Application of microwave against Protoscoleces of Echinococcus granulosus in vitro and Secondary Hydatid Disease in BALB/c Mice

Aplicación de Microondas contra Protoscoleces de Echinococcus granulosus in vitro y e Hidatidosis Secundaria en Ratones BALB/c

Seham Ibrahim Khalil, Department of Biology Education College for Pure Sciences University of Mosul, Email: seham.esp57@student.uomosul.edu.iq
Asmaa Abdulaziz Ali Email: dr.asmaa_abdulaziz@uomosul.edu.iq
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Abstract

The present research examines the impact of microwave of (230-240V-50Hz 900W) on the viability of *Echinococcus granulosus* protoscoleces in vitro for different exposing periods, 3, 5, 7, 10, 12, 13, and 15 seconds, respectively. Subsequently, the study investigates the prevention of mice against infestation with secondary hydatid cysts, compared with the control animals infested with protoscoleces without exposure to microwaves over four months, depending on many criteria, including the numbers, weights, and diameters of developed hydatid cysts. The results revealed an obvious infl of microwaves on *Echinococcus granulosus* protoscoleces viability by an increase of exposure period in vitro, 90%, 80%, 77%, 70%, 50, 30, and 0%, respectively. Plus, the results showed a decrease of 97%, 92.5% of hydatid cysts numbers in the mice injected with exposed protoscoleces with 30% fertility exposed for 13 seconds, four months post-infection, followed by 92.5% and 87.2% in the same group, two and three months post-infection.

Keywords: *Echinococcus granulosus*, microwave`, Hydatidosis, BALB/c Mice

Resumen

La presente investigación examina el impacto de microondas de (230-240V-50Hz 900W) sobre la viabilidad de *Echinococcus granulosus* protoscoleces in vitro para diferentes períodos de exposición, 3, 5, 7, 10, 12, 13 y 15 segundos, respectivamente. Posteriormente, el estudio investiga la prevención de la infestación con quistes hidatídicos secundarios en ratones, en comparación con los animales de control infestados con protoscólices sin exposición a microondas durante cuatro meses, dependiendo de muchos criterios, incluidos los números, pesos y diámetros de los quistes hidatídicos desarrollados. Los resultados revelaron una influencia obvia de microondas en la viabilidad de *Echinococcus granulosus* protoscoleces por un aumento del período de exposición in vitro, 90%, 80%, 77%, 70%, 50, 30 y 0%, respectivamente. Además, los resultados mostraron una disminución del 97 %, 92,5 % del número de quistes hidatídicos en los ratones inyectados con protoescólices expuestos con un 30 % de fertilidad expuestos durante 13 segundos, cuatro meses después de la infección, seguidos por un 92,5 % y un 87,2 % en el mismo grupo, dos y tres meses después de la infección.

Palabras clave: *Echinococcus granulosus*, microondas`, hidatidosis, ratones BALB/c

Introduction

Cystic echinococcosis (CE, also known as hydatid disease) is a neglected zoonotic tropical illness that is predicted to cause the death of millions of people each year[1]. Infection with the larval stages of platyhelminthes (flatworms) belonging to the taenidae family, notably the genus Echinococcus, causes it. The condition is characterized by the formation of fluid-filled, bladder-like larvae within internal organs. These are referred to as hydatid cysts, or more accurately, hydatids, because the term 'cyst' refers to both the parasite and the local host reaction. At least three Echinococcus species cause human CE, and only molecular biology methods can tell them apart. *E. granulosus* (*sensu stricto*) and E. canadensis are responsible for about 90% and 10% of cases, respectively. *E. granulosus sensu lato* refers to the species cluster (which also includes two other species). The genus Echinococcus contains several species, the most important of which is Echinococcus multilocularis, which causes alveolar echinococcosis, in which the larva invades the liver parenchyma aggressively[2]. Echinococ-

cus species have two life-cycles: one with one host and one with the other host[3]. Adult stages of E. granulosus sensu lato (worms measuring only a few millimeters in length) develop in the small intestine of carnivore hosts, which are usually dogs. The so-called intermediate hosts are infected by eggs transmitted out with dog feces. Sheep, cows, pigs, camels, goats, buffaloes, and cervids, as well as humans, make up the intermediate host range. Eggs hatch in the intestine, releasing oncospheres that puncture the gut wall, travel by blood or lymph, and settle in internal organs, eventually forming hydatids. Hydatids can generate protoscoleces, which are parasitic forms, within months or years. When dogs eat infected tissues from intermediate hosts, the parasites' life cycles are completed: eaten protoscoleces mature into adult worms in the gut. Within E. granulosus sensu lato, each species (and subspecies) has a favored intermediate host species range[3]. Sheep are E. granulosus sensu stricto's favored intermediate hosts. Because human infection plays no role in the life-cycles, humans are considered incidental or aberrant intermediate hosts. Except for Antarctica, CE is found on every continent. The infection is highly related with livestock rearing in undeveloped situations, as expected given the life-cycle pattern outlined. North Africa, the Near and Middle East, Central Asia, eastern Russia, and western China have the highest disease burdens; South America, while still heavily afflicted, has seen the prevalence of CE decline in recent decades[4,5].

