AJEMA GLOBAL RESEARCH NETWORK

AMERICAN Journal of Engineering, Mechanics and Architecture
Volume 2, Issue 1, 2024 ISSN (E): 2993-2637

The Effect of Electrical Current on Hydatid Cyst Protoscolices in Vitro

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Abstract: Hydatidosis is a parasitic disease in human and animals caused by Ehcinococcus granulosus. The current study investigated the effect of direct electric current on the vitality of protoscoleces of Echinococcus granulosus in the laboratory using different voltages (9v,11v) for three minutes. The results showed that the effect of direct electric current on the viability of the scoleces in vitro increases with increasing voltage and exposure time. Where the final results were obtained after the exposure and compared the numbers of cells before and after the exposuring using a light microscope, where the results proved the effectiveness of electric current on the viability of the living cells and killing them in an appropriate time depending on the given voltage values. Based on the foregoing, it is possible to apply this experiment to eradicate granulomatous echinococcosis in order to reduce the problems associated with surgical operations, in less time, cost and less side effects.

Keywords: Malaysia, employees, social interaction, workplace.

1. INTRODUCTION

The first history of hydatids goes back to antiquity, from Hippocrates time (377 BC) who wrote in his scripts (Seventh, 55): “In those whose liver is stuffed with water open into the omentum, the belly is filled with water, and they die" [1,2]. In 200 BC, Galen considered the liver as the main site of hydatids in animal's slaughters. Later, the presence of hydatids in animals and humans was reported frequently. Until the early modern age, the true nature of hydatids was still unknown. In 1685, Philip Hartmann emphasized the animal nature of cysticerci when he described a small, spherical structure which was connected with the bladder. Peter Pallas arranged the hydatids as a separated group (bladder worms) and described them in his medical thesis (in 1760) as small bodies located on the inner wall of the bladders. Carl Asmund Rudolphi (1801) introduced the Echinococcus name to zoology. Echinococcus granulosus is distributed worldwide and more frequently in rural areas. The geographic distribution of E. granulosus is variable due to deficiency of accurate case reporting; thus, it is difficult to assign a true specific map of the epidemiologic. In general, there are highly endemic areas in the eastern part of the Mediterranean region, at the southern tip of South America, Southern and Eastern Europe, Northern Africa, in Central Asia, Siberia, and Western China. E. granulosus is restricted to the northern hemisphere, in particular to the regions of China, the Russian Federation, and countries in continental Europe and North America [1].

Hydatid disease in people is mainly caused by infection with the larval stage of the dog tapeworm Echinococcus granulosus. It is an important pathogenic, zoonotic and parasitic infection (acquired from animals) of humans, following ingestion of tapeworm eggs excreted in

the faeces of infected dogs. Hydatid disease is a major endemic health problem in certain areas of the world. Cystic hydatid disease usually affects the liver (50–70%) and less frequently the lung, the spleen, the kidney, the bones, and the brain. Liver hydatidosis can cause dissemination or anaphylaxis after a cyst ruptures into the peritoneum or biliary tract. Infection of the cyst can facilitate the development of liver abscesses and mechanic local complications, such as mass effect on bile ducts and vessels that can induce cholestasis, portal hypertension, and Budd-Chiari syndrome.

Treatment of hydatid liver cyst has to be considered mandatory in symptomatic cysts and recommended in viable cysts because of the risk of severe complications. The modern treatment of hydatid cyst of the liver varies from surgical intervention to percutaneous drainage or medical therapy. Surgery is still the treatment of choice and can be performed by the conventional or laparoscopic approach. However, laparoscopic approach leads to an important rate of recurrence of the disease. Percutaneous Aspiration-Injection-Respiration Drainage (PAIR) seems to be a better alternative to surgery in selected case.Figure (1.1) show Hydatid cysts in the livers of sheep origin[2].

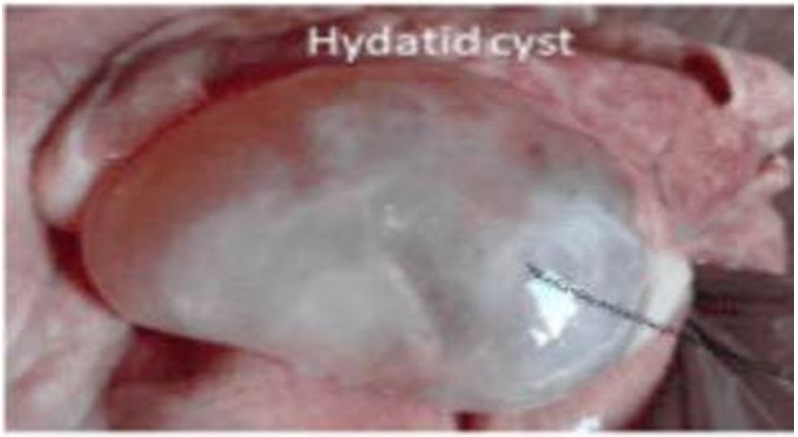


Figure (1.1) Hydatid cysts in the livers of sheep origin [3].

Several studies have appeared searching for different therapeutic methods for hydatid cyst, as shown in the following literature view:

1.1. Literature Review

In 2005 A Dalimi et al. [4]. They have studied a small-scale method for killing hydatid cyst protoscoleces using low voltage direct electric current. After collecting hydatid cysts from infected organs of slaughtered animals, protoscoleces were cultured in four different media: hydatid cyst fluid, RPMI, normal saline, and Tris buffer, respectively. Protoscoleces from each of the above media were then transferred to an electrolysis device (Which was designed using fiberglass in a rectangular shape having dimensions of 2cm (length) x 2 cm (width) × 7 cm (height). Two flat carbon electrodes 2 x 7 cm were installed parallel to each other on the opposite sides of the device) through which different electric current densities were applied. For measuring the survival rate of protoscoleces, flame cell movement and eosin staining was used. The results show that the survival rate of protoscoleces in hydatid fluid was dependent on the electric current density and the time of the applied current. Current densities of 62.5 mA/cm2 (11 V), 53.71 mA/cm2 (10 V), and 18.18 mA/cm2 (5 V) after 1, 2, and 3 min, respectively, killed all the scoleces he hydatid fluid. However, a current density of 7 mA/cm2 (9 V) in RPMI medium after 3min was most effective.

In 2010 Sadam Salim Yaseen, [5]. This study investigates the cellular immune response in BALB/c mice immunized with protoscoleces (PSC) of Echinococcus granulosus of sheep origin, treated with direct electrical current which was obtained by an electrolysis device was designed in a rectangular shape having dimension of 2cm (length) x0.26cm (width) x7cm (height). Two

flat carbon electrodes 2xcm were installed parallel to each other on the opposite side of the device, a distance of 1.72 cm between electrodes was selected as a potential, depending on some immunological criteria including delayed type hypersensitivity (DTH) and mitotic activity of cells obtained from lymphoid organs, spleen and bone marrow. The results revealed an increase in cellular immune response in immunized groups compared with the control group infected for 3 months. An obvious significant increase ($P < 0.01$) occurred in rate of foot pad thickness 1.83 mm in immunized group with treated PSC with direct electrical current with potential 5.77mA/cm2 for 2 min. whereas, mice immunized with PSC treated with direct current potential 11.82 mA/cm2 for 2min. too, led to significant increases ($P <0.01$) in rate of mitotic activity of spleen and bone marrow cells, 61.8%, 70.1% for each, respectively.

In 2011 M Moazeni1 et al. [6] their study, was to evaluate the scolicidal activity of methanolic extract of Zingiber officinale (Rosc.) family Zingibe - raceae, against protoscolices of hydatid cyst. Protoscolices were collected aseptically from sheep livers containing hydatid cyst and were exposed to different concentrations of ginger extract for various exposure times. Scolicidal activity of Z. officinale extract at concentration of 25 mg/mL was 25.6%, 39.1%, 56.7%, 83.7%, 98.1% and 100% after 10, 20, 30, 40, 50, and 60 min of exposure respectively. Scolicidal effect of this extract at concentration of 50 mg/mL was 52%, 85.8 %, 99.6% and 100% after 10, 20, 30 and 40 min of exposure respectively. Z. officinale extract at concentration of 100 mg/mL killed 76.5 %, 87% and 100 % of protoscolices after 10, 20 and 30 min respectivly. The results of this study showed that the methanolic extract of Z. officinale has high scolicidal activity and might be used as a natural scolicidal agent.

