Effect of electric current on the activity of the protoscolices of the Echinococcus granulosus

Jasim Hameed Rahma (Kufa college of Education for girls); **Najim Abdul-Wahid** (Al-Qadisiya college of science); **Fatima Adnan Ali Al-Zubaidi** (Kufa College of Pharmacy); **Najah R. Hadi Al-Mousawi** (Kufa college of Medicine)

Abstract

The present study was undertaken to asses the effect of different direct electric current (200mA, 400mA and 600mA) on the activity of protoscolices of the larval stage of Echinococcus granuosusin vitro and in vivo. The hydatid cysts were collected from the sheep livers in the Al-Najaf Al-Ashraf carnage, they were divided into 5 groups(5 cysts in each group), the first group was considered as the control group where there was no electric current used, in the other 4 groups an electric current 200mA,400mA, 600mA and 800mA passed through the hydatid cysts respectively, each cyst then was opened separately and its fluid was collected in sterile test tubes, then the protoscolices were isolated to evaluate their activity. 20 rats were enrolled in this study (5 rats in each group). The first group was considered as the positive control group in which rats were injected with 1ml of protoscolices from the group that did not expose to electric current. The second group was considered as the negative control group in which rats were injected with 1ml of normal saline not exposed to electric current. In the third and forth groups rats were injected with 1ml of protoscolices that were exposed to electric current 600mA and 800mA respectively. After 3 months the rats underwent laparatomy and biopsies from the liver, spleen and lung were taken to study the histopathologic changes.The in vitro study showed that there were significant effects and the percent of killing of the peotoscolices reached 100% both at zero time (at 800mA) and at the fourth minute (at 600mA). While the in vivo study showed that the number and size of the cysts in the body organs like liver, lung and spleen were decreased in the third and forth group when compared with the positive control group. Microscopically, there were inflammatory cells and cysts in the infected organs of the positive control group which were disappeared when treated with the electric current without any histological changes.

Introduction

Echinococcus also called hydatid disease or echinococcal disease is a widely spread disease that is transmitted between huma and animals as sheep, dogs, rodents and horses (1), it is caused by the larval/hydatid cyst stage of the Echinococcus granulosus, its eggs contain an embryo that is called an oncosphere, From the embryo released from an egg develops a hydatid cyst, which grows to about 5-10 cm within the first year and is able to survive within organs for years (2). Cysts sometimes grow to be so large that by the end of several years or even decades, they can contain several liters of fluid. Once a cyst has reached a diameter of 1 cm, its wall differentiates into a thick outer, non-cellular membrane, which covers the thin germinal epithelium. From this epithelium, cells begin to grow within the cyst. These cells than become vacuolated and are known as broad capsules, which are the parts of the parasite from which protoscolices bud. Over times, daughter cysts will also form within cysts (3). **Carl von Siebold** in 1850 was the first

who describe the life cycle of the parasite and determine the nomenclature of it as E.granulosus at the end of the 18 century (4). The worm has a life cycle that requires definitive hosts and intermediate hosts. Definitive hosts are normally carnivores such as dogs, while intermediate hosts are usually herbivores such as sheep and cattle. Humans function as accidental hosts, because they are usually a 'dead end' for the parasitic infection cycle. Life cycle: 1) An adult worm resides in the small intestine of a definitive host. 2) Afterwards, gravid proglottids release eggs that are passed in the feces of the definitive host. The egg is then ingested by an intermediate host. 3) The egg then hatches in the small intestine of the intermediate host and releases an oncosphere that penetrates the intestinal wall and moves through the circulatory system into different organs, in particular the liver and lungs. Once it has invaded these organs, the oncosphere develops into a cyst. 4) The cyst then slowly enlarges, creating protoscolices and daughter cysts within the cyst. The definitive host then becomes infected after ingesting the cyst-containing organs of the infected intermediate host. 5) After ingestion, the protoscolices attach to the intestine. 6) They then develop into adult worms and the cycle starts all over again (5). Echonococcus is an epidemic disease in Iraq (6,7), its widely spread in the rural areas where there are sheep, cattle and dogs that provide a suitable hosts for the parasite to be transmitted (8).