Chemotherapy and surgery are now used to treat hydatid illness. Because of the numerous layers of cyst, antiparasite therapy has a hard time penetrating and killing protoscoleces in cystic echinococcosis[6,7]. As a result, the antiparasite medication can only be taken as a last resort. The most common treatment is still surgery[8, 9]. To aspirate daughter cysts and inject antihelminthic agent, the parasite cyst must be opened.

Surgical methods have increasingly evolved from destructive to constructive in recent years. The minimally invasive treatment has a high cure rate and low morbidity and fatality rates[10]. WHO has advocated PAIR (puncture aspiration injection reaspiration) as an alternate treatment for hydatid cysts. In terms of disease recurrence, morbidity, and death, PAIR plus chemotherapy outperforms open surgery[11,12]. New minimally invasive therapeutic approaches for hydatid cyst ablation have been introduced in recent years, such as radio frequency[13], microwave[14], cryoablation[15], and HIFU[16–19].

As a result, it appears that microwave radiation may impact protoscoleces and may be useful in the treatment of hydatidosis or the prevention of secondary cyst recurrence. Microwaves are non-ionizing electromagnetic radiation that are very brief waves of electromagnetic energy[20]. Many studies have shown that microwaves are effective at killing bacteria[21-23]. Microwaves can create two types of impacts, according to the literature: thermal and non-thermal[23]. Microwave therapy makes advantage of the thermal effects of microwaves[24]. Microwave irradiation is used to heat bodily tissues and kill cancer cells, or to make cancer cells more sensitive to the effects of other types of radiation and/or particular anticancer medications, in this type of treatment[25,26].

In this study the effect of microwave radiation on hydatid cyst has been investigated, we evaluated the effects of this kind of waves on protoscoleces in vitro, and on growth and development of hydatid cysts in vivo.

Materials and methods

Isolation and testing hydatid cysts

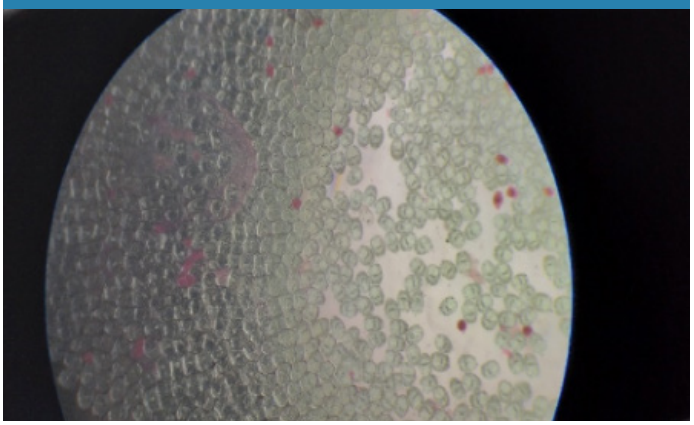
Hydatid cysts were obtained (Figure 1) after being separated from livers of slaughtered sheep in the main abattoir in Mosul, upon arrival to the laboratory, hydatid cysts were examined to accentual the vitality of protoscoleces.

Figure 1. Larval stages (hydatid cysts) in the liver of sheep.

Collection and assessment of E. granulosus protoscoleces

Sheep cysts containing protoscoleces were obtained under sterilized conditions, a live protoscoleces, 20 μl of protoscoleces suspension were added to 20 μl of 0.1% of eosin pigment on a slide, then tested under light microscope. Bright green protoscoleces were considered as alive because of eosin exclusion by protoscoleces membranes, whereas red protoscoleces were regarded as dead regarding to the acceptance of the pigment (Fig 2). Gained and counted according to authenticated manner [27].

Fig 2. Protoscoleces of Echinococcus granulosus



Experimental animals

120 female Albino mice, BALB/c (three to four weeks old) were used and cared for in the animal house, College of Veterinary Science, University of Mosul, according to the conditions required for the experiments.

The system used in the experiment

Microwave system (230-240V-50Hz 900W) was used.

