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Effect of Direct Electrical Current on Protoscoleces of Echinococcus Granulosus in vitro and on Immune Response of BALB/c Mice Against Infection with Secondary Hydatid Disease

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Abstract: The present study investigated the effect of the direct electrical current on the viability of the protoscoleces of Echinococcus granulosus in vitro using different voltages of electrical current 3, 6, 9, 12 and 15, for different durations 3, 6, 9, 12 and 15 minutes, and on the immune response in BALB/c mice against infection with secondary hydatid cysts by injecting the mice with protoscoleces treated with direct electrical currents with viability 75%, 50%, 25% and 0%, in comparison with the control group, through six months, depending on many criteria included numbers, weights and diameters of the hydatid cysts and the percentage of reduction of their numbers, non-specific immune response represented by changes in the phagocytic index and specific immune response represented by delayed type hypersensitivity test (DTHT).The results revealed that the effect of direct electrical current on protoscoleces viability in vitro increased with the increase in voltage and exposure time. the reduction in the numbers of hydatid cysts in treated mice was 100% , in groups injected with treated protoscoleces with viability 75%, 50% and 25%, respectively. A significant increase ($p<0.01$) in innate and cellular immune response in treated mice , represented by elevation in the rates of phagocytic index and delayed type hypersensitivity (foot pad thickness).

Keywords: Direct electrical current, Echinococcus granulosus, Hydatid disease, Cell-mediated immunity

Introduction

Cystic echinococcosis (CE) is a parasitic disease of global importance caused by the larval stage of *Echinococcus granulosus*. *E. granulosus* belongs to the family Taeniidae and comprises different genotypes: genotypes G1-G3 (*E. granulosus* sensu stricto), G4 (*E. equinus*). G5 (*E. ortleppi*). G6-G7, GS, G10 (*E. canadensis*) and *E. felidis* ("lion strain") (Romig *et al*.2015). E. *granulosus* sensu stricto (s.s.). and in particular the G1 genotype, is responsible for the vast majority of human CE cases worldwide (88.44%) (Alvarez Rojas *et al*., 2014). The *E. granulosus* life cycle is indirect and includes definitive and intermediate hosts. The adult tapeworm resides in the small intestine of wild and domestic carnivores. Intermediate hosts usually ungulates are infected by ingesting eggs released in the feces of definitive hosts. The carcases of infected intermediate hosts. Accidentally. Aberrant hosts can also ingest eggs and acquire infection (Eckert and Deplazes, 2004; Deplazes *et al*. 2017).

There is evidence that low voltage direct current (DC) (less than 10 V) is bactericidal and parasitocidal *in vitro* (Hejazi *et al*., 2004; Izadpanah *et al*., 1997; Cinar *et al*.2009). Electric currents may destroy cell physiological action by altering the passage of molecules through cell membrane (Kilbane and Bielaga, 1991). Sharquie *et al*. (1998) treated dermal leishmaniasis using direct current with various current intensities. The killing effect of diferent DC electric potentials against *Leishmania major in vitro* and *in vivo* has been further investigated (Hejazi *et al*., 2004). The complete destruction of human hydatid cyst protocoleces by electrolysis device has also been reported (Izadpanah, 1997).

In vivo treatments with different electric pulses on tissues and organs have developed rapidly in the past decade. Impermeable molecules gain access to cytosol using short, intense, electric pulses. Electrical stimulation (ES) has been stated as being a cell migration promoter in a study (Faurie *et al*., 2005). Studies put emphasis on the fact that electrical fields stimulate the migration of macrophages, corneal epithelial cells, and fibroblasts (Cho *et al*., 2000). Electric currents applied to wounded tissue increase neutrophil and macrophage migration (Fberhardt *et al*., 1986). Membrane permeability increases under exposure to an external electric field pulse of power frequency (Aydin *et al*., 2006). Critical field intensity may cause alteration of local membranes. This previously unknown phenomenon is called electropermeabilization or electroporation (Neumann *et al*., 1989; Teissie,2002).

In the present work, we attempted to optimize the voltage and time of induction for killing hydatid cysts protoscoleces, using low voltage direct electric currents *in vitro*, and the effect of these voltages on hydatid disease in BALB/c mice.

Materials and Methods

Laboratory Animals

3-4 weeks old, laboratory- bred, helminth- free, males of *Mus musculus* (BALB/c) mice were used.

Cyst Collection and Viability Assessment

Hydatid cysts of sheep origin (image 1) were obtained from infected livers of sheep slaughtered in Ninevah Slaughter House. Protoscoleces were removed from the cysts, aseptically, according to Smyth (1985). Viability was estimated according to Smyth and Barett (1980). Protoscoleces, approximately 2000/ mouse were injected intraperitoneally according to Wangoo *et al*. (1989).