The most common form of treatment is surgical removal of the cysts combined with chemotherapy using albendazole and/or mebendazole before and after surgery. However, if there are cysts in multiple organs or tissues, or the cysts are in risky locations, surgery becomes impractical. For inoperable cases such as these, chemotherapy and/or PAIR (puncture-aspiration-injection-reaspiration) become alternative options of treatment using chemotherapy albendazole and mebendazole (9). The use of electric current in the treatment of this disease is considered to be the first trial in this field by applying a direct electric current on the cyst surface without harming the tissue and organs surrounding the cysts, so the protoscolices will be destroyed before the rupture of the cyst and the release of the protoscolices to the body, so a new electric instrument was modified as in figure (1). An Iraqi team used this instrument to kill the protoscolices inside the cyst by using the effect of heat from conical heater (40-70 c°) and 3 mm in diameter that supply heat inside the cyst. First the experiments were performed on the infected sheep and cow then the instrument was used to treat a number of patients. Results showed that the optimal degree to kill the protoscolices is 50 c° for 5 min. without any unwanted effect on the infected tissue.

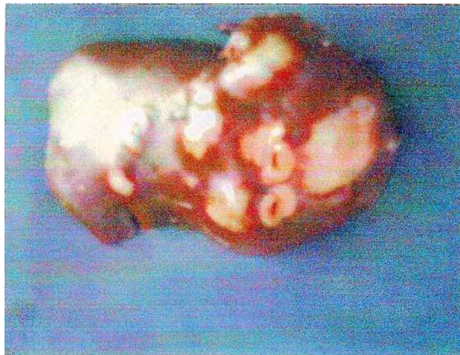
The aim of this study is to find a new method to treat Echinococcosis since the traditional drugs are not effective in the treatment. This study tried to use direct electric current to kill the protoscolices and to determine the potency of the current and the duration that are required to treat the disease without any adverse effects.

Materials and method

Twenty Sprague dawely rats were enrolled in this study. Their weight was between 170-250 g and aged between 3-6 months. The rats were housed in the animal house that belong to the department of biology/ Kufa college of Education for girls,5 rats in each cage and kept at 25 c° and 12 hours light-dark cycles with 12.00 AM being the mid-dark period. Rats had free access to drink water ad libitum.

The samples of hydatid cysts were collected from the livers of the infected sheep in Al-Najaf Al-Ashraf carnage; and put in cork containers and were sent immediately to

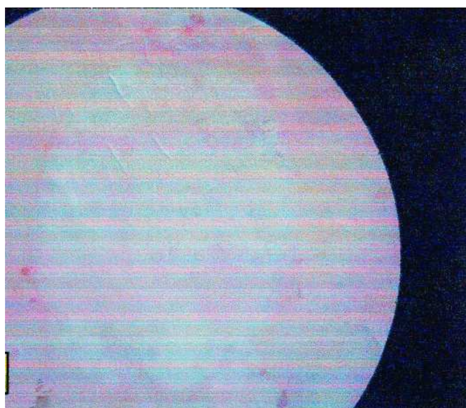
the laboratory to separate the protoscolices according to Smyth method (10), as in **figure(1,2)**.



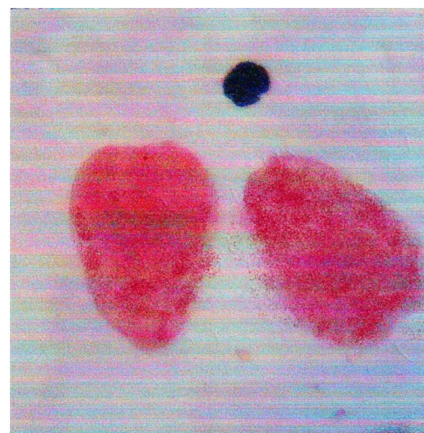
Figure(1):Piece of sheep liver infected with hydatid cysts

Figure(2): hydatid cyst isolated from sheep liver to be used for collection of protosclices.