1. Power
2. Minute, second
3. Start
4. stop

Experimental Design

Effect of the microwave on the vitality of sheep protoscoleces in vitro

To determine the effect of microwave on the vitality of protoscoleces in vitro within different durations, protoscoleces with a 98-100% viability were taken at a concentration of 2000 protoscoleces /ml, and then exposed continuously to the microwave waves, in test tubes, Samples were exposed, in a continuous manner, for 3, 5, 7, 10, 12, 13, 15 seconds, respectively. the post exposure vital percentages were 90%, 80%, 77%, 70%, 50, 30 and 0%, respectively (Table 1, 2). The protoscoleces were examined under a light microscope for viability taking into consideration the flame cells movement, morphological abnormalities, and exclusion of eosin pigment.

Table 1. Effect of microwaves on the vitality protoscoleces of sheep origin in virto

Frequency/ Time	Control	3 Sec	5 Sec	7 Sec	10 Sec	12 Sec	13 Sec	15 Sec
50Hz 900W		90%	80%	77%	70%	50%	30%	0%

Table 2. Effect of microwaves on the response of the vitality ratio to exposure Periods

Source	DF	SS	MS	F	P
Treated	6	17937.909	2989.651	2907.33**	0.0001
Error	28	28.792	1.028		
Total	34	17966.702			

** mean significance at P<0.01, while * mean significance of P<0.05

Effect of ultrasound waves on secondary hydatid disease in mice

Protoscolices with vitality 30%, 50%, 70%, were selected for mice injection in addition to the control with 100% vitality. A total of 60 female mice, aged 5-3 weeks, were divided into four groups (fifteen mice/ each group). The 1st group was injected with 2000 protoscoleces of vitality 30%. The 2nd was injected with 2000 protoscoleces of vitality 50%. The 3rd was injected with 2000 protoscoleces of vitality 70%. The 4th group was injected with 2000 protoscoleces of vitality 100% (control group). All groups were all dissected after two, three, four months post injection, respectively.

After dissection, the numbers, weights and diameters of hydatid cysts were detected, in addition to their percentage of reduction according to the equation:

Percentage reduction of hydatid cysts:

100 – number of hydatid cysts in treated mice/ number of cysts in control group *100

Statistical analysis

Results of this study were analyzed statistically based on Completely Randomized Design (CRD) to study the effect of intervals and exposures and the integration between them, then the differences between the averages of each factor and the compatibility between factors were estimated depending on Duncan's Multiple Range Test (DMRT). Statistical Analysis System (SAS version 9) was utilized for applied for data analysis[28].

Results

Table 3 reveals the changes in the number of hydatid cysts, their diameters, and weights, as well as the significance of F in the ANOVA analysis. the DMRT for averages test (Table 4) explained a decrease in the average number of in the treated groups (Figure 3), When compared to the control animals (Figure 4). The number of cysts in treated mice decreased by 1.0 after 13 seconds of exposure, while the control animals had the highest average of 42.2, 4 months after infection. Table 5-7 demonstrated the highest percentage reduction of hydatid cysts 97% in treated mice followed by 92.2% and 87% after 13 seconds exposure respectively.

Table 3. Effect of microwaves on numbers, weights and diameters of hydatid cysts in all experience animals

Sources of difference		Periods			Periods Treatment×	
		df	2		3	48
Cysts number	1733.6 Mean squares	5945.25 1344.46**	1981.75**	2688.93 288.93**		2336.40 48.6750
Cysts weight(gm)	Sum of squares Mean squares F value	0.020721 0.010360** 5.20	0.02917 0.00972** 4.88	0.02708 0. 00451* 2.26		0.095704 0.001993
Cysts diameters (mm)	Sum of squares Mean squares F value	20.1241 10.0620** 85.85	17.1953 5.73177** 48.9	7.5188 1.2531** 10.69		5.6259 0.11720

** mean significance at P<0.01, while * mean significance of P<0.05

Table 4. Effect of microwaves on the number of hydatid cysts in treated animals

periods	2 months	3 months	4 months	Average
Control	8.000 bcd	35.800 a	42.200 a	28.667 a
10 second	2.000 d	8.000 bcd	17.800 b	9.267 b
12 second	1.200 d	10.200 bcd	14.000 bc	8.467 b
13 second	0.600	4.600 cd	1.000 d	2.067 c
Average	2.950 b	4.650 a	8.750 a	

The different letters indicate significance, while the similar letters indicate insignificancy.

Table 5. Percentage of hydatid cysts reduction in treated animals for all months

Periods	2 months	3 months	4 months
10 Sec	75%	77.7%	57.9%
12 Sec	85%	71.6%	66.9%
13 Sec	92.5%	87.2%	97%

Table 6. Effect of microwaves on the weight of hydatid cysts in treated animals

Periods	2 months	3 months	4 months	Average
Control	0.00140 b	0.04000 b	0.13200 a	0.05780 a
10 Sec	0.00080 b	0.01000 b	0.02600 b	0.01227b
12 Sec	0.00080 b	0.00820 b	0.02200 b	0.01033 b
13 Sec	0.00040 b	0.00100 b	0.00140 b	0.00093 b
Average	0.00085 b	0.01480 b	0.04535 a	

The different letters indicate significance, while the similar letters indicate insignificancy.

