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Evaluation of lethal effect of microwave exposure on protoscolices of hydatid cyst *in vitro*Zahra Eslamirad<sup>1</sup>, Homa Soleimani<sup>2\*</sup>, Reza Hajhossein<sup>1</sup>, Fatemeh Rafiei<sup>3</sup><sup>1</sup>Department of Parasitology, School of Medicine, Arak University of Medical Sciences, Arak, Iran<sup>2</sup>Department of Medical Physics and Physiology, School of Medicine, Arak University of Medical Sciences, Arak, Iran<sup>3</sup>Thyroid Disorders Research Center, Arak University of Medical Sciences, Arak, Iran

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## ABSTRACT

**Objective:** To investigate the lethal effect of microwave radiation on protoscolices of hydatid cyst.**Methods:** The protoscolices were divided in two separate groups. The first group received continuous irradiation while the second group received repetitive irradiation. According to the exposure time, the first and the second groups were divided into 8 subgroups. Non-treated protoscolices were considered as the control in each experiment. The protoscolex mortality rate was calculated, and changes in temperature difference in protoscolex suspension before and after the irradiation and the mortality rate with the increase of exposure time were recorded.**Results:** The results showed that microwave was able to increase the mortality rate of protoscolices in hydatid cyst. The mortality rate from 20% in 20 s of continuous exposure was increased to 100% in 50 s. Also, the differences between the mortality rates in subgroups of the first and the second groups and the control were significant ( $P < 0.001$ ). Although the effect of temperature change in repetitive irradiation was not significant, non-thermal repetitive irradiation effects were obviously stronger than the thermal continuous irradiation effects.**Conclusions:** It seems that, microwaves especially in the repetitive mode, may be used as a supplementary measure for both treatment and prevention of hydatidosis.

## 1. Introduction

Microwaves are very short waves of electromagnetic energy classified as non-ionizing electromagnetic radiation[1,2]. The efficacy of microwaves in microbial destruction has been reported in many studies[3-6]. According to the literature, two types of effects can be produced by microwaves, thermal and non-thermal[7]. The thermal effects of microwaves are used for microwave therapy[7]. In this type of treatment, microwave irradiation is used to heat body tissues and damage cancer cells or to make cancer cells more sensitive to the effects of other forms of radiation and/or certain anticancer drugs[8,9]. Additionally, microwaves are primarily used in other medical situations as an alternative to surgery. For example, they are used to treat prostate enlargement[10-13]. Hydatidosis is a zoonotic infection caused by the larval stage of *Echinococcus*[14,15]. *Echinococcus* at larval stage can infect both animals and humans[14]. There is currently no agreement about the ideal therapy for hydatidosis. Surgery is a common treatment for the hydatid

cyst and drug treatment is used to prevent recurrence of secondary cysts[16]. All surgical techniques, however, are invasive and pose a risk of recurrence[17]. Therefore, new therapeutic options for hydatidosis are urgently needed. In recent years, minimally invasive techniques, such as ultrasound waves, were used for destruction of protoscolices. The researchers found that high intensity focused ultrasound can damage protoscolices and inhibit their growth *in vitro* and *in vivo*[18]. Hence, it seems that the microwave radiation may affect protoscolices and also can be beneficial in the treatment of hydatidosis or used to prevent recurrence of secondary cysts. Considering that so far, the effect of microwave radiation (thermal and non-thermal) on hydatid cyst has not been investigated, we evaluated the effects of this kind of waves on protoscolices.

## 2. Materials and methods

This is an experimental study that was done on protoscolices obtained from hydatid cyst.

2.1. *Echinococcus granulosus* protoscolices

Hydatid cysts were obtained from sheep that were naturally infected and had been slaughtered a maximum of 2 h before transferred to Parasitology Laboratory in Arak University of

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Medical Sciences. The content of cysts was completely removed by a sterile syringe. Exclusion criteria for this study were number of protoscolices per milliliter of hydatid fluid (< 9000) and mortality rate of them per milliliter of hydatid fluid (> 10%). Therefore, the protoscolex suspension containing 9000–10000 protoscolices per milliliter was provided. The viability of protoscolices was determined by the eosin stain method[19]. When more than 90% of protoscolices were viable in the suspension, it was considered to be suitable for further examination. The protoscolex suspension was aliquot into identical tubes and in equal volumes (20 µL).

2.2. Microwave treatment

Microwave irradiation was performed by using a microwave oven (ME3410W; Samsung Co., South Korea). During the irradiation, the tubes containing protoscolices were placed in the center of irradiation plate. At this point, the microwave power was 1550 W with a frequency of 2450 MHz. Experiment was conducted in two separate groups. These groups received continuous and repetitive irradiation, respectively. According to the exposure time, both groups were divided into 8 subgroups. All the experiments were performed in triplicate. Non-treated protoscolices were considered as the control in each experiment.