In 2011 JH Rahma et al. [7]. The present study was undertaken to asses the effect of Different direct electric current.Direct current was obtained by 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 (200mA, 400mA and 600mA) on the activity of protoscolices of the larval stage of Echinococcus granuosus in vitro. 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 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).

In 2016 HAA AL-Aquli. [8]. They studied the inactivation of the parasite with protoscolicidal agents which is a crucial part in the treatment. So, this study was to evaluate the protoscolicidal effect of cetrimide and povidone-iodine in hydatid cyst disease. Fifty intact cysts of pulmonary hydatid disease of patients not received preoperative antihelmenthic were included in the study. While those cysts of patients who received preoperative antihelmenthic were excluded from the study. The protoscolicidal effect of cetrimide (0.05%, 0.1%, 0.5%) and povidone-iodine (10%) were assessed in this in vitro study using 1, 2 and 5 minutes as exposure time. Cetrimide (0.1%, 0.5%) have a higher protoscolicidal effect than 10% povidone-iodine that is statistically significant after different exposure time. The data concluded that cetrimide (0.1%) is a very effective protoscolicidal agent even with short exposure time, so it is the least concentration dependent and the least time dependent to achieve its protoscolicidal effect.

In 2016 M Shahnazi et al. [9]. Hydatidosis is one of the most important zoonotic diseases and surgery is still the main treatment for this problem. One of the side effects of hydatid cyst surgery is recurrence, thus, searching and assessment of some new agents or methods to solve this problem. In this study, the scholicidal effect of ethanolic extract of Ziziphora tenuior (Z. tenuior) protoscolices were aseptically collected from sheep livers containing hydatid cyst and used in the experiments. Z. tenuior extract was used at concentration of 3-100 mg/ml for 10-60 min. Viability of protoscolices was determined by 0.1% eosin staining. Medicinal plant extracts are very important. Based on their results, Z. tenuior extract at concentration of 10 mg/ml killed all protoscolices after 20 min.However, this medicinal plant at concentration of 25 mg/ml destroyed all protoscolices in a shorter exposure time (10 min) therefore, the scolicidal activity of the extract at 10 and 25 mg/ml concentration was considerably effective in lower concentrations and shorter exposure.

In 2018 Asmaa Abdelazeez Ali and Fouad Alrubee,[10].Their study investigated the effect of the direct electrical current on the viability of the protoscoleces of Echinococcus granulosus in vitro using the electrical device which consists of power supply unit, current measurement unit, and exposure unit, five voltages were applied on protoscoleces , 3(10mA/cm2), 6 (120mA/cm2, 9(270mA/cm2), 12(480mA/cm2), 15(580mA/cm2), suspension in Phosphate Buffer Saline(PBS) in vitro for different durations 3, 6, 9, 12 and 15 minutes, and injected in to BALB/c mice to assess their effects on the immune response against infection with secondary hydatid cysts by injecting the mice with protoscoleces treated with direct electrical currents with viability 75%, 50%, 25% and 0%, respectively 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% ,75%, 50% and 25%, in groups injected with treated protoscoleces 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).

1.2. Aim of the project

- Design an electrical circuit to get different direct electrical currents.
- Applying the different direct electrical current with an exposuring time on hydatid cyst protoscoleces.
- Evaluating their scolicidal effect, to be considered an alternative for treatment secondary hydatidosis with less time, less side effects and less cost instead surgery due to the presence of some problems when there is surgical intervention.

1.3. Study Layout

This project has been organized in five chapters as follows:

Chapter One: presents a general introduction about the secondary hydatidosis, with literature review and aim of the project.

Chapter Two: includes a theoretical background of the hydatid cyst with protoscolices and Its transmission, therapeutic effect and the effect of electric current on their viability.

Chapter Three: Includes the experimental work by design electrical circuit to apply the electric current on the protoscolices in vitro.

Chapter Four: includes the obtained results and their discussion

Chapter Five: includes the final conclusion of the project and gives suggestions for future work.

2. THEORETICAL BACHGROUND

2.1. Introduction

Hydatid disease in people is mainly caused by infection with the larval stage of the dog tapeworm *Echinococcus granulosus*. It is an important pathogenic, zoonotic and parasitic infection (acquired from animals) of humans, following ingestion of tapeworm eggs excreted in the faeces of infected dogs. Hydatid disease is a major endemic health problem in certain areas of the world [11].

2.2. Life cycle of Echinococcus granulosus

The life cycle of E. granulosus requires both an intermediate host usually (a sheep, a cattle, or a swine), and a primary canine host. A man becomes both an accidental and an intermediate host through contact with infected dogs or by ingesting food or water contaminated with eggs of the parasite. One can never be surprised to find out that this disease is most commonly found in the temperate and sheep-raising areas of the world.

Once the eggs are ingested, they release larvae into the duodenum. The larvae migrate through the intestinal mucosa and gain access to mesenteric vessels which carry them to the liver. The liver is the site of up to 70% of echinococcal lesions. Larvae that escape hepatic filtering are carried to the lung, the site of an additional 15-30% of lesions. From the lungs, larvae may be disseminated to any part of the body, (figure2.1). Larvae that escape the host's defenses and persist in a host organ develop into small cysts surrounded by a fibrous capsule. These cysts grow at a rate of 1-3 cm/year and may remain undetected for years. Thus; they can reach very large sizes before they become clinically evident. The cyst wall contains an outer chitinous layer and an inner germinal layer. The germinal layer may develop internal protrusions and eventually form daughter cysts within the original cyst,this final stage rarely occurs. In general, the cysts are locally growing, not disseminating. Extrahepatic and extrathoracic involvement of the hydatid disease including bones, heart, spine, pelvic-perianal region, muscles, subcutaneous space, adrenal, ovaries, retroperitoneum, breast, and cranium have been reported [12,13].

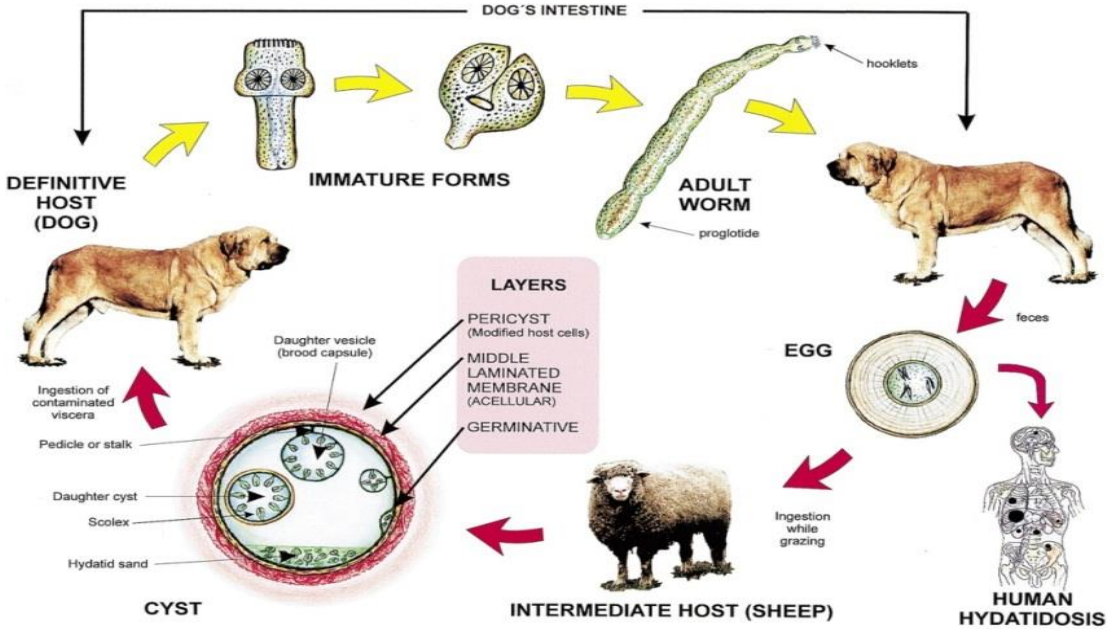


Figure (2.1) The Life cycle of Echinococcus granulosu [12].