Table 7. Effect of microwaves on the diameters of hydatid cysts in treated animals

Periods	2 months	3 months	4 months
Control	0.8840 d	1.3640 c	2.8200 a
10 Sec	0.6200 def	0.8820 d	2.3000 b
12 Sec	0.4200 def	0.7000 de	2.2000 b
13 Sec	0.1400 f	0.3600 ef	0.1600 f
Average	0.5160 c	0.8265 b	1.8700 a

The different letters indicate significance, while the similar letters indicate insignificancy.

Fig 3. hydatid cysts in t mice injected with exposed protoscoleces for 13 seconds, three months post infection

Fig 4. Hydatid cysts in mice injected with un exposed protoscoleces (control group) three months post infection

Discussion

MWA is a common interventional technique that uses a heat action to cause coagulation necrosis in the target lesion. During MWA, the active tip produces an oscillating electromagnetic field that radiates into the tissue around the antenna, forcing polar molecules (mainly H_2O) to realign continually, boosting their kinetic energy and, as a result, causing direct tissue heating[29].

MWA was first described by Seki et al[30], and it has since been routinely used to treat primary hepatocellular carcinoma. Despite the fact that the friction of intracellular and extracellular charged ions is caused by microwave frequency and collisions with other molecules, the local temperature rises above 65°C in a short amount of time, resulting in coagulation necrosis. MWA may also boost immunological function by promoting the generation of Th1 cells[31].

Yangdan Cairang (2017) Efficacy and safety of ultrasound – guided percutaneous

The hepatic alveolar echinococcosis HAE lesions gradually calcify after microwave treatment, according to Yangdan Cairang et al. (2017)[32], with no major problems or recurrence after surgery.

The advancement of numerous medical technology has enabled radio frequency electromagnetic as a non-invasive method[33]. The influence of an electromagnetic field (REF) on a cell membrane causes alterations in ion transport across the membrane, according to the researchers[34]. A cell's membrane might also become more fluid[35],[36]. The cell's permeability was altered, perhaps allowing certain potentially hazardous molecules to enter as well as greater protein and other molecule mobility in and through the membrane[37]. High tem-

peratures can damage both integral and peripheral proteins in the membrane, and excessive heat can cause these proteins to break down, or denature[34].

Hyperthermia, either local or whole-body, has been suggested as a treatment for various malignant tumors in several studies. Microwave application is one of the most effective means of producing hyperthermia. In regions with a shallow depth, these waves quickly elevated temperature[32]. Eslamirad et al[38] employed a different method called shortwave diathermy (SWD) to heat deep laying tissues and a big volume of tissue. In length deep, SWD can cause heat.

The current study found that microwave irradiation can harm protoscoleces, and that this effect is depending on the duration and manner of irradiation. Electromagnetic radiations like UV, gamma rays, lasers, and microwaves have recently been employed to cure ailments. In addition, some studies have found that electromagnetic waves alter cell proliferation, cell function, chromosomal number, and cell membrane permeability[39,40]. Microwave electromagnetic radiation has been found in various studies to have harmful effects on biological tissue, However, the intended effects of these waves in the therapy of certain disorders have been demonstrated and have been used in diathermy (38). In another study, the thermal effects of microwave radiation on bacteria's ultrastructure were investigated. Microbiological investigations have demonstrated that cells die when exposed to microwaves. Temperature increases have been identified as the primary cause of cell death in these situations[22].

Although most scientists are concerned about the dangers of microwave waves, some have concentrated on using them to kill pathogenic organisms and treat ailments like hydatid cysts and protoscoleces. LA Monaca et al[13] found that RF can be used to necrotize the germinal layer of a hydatid cyst. Then, by raising the temperature of the cyst to 95 °C, Saricik et al[41] discovered that the RF technique might harm the germinal layer of a hydatid cyst (after 4-minute exposure). Although the germinal layer of hydatid cysts must be damaged with intense heat, Other organs and blood arteries may be damaged as a result of the heat[42]. Protoscoleces exposed to mobile phone radiation exhibited a greater mortality rate than those not exposed, according to Soleimani et al[43]. The parasite's mortality rate is also affected by its distance from the mobile phone and the length of time it is exposed to its radiation[42-45].

Conclusion

It may well be concluded ultrasound has a significant impact on the viability of protoscoleces of Echinococcus granulosus in vitro and in BALB/c mice, and against growth and development of hydatid disease in experimental animals, so it may be used in the future as a promising therapeutic method for cystic echinococcosis.

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Conflict of interests
The authors declare no conflict of interest

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