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Research Article

The Effect of Extracts of Citrus Sinensis Peel on Protoscolices in Echinococcus Granulosus.

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ABSTRACT

The present study aims to evaluate the scolicidals effects of Citrus *sinensis peel* extract on the protoscolices of hydatid cysts on an in vitro and in vivo. Protoscolices were aseptically aspirated from the livers of naturally infected sheep. Various concentrations of extract were used for 1-24h. Eosin exclusion test was used to determine the viability of protoscolices. Findings showed that extract at the concentrations of 100, mg/mL killed 100% protoscolices after 6 hour , but the concentration 75 and 50 mg/mL killed the protoscolices after 12 hours and 24 exposure, respectively. Obtained results in this investigation for demonstrated that sweet orange might be a natural source for the production of new scolicidals agents.

Keyword: Hydatidosis; Echinococcus; granulosus; protoscolices; Citrus; Sinensis.

INTRODUCTION

Hydatidosis is a very serious health problem and epidemic in most countries of the world. The larval stage of granulosus parasite is the cause of the disease, which belongs to the Platyhelminthes phylum of the Cestoda species infect the small intestine of the carnivorous [1]. Human beings are accidental intermediate hosts and get infected due to food or water contaminated with feces of dog containing eggs of parasite or with direct contact with dogs [2].

Therefore, the studies in recent years have been directed to investigate many diseases using the folk medicine known as a herbal medicine for being effective, safe and economical factors [3]. The significance of this study is to show the effect of the extracts of the plant Citrus *sinensis* in the vitality of the protoscolices of Echinococcus granulosus of sheep origins in vitro and their growth in vivo.

Citrus *sinensis* (Sweet orange) plant belonging to the family Rutaceae. It is one of the most popular world fruit crops, contains active phytochemicals that can protect health [4]. (sinensis) refers to its Chinese origin [5]. Economically, oranges are important fruit crops, with an estimated 60 million metric tonnes produced worldwide as at 2005 for a total value of 9 billion dollars. Of this total, half came from Brazil and the United States of America [6,7].

Oranges as an excellent source of vitamin C, consist of powerful natural antioxidant, folate, dietary fibre and other bioactive components, such as carotenoids and flavonoids that prevent cancer and degenerative diseases [8]. Orange peel and juice play an anti-diabetic role by inhibiting the alpha-amalase enzyme responsible for converting complex carbohydrates into glucose, stimulating insulin secretion, and repairing defects of beta-pancreatic beta secretions [9,10]. It is also used against obesity, because it contains low calories and is rich in dietary fiber [11]. Orange fruit also works against typhoid fever [12]. Orange peel contains antioxidants and plays an important role against inflammation as well as the use of orange odors as an anti-anxiety agent [13]. Lemonin, linoleic and mersin in orange are effective against fungi [14].

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MATERIALS AND METHODS

Plant source

C. *sinensis* fruits were obtained from the market of the Al-Mansoura town- Aden governorate – Yemen.

Preparation of plant extracts and aqueous extracts

The plant extracts were prepared according to the method adopted by [15], Put (400 ml) of distilled water in a clean glass beaker and (40 gm) of plant powder was added, then it was placed the magnetic stirrer solution for (24 hours) at room temperature, Then solution was filtered by using stirred four layers of gauze medical and then placed in a centrifuge for (10 min) at a speed of (3000 rpm). Then it was placed in an electric oven at (40-60) degrees Celsius for evaporating water and then obtaining a dry powder which was scraped and stored in refrigerator in sealed bottles until use[16].

Alcoholic extract was prepare

The plant leaves powder (40 gm) was taken in Soxhlet apparatus. Then (400 ml) ethyl alcohol (70%) was added. The plant materials are extracted till the colour extract disappears it was left undisturbed. The solvent was evaporated by rotary evaporation .The mixture was finally placed in clean Petri dishes. Then the dry mass was transferred to an incubator and kept for (24 hours) at (50°C). It was weighed and kept in refrigerator in sterile and dark colored containers until use [17].

The source of the hydatid cyst

The hydatid cyst were obtained from the sheep of the butchery of Al-Basateen, of Aden-Yemen. The cysts were then transferred to the laboratories of the Faculty of Science, University of Aden-Yemen (Figure 1).