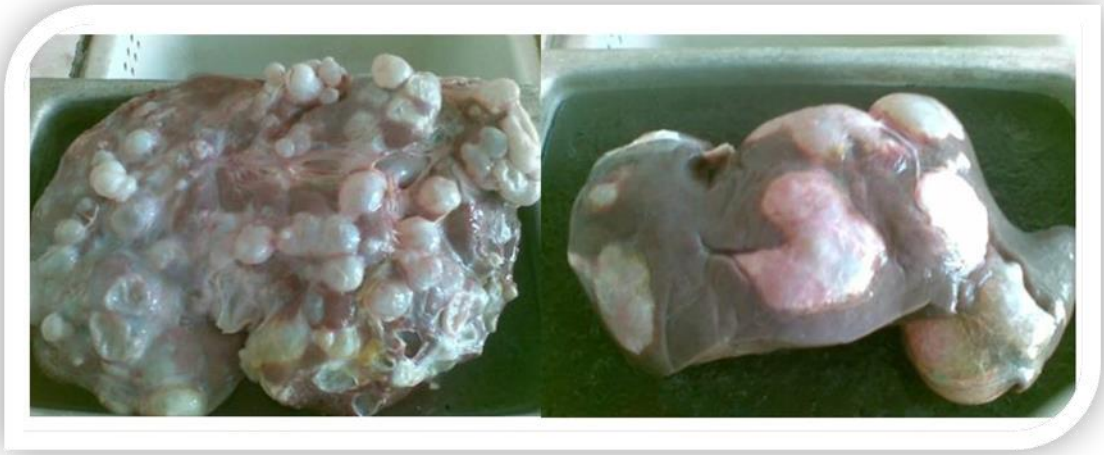

Image 1. Hydatid cysts in the livers of sheep origin

Electrical Device Design

The electrical device (image 2) consists of power supply unit, current measurement unit, and exposure unit (Fig 1). Five voltages were applied on protoscoleces *in vitro*, 3(10mA/cm^2), 6(120mA/cm^2, 9(270mA/cm^2), 12(480mA/cm^2), 15(580mA/cm^2), in Phosphate Buffer Saline(PBS) for 3, 6, 9, 12, 15 minutes.Protoscoleces (alive and dead) wree tested by eosin pigment under light microscope according to the movement, abnormalities, and staining with the pigment.

Image 2. Direct electrical current device

Effect of electric Current *in vivo*

After exposure to electric current and assesment of Protoscoleces viability , 75%, 50%, 25%, 0%, viable Protoscoleces were chosen foe experiments in vivo as treated groups, in addition to the control group (untreated with electric current) with viability 98%. 2000 protoscoleces treated with E.C.with viability 75%, 50%, 25%, 0%, were injected intraperitoneally in mice(5 for each experiment) as group (1,2, 3, 4,), 5 mice were injected intraperitoneally with 2000 untreated protoscoleces with E.C. as control goup (group 5). Treated and un treated groups were dissected 1, 2, 3, 4, 5, 6, months post infection.

Phagocytosis

Mice were anesthetized and blood was pulled from eyes according to Waynforth (1980). Phagocytosis was applied by nitro-blue tetrazolium (NBT) according to Park *et al*. (1968) and the phagocytic index was estimated as follows:

$$\text{Phagocytic index} = \frac{\text{No. of neutrophils reduce pigments} \times 100}{\text{Total no. of neutrophils}}$$

Preparation of Protoscoleces Antigen and Footpad Thickness

Protoscoleces antigen was prepared according to Dottorini *et al.*(1985). Protein was estimated according Schacterle and Pollack (1973). Footpad response to parasite antigen was estimated according to Ali-Khan (1978).

Stastistical Analysis

Resulsts analyzed statistically by Complete Randomized Design (CDR), and Duncan's Multiple Range Test was applied to detect the significant differences between different voltages and periods of the electric currents effect on the viability of protoscoleces *in vitro*, and between mice injected with protoscoleces treted with direct electric current *in vivo* in comparison with the untreated control group (Gomez and Gomez, 1983).

Results

DC treatment had a marked effect on the cellular viability of protoscoleces, induced an identical and significant ($P < 0.01$) decrease in the number of viable cells in phosphate buffer saline, which was proportional to the

duration of treatment, and the voltage culminating in killing around 100% of the protoscoleces at 6 volt after 9, 12,15 minutes , and at 9, 12, 15 volt after 3, 6, 9, 12 and 15 minutes (Table 1, images 3,4).

Table 1. Effect of direct electric current on the viability of protoscoleces (sheep origin) in phosphate buffer saline *in vitro*

Volt	**Time (minutes)**					
	0	**3**	**6**	**9**	**12**	**15**
	(control)					
3		94a±0.707	89b±0.805	86c±0.53	83d±1.003	81±0.715
6		75f±0.803	20g±0.77	0h	0h	0h
9	98	0h	0h	0h	0h	0h
12		0h	0h	0h	0h	0h
15		0h	0h	0h	0h	0h

Similar letters = no significant differences, Different letters= significant differences, Numbers below= standard deviation. ± means the standard deviation