2.3. Pathology- Hydatid cyst structure

A primary cyst in the liver is composed of three layers as manifested in figure (2.2):

1. Adventitia (pericyst): consisting of compressed liver parenchyma and fibrous tissue induced by the expanding parasitic cyst.

2. Laminated membrane (ectocyst): is elastic white covering, easily separable from the adventitia (Figures 2.3-2.4).
3. Germinal epithelium (endocyst) – is a single layer of cells lining the inner aspects of the cyst and is the only living component, being responsible for the formation of the other layers as well as the hydatid fluid and brood capsules within the cyst [12].

Daughter vesicles (brood capsules) are small spheres that contain the protoscolices and are formed from rests of the germinal layer. Before becoming daughter cysts, these daughter vesicles are attached by a pedicle to the germinal layer of the mother cyst. At gross examination, the vesicles resemble a bunch of grapes (Fig 2.5). Daughter cysts may grow through the wall of the mother cyst, particularly in bone disease [14].

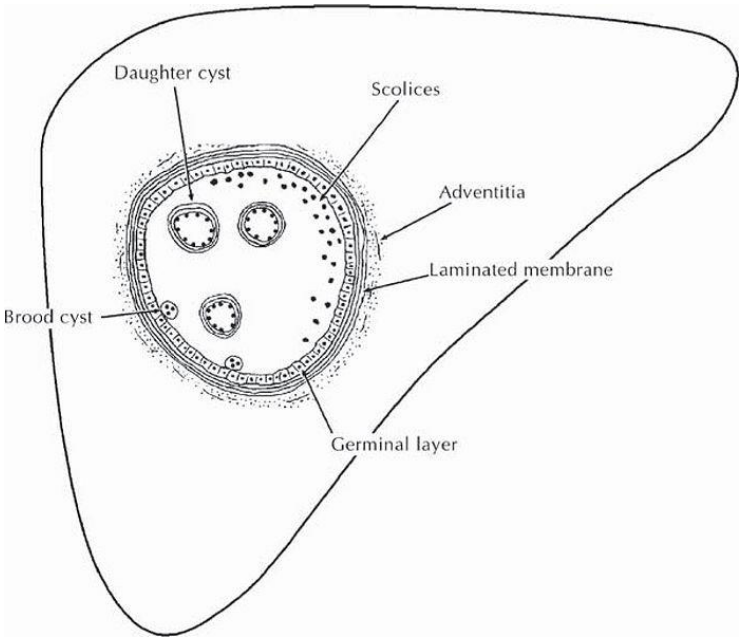


Figure (2.2) Hydatid cyst of the liver [12]

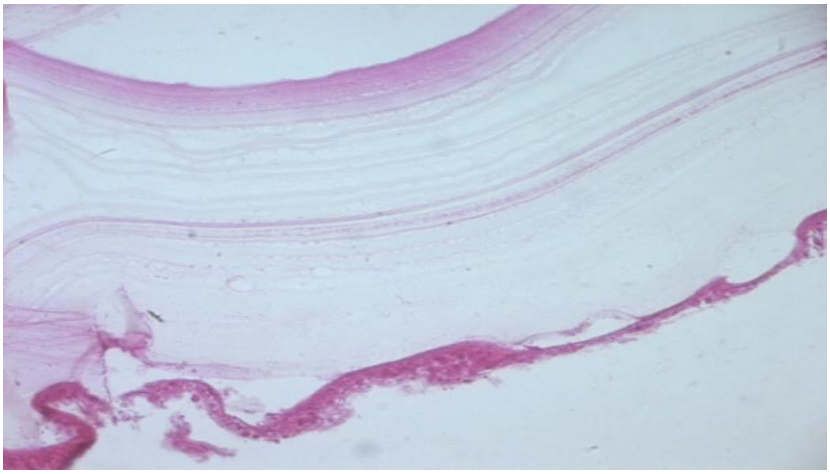


Figure (2.3) Cystic structures with laminating fibrous wall and inner germinal layer.

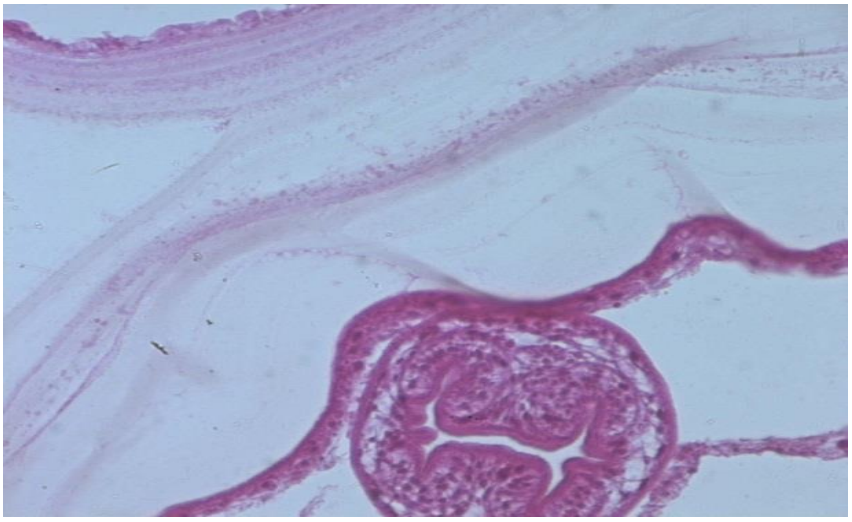


Figure (2.4) Tissue section of a hydatid cyst showing daughter cyst.

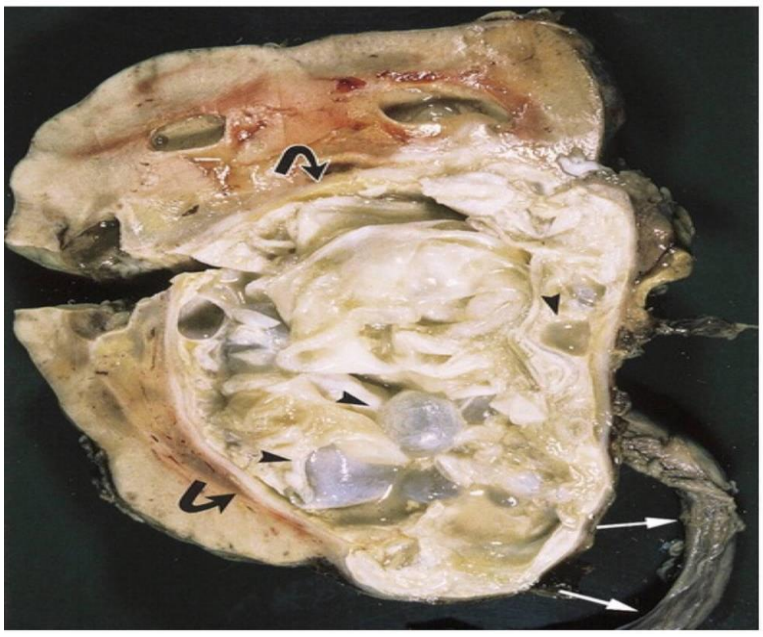


Figure (2.5) Multivesicular cyst. Photograph of a human kidney that has been sectioned along the midcoronal plane demonstrates a large cyst with the typical "bunch of grapes" appearance (black arrows) due to the presence of daughter cysts (arrowheads). White arrows indicate the ureter [14].