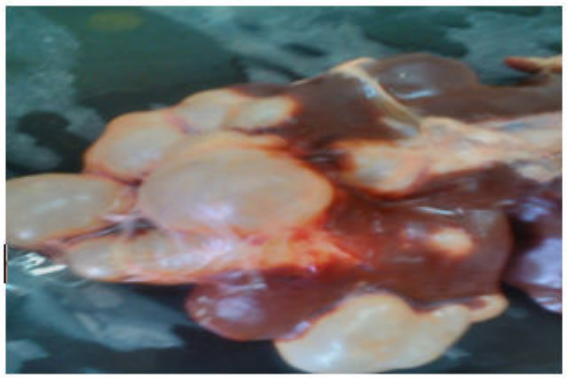

Figure1: Hydatid cysts in liver of Sheep

Collecting the protoscolices

Smyth's [18] method was used to obtain the protoscolices where the hydatid cyst was sterilized twice with ethyl alcohol(70 %), then the cyst fluid was removed by a sterile syringe The cyst was washed internally with (pH 7.2) and the antibiotic was penicillin IU20000 and Streptomycin 1 g/liter, the liquid was discarded in the test tubes, then it was centrifuge to at 3000 cycles/minute, and the protoscolices were examined under the microscope.

Evaluating the vitality of the protoscolices

Protoscolices were estimated using aqueous eosin stain (1%) 20 microliters of the primer suspension was taken and same amount of the eosin stain was added to a clean glass slide .It was examined under the microscope. Which kept the colour oblique of the green and prevented the entry of the stain they are a live protoscolices whereas the red was counted dead for its color pigmentation. The percentage of live protoscolices in the sample was calculated by dividing the number of live protoscolices in the sample to the total number of calculated headings x 100. The procedure was repeated three times in a row and the survival rate was taken. The percentage of the vitality of the protoscolices was calculated after each exposure period [19].

Laboratory animals

In this study, white albino rat Rattus norvegicus were used. The rats grew up in the Animal House laboratory at the Faculty of Science, University of Aden-Yemen [20].

ANATOMY OF RATS

The rats that were injected with the protoscolices of sheep origin were treated with the extracts under study after three months of the secondary hydatid cysts were investigated in the peritoneum , liver, lungs, kidneys and other areas of the body using a magnifying lens. Pictures were taken for the rats of two groups [21].

Statistical analysis

The results of the present study were analyzed by GenStat 5.2 using by general treatment structure (no blocking), factorial experiment, with 3 replications. Least Significant Different test (LSD) was used to test the difference between means (groups) at $P \leq 0.05$ and was considered significant [22].

RESULTS

Effect of aqueous and alcoholic extracts of C. sinensis plant on protoscolices in vitro.

The analysis of the variance table (1) shows a clear effect of the plant extracts of the peel of Citrus *sinensis* fruits of killing protoscolices. The table shows that there are significant differences between concentrations and exposure periods at the probability level $P < 0.05$. The concentration of 100 mg/ml of both alcoholic and aqueous extract had shown its superiority in reducing the vitality of the protoscolices to zero at 6 hours compared with the control group which was vital 94.67% and 93.33% respectively. Then the concentration of 75 mg/ml followed [23].

The lowest rate of killing protoscolices at the concentration was50 mg/ml at 1 hour, where the rate reached 72% and 62% respectively compared to the control group. The aqueous extract

exceeded at the concentration of 50 mg/ml than the alcohol extract, especially at exposure periods 1, 6 and 12 hours. (Figure 2) The exposure periods exceeded 24 hours from the rest of the periods where the death-rate of protoscolices was 100%.

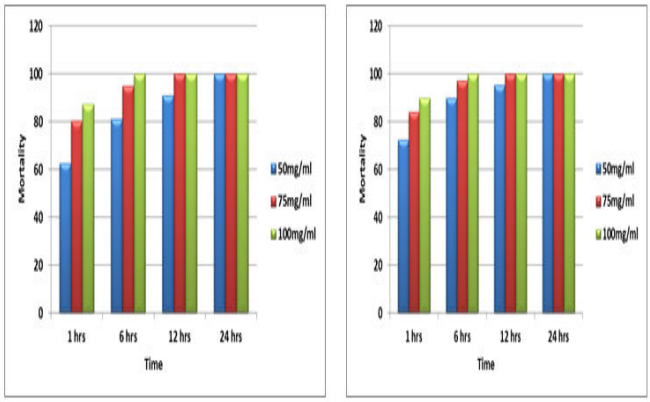

Figure. 2: Effect of extract alcoholic with aqueous of plant C. sinensis on protoscolices sheep origin in vitro.