Image 3. Untreated protoscoleces with direct electric current, magn. 40x

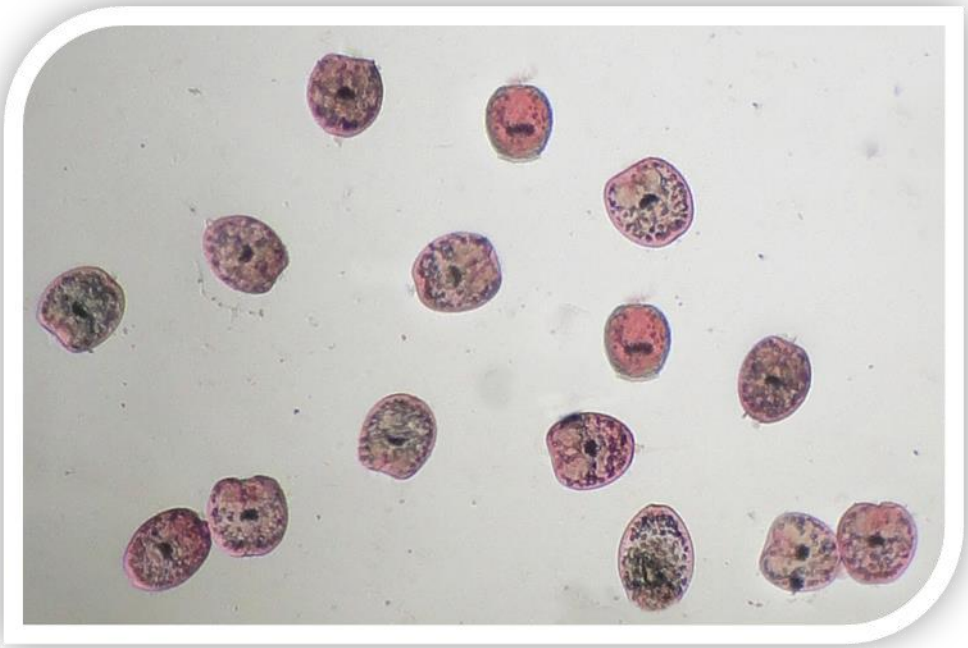

Image 4. Treated protoscoleces with direct electric current, magn. 40x

Table 2. Effect of direct electric current on means, diameters, weights and percentage reduction of hydatid cysts in mice infected with treated protoscoleces in comparison with control for six months

Months	Hydatid cysts (control)			Weight mean(mg)	Hydatid cysts (treated)	Percentage reduction
	No. mice	Number mean	Diameter mean (mm)			
1	5	4.6 0.8944±	1.34±0.183	1.54 0.326±	0	**100 %**
2	5	7.4 1.816±	1.99±0.314	2.82 0.457±	0	**100 %**
3	5	15 6.164±	2.93±0.491	29.2 5.452±	0	**100 %**
4	5	26.8 2.683±	3.31±0.338	20.6 7.162±	0	**100 %**
5	5	15.8 10.756±	5.61±0.662	78 23.657±	0	**100 %**
6	5	12.4 4.098±	6.81±0.368	136 48.437±	0	**100 %**

Protoscoleces with different viability didn't grow to hydatid cysts in treated group, therefore, No cysts were seen in mice infected with protoscoleces treated with electic current, and the percentage reduction was 100%, along six months, in comparison with the control group (Table 2, images 5,6,7). ± means the standard deviation

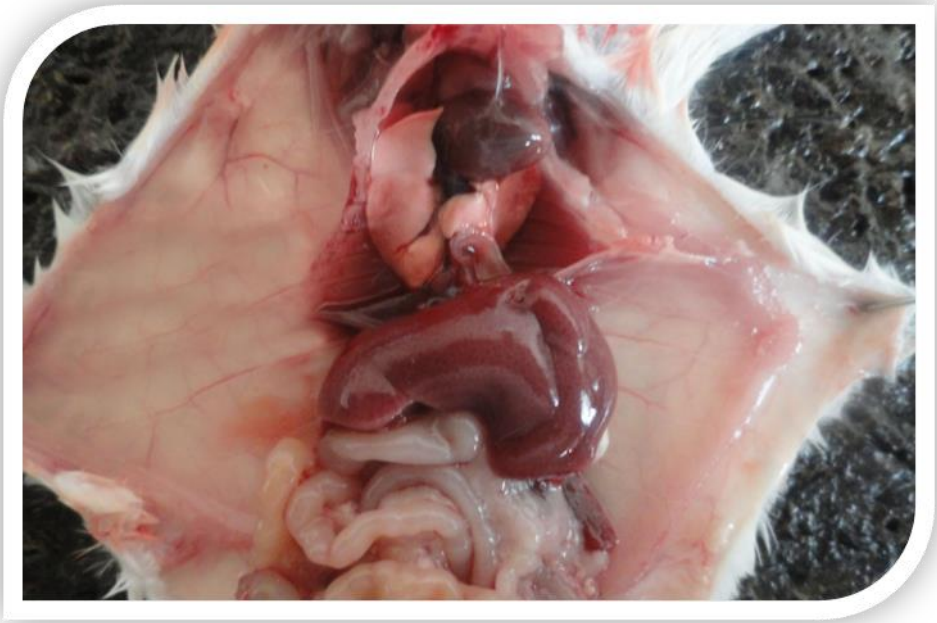

Image 5. Mouse infected with protoscoleces treated with direct electric current (free of hydatid cysts)