2.4. Complications of hydatid cyst of the liver

- The most common complication is the intrabiliary rupture of the hydatid cyst.
- Other less common complications are:

1. The rupture of the cyst into the peritoneal cavity.
2. Rupture into the thoracic cavity through the diaphragm and toward organs of the gastrointestinal tract.
3. The secondary bacterial infection of the cyst [15].

Intrabiliary rupture of the hydatid cyst is a rare but serious complication of hydatid disease and it should be considered as a differential diagnosis in patients with hydatid disease who were admitted with complaints of abdominal pain, fever, and jaundice.

Early detection of complications and aggressive treatment is vital.

The presentation of intrabiliary rupture of the hydatid cyst can range from asymptomatic to obstructive icterus, cholecystitis, cholangitis, pancreatitis, or septicemia. Clinical findings depend on the size of cystobiliary communication and the incidence of complications is increased in cases with large size of cystobiliary communication [15].

The authors classify rupture of echinococcal cysts into three types: contained, communicating, and direct. Contained rupture occurs when only the parasitic endocyst ruptures and the cyst contents are confined within the host-derived pericyst. When cyst contents escape via biliary or bronchial radicles that are incorporated in the pericyst, the rupture is communicating. Direct rupture occurs when both the endocyst and the pericyst tear, spilling cyst contents directly into the peritoneal or pleural cavities or occasionally into other structures. Communicating and direct forms have more serious clinical implications than contained rupture, but even contained rupture should have prompt surgical attention to prevent it from developing into one of the other forms. Untreated communicating rupture of a liver cyst can lead to obstruction of the biliary system with a 50% mortality rate. Direct rupture may cause anaphylaxis, and it should be managed surgically, possibly with adjunctive treatment with antihelminthic drugs to decrease the possibility of metastatic hydatidosis [16].

2.5. Diagnosis

Early diagnosis of CE can result in significant improvements in the quality of the management and treatment of a disease. In most cases, the early stages of infection are asymptomatic, so that methods that are cheap and relatively easy to use are required for large-scale screening of populations at high risk. Immunodiagnosis provides such an approach and can also, confirm clinical findings [17].

2.5.1. Clinical feature

Studies have shown that liver hydatid cysts in humans grow very slowly, with more than half of the cysts differ in size within 10 years, and one- third are growing less than 3 cm. The average cyst growth in patients with long-term follow-up is 0.7 cm. Various clinical findings such as cholangitis with bile rupture, portal hypertension, bile obstruction, fistula and abscess formation are observed depending on the cyst localized organ [18].

A/ In Non complicated cysts:

Hydatid cyst of the liver is frequently silent and only diagnosed incidentally during abdominal investigation for other pathology. The clinical signs appear gradually with the increase volume of the cyst. The most common symptom, when it occurs, is right upper quadrant or epigastric pain and the most common findings on examination are an enlarged liver and a palpable mass. Pressure effects are initially vague. They may include non-specific pain, cough, low-grade fever, and the sensation of abdominal fullness. As the mass grows, the symptoms become more specific because the mass impinges on or obstructs specific organs [12].

B/ In Complicated cysts:

Patients may also present with complications of the cyst such as biliary communication, intraperitoneal rupture (spontaneous or post-traumatic) and, rarely, intrathoracic or intrapericardial rupture.Cyst rupture can be associated with anaphylaxis secondary to the highly antigenic content of the cyst fluid or may be silent and present with multiple intraperitoneal cysts. With secondary infection, tender hepatomegaly, chills, and spiking temperatures occurs. Urticaria and erythema occur in cases of generalized anaphylactic reaction. With biliary rupture the classic triad of jaundice, biliary colic and urticaria occurs. the diagnosis is most easily set by ultrasound or other imaging techniques such as CT-scan or MRI, combined with case histo. A great part of the patients treated for hydatid disease get their diagnosis incidentally, seeking medical care for other reasons. The time at when a previously silent cyst gives rise to pathology

depends both on the size of the cyst, but also on its location, making presenting symptoms of cystic echinococcosis highly variable. Most presenting features are caused by the pressure that the enlarged cyst expels on its surroundings, but may also appear if there is a rupture of a cyst. Symptoms leading to diagnosis mostly include Liver cysts may eventually cause abdominal pain or a palpable mass. Jaundice may occur if the bile duct is obstructed. Rupture into the bile duct, peritoneal cavity, or lung may cause fever, urticaria, or a serious anaphylactic reaction, pulmonary cysts can rupture, causing cough, chest pain, and hemoptysis [12,19].

2.5.2. Investigations

Considering that the early stages of infection are usually asymptomatic, the diagnosis of liver hydatid cyst may often be incidental, associated with an abdominal ultrasonography performed for other clinical reasons. In endemic areas, the presence of symptoms suggestive of hydatid liver cyst in a person with a history of exposure to sheep and dogs supports the suspicion of hydatidosis. Serology tests such as ELISA or immunoblotting can be used in addition, being 80-100% sensitive for liver cysts but only 50-56% for lungs and other organs [12].

False positive reactions may occur in persons with other tapeworm infections, cancer, or chronic immune disorders. Whether the patient has detectable antibodies depend on the physical location, integrity and viability of the cyst. Patients with senescent, calcified or dead cysts usually are sero-negative. Patients with alveolar echinococcosis have most of the time detectabl antibodies. Fine needle biopsy should be avoided if dealing with E. granulosus since there is a great danger of leakage with subsequent allergic reactions and secondary recurrence[12].

Imaging of hydatid disease

The imaging methods used for diagnosis and evaluation of the extent of HD are ultrasonography (USG), computed tomography (CT), magnetic resonance imaging (MRI), and less commonly radiography and urography. USG is screening modality of choice and is also used to monitor the efficacy of treatment. It clearly demonstrates the hydatid sand, floating membranes, daughter cysts, and vesicles inside the cyst. CT has high sensitivity and specificity for HD. CT is an important diagnostic modality in detecting cyst wall or septal calcification, demonstrating internal cystic structure posterior to calcification, assessing complications, depicting osseous lesions and in cases where USG has limitations (obesity, excessive bowel gases, abdominal wall deformities, and previous surgery).MRI is superior for demonstrating cyst wall defect, biliary communication, and neural involvement.

Recently, MRI has been shown to be important in differentiating liver hydatid cysts from other simple cysts.

The imaging findings depend on the organ involved, host reaction, stage of evolution, and maturity of disease. The findings can range from purely cystic lesions to solid-appearing masses. The cysts may be solitary or multiple, unilocular or multivesicular, and with or without calcification. Presence of daughter vesicles and membranes within the cyst, peripheral cyst wall, or internal matrix calcification are important findings for differential diagnosis of HD [20].

2.6. Therapeutic strategies

The treatment of echinococcosis involves many options depending on the experience of specialists, the availability of abilities, the size, and location of the hydatid cyst and the appearance of complications. Surgery has played an effective role in the therapy of echinococcosis including cysts removed and liver transplant. Surgery is the preferred treatment when hydatid cysts are large (eg. liver hydatid cysts >10 cm in diameter) or when they are located in certain organs (e.g, brain, lung, or kidney), but in some cases, surgery becomes ineffective, especially in patients who have multiple cysts and it is so difficult to acces. It is worth mentioning that the surgery should be done carefully with avoidance of the adverse effects of leakage of hydatid cyst fluid [1].

There is evidence that low voltage direct current (DC) (less than 10 V) is bactericidal and parasitocidal in vitro. Electric currents may destroy cell physiological action by altering the passage of molecules through cell membrane in 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. The complete destruction of human hydatid cyst protocoleces by electrolysis device has also been reported. 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. Studies put emphasis on the fact that electrical fields stimulate the migration of macrophages, corneal epithelial cells, and fibroblasts. Electric currents applied to wounded tissue increase neutrophil and macrophage migration. Membrane permeability increases under exposure to an external electric field pulse of power frequency. Critical field intensity may cause alteration of local membranes. This previously unknown phenomenon is called electro permeabilization or electroporation that attemped 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 [10].