The principle of the work depends on the diffusion of the aqueous eosin into the protoscolices to measure their viability, because eosin diffusion is a physical process that depend on the protoscolices membrane permeability that is any physiologic changes can lead to increase permeability so increase the diffusion of the stain while the viable protoscolices maintain their normal color. The activity was assessed by adding a drop of eosin stain (0.1%) to an equal volume of the protoscolices suspension using a micropipette then after shaking well a drop was taken and examined under the microscope (Olympus), the per cent of activity was measured for the viable protoscolices which were stained with green color and for dead protoscolices which were stained with red color as in **figure (3 a, b)**. The number of protoscolices was assessed by taken a constant volume of by a micropipette (10 µl) and examined it under the microscope.



Figure(3): A)viable protoscolices stained with Green color (aqueous eosin stain, X10)



B) Dead protoscolices stained with red color (aqueous eosin stain, X10)

The electric instrument design used in this study

It is known that the electric current is already an alternative current, while the experiment required a direct current. A group of diodes was connected which converts the alternative current to a direct current, so get the suitable current range(0-1000) mA and voltage 30 volt; then electric poles were used through which the current passes to the cysts. The potency of the electric current was controlled by galvanometer. The activity of protoscolices was measured after their isolation from the cysts and exposure to different potencies of current over different periods of time.

Effect of electric current on the activity of protoscolices in vitro

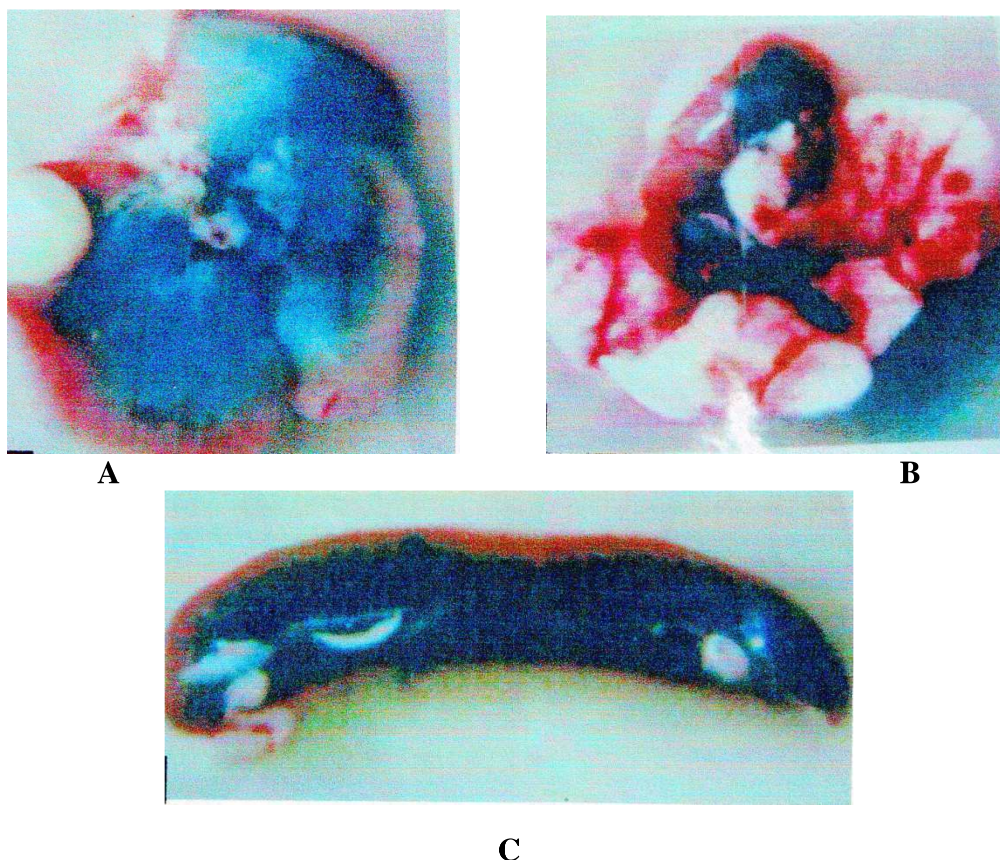
collected hydatid cysts were divided into 5 groups (5 cysts in each group). The first group was considered as the control group where there was no electric current used, in the second group 200mA electric current passed through the hydatid cysts; in the third group 400mA electric current passed through the hydatid cysts; in the forth group 600mA electric current passed through the hydatid cysts; in the fifth group 800mA electric current passed through the hydatid cysts. Each cyst then was opened separately and its fluid was collected in sterile test tubes, then the protoscolices were isolated and their activity was evaluated at different periods of time as in **table (1).**