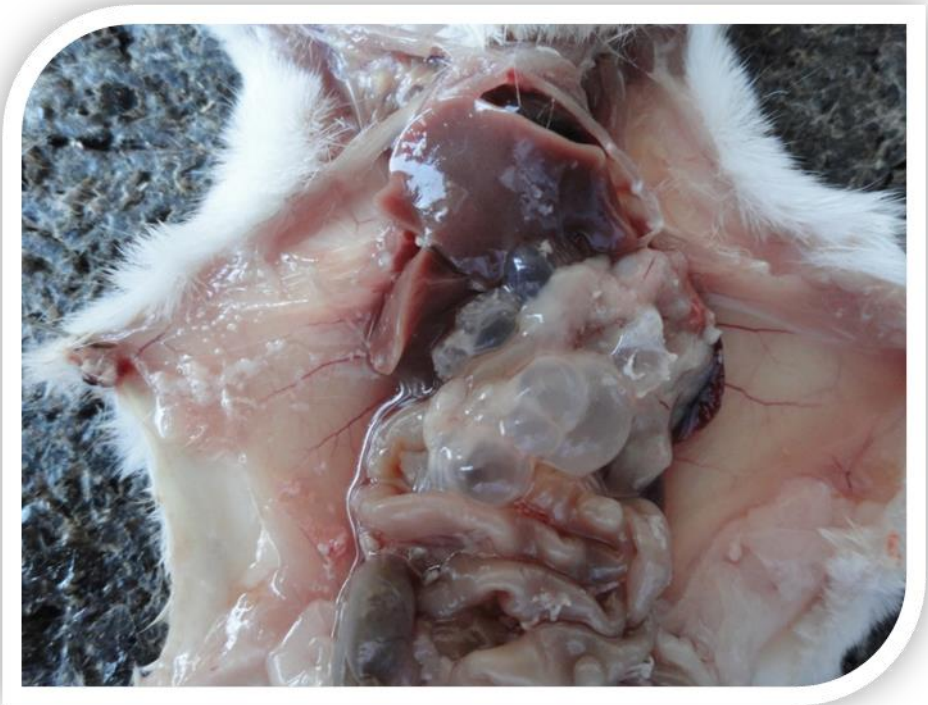

Image 6. Secondary hydatid cysts in mouse infected with protoscoleces untreated with direct electric current (5 months postinfection).

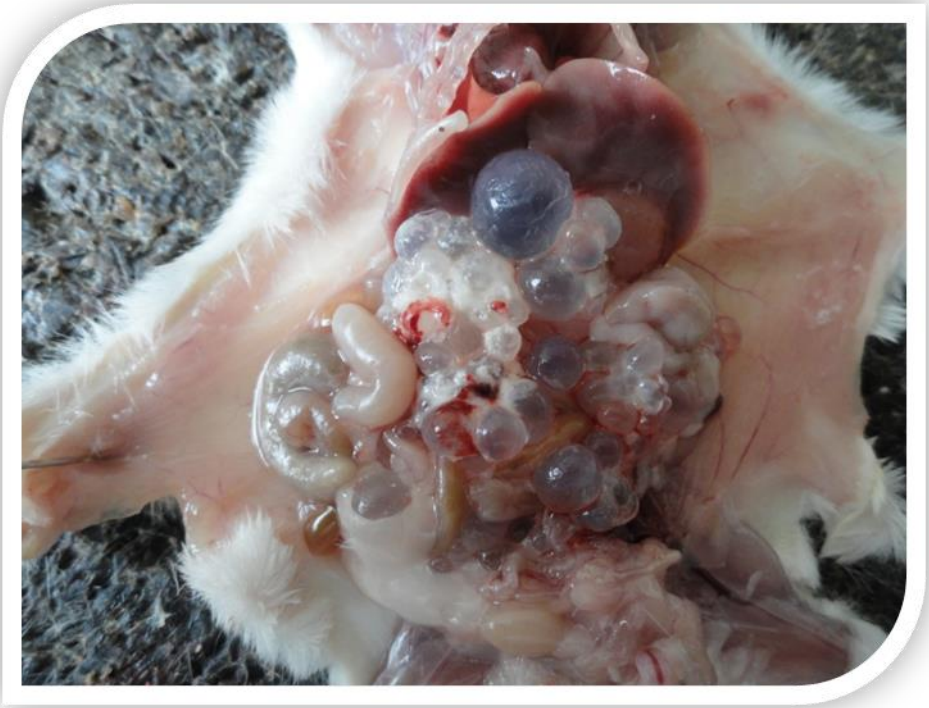

Image 7. Secondary hydatid cysts in mouse infected with protoscoleces untreated with direct electric current (6 months postinfection)

Table 3. Effect of direct electric current on phagocytic index (%) in mice infected with treated protoscoleces in comparison with control for six months

	Months	**Viability**			
	c	**75%**	**50%**	**25%**	**0%**
1	41.2 c	56.8 b	64.6 a	62.4 ab	61.8 ab
	3.114±	5.31±	2.88±	1.516±	7.049±
2	40.8 c	56.6 b	65 a	67 a	60.6 ab
	4.816±	4.56±	2.828±	3.464±	8.018±
3	35 d	49.4 c	58.2 b	62.4 ab	66.2 a
	3.674±	1.516±	3.898±	3.209±	4.658±
4	43.6 c	55 b	60.4 ab	64 a	65.2 a
	4.159±	3.674±	4.669±	5.958±	7.661±
5	42 b	67.6 a	69.8 a	70.6 a	67.4 a
	4.427±	3.781±	2.683±	3.646±	4.335±
6	37.4 c	54.8 b	59.8 a	63 a	61.8 a
	2.302±	3.898±	2.387±	4.636±	3.563±

Similar letters = no significant differences, Different letters= significant differences. ± means the standard deviation

Phagocytic index increased in all treated groups along six months, with significant differences at (P≤0.01) , reached the maximum 70.6(for viability 25%) five months postinfection, in comparison with the control group 42 (Table 3).