2.7. Electrical Component Over View

An electric circuit is simply an interconnection of the elements. Circuit analysis is the process of determining voltages across (or the currents through) the elements of the circuit. There are two types of elements found in electric circuits: passive elements and active elements. An active element is capable of generating energy while a passive element is not. Examples of passive elements are resistors, capacitors, and inductors. Typical active elements include generators, batteries, and operational amplifiers. The most important active elements are voltage or current sources that generally deliver power to the circuit connected to them [21].

2.7.1. Type of Source

1-Voltage Sources: The term dc is an abbreviation for direct current, which encompasses all systems where there is a unidirectional (one direction) flow of charge.

In general, dc voltage sources can be divided into three basic types:

1. Batteries (chemical action or solar energy).
2. Generators (electromechanical).
3. Power supplies.

2-Current Sources: Two types of current are readily available to the consumer today. One is direct current (dc), in which ideally the flow of charge (current) does not change in magnitude (or direction) with time. The other is sinusoidal alternating current (ac), in which the flow of charge is continually changing in magnitude (and direction) with time [22].

2.7.2. Simple electrical circuit components

The simple electrical circuit used in this study consists of the following:

1. Power supply.
2. Mulitimeter.
3. Electrodes.

2.7.2.1. Power Supply

Virtually every piece of electronic equipment, e.g., computers and their peripherals, calculators, TV, and instruments, is powered from a DC power source, (a battery or a DC power supply). Most of this equipment requires not only DC voltage but voltage that is also well filtered and regulated. Power supplies are so widely used in electronic equipment. There are three types of electronic power conversion devices in use today which are classified as follows according to their input and output voltages:

1. The AC/DC power supply (this is the type we used in the current experiment).
2. DC/DC converter.
3. The DC/AC inverter.

A power supply converting AC line voltage to DC power must perform the following functions at high efficiency and at low cost:

1. Rectification: Convert the incoming AC line voltage to DC voltage.
2. Voltage transformation: Supply the correct DC voltage levels.
3. Filtering: Smooth the ripple of the rectified voltage.
4. Regulation: Control the output voltage level to a constant value irrespective of line, load and temperature changes [23].

The used power supply is shown in Figure (2.6)

Figure (2.6) The power supply.

2.7.2.2. Multimeter

Multimeters are used worldwide by many people and in different professions. Since their invention, they have proved to be invaluable tools for technicians, electricdans, engineers, and homeowners. Multimeters can be used to troubleshoot egaipment, verify proper operation of equipment, and determine valves of electrical components.

Digital multimeters combine the testing capabilities of single-task meters the voltmeter (for measuring volts), ammeter (amps) and ohmmeter (ohms). Often, they include several additional specialized features or advanced options. Technicians with specific needs, therefore, can seek out a model targeted to meet their needs.

Digital measurement and display technologies developed in parallel,with digital integrated circuit technology until finally, in the late 1960s, portable digtal versions of the VOM (Volt-Ohm-Milliammeter) appeared. The used form of meter shown in Fig (2.7), has since becom known as the digital multimeter (DMMT)[24].

Figure (2.7) Digital Multimeter.

2.7.2.3. Electrodes

Using Banana Plug to Alligator Clip Test Lead cable as shown in figure(2.8) is considered one of the types of disposable electrodes and can be used in electrical or laboratory electric testing work. It is frequently used in school physics laboratories to quickly and cheaply assemble circuits. They are useful for connecting components to wires. They are good, useful for connecting components or others to wires. Easy for installation and safety.

Figure (2.8) Banana Plug to Alligator Clip Test Lead cable.

2.8. Application of Biomedical Circuits

A biomedical engineering is today one of the most important research fields in the world. This fact is parallel with the health challenges surrounding the improvement of human health as one of the main vehicles to increase the quality of life. The maturity of many technologies, such as microelectronic, biomaterial, microfluidic, together with progress in the biology and medicine fields, develop alternative solutions for medical evaluation, diagnosis, therapy and research in general, opening the opportunity for new medical devices, e.g., lab-on-a-chip, wearable technology, and implants. The biomedical electronic industry supports the development of many of these new devices, as the main technologies for bio-signal acquisition, processing, and communication [25].

The development of new sensing technologies, biomaterials, microelectronic devices, microfluidic systems and micro-electro-mechanical systems (MEMs) etc., opens the window to new biomedical circuits and system opportunities to measure “better”, and to develop “alternative” methods to find relevant information for physician and biologist teams, in

applications such as diagnosis, therapy, clinical tests and bio-signal monitoring. However, the accomplishment of new medical equipment for specific tests in the health field poses significant challenges regarding the electronic circuits [26].

3. EXPERIMENTAL WORK

3.1. Introduction

This chapter contains the design of the electrical circuit and clarification of its basic components, as the electrical circuit will be tested and its effect on the primary particles of hydatid cysts prepared in the laboratory will be studied.

3.2. Components of the Electrical Circuit

The block diagram shown in Figure (3-1) illustrates the components of the electrical circuit, the connection and checking the circuit performed by using simple solution:

1. power supply.
2. mulitimeter (for direct electrical current as an ammeter).
3. Electrodes (type of probe: Nickel).
4. Glass container for simple solution (water and salt) :((0.1g from salt) / (100ml from distill water)).

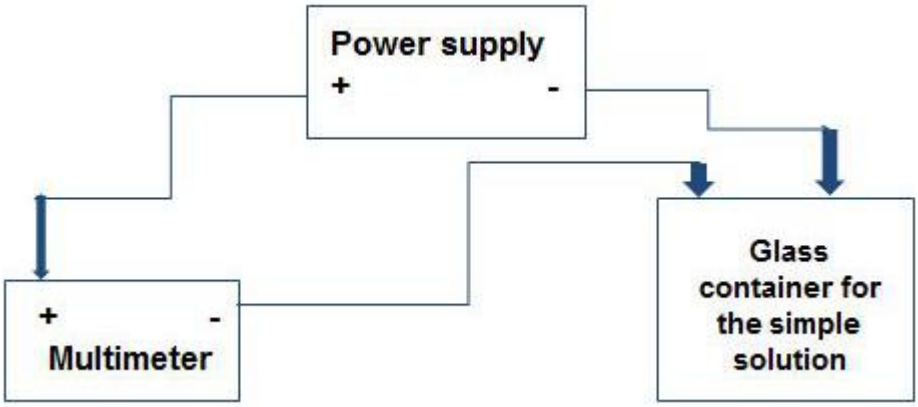


Figure (3.1) Block Diagram of electrical circuit

3.3. Expermintal Electrical Work

The positive electrode of the power supply was connected with the positive electrode of the multimeter and the reading of the power supply was fixed at (9v,11v) respectively. The negative electrodes were placed for each of them in the container containing the salt solution in order to test the conductivity of the electrodes through the solution and read the multimeter results obtained after Run the circuit later. The figure(3.2) below shows the connection of the circuit.

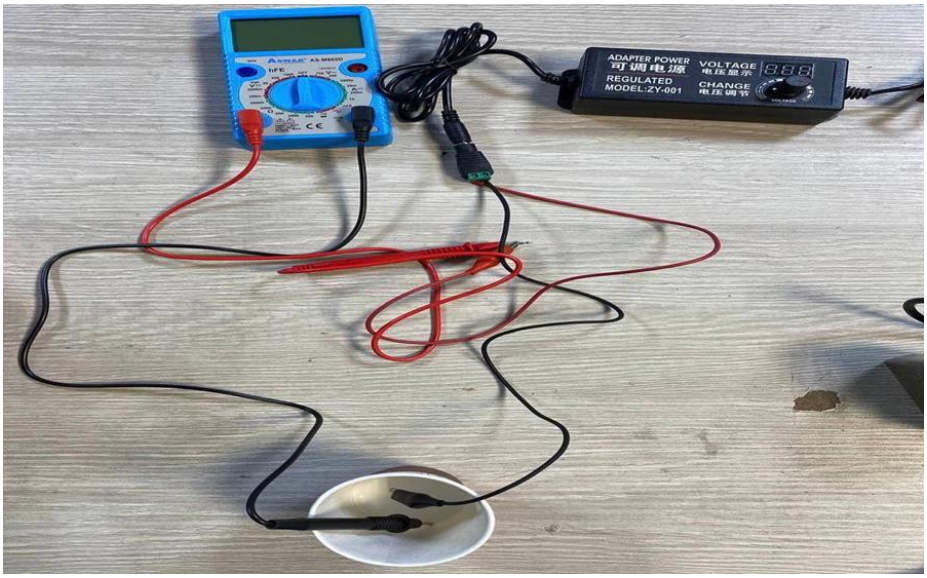


Figure (3.2) Serial Connection Circuit

3.4. Sample preparation and laboratory work

The devises and tools were used in this part of the project shown in table (3.1).