Effect of electric current on the activity of protoscolices in vivo

Rats were divided into four groups (5rats in each one); the first group was considered as the positive control group in which rats were injected with 1ml of protoscolicesfrom the group that did not expose to electric current. The second group was considered as the negative control group in which rats were injected with 1ml of normal saline not exposed to electric current. In the third group rats were injected with 1ml of protoscolices that were exposed to electric current 600mA, while in the forth group rats were injected with 1ml of protoscolices that were exposed to electric current 800mA. After 3 months the rats underwent laparatomy to take biopsies from the liver, spleen and lung to study the histopathologic changes.

Histopathologic changes

After 3 monthsthe infected rats were anesthetized with ether and underwent laparatomy to determine the spread and number of hydatid cysts in the dufferent organs macroscopically as in figure (4). Biopsies from liver, spleen and lung were taken and kept in formalin 10% to study the histopathologic changes according to Bancroft and Steven method (14).



C

Figure (4):Gross section of the liver (A); lung (B); spleen (C), of the positive control group showing spread of hydatid cyts.

Statistical analysis

Statistical analysis has been done by using completely randomized design (CRD) and least significant difference (LSD). Significant difference was set at α=0.05.

Results

1- **Effect of different potencies of electric current on the protoscolices viability.**
The number of protoscolices decreased significantly $P< 0.05$ at the different periods and potencies **(table 1).**

Table (1): Effect of different potencies of electric current on the protoscolices viability at different periods at 20 c° and PH 7.4.

Period of exposure / Electric current Potencies	the mean of protoscolices viability / time (min.)						P value
	0 min.	4min.	6min.	8min.	10min.	15min.	
Control	98	97	95	94	94	91	
200mA	55	42	30	22	6	0	< 0.05
400mA	27	18	10	0	0	0	< 0.05
600mA	8	0	0	0	0	0	< 0.05
800mA	0	0	0	0	0	0	< 0.05

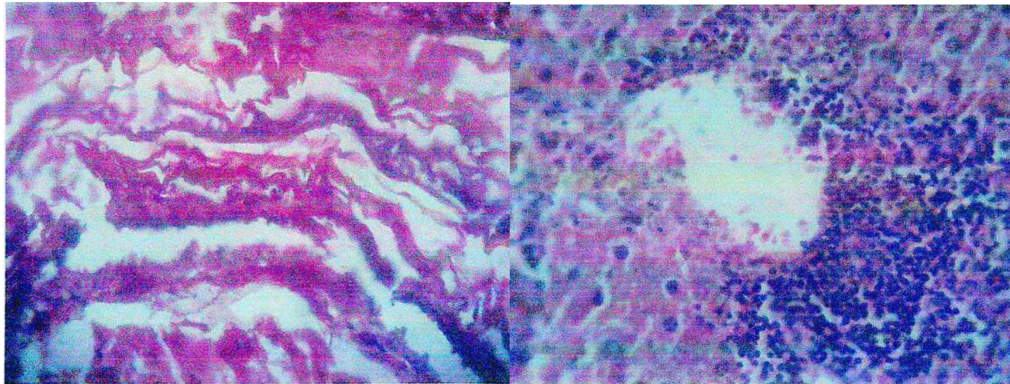
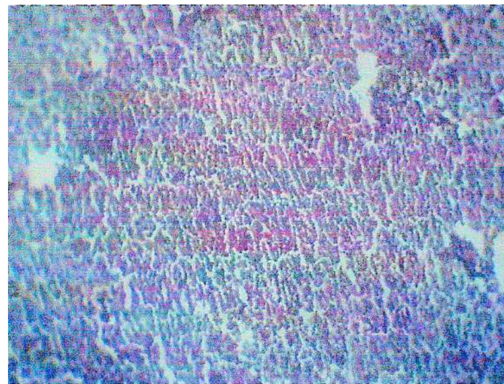
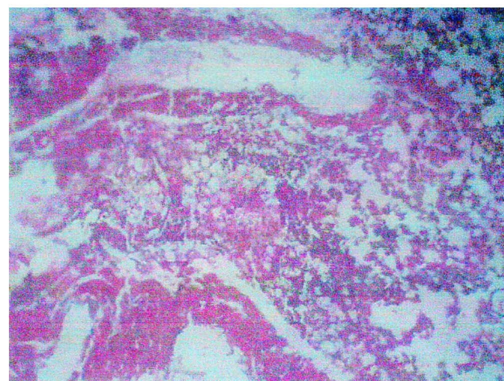