Table 4. Effect of direct electric current on foot pad thickness in mice infected with treated protoscoleces 3 hours post injection with antigen in comparison with control for six months

Months	**Viability**				
	c	**75%**	**50%**	**25%**	**0%**
1	0.966 a	1.114 a	1.41 a	1.296 a	1.306 a
	0.576±	0.279±	0.175±	0.25±	0.218±
2	1.254 a	1.578 a	1.328 a	1.43 a	1.412 a
	0.06±	0.28±	0.66±	0.321±	0.307±
3	0.95 a	1.092 ab	1.152 ab	1.212 ab	1.304 a
	0.24±	0.196±	0.121±	0.232±	0.117±
4	0.632 b	0.734 ab	0.882 a	0.766 ab	0.886 a
	0.114±	0.111±	0.097±	0.083±	0.213±
5	0.564 c	0.722 b	0.822 a	0.686 b	0.748 ab
	0.06±	0.067±	0.075±	0.051±	0.071±
6	0.788 b	1.024 a	0.994 a	0.872 ab	0.92 ab
	0.064±	0.1±	0.072±	0.125±	0.26±

Similar letters = no significant differences, Different letters= significant differences, ± means the standard deviation.

Delayed- type hypersensitivity represented by Foot pad thickness reached the maximum in treated group five months postinfection, three hours postinjection with antigen, 0.722 mm(for viability 75%), 0.822mm(for viability 50%), 0.686mm (for viability 25%) and 0,748mm(for viability 0%), with signicant diffences at (P≤0.01), in comparison with the control group 0.564mm (Table 4).

Table 5. Effect of direct electric current on footpad thickness in mice infected with treated protoscoleces 24 hours post injection with antigen in comparison with control for six months

Months	**Viability**				
	c	**75 %**	**50%**	**25%**	**0%**
1	0.604 b	0.776 ab	0.716 ab	0.846 a	0.906 a
	0.124±	0.281±	0.135±	0.126±	0.117±
2	0.296 c	0.564 b	0.878 a	0.358 c	0.554 b
	0.113±	0.155±	0.061±	0.093±	0.165±
3	0.448 b	0.738 a	0.684 a	0.624 ab	0.71 a
	0.192±	0.134±	0.169±	0.189±	0.075±
4	0.236 c	0.5 a	0.372 b	0.358 b	0.334 b
	0.041±	0.089±	0.054±	0.05±	0.085±
5	0.216 b	0.416 a	0.354 a	0.44 a	0.364 a
	0.075±	0.074±	0.055±	0.121±	0.123±
6	0.462 b	0.646 a	0.626 a	0.672 a	0.586 ab
	0.122±	0.085±	0.127±	0.16±	0.055±

Similar letters = no significant differences, Different letters= significant differences. ± means the standard deviation

Whereas the maximum foot pad thickness in treated group was 0.564mm(viability 75%), 0.878mm(viability 50%), 0.358mm (viability 25%) and 0.554mm (viability 0%), two months postinfection, 24 hour postinjection with antigen, in comparison with the control group 0.296mm, with significant differences at ($P \leq 0.01$)(Table 5).

Table 6. Effect of direct electric current on foot pad thickness in mice infected with treated protoscoleces 48 hour post injection with antigen in comparison with control for six months

Months	**Viability**				
	c	**75%**	**50%**	**25%**	**0**
1	0.17 b 0.079±	0.462 a 0.039±	0.244 b 0.085±	0.298 b 0.156±	0.582 a 0.173±
2	0.092 c 0.105±	0.34 ab 0.173±	0.478 a 0.094±	0.482 a 0.119±	0.22 bc 0.05±
3	0.266 b 0.115±	0.45 ab 0.204±	0.53 a 0.166±	0.336 ab 0.106±	0.36 ab 0.158±
4	0.146 b 0.091±	0.31 a 0.161±	0.23 ab 0.089±	0.334 a 0.048±	0.346 a 0.054±
5	0.17 b 0.054±	0.25 ab 0.083±	0.302 a 0.052±	0.28 a 0.046±	0.326 a 0.06±
6	0.118 b 0.084±	0.23 a 0.083±	0.226 a 0.015±	0.208 ab 0.074±	0.212 ab 0.074±

Similar letters = no significant differences, Different letters= significant differences. ± means the standard deviation

The maximum foot pad thickness in treated group was 0.462mm (viability75%), 0.244mm(viability 50%), 0.298mm(viability 25%) and 0.582mm(viability 0%) , one month postinfection with treated protoscoleces, 48 hour postinjection with antigen, at signicant differences ($P \leq 0.01$), in comparison with control group 0.17mm (Table 6).

Discussion

Electrical current was applied in the last decades for treatment of some parasitic infections (Dalimi *et al*. 2005; Jennings, 2010; Habel, 2010), bacterial infections and wound healing (Cinar *et al*., 2009; Champman-Jones *et al*.,2010; Szkotak *et al*., 2011; Wei *et al*., 2011), fungal infections (Kalinowski, 2004; Stoica *et al*., 2011), and some neurological diseases (Brunoni *et al*. 2013; Datta *et al*., 2013). Gomez *et al*.,(2012) mentioned that the direct electric current inhibits cells viability and this effect is in prportion with the time of exposure.