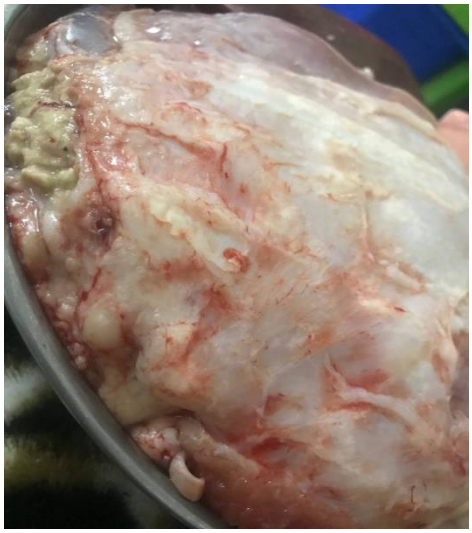
Table (3.1) shows each of the companies and models of devices and tools used in this experimental work.

Name of the devices and tools	Name of the companies and models
Hood	CERTIFIED Model:LCB-1201V
Light Microscope	OPTIKA Model:N-400M
Centrifuge	HETTICH Model:D-78532
Pipettors	EPPENDORF

The preparation was performed according to Asmaa Abdelazeez Ali and Fouad Alrubee in may 2019[10].

1- Infected Liver sheep with hydatid cysts as shown in Figure(3.3) was obtained from Al-Shula slaughterhouse in Baghdad and transferred to the laboratories of the Research Center of Al-Nahrain University.

2-In the slaughterhouse the sample was placed in a container containing normal saline to preserve it until starting the expreiment. The work was done inside a sterile hood.

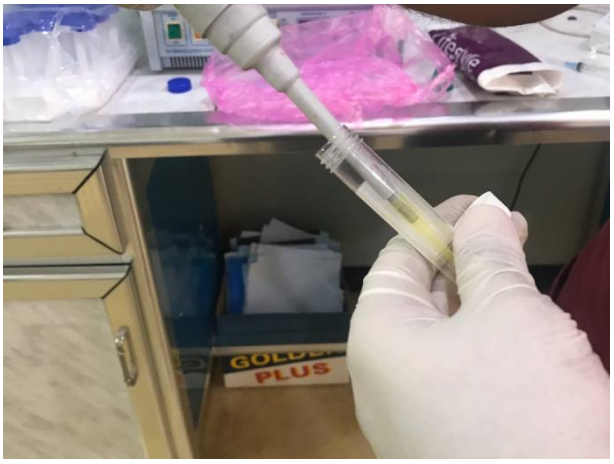


Figure(3.3) Liver of a sheep infected with hydatid cysts.

3- The hydatid cyst fluid was withdrawn using a sterile needle (5ml) (guage 18), in order to preserve the protoscolices from damage during withdrawal as shown in Figure(3.4).

Figure(3.4) Hydatid cyst fluid withdrawal.

4- The hydatid fluid was diluted with the addition of normal saline and mixed by a pipette as shown in the Figure(3.5).

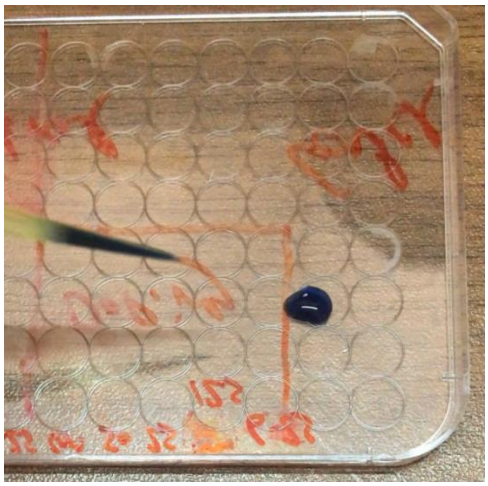


Figure(3.5) Mixing hydatid fluid with normal saline,by a pipette.

5- The diluted liquid was placed inside the centrifuge at a speed of 1500 rpm for 3 minutes in order to separate the protoscolices from the liquid components as shown in figure(3.6).

Figure(3.6) Putting the tube inside the centrifuge.

6- The protoscolices were washed 2 times using normal saline final precipitate diluted in 1 ml and taking 100µl of the liquid was stained with 100µl of 0.1% trypan blue dye and placed on the slide (measuring chamber hemocytometer) to be examined under the light microscope distinguishing and counting the live cells as shown in the three Figures(3.7,3.8,3.9)



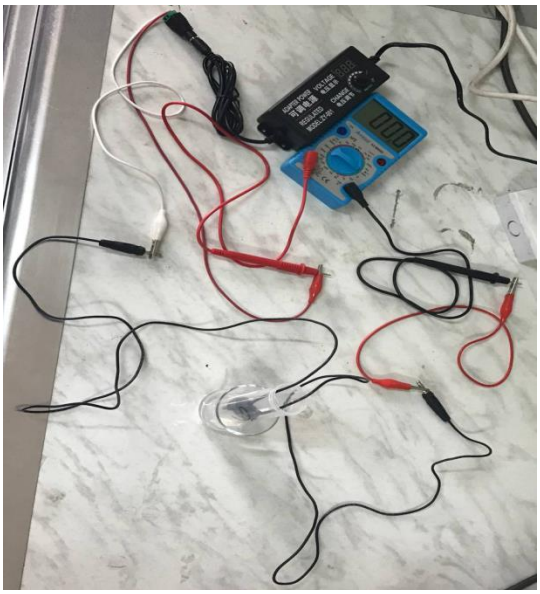
Figure(3.7) Trypan blue staining of hydatid cyst protoscoleces.

Figure(3.8) Put suspention on the counting champer (hemocytometer).

Figure(3.9) Examination of the sample under the light microscope.

7- The number of viable protoscolices was calculated, as the total number of protoscolices reached 320000cell/ml (stock),depending on the following equation(Law of cell concentration cell/ml = average number of viable cells in the large square ×dilution factor × conversion factor of champer).

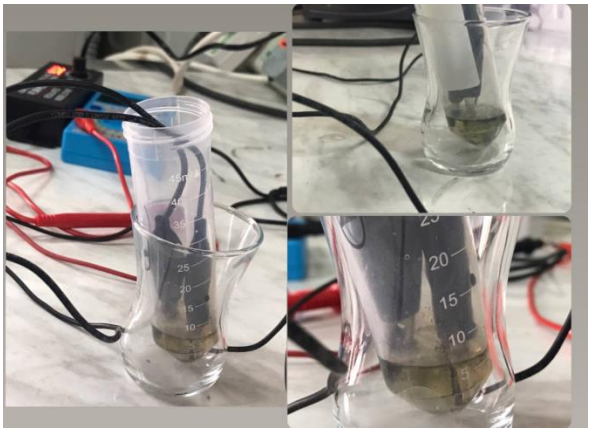
8- 5ml of normal saline was placed in two tubes and each mixed with 30µl from the stock to be the concetration 2000cell/ml in each tube then expose them to the electric current as shown in the Figures(3.10,3.11).