Figure (5): Histlopathological section of hepatic tissue of the positive control group showing **:(A)** The Layers of the cyst**; (B)** inflammatory cells infiltration. (eosin-haematoxylin X100)



Figure(6): Histlopathological section of spleen tissue of the positive control group showing no differentiation of the white core from the red core (eosin-haematoxylin X100)



Figure(7): Histopathological section of the lung tissue of the positive control group showing hydatid cysts with calcification inside the cysts (eosin-haematoxylin X100).

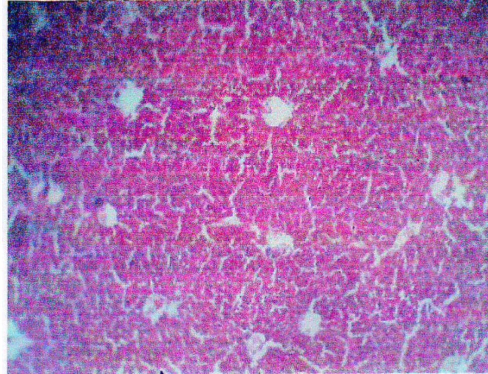


Figure (8): Histopathological section of infected hepatic tissue with protoscolices exposed to electric current 800mA showing normal tissue without any histological changes. (eosin- haematoxylin X100)

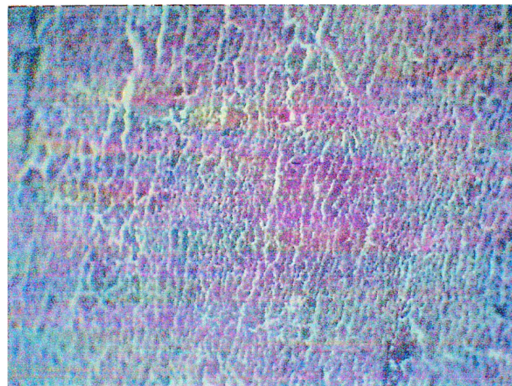


Figure (9): Histopathological section of infected spleen tissue with protoscolices exposed to electric current 800mA showing normal histological properties with differentiation between the white and red core. (eosin- haematoxylin X100)

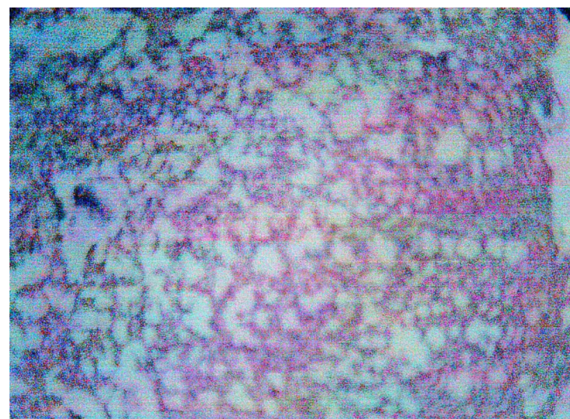


Figure (10): Histopathological section of infected lung tissue with protoscolices exposed to electric current 800mA showing normal tissue without any histological changes. (eosin- haematoxylin X100)