DC can produce several biological effects on living systems, which are largely studied for therapeutic purposes. Generation of oxidative/reductive electrolysis products and pH variation are thought to be the main events responsible for cell and tissue destruction after DC application [Veiga *et al*., 2000; Holandino *et al*., 2001; von Euler *et al*., 2002).

Electrical stimulation was used by Sharquie *et al.* [1998] for the treatment of cutaneous Leishmania lesions in 146 patients in 54 weekly, 10 min sessions of DC between 5 and 15 mA. Of the 146 injuries, 135 (92.5%) had complete or partial improvement after 4-6 weeks. In 2004, an *in vitro* and *in vivo* study with Leishmania major showed loss of protozoan viability and healing of lesions in infected mice after DC exposure [Hejazi *et al*., 2004).

The present study reported a dose-dependent antiparasitic effect of DC on protoscoleces of *Echinococcus granulosus in vitro*, These results are similar to the study of Gomes *et al*. (2012) on the effect of Dc as anti-protozoan on *H. samuelpessoai*. The 2 mA intensity applied caused a decrease in cell viability that was directly proportional to the time of exposure. Considering the morphological surface changes observed in *H. samuelpessoai*, it is likely that molecules involved in parasite-host cell interactions would be affected by DC, which could represent an interesting effect in a therapeutic context (Gomes *et al*.2012). The t study showed that DC killed *H. samuelpessoai* cells by multiple mechanisms, depending on the time of exposure and the current potential.

In this *in vitro* study, it is clear that killing the parasites by direct electric current is dependent on the applied electric current and potential. The higher current can affect the ion transport of parasite membrane to a greater extent resulting in destruction of its membrane and leading to loss of the viability of the parasites (Dalimi *et al*. 2005). In fact, biological membranes transport groups of ions and polar molecules that can maintain potentials and electrostatic charges, and the integrity of the cell membrane is related to these effects (Kilbane and Bielaga, 1991; McLaughlin, 1989). External electrical currents may also draw vital compounds from the cell cytoplasm and so inhibit the cell's normal physiological activity (Kilbane and Bielaga, 1991).

In the present study, there is a direct relation between the death of protoscoleces and the time and intensity of the electric current that is applied. Temperature and pH may also affect the experimental results. In this study, the impact of the temperature on the inactivation of protoscoleces was limited because the intensity of the current applied was low (in the mA range), and by using carbon electrodes the temperature alteration can be minimized. Changes in the pH of the medium or eosin vital dye can have a great influence on the protoscolex viability..(Dalimi *et al*., 2005). The electrolysis products generated near the electrodes are the main agents responsible for cell killing [Veiga *et al*., 2005; Holandino *et al*., 2001).

Many of the electric current effects observed in -treated cells may be directly or indirectly associated with changes in the physical and chemical properties of membrane lipids. These changes can be caused by oxidation or reduction of these molecules by the electrolytic products generated by electrodes. On the other hand, Gomes *et al*. (2012) explained that AF clearly caused a marked destabilization on the surface structure of the cell, resulting in an irregular pattern with many membrane projections and detaching fragments.

In vivo the electric current act to inhibit or stop the physiological reactions of the cell by changing the pass pathway of the molecules through the cell membrane (Kilbane and Bielage, 1991). In 2004, an *in vitro* and *in vivo* study with *Leishmania major* showed loss of protozoan viability and healing of lesions in infected mice after DC exposure [Hejazi *et al*., 2004). It is also known that electrical stimulation may play an important role in promoting the healing of intractable ulcers and other cutaneous lesions, leading to skin regeneration [Huckfeldt *et al*., 2007; Talebi *et al*., 2007].

Results of the present study revealed an obvious increase in phagocytic index in treated animals in comparison with the control group, this attributed to the effect of the direct electric current which causes division and infiltration of neutrophils and monocytes the main phagocytic cells (Eberhardt *et al*., 1986; Cho *et al*., 2000; Faurie *et al*., 2005). DC-treated parasite cells confirmed the presence of autophagosomes, vesicles containing concentric membranes and organelle leftovers, as well as vacuoles surrounding the endoplasmic reticulum. These features are both suggestive of autophagy, an attempt of the cell to recycle damaged structures (Gomes *et al*., 2012).

Results of cellular immunity represented by delayed type hypersensitivity revealed an obvious increase in foot pad thickness in animals treated with electrical current reached the maximum 3 hours post injection with the antigen in comparison with the control group, then the thickness declined 24 , 48 hours post injection. Increase in cellular immunity is attributed to the ability of electric current to stimulate cellular immunity (Song *et al*., 2007; Cinar *et al*., 2009), ,activate phagocytes and lymphocytes (Weiser *et al*. 1991), and increase lymph compounds secretion which regulate immune response (AbulAbbas and Lichtman,2005; Male *et al*., 2007). Studies revealed that electrical fields stimulate phagocytes, neutrophils migration and inflammatory reactions by inflammatory cells migration (Faurie *et al*., 2005; Cinar *et al*., 2009). Electrical current also stimulates INF-γ, TNF-α,Willey *et al*.(2008) explained that IL-2, INF-γ and TNF-α secreted by TH1 are responsible for delayed hypersensitivity reactions. Footpad thickness is an indicator of cellular immunity and it is attributed to the filtration of neutrophils, phagocytes, monocytes and lymphocytes (Ryu and Kim, 2000).