Figure(3.10) The connected electrical circuit.

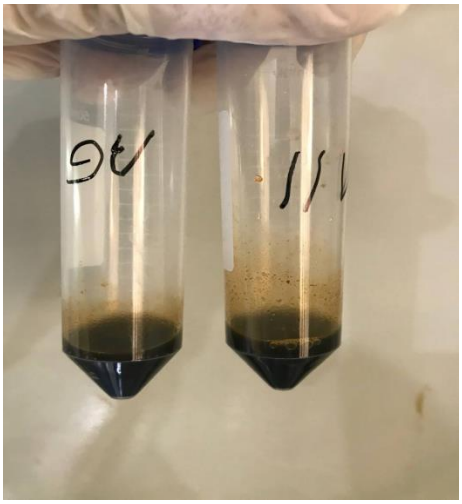
Figure(3.11) Tubes contains a suspension of hydatid cyst protoscoleces in normal saline.

9- The reading of the power supply was set at (9v), the electrodes were fixed, and the fluid was exposed to the voltage for 3 minutes, as shown in the Figure(3.12).



Figure(3.12) Clarify the installation of the electrodes.

10- Repeat step 9 with another voltage (11v) and the same steps in point 9,Figure(3.13) shows the effect of electric current on the mixture.



Figure(3.13) The mixture after exposing to the current at(9v,11v).

11- A drop(100µl) of the liquid was taken after the exposure and stained with trypan blue dye to distinguish the cells under the light microscope to observe the effect on the viability of the protoscoleces at (9v,11v).

12-Perfect results were obtained after examination under the light microscope, the rate of cell killing reached 100% at each of the two voltages for the same time.

4. RESULT AND DISCUSSION

4.1. Introduction

This chapter presents an analysis and discussion of the results of the current study.

4.2. The Result

4.2.1. Test the circuit that mentioned before (in chapter 3) simple solution

The electrical circuit was tested on the solution, and the obtained results are shown in Table (4.1) below were obtained.

Table (4.1) Test the circuit on the aforementioned simple solution

Trial	Electrical voltage(volts)	Electrical current(mA)
1	11	6.5
2	9	5.2

4.2.2. Counting of viable cells (protoscoleces):

Depending on equation of cell concentration, the number of viable cells befor exposure was calculated, as shown below and as indicated in Figure (4.1):

cell concentration (cell/ml) = average number of viable cells in the large square × dilution factor × conversion factor of champer (equation 1)

cell concentration (cell/ml) =1×16×2×10000=320000 cell/ml (stock)

The number of viable cells based on the equation (1) was 320000cell/ml of the stock (cells suspension)

Taking 30µl from the stock containing 2000 protoscoleces in two tubes put in each one 30µl of the stock with 5ml of normal saline (so the concentration in each tube 2000cell/ml) till expose them to the electrical current (9v,11v).

4.2.3. Test the electrical circuit on the mixture of normal saline and with the previously prepared hydatid cyst protoscoleces

The voltages were applied to the mentioned above mixture (suspension of prepared protoscoleces in normal saline) and the amount of currents resulting from this application were obtained and the numbers of cells were counted before and after the application and the viability ratios were clarified as shown in Table (4.2) and Figures (4.2,4.3) respectively below:

cell concentration cell/ml =0×16×10000×2=0cell/ml

Table (4.2) Shows the effect of applying voltages(9v,11v) on the hydatid cysts protoscoleces

Tubes	Electrical voltage(volts)	Electrical current(mA)	Number of cells before exposure	Number of cells after exposure	Percentage of killing(mortality)
1	11	64.3	2000cell/ml	0cell/ml	100%
2	9	43.5	2000cell/ml	0cell/ml	100%

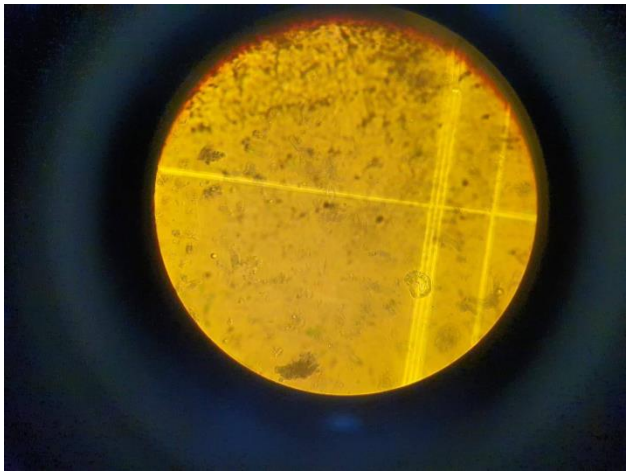


Figure (4.1) Shows the cells (protoscoleces) in the hydatid cyst under a light microscope, (40× magnification).

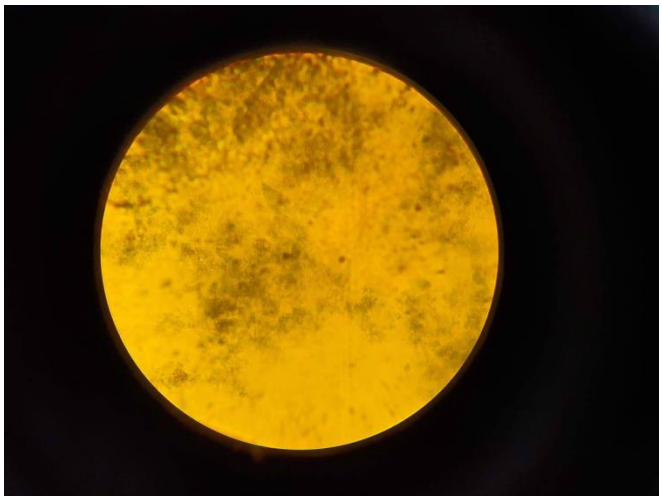


Figure (4.2) Shows the killing of viable hydatid cyst scoleces under a light microscope after applying a voltage of (9v),100% killing, (10× magnification).

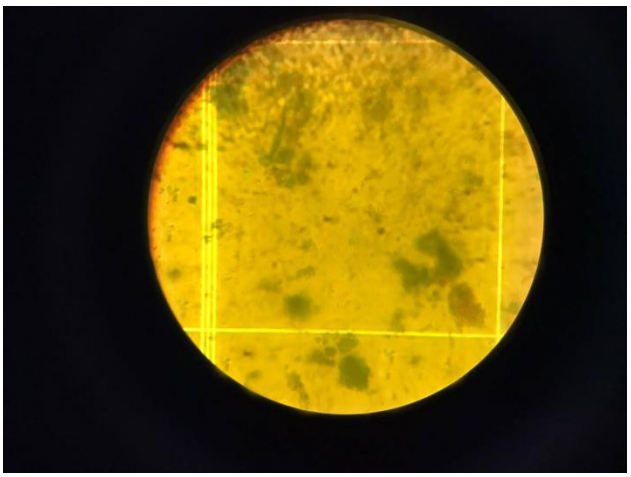


Figure (4.3) shows the rates of killing of viable hydatid cyst cells under a light microscope after applying a voltage of (11v),100% killing, (40× magnification).

4.3. Discussion

The echinococcosis is an endemic zoonotic disease caused by the dog tape-worm Echinococcus granulosus. So, we need for new therapeutic stratigies in addition to the Surgical treatment.