Discussion

The results showed that the electric current had a potent effect in the treatment of hydatid disease in vitro by killing all the protoscolices over a short period especially at a potency of 600mA and 800mA as in the figure (9,10,11). This my be due to the destruction of the spiral structure of the DNA by the electric shock so lead to the death of he protoscolices or by the destruction of the enzymes that play a role in the transcription and multiplication of the DNA, so inhibit nucleic acid and protein synthesis leading to death of protoscolices, **Chrometuna (2005)** used a direct electric current with very low voltage to determine the activity of the viruses, he found that the viruses was not be destroyed directly by the electric current but their membrane was affected and the virus became unable to synthesize the enzyme reverse transcriptase which has important role in invading the host cells and allowing the virus to inhibit the immune system (12).

The macroscopic studies showed infection of the liver, spleen and lung of the rats injected with protoscolices only, this may be due to the injected dose of the protoscolices (13), the injection lead to the growth of hydatid cysts in the infected organs, this finding in agreement with that found by Heath (14).

The microscopic studies of the rats injected with protoscolices treated with electric current showed that the current has a potent effect in killing and destroying the protoscolices and this finding in agreement with that found by Clark 2004 who discovered an electronic technique to kill viruses, germs, fungi and parasites; she designed an electronic machine called **zapper parasite** used in electrotherapy and she explained the effect of a weak electric current in killing microorganisms and parasites by that all parasites and infected tissues carry a positive charge and the electric curt diffuse the negative charge through the skin and viable tissues so the polarity of the parasite will be changed and that lead to its death (15).

The histopathological studies showed no changes in the tissues of the infected rats after exposure to the electric current, this may be due to that the electric current after entering the body may stimulate the growth of the cells by increasing ATP and stimulate DNA and protein synthesis (15).

References

1- Karebov, A.; Nahmias,J. and El-on,J., (1997). Cellular and humoral immune response of hydatidosis patients to E.granulosus. purified antigens. Am.J. Trop.Med. Hyg., 57(5): 619- 625.
2- Mandell, Gerald L. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, PA: Elsevier Inc., 2010. Ch. 290. Print.
3- John, David T. and William A. Petri. Markell and Voge's Medical Parasitology. 9th ed. St. Louis, MI: Saunders Elsevier, 2006. 224-231. Print.
4- Frayha, G.I., (1970). Studies on hydatid disease in London. Leb.Med,J.,23(2): 135-140.
5- Bgh

6- Taha, A.S., (1999). Diagnosis of ruptured pulmonary hydatid cyst by flexible fibroptic bronchoscopy, a report of 6 cases. BIS.,5: 86-92.
7- Al-Abbassy, S.N.; Al-Taif, K.L.; Jawad, A.K. and Al-Saqur, I.M. ;(1980). The prevalence of hydatid cyst in slaughtered animals in Iraq.Ann,Trop.Med,parasitolo,74 (2):185-187.
8- Kadir, M.A. and Aziz,L.J., (1999). Comparative study on chemical composition of liver hydatid cysts in some intermediate hosts. Iraq. J.Vet. Med. 23(2): 117-113.
9- Eckert, Johannes, and Peter Deplazes. "Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern." Clinical Microbiology Reviews 17.1 (2004): 107-135. Web. 5 Feb 2010.
10- Smyth, T.D.,(1985).In vitro culture of Echinococcus species in producing of the 13th Int. Congr. Of hydatidology. Madrid. pp, 84-89.
11- Banerofti, J. and Steven, A., (1982).Theory and practice of histological technique. 2nd Churchill Livingstone Edinburgh and London.
12- Chrometuna, (2005). The story of blood electrification. Topeka. DIY News.V.(11) : 1-5.
13- Colli, C.W. and Schantz, P.M., (1974). Growth and development of E.granulosus. from embryophores in an abdominal host (Mus. Muscularis). I. Parasitol, 60 :53-58.
14- Heath, D.D., (1970). The development of E.granulosus larval in laboratory animals. Parasitol., 60: 449-456.
15- Clark, H.R., (2004). Technical book of electrotherapy. London.