonged therapy is often necessary due to the difficulty of drug penetration into the cysts, the resilience of the parasite's layers, and the need to ensure complete eradication of the disease [16,25,26]. Liver transplantation has been explored as a complementary treatment option alongside surgery and chemotherapy. However, this method carries significant risks, including the possibility of reinfection with alveolar echinococcosis, which limits its widespread clinical use [27,28]. The hydatid cyst and its fluid components of a protoscoleces, daughter cysts, germinal layer, and brood capsule demonstrate infective potential. In the event of cyst rupture or

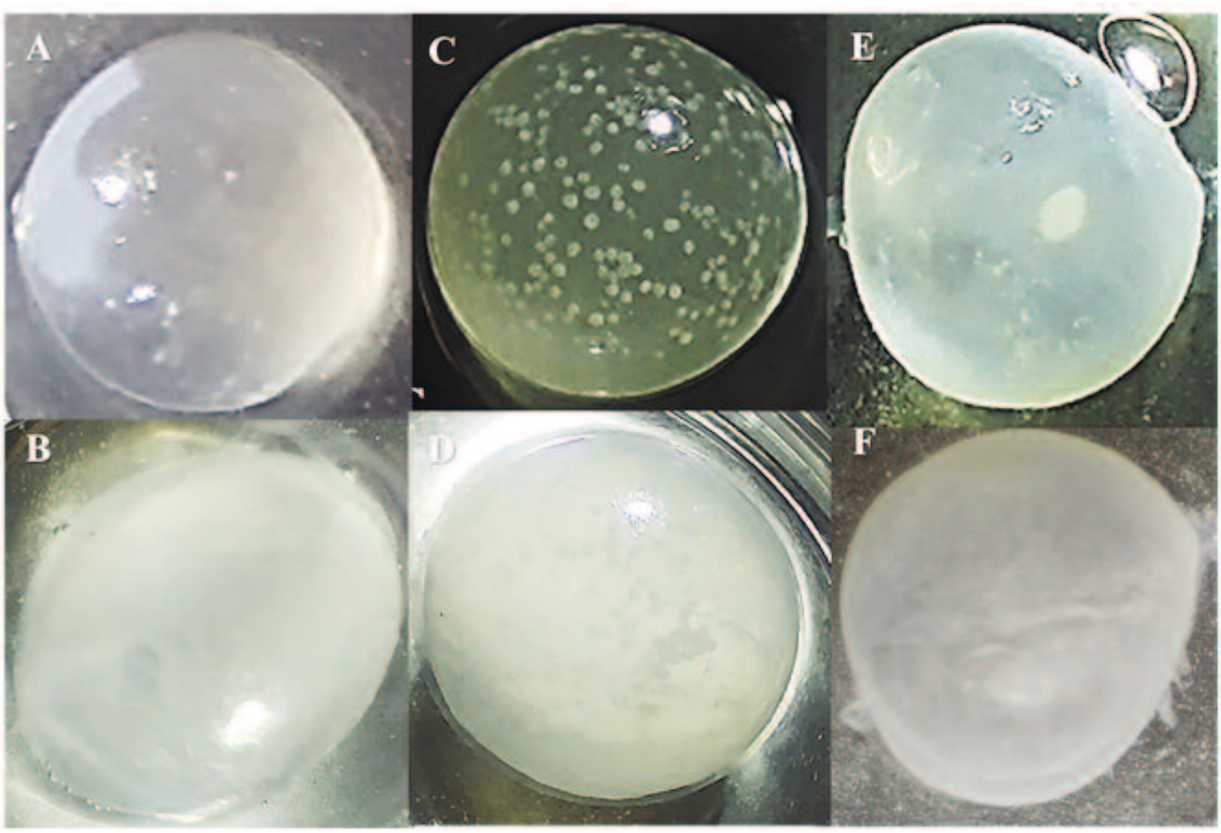

Figure 3. Exposure of daughter cysts to albendazole (100 µg/ml) and PEF at 70, 150, and 1300 V/cm. A. healthy cyst, B. cyst after 70 V/cm exposure, C. healthy cyst, D. cyst after 150 V/cm exposure, E. healthy cyst, F. cyst after 1300 V/cm exposure

perforation, these contents may be released, posing a risk of anaphylactic shock or reinfection in other areas of the body [29–31].