The dead protoscolices were counted in microwave-irradiated suspensions and in controls. The protoscolex mortality rate was calculated as follows:

$$\text{Mortality rate (\%)} = \frac{\text{number of dead protoscolices in each subgroup} - \text{number of dead protoscolices before irradiation}}{\text{total protoscolices}} \times 100$$

The temperature change in the protoscolex suspensions was monitored before and after the irradiation with a thermocouple (Tp-01, Lutron Electronic Enterprise Co., Taiwan). The temperature of the suspension was measured with an accuracy of 0.1 °C when the probe was inserted into the suspension. The temperature was shown as ΔT, representing temperature difference in protoscolex suspension before and after the irradiation. The mean of initial temperature of protoscolex suspension in the both groups was (26.0 ± 0.9) °C.

Note that in the second group which received repetitive irradiation, during the interval between the irradiations the temperature inside the tube was allowed returning to the initial temperature.

2.3. Statistical analysis

Statistical analyses were performed by using SPSS version 16.0. The data were presented as mean values in three separate experiments and expressed as mean ± SD. Differences between the subgroups and the control were analyzed with One-way ANOVA test. Statistical significance was defined as P < 0.05. Also, to determine the lethal dose and percentage of mortality, probit analysis and linear equations were used, respectively.

3. Results

Table 1 shows the mortality rate and ΔT for different exposure time in the first group which received continuous irradiation. As it can be seen in this table, mortality rate of protoscolices in this group was suddenly increased from 45% in the 7th subgroup to 100% in the 8th subgroup.

Statistical analyses showed that differences between the mortality rates in all subgroups of the first group and the control were significant (P < 0.001). As it can be seen in Figure 1, when the exposure time increased, the mortality rate and ΔT were increased.

Table 1

Mortality rate of protoscolices and ΔT in subgroups of the first group.

Subgroups	Exposure time (s)	ΔT (°C)	Mortality rate (%)
1	15	4.0 ± 1.0	20.0 ± 2.0*
2	20	5.0 ± 1.0	23.7 ± 2.1*
3	25	7.7 ± 0.6	25.3 ± 1.5*
4	30	8.3 ± 0.6	27.7 ± 2.5*
5	35	18.0 ± 1.7	34.7 ± 2.5*
6	40	20.3 ± 2.5	41.0 ± 2.0*
7	45	25.7 ± 1.6	45.0 ± 3.6*
8	50	30.7 ± 2.1	100.0 ± 0.0*
Control	0	0.0 ± 1.0	8.3 ± 1.5

The mean of minimum temperature recorded was 26 °C; data were expressed as mean ± SD. \*: Significant differences between the mortality rates in the subgroups and the control.

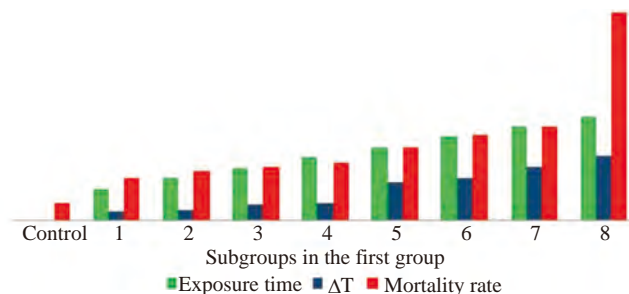


Figure 1. The changes of exposure time (s), ΔT (°C) and mortality rate (%) in the subgroups of the first group.

The results of probit analysis for the first group were shown in Table 2. As regards the LD<sub>50</sub> occurred in 36.86 s, probit regression equation for the first group was estimated as: PROBIT (p) = -1.848 + 0.05 × exposure time.

Table 2

Estimated time of deadly radiation with confidence interval of 95% for the first group.

95% Confidence limits for time		Time estimate	Probability
Lower bound	Upper bound	(s)	
17.72	27.18	23.40	0.25
33.62	40.60	36.86	0.50
45.62	58.00	50.31	0.75
55.41	74.64	62.42	0.90
71.83	103.70	83.27	0.99

As it can be observed in Figure 2, with increasing exposure time, the probit index was increased and there was a directly significant correlation between these two variables.