It may well be concluded that direct electrical current has an obvious effect on protoscoleces viability *in vitro*, and against secondary hydatid disease *in vivo*.

References

Abul Abbas K. , and Lichtman A. H. (2005) Cellular and molecular immunology . 5th.ed. Elsevier sanders . Philadelphia USA .

Ali Khan Z. (1978) *Echinococcus multilocularis* : Cell mediated immune response in early and chronic alveolar murine hydatidosis. Exp. Parasitol. , 46 :157-165 .

Alvarez Rojas CA, Romig T, Lightowlers MW. 2014. Echinococcus granulosus sensu lato genotypes infecting humans - review of current knowledge of current knowledge. International Journal for Parasitology, 44, 9-18.

Aydin MA, Comlekci S, Ozguner M, Cesur G, Nasir S, Aydin ZD (2006) The influence of continuous exposure to 50 Hz electric field on nerve regeneration in a rat peroneal nerve crush injury model. Bioelectromagnetics 27:401-413. doi:10.1002/bem.20221

Brunoni, A.R. (2013) The Sertraline vs Electrical current Therapy for Treating Depression Clinical study Result From a Factorial, Randomized, Controlled Trial. Journal of the American Medical Association. 32:1-9.

Champman-Jones, D., Young, S. and Tadej (2010) Assessment of wound healing following electrical stimulation with accel-heal. 6 (3): 67-71.

Cho MR, Thatte HS, Lee RC, Golon DE (2000) Integrin dependent human macrophage migration induced by oscillatory electrical stimulation. Ann Biomed Eng 28:234-243. doi:10.1114/

Cinar, K., Comlekci, S. and Senol, N. (2009) Effects of a specially Effects of a specially pulsed electric field on an animal model of wound healing. Lasers Med Sci. 24:735–740.

Dalimi A. , Ghasemikhah R. and Hashemi Malayer B. (2005) *Echinococcus granulosus* : Lethal effect of low voltage direct electric current on hydatid cyst protoscoleces . Exp. Parasitol. , 109 : 237-240.

Datta, A., Dmochowski, J.P., Guleyupoglu, B., Bikson, M. and Fregni, F. (2013) Cranial electrotherapy stimulation and transcranial pulsed current stimulation: A computer based highresolution modeling study. NeuroImage 65:280–287.

Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, Antolova D, Schurer JM, Lahmar S, Cringoli G, Magambo J, Thompson RCA, Jenkins EJ. 2017. Global distribution of alveolar and cystic echinococcosis. Advances in Parasitology, 95, 315-493.

Dottorini S. , Sparvoli M. , Bellucci C. and Magnini M. (1985) *Echinococcus granulosus* : Diagnosis of hydatid disease in man . Am. Trop. Med. Parasitol. , 74: 43-49.

Eberhardt A, Szczypiorski P, Korytowski G (1986) Effects of transcutaneous electrostimulation on the cell composition of skin exudate. Acta Physiol Pol 37:41-46.

Eckert J, Deplazes P. 2004. Epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. Clinical Microbiology Reviews, 17, 107-135.

Faurie C, Golzio EP, Teissie J, Rols MP (2005) Electric field induced cell membrane permeabilization and gene transfer: theory and experiments. Eng Life Sci 5:179–186.

Gomes M. N. , Teixeira C. A. A. , Barbosa G. M. , Froes M. T. , Silva M. B. , Veiga V. F. , Soares R. M. A. , Santos A. L. S. and Holandino C. (2012) Effects of direct electric current on *Herpetomonas samuelpessoai* : An Ultrastructural Study . Bioelectromagnetics 33 : 334-345 .

Gomez, K. A. and Gomez, A. A. (1983) Statistical procedures for agricultural. Research 2nd ed., John Wiley and sons New York.

Habel A. (2010) Electrokinetic management of biosolids for the inactivation of helminth ova . Concordia University . ProQuest® Dissertations and Theses . 1 : 165 .

Hejazi H. , Eslami G. and Dalimi A. (2004) The parasiticidal effect of electricity on *Leishmania major*, both *in vitro* and *in vivo* . Ann. Trop. Med. Parasitol. , 98(1) : 37-42 .

Holandino C. , Veiga V. F. , Rodrigues M. L. , Morales M. M. , Capella M. A. M. , Alviano C. **S.** (2001) Direct current decreases cell viability but not P-glycoprotein expression and function in human multidrug resistant leukemic cells. Bioelectromagnetics 22 : 470-478 .

Huckfeldt R, Flick A, Mikkelson D, Lowe C, Finley P. 2007. Wound closure after split-thickness skin grafting is accelerated with the use of continuous direct anodal microcurrent applied to silver nylon wound contact dressings. J Burn Care Res 28:703-707.

Izadpanah, A., 1997. The effect of direct current on scolices. In: 18th International Hydatidology Congress, Lisbon, Portugal.

Jennings J. A. , Chen D. and Feldman D. S. (2010) Upregulation of chemokine (C–C motif) ligand 20 in adult epidermal keratinocytes in direct current electric fields . Arch. Dermatol. Res. 302:211-220 .