Effect of aqueous and alcoholic extracts of C. sinensis indica plant on protoscolices in vivo.

After performing the above mentioned experiments related with effect of alcoholic and aqueous extracts of C. *sinensis* plant on the vitality of the protoscolices in vitro and the observation of the mortality rate of the protoscolices in vitro, The protoscolices treated with the extracts under this study were injected into the peritoneum of the laboratory rats to check the effect of these substances on the mortality of the protoscolices in vivo [24].

Three months later the rats were dissected to investigate the presence and growth of secondary hydatid cysts in different of the effect of the extract under this study on the mortality of the protoscolices as shown (Figure 4). The hydatid cysts were clearly visible in the laboratory rats injected with the non-treated extracts using the substances used in this study, the control group as shown the (Figure 3 and 4)

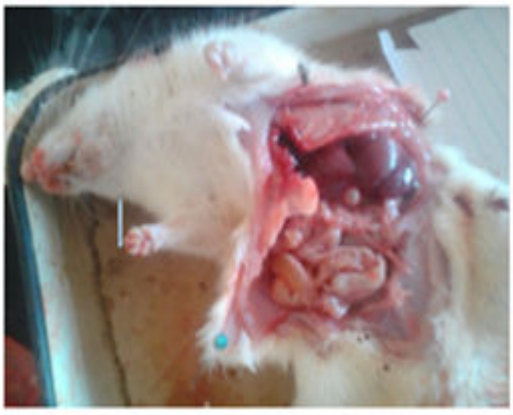

Figure 3: Rat control

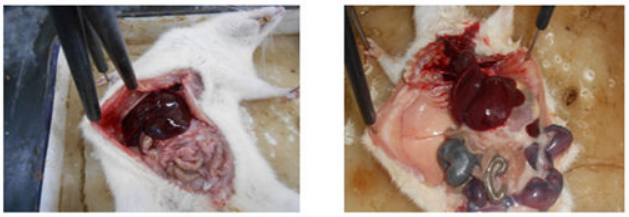

Figure 4: Rat treated alcoholic with aquous extract of Citrus sinensis.

DISCUSSION

The results were found similar to the result of [20].when they used endophytic Eupencillium and Chaetomium isolates from Azadirchta indica as they obtained complete death of the heads at 6 hours exposure time. This result was similar to the result obtained by when they used fruit and leaf extractions for the Pistacia atlantica plant at a concentration of 0.1 mg/ml where it resulted in the death of protoscolices after 6 hours of treatment [25].

The results of this study outperformed the period of exposure to protoscolices over the results of the study conducted by, when they used Osthole at a concentration of 120 µg/ml, it led to the death of protoscolices after 3 days of treatment. The results of present study outperformed the results obtained by, when cold water extractions were used for Zizphus plant at a concentration of 30%, as it obtained a complete death of protoscolices after 48 hours of exposure[26]. Citrus *sinensis* peel extractions were used in the cotton web industry to repel mosquitoes. It has also been used against Entamoeba histolytica and Giardia lamblia parasites.The aqueous extraction of orange peel led to death of adults of the insect, Coleoptera: Teneberionidae, where the death rate reached 100% three days after exposing adults to a concentration of 10% [27].

The mortality rate of the protoscolices treated by C. *sinensis* plant extracts can be attributed to its inclusion of active substances such as: Alkaloids whose effect is a consequence of its reaction with the metabolic protein reaction required for the vitality of the protoscolices. Then this leads to the destruction of the cell wall and its proteins and fats till the protoscolices die. The mortality rate of the protoscolices by C. *sinensis* death of the primates when treated with aqueous and alcoholic extracts of peel of C. *sinensis* plant can also be attributed to the tannins which may penetrate the cell membrane and block the active sites of some enzymes inside the cell which are necessary for the growth of parasites [28].

The death of the parasite can be due to Phenol substance which has an effect on the acetyl cholinesterase enzyme that controls the flexibility and permeability of the cell membrane. Phenols make the membrane lose its which result in passing of various toxic substances without regulating and this leads to the death of parasite [29].

The death of the parasite is attributed to flavonoids, which can reduce sugars, leading to a reduction of carbohydrate metabolism and thus a decrease in ATP [30].

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