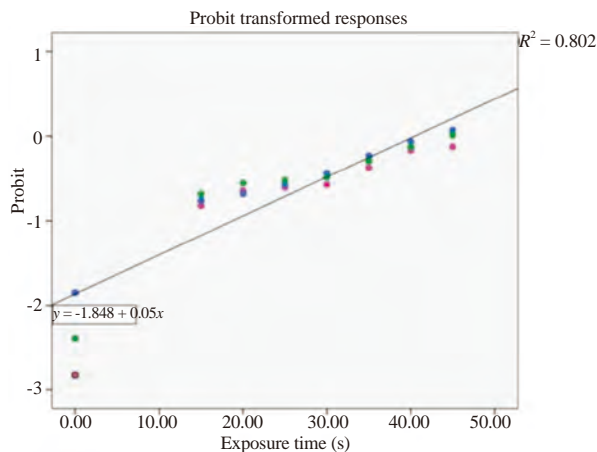


Figure 2. Probit transformed responses based on the exposure time (s) for the first group.

Pink: First experiment; Blue: Second experiment; Green: Third experiment.

Table 3 shows the mortality rate and  $\Delta T$  for different exposure time in the second group which received repetitive irradiation. As it can be seen in this table, the mortality rate of protoscolices was 50.3% after 40 s of exposure to repetitive microwave radiation. Also, mortality rate of protoscolices in this group was suddenly increased from 50.7% in the 2nd subgroup to 82.0% in the 3rd subgroup. Statistical analyses showed that differences between the mortality rates in all subgroups of the second group and the control were significant ( $P < 0.05$ ).

As it can be seen in Figure 3, when the exposure time increased, the mortality rate was also increased, but  $\Delta T$  was not affected significantly.

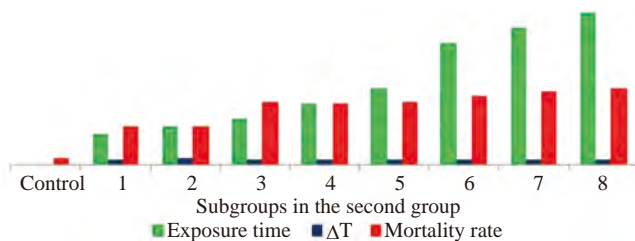
The effects of microwave radiation on structure of protoscolices are shown in Figure 4.

**Table 3**

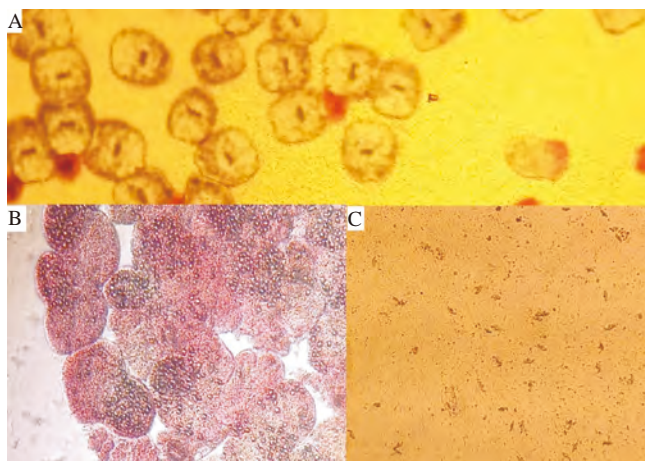
Mortality rate of protoscolices and  $\Delta T$  in subgroups of the second group.

Subgroups	Exposure frequency $\times$ time (s)	$\Delta T$ ( $^{\circ}C$ )	Mortality rate (%)
1	4 $\times$ 10	6.30 $\pm$ 0.30	50.3 $\pm$ 1.5*
2	5 $\times$ 10	7.70 $\pm$ 0.10	50.7 $\pm$ 1.5*
3	6 $\times$ 10	6.00 $\pm$ 0.50	82.0 $\pm$ 1.4*
4	8 $\times$ 10	6.10 $\pm$ 0.35	80.0 $\pm$ 4.2*
5	10 $\times$ 10	7.45 $\pm$ 0.60	83.0 $\pm$ 4.2*
6	16 $\times$ 10	6.93 $\pm$ 1.24	91.0 $\pm$ 5.1*
7	18 $\times$ 10	7.10 $\pm$ 1.23	97.0 $\pm$ 5.2*
8	20 $\times$ 10	6.60 $\pm$ 0.11	100.0 $\pm$ 0.0*
Control	0	0.00 $\pm$ 1.00	8.3 $\pm$ 1.5

The mean of minimum temperature recorded was 26  $^{\circ}C$ ; data were expressed as mean  $\pm$  SD; \*: Significant differences between the mortality rates in the subgroups and the control.



**Figure 3.** The changes of exposure time (s),  $\Delta T$  ( $^{\circ}C$ ) and mortality rate (%) of protoscolices in the subgroups of the second group.



**Figure 4.** The effects of microwave radiation on structure of protoscolices. A: Living protoscolices in control group ( $\times 40$ ); B: Protoscolices exposed to repetitive microwave for 20  $\times$  10 s ( $\times 40$ ); C: Protoscolices exposed to continuous microwave for 50 s ( $\times 40$ ).