Nano-second Pulsed Electric Fields (nsPEF) has proven to be a novel technology for tumor removal [32]. In recent years, minimally invasive techniques, such as ultrasound waves, have been used to disrupt protoscoleces. Researchers have found that ultrasound waves can damage protoscoleces and inhibit their growth under laboratory conditions [33]. Therefore, utilizing of nsPEF is an effective therapeutic strategy for hydatid cysts, designed to inhibit cyst growth and disrupt the germinal layer and protoscoleces. The damage caused by nsPEF occurs gradually over time [22].

This study investigated the effects of PEF and albendazole on the morphology and structure of daughter cysts in the first, second, and third groups. Pulses applied in the first group, with intensities of 70 and 150 kV/cm, caused detachment of the germinal layer from the laminated layer in three out of five cysts. These structural changes suggest that PEF, even at low intensities, can disrupt cyst membrane integrity, leading to destructive effects. In hydatid cyst treatment, detachment of the germinal layer is particularly important, as this layer is vital for cyst reproductive potential [34]. At a pulse intensity of 1300 kV/cm, complete detachment of the germinal layer from the laminated layer occurred in all cysts. It shows that PEF exhibits a dose-dependent effect. The potential of PEF to inflict irreversible damage on cysts positions it as a promising non-invasive therapeutic approach. However, the destructive effects of PEF were observed one-hour post-exposure, indicating a time-dependent mechanism of action. Some studies showed this delayed effect has significant implications for therapeutic protocols and highlights the need for extended monitoring periods following nsPEF exposure [35]. Findings from the second group provided further insights into albendazole role in altering cyst structure. One-hour post-exposure to albendazole, daughter cysts displayed significant opacity, indicative of structural changes. This opacity likely reflects cellular damage or biochemical alterations, suggesting that albendazole induces cellular stress or disruption [35–38].

However, the combination of albendazole with PEF in the third group did not show significant immediate morphological changes compared to first group. Although interaction albendazole with PEF warrants further investigation to determine potential synergistic effects under in vivo conditions [22]. While albendazole independently induces structural changes and PEF alone induce germinal layer detachment, albendazole appears to reduce the effect of electroporation. The results of this study indicate that utilizing of PEF, particularly at higher pulse intensities, is a promising non-invasive therapeutic modality for hydatid cysts. By disrupting the germinal layer, PEF may prevent further cyst development and offer an alternative to conventional surgical interventions. Combination of chemotherapy (albendazol, mebendazole, etc) and PEF, and surgery may increase chance of a successful treatment and decrease risk of relapse. The observed delayed effects of PEF alone and in combination with albendazole emphasize the importance of optimizing treatment timing in clinical settings. In support of these findings, an *ex vivo* study by Chen et al. [39] demonstrated that treatment with nsPEF caused destruction of various cyst wall layers, including the germinal and laminated layers, and also led to damage of the protoscoleces. Furthermore, significant changes in the cyst fluid composition, including a reduction in protein and glucose levels and a decrease in pH, were observed, indicating a disruption in the parasite's metabolism and nutrition [39]. Further research is needed to explore the long-term effects of PEF and albendazole, particularly on cyst component viability (specially protoscoleces) and new cyst formation or recurrence. Investigating the combined effects of PEF with other antiparasitic agents or immune-modulating therapies could provide valuable insights for refining treatment strategies. Additionally, imaging techniques to quantitatively assess changes in cyst volume and structural integrity over time could offer a more precise understanding of PEF dynamic effects on cysts.

The findings of this study underscore the potential of PEF as a promising therapeutic modality for hydatid disease, with the capacity to disrupt the structural integrity of cysts and potentially prevent further cyst formation. However, additional research is required to fully understand the underlying mechanisms and to establish optimal treatment protocols for clinical application.

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