This work based on the circuit test in the aforementioned simple solution, it was found that fixing the electrodes in the solution has a very important role in the accuracy of the results

obtained (9v(5.2mA)) and (11v(6.5mA)). When the reading of the power supply was fixed at a voltage of (9v), the reading of the multimeter (43.5mA) was obtained and for an exposure period that lasted (3min). The cells, as mentioned above, died due to the applied voltage to them. When the previous steps were repeated for a new voltage (11v), a new current reading of (64.3mA) was obtained, and for the same exposure period, the same result was obtained in terms of the killing ratio of 100% for killing the same cell concentration. From the results presented above, the killing effect of the electric current on the living cells can be confirmed depending on the amount of electric voltage and the duration of exposure. The mechanism of current for killing the viable cells can be explained as following: Electricity breaks down the osmotic membrane of the cell wall and thus the process of diffusion and osmosis ends and as a result the cell dies. If the plasma membrane ruptures or breaks down, the cell will not be able to exchange material from its surroundings by diffusion or osmosis because it acts as a mechanical barrier. Thereafter, the protoplasmic material will be disappeared, and the cell will die. However, the answer is not very simple as the amount of current/voltage to kill a single cell sometimes differs among cell types and even among individual cells. Also, the duration, frequency, distance etc., of application determines the resistance of the membrane. Basically, cellular membranes can be broken down with electrical pulses, called "dielectric membrane breakdown". This is the mechanism for killing cells by electric current, and this is conferred by Tsong and SU in 1999[27].

5. CONCLUSION AND FUTURE WORK

5.1. Introduction

This chapter presents the conclusion of the current study and clarifies some suggestions for future work regarding the study of the effect of electric current on hydatid cysts protoscoleces in vivo.

5.2. Conclusion

After completing current experiment, we conclude that the effect of electric current is very significant on the vitality of the cells, killing the cells (protoscoleces) based on the effect on the cell wall. We conclude that the percentage of killing the living cells increases with the increase in voltage (the amount of voltage applied to the on suspension) and exposure time, as the higher the voltage in an exposure time the higher the killing ratio based on the results obtained above. Based on the foregoing, we can say that this experiment proved successful results with simple capabilities in terms of the components of the circuit and the time it took in addition to the cost, considered as a good strategy. So, it is a good choice for treating secondary hydatidosis in addition to the traditional (surgical) therapeutic strategies.

5.3. Future Work

1. The possibility of applying electrical current in the laboratory on lab animals (in-vivo).
2. With the development of biomedical technology and capabilities, it is possible to manufacture suitable electrodes through which an electric current can be delivered to the patient in a safe and useful manner for killing hydatid cyst protoscoleces, applying lower voltages for different exposure periods to assess their effect on the protoscoleces with low side effects in the host.
3. It is possible in the future that this experiment will be applied to humans depending on the selection of safe voltages that do not cause significant harm to the human body to solve the problems related to surgical operations for hydatid cyst disease and protect against recurrence of infection in the shortest time, at the lowest cost and with the best result (high killing ratio).

REFERENCES

1. AL-Tameemi, Kanaan and Kabakli, R., 2019. Hydatid cyst with focus on manifestations, diagnosis,and management. Asian J Pharm Clin Res, 12(12), pp.6-9.

2. Derbel, F., Mabrouk, M.B., Hamida, M.B.H., Mazhoud, J., Youssef, S., Ali, A.B., Jemni, H., Mama, N., Ibtissem, H., Nadia, A. and El Ouni, C., 2012. Hydatid cysts of the liver-diagnosis, complications and treatment. Abdominal Surgery, 5, pp.105-38.
3. Joanny, G., Mehmood, N., Dessì, G., Tamponi, C., Nonnis, F., Hosri, C., Saarma, U., Varcasia, A. and Scala, A., 2021. Cystic echinococcosis in sheep and goats of Lebanon. Parasitology, 148(7), pp.871-878.
4. Dalimi, A., Ghasemikhah, R. and Malayeri, B.H., 2005. Echinococcus granulosus: lethal effect of low voltage direct electric current on hydatid cyst protoscoleces. Experimental parasitology, 109(4), pp.237-240.
5. Yaseen, S.S., 2010. The Effect of Protoscoleces Treated With Direct Electrical Current on the Cellular Immune Response in Mice Infected With Secondary Hydatid Disease. Journal of Education and Science, 23(1), pp.15-23.
6. Moazeni, M. and Nazer, A., 2011. In vitro lethal effect of Zingiber officinale R. on protoscolices of hydatid cyst from sheep liver. Microbiology Research, 2(2), pp.91-94.
7. Rahma, J.H., Abdul-Wahid, N., Al-Zubaidi, F.A.A. and Al-Mousawi, N.R.H., 2011. Effect of electric current on the activity of the protoscolices of the Echinococcus granulosus. Kufa Medical Journal, 14(1).
8. AL-Aquli, H.A.A., Share this article with your colleagues. American Journal of Bio Medicine Volume, 4(1), pp.11-18.
9. Shahnazi, M., Azadmehr, A., Latifi, R., Hajiaghaee, R., Saraei, M. and Alipour, M., 2016. In vitro protoscolicidal effects of various concentrations of Ziziphora tenuior L. extract at different exposure times. Avicenna journal of phytomedicine, 6(4), p.376.
10. Ali, A.A. and Salim, F., 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. The Eurasia Proceedings of Science Technology Engineering and Mathematics, (3), pp.90-102.
11. Arandes, A.S. and Bertomeu, F.G., 2012. Echinococcosis/Hydatidosis. Current Topics in Tropical Medicine, 299.
12. Derbel, F., Mabrouk, M.B., Hamida, M.B.H., Mazhoud, J., Youssef, S., Ali, A.B., Jemni, H., Mama, N., Ibtissem, H., Nadia, A. and El Ouni, C., 2012. Hydatid cysts of the liver-diagnosis, complications and treatment. Abdominal Surgery, 5, pp.105-38.
13. Celik, A., Turanli, M., Kutun, S., Delibasi, T., Mengi, N., Comert, E., Aslan, S., Altundag, K. and Cetin, A., 2006. Unusual location of hydatid cyst: soft tissue mass in the neck. European Archives of Oto-Rhino-Laryngology and Head & Neck, 263(12), pp.1147-1150.
14. Pedrosa, I., Saíz, A., Arrazola, J., Ferreirós, J. and Pedrosa, C.S., 2000. Hydatid Disease: Radiologic and Pathologic Features and Complications 1: (CME available in print version and on RSNA Link). Radiographics, 20(3), pp.795-817.
15. Mehta, P., Prakash, M. and Khandelwal, N., 2016. Radiological manifestations of hydatid disease and its complications. Tropical parasitology, 6(2), p.103.
16. Ufuk, F. and Duran, M., 2018. Intrabiliary rupture of hepatic hydatid cyst leading to biliary obstruction, cholangitis, and septicemia. Journal of Emergency Medicine, 54(1), pp. e15-e17.
17. Zhang, W., Li, J. and McManus, D.P., 2003. Concepts in immunology and diagnosis of hydatid disease. Clinical Microbiology Reviews, 16(1), pp.18-36.
18. Ün, M., Yaman, S.S. and Erbaş, O., Hydatid cyst and treatment.

19. Moro, P. and Schantz, P.M., 2009. Echinococcosis: a review. International journal of Infectious diseases, 13(2), pp.125-133.
20. Mehta, P., Prakash, M. and Khandelwal, N., 2016. Radiological manifestations of hydatid disease and its complications. Tropical parasitology, 6(2), p.103
21. Alexander, C.K., 2009. Fundamentals of electric circuits. McGraw-Hill.
22. Edition, E. and Boylestad, R.L., 2007. Introductory Circuit Analysis.
23. Locher, R.E., 1988. Introduction to power supplies. Proc. National Semiconductor Application Note, 556.
24. Melhorn, C.J., 2004. Digital Multimeters. Wiley Survey of Instrumentation and Measurement, p.286.
25. King, P.H., Fries, R.C. and Johnson, A.T., 2014. Design of biomedical devices and systems. Crc Press.
26. Benam, K.H., Gilchrist, S., Kleensang, A., Satz, A.B., Willett, C. and Zhang, Q., 2019. Exploring new technologies in biomedical research. Drug discovery today, 24(6), pp.1242-124
27. Tsong, T.Y. and SU, Z.D., 1999. Biological effects of electric shock and heat denaturation and oxidation of molecules, membranes, and cellular functions. Annals of the New York Academy of Sciences, 888(1), pp.211-232.