#### 4. Discussion

The results of current study show that irradiation of microwave is able to damage protoscolices and this effect is dependent on the

duration and mode of irradiation. For example, repetitive irradiation for 200 s led to 100% death and continuous irradiation for 50 s led to destruction of some protoscolices and scattering of hooklets.

Recently, the electromagnetic radiations such as ultraviolet, gamma rays, laser and microwave have been used to treat certain diseases. Also, the results of some researches have shown that the electromagnetic waves affect on cell growth, cell function, number of chromosomes and permeability of cell membrane[20-22]. Different studies have shown that microwave electromagnetic radiation could carry undesirable effects on living tissue, but the desired effects of these waves in the treatment of some diseases are proven and have already been used in diathermy.

Many previous studies have investigated the lethal effects of microwave on various bacterial species[3-6]. In most of these studies, the thermal effects of microwave radiation on the ultra structure of the bacteria have been studied[3-5]. Microbiological studies have shown that cells die during microwave irradiation. The main causes of cell death in these cases have been described to be an increase in temperature[7].

Wu and Yao reported that microwave radiation only inactivated microorganisms that contain water[23]. Gedikli *et al.* also reported that the reduction of water activity in bacteria reduced the effect of microwave radiation. He also reported that continuous exposure to microwave radiation might produce lethal effects on bacteria by generating heat[24]. We know that microwave radiation increases the temperature, and the temperature is involved in the death of many microorganisms containing water[7]. However, so far, the effect of microwave radiation on hydatid cyst has not been investigated. In the present study, the lethal effects of microwaves on protoscolices of hydatid cyst was proven. When continuous irradiation was used, the increase of the mortality rate was found to be directly correlated with the increasing exposure time and also directly related to increasing temperature. It can be concluded that significant increase in temperature has a major role in the death of all protoscolices. Moazeni and Alipour-Chaharmahali reported that warm water had a lethal effect on protoscolices of hydatid cyst. The maximum lethal effects of warm water was obtained after 5, 2, and 1 min at 50, 55, and 60  $^{\circ}C$ , respectively[25]. Also, during 60 s, the mortality rates in warm water-treated groups at 50 and 55  $^{\circ}C$  in comparison with the control (13.8% and 15.7%) were increased to 24.0% and 78.0%, respectively. In the present study, in comparison with warm water, the lethal effects of continuous microwave irradiation was associated with large variations in temperature ( $\Delta T = 30$   $^{\circ}C$ ). Also, during 50 s of continuous irradiation, the mortality rate in microwave-treated group at mean temperatures of 50  $^{\circ}C$  ( $\Delta T = 25$   $^{\circ}C$ ) and 55  $^{\circ}C$  ( $\Delta T = 30$   $^{\circ}C$ ) in comparison with the control (8%) were increased to 45% and 100%, respectively. These results may indicate non-thermal effects of microwave radiation on protoscolices.

In our study, in order to reduce the thermal effects of microwaves, a repetitive microwave irradiation protocol was used. In repetitive irradiation, the change in temperature is much lower than in continuous irradiation. When repetitive irradiation was used, the maximum  $\Delta T$  was only 7.7  $^{\circ}C$ ; however, longer exposure time was needed to kill all protoscolices.

As some studies have shown, ultrasound, as a non-invasive technique, destroys the protoscolices of hydatid cyst[18,26,27]. The results of study conducted by Zou *et al.* showed that the death of protoscolices was accrued in low post-exposure temperature ( $\Delta T = 20.0$   $^{\circ}C$ ), but in our study, change of temperature was much lower ( $\Delta T = 7.7$   $^{\circ}C$ ) in repetitive mode[18]. Therefore, it seems that microwave radiation can also act as ultrasound. Nevertheless, to determine its effectiveness in comparison with ultrasound, more studies are needed.



The non-thermal effects of microwave on protoscolices are not clear, but the results of present study showed the increase of  $\Delta T$  from 25 °C to 30 °C led to an increase of mortality rate from 45% to 100%, respectively.

To date, many scolicidal agents have been used for inactivation of protoscolices in hydatid cyst. An ideal scolicidal agent might produce its lytic effect at a low concentration, within a short time, and with the least side effects[28]. Significant side effects caused by agents such as hypertonic saline 20%, silver nitrate 20%, cetrimide 0.5%–1.0% and ethyl alcohol 95% have limited their use and applicability[25,29]. It seems that microwaves, especially in the repetitive mode, may be used as a supplementary measure for both treatment and prevention of hydatidosis. This method is noninvasive, and therefore, promises to minimize the risk of cyst rupture. However, extensive application of the method requires additional research in this issue.

### Conflict of interest statement

We declare that we have no conflict of interest.

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