Kalinowski D. P. , Edsberg L. E. , Hewson R. A. , Johnson R. H. and Brogan M. S. (2004) Low-voltage direct current as a fungicidal agent for treating onychomycosis . J. Am. Podiat. Med. Assoc. , 94(6) : 565-572.

Kilbane, J.J., Bielaga, B.A., 1991. Instantaneous gene transfer from donor to recipient microorganism via electroporation. Biotechniques 10, 354–365.

Male D. , Brostoff J. , Roth D. and Roitt I. (2007) Immunology 7th ed. Inter. Edith Mosby Elsevier ltd.

McLaughlin, S., 1989. The electrostatic properties of membrane. Annual Review of Biophysics and Biophysical Chemistry 18, 113-136.

Neumann E, Sowers AE, Jordan CA (1989) Electroporation and electrofusion in cell biology. Plenum Press, New York, pp 100 -120

Park P. H. , Filkring S. M. and Smith W. E. M. (1968) Infection and nitro blue tetrazolium reduction by neutrophils . Lancet , 2: 532-534 .

Romig T, Ebi D, Wassermann M. 2015. Taxonomy and molecular epidemiology of Echinococcus granulosus sensu lato. Vet. Parasitol. 213, 76-84.

Ryu H. and Kim C. (2000) Immunologic reactivity of a lipopoplysaccharide-protein complex of type A *Pasteurlla multocida* in mice . J. Vet. Sci. , 1 (2): 87-95 .

Schacterle G. R. and Pollack R. L. (1973) A simplified method for the quantitative assay of small amount of protein in biologic material . Anal. Biochem. , 51: 654-655 .

Sharquie, K.E., al-Hamamy, H., el-Yassin, D., 1998. Treatment of cutaneous leishmaniasis by direct current electrotherapy: The Baghdadin device. Journal of Dermatology 25, 234-237.

Smyth J. D. (1985) *In vitro* culture of *Echinococcus spp*. Proc. 13th ed., Int. Cong. Hydit. Madrid , 84-95 .

Smyth J. D. and Barett N. J. (1980) Procedure for testing the viability of human hydatid cysts following surgical removal especially after chemotherapy . Trans. Roy. Soc. Trop. Med. Hyg. , 74: 649-652.

Song B. , Gu Y. , Pu J. , Reid B. , Zhao Z. and Zhao M. (2007) Application of direct current electric fields to cells and tissues *in vitro* and modulation of wound electric field *in vivo* **.** Nature protocols , 2 (6) : 1479-1489 **.**

Stoica, M., Bahrim, G. and Carac, G. (2011) Factors that influence the electric field effects on fungal cells. Science aginst microbial pathogens: communication current research and technological advances. A. Mendz-vilas(Ed) ©Formatex.291-302.

Szkotak, R., Niepa, T.H.R., Jawrani, N., Gilbert, J.L., Jones, M.B. and Ren, D. (2011) Differential Gene Expression to Investigate the Effects of Low-level Electrochemical Currents on *Bacillus subtilis*. AMB Express 2011, 1(39) 1-12.

Talebi G, Torkaman G, Firoozabadi M, Mofid M, Shariat S, Kahrizi S. 2007. Effects of micro-amperage direct current stimulation on injury potential and its relation to wound surface area in guinea pig. Conf Proc IEEE Eng Med Biol Soc 2007:3516-3519.

Teissie J (2002) Recent biotechnological developments of electropulsation. A prospective review. Bioelectrochemistry 55:107-112. doi:10.1016/S1567-5394(01)00138-4. U.K.

Veiga V. F. , Nimrichter L. , Teixeira C. A. A. , Morales M. M. , Alviano C. S. , Rodrigues M. L. , Holandino C. (2005) Exposure of human leukemic cells to direct electric current : Generation of toxic compounds inducing cell death by different mechanisms . Cell Biochem. Biophys. 42 : 61-74 .

Vijk AK. 2004. Electrochemical treatment (ECT) of cancerous tumours: Necrosis involving hydrogen cavitation, chlorine bleaching, pH changes, electroosmosis. Int J Hydrogen Energ 29:29663–29665.

von Euler H, Soderstedtb A, Thornec A, Olssonb J, Yongqingb G. 2002. Cellular toxicity induced by different pH levels on the R3230AC rat mammary tumour cell line. An in vitro model for investigation of the tumour destructive properties of electrochemical treatment of tumours. Bioelectrochemistry 58:163–170.

Wangoo ,A., Ganguly N.K. and Mahgjan R.C. (1989). "Phagocytic activity of monocytes in murine model of *Echinococcus granulosus* of human origin". J. Med. Res., 89: 40-42. Waynforth H. B. (1980) Experimental and Surgical Technique in the Rat . Academic Press Inc. , (London) LTD , NWI , 29.

Wei, V., Elektorowicz, M. and Oleszkiewicz, J.A. (2011) Influence of electric current on bacterial viability in wastewater treatment. Water research. 45:5058-5062.

Weiser, W. Y., Pozzi, L. A. and David, J. R. (1990) Human recombinant migration inhibition factor activates human macrophages to kill Leishmania donovani. J. Immnunol., 174: 2006-2011.

Willey J. M. , Sherwood L. M. and Woolverton C. J. (2008) Prescott , Harley and Klein's Microbiology. 7th ed. , McGraw-Hill Companies , Inc. , USA , 782-788 .

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