INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

AN EVALUATION OF THE EFFECT OF *HYPHAENE THEBAICA* SAP AGAINST HYDATID CYSTS PROTOSCOLECES *IN VIVO* AND *IN VITRO*

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Article Received on: 09/11/18 Approved for publication: 09/01/19

DOI: 10.7897/2230-8407.100124

ABSTRACT

In the present work investigated the effect of the sap of the plant *Hyphaene thebaica* in the vitality of the protoscolices of *Echinococcus granulosus* of sheep origins in vitro and their growth *in vivo*. The results of this study showed that the sap of the *Hyphaene thebaica* stem has fatal effects on the protoscolices of the *Echinococcus granulosus* of sheep origin. The 100% concentration led to complete death of the protoscolices over the time period of 10 min. There were also significant differences among concentrations used at $p < 0.05$. When injecting the laboratory animals with the treated protoscolices they failed to formation secondary hydatid cysts.

Keywords: Hydatid cysts, Echinococcosis, protoscolices, *Hyphaene thebaica.*

INTRODUCTION

Hydatidosis is a very serious healthy and epidemic problem in the countries where it spreads.[1] *E. granulosus* requires dog and other canids as definitive hosts and livestock as an intermediate plete its life cycle.[2] It does not appear early and its symptoms are not clinically clear, but after arriving in an advanced stage and due to its evolution increase in size, it causes pressure on the organ adjacent to its location.[3] It has two developmental stages in its life cycle: the adult tapeworm, which is the sexual stage, and the cystic larval metacestode, which is reproduced asexually.[4] 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.[5] Therefore, the studies in recent years have been directed to investigate many diseases using folk medicine known as a herbal medicine for being effective, safe and economical factors.[6] The significance of this study is to show the effect of the sap of the plant *Hyphaene thebaica* in the vitality of the protoscolices of *Echinococcus granulosus* of sheep origins *in vitro* and their growth *in vivo*.

Hyphaene thebaica has local names such as (doum) in Arabic ; (gingerbread palm) in English, and (palmier doum*)* in French. *Hyphaene thebaica* belongs to the family of Arecaceae, which is a deciduous palm of 10-17 m height, with a girth of 90 cm. The trunk is Y-shaped, and the tree is easily recognizable by the dichotomy of its stem forming up to 16 crowns.[7] *Hyphaene thebaica* is one of the 11 species of the genus found in Africa. Widespread in the Sahel, it grows from Mauritania to Egypt, from Senegal to Central Africa and east to Tanzania. In Egypt, the doum palm has been cultivated since ancient times and has long been considered a sacred tree, symbolizing masculine strength. It was also planted in the belief that it protects and supplies people with shade, water and food after their death.[8] In medicine, its roots are used in the treatment of bilharzia.[7] The fruit pulp extract is also used in the treatment of bleeding especially after childbirth.[9] It is also used as a treatment to lower blood pressure.[10] The anti-cancer, antioxidant and antimicrobial activities of the fruit pulp has also been well documented.[11, 12]

MATERIALS AND METHODS

Plant source

The plant sap of *Hyphaene thebaica* was obtained from Al-Haswa Area, Aden-Yemen.

Preparation of plant sap

The first stage is to peel the scales that grow in the stem of the tree softly so that it becomes free of the leaves and scale. Then, a highly accurate stage comes which is amputation of a specific part of the tree's neck to separate its head from the rest of the trunk. After that, the most dangerous step comes on the life of the tree which is the digging of a hole on the neck(the heart) where a light layer is peeled superficially to the level that let the transparent liquid (Taari) come out without causing death to the tree. In the above-mentioned stages, various kinds of knives are used, and a natural tongue made of a piece of light plastic is set up away from the heart bark to regulate the flow of the liquid and maintain it without loss and leakage out of the box that is placed in the lowest neck. This process of (peeling and collecting liquid sap) is repeated three to four times per day in the early morning, in the afternoon and then in the middle of the night.

This fluid is called in its fresh state (Taari) and is characterized by sugar taste and smell similar to the smell of palm fluid of coconut “Nargil", and is used for the treatment of some types of worms that infect human intestines.

This liquid is collected in clean containers for the maximum of 40 days until it becomes mature vinegar. Before its 40 days age, this sap is characterized as a narcotic substance that affects humans' mind. If this liquid is exposed to a simple degree of air pollution

or even transient contact, it will spoil, decompose and becomes unusable. After this period, i.e. after forty days pass, it becomes more mature and the longer it is stored, the more effective its quality becomes. It will be finally stored in special containers.

The source of the hydatid cyst

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

Collecting the protoscolices

According to Smyth's method [13] used to obtain the protoscolices, the hydatid cyst was sterilized twice with ethyl alcohol (70%), and then the cyst fluid was removed by a sterile syringe. The cyst was washed internally with pH 7.2 and the antibiotic was penicillin 20000 IU and Streptomycin 1 g/liter. The liquid was discarded in the test tubes, then it was centrifuge of at 3000 cycles/minute, and the protoscolices were examined under the microscope.

Evaluating the vitality of the protoscolices

According to Smyth and Barret's [14] methods used for evaluating the vitality of protoscolices, These protoscolices were estimated using aqueous eosin stain (1%). 20 microliters of the primer suspension were taken and the same volume of the eosin stain was added to a clean glass slide and examined under the microscope. The protoscolices which maintained the oblique green color and prevented the entry of the stain are alive protoscolices whereas the red ones were counted as dead for its color pigmentation. The movement of the protoscolices was taken into consideration because they are important signs to examine the vitality (Fig. 2). The percentage of alive protoscolices in the sample was calculated by dividing the number of alive protoscolices in the sample to the total number of the calculated headings x 100. The process 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.

Solutions used

Phosphate buffer solution (PBS), Hank's solution and aqueous eosin stain were used for present investigation.

Laboratory animals

In this study, white albino rats *Rattus norvegicus* were used. A permission of ethics was granted to the researchers by the faculty of science, University of Aden. The rats grew up in the laboratory of the Faculty of Science, University of Aden-Yemen. They grew and reproduced in the conditions of the animal house and were provided with water and food, which was a concentrated mash of added protein and dry milk. The floors of the plastic cages were covered with sawdust that were usually changed weekly to keep the rats clean.

Implantation in laboratory animals

In order to determine the effect of the plant extract used in this study on the vitality of the protoscolices of sheep origin, on the growth and development in v*ivo* [15], rats were injected with the protoscolices treated with plant sap at the highest concentration in specific period of time. This was based on the results of screening the effect of this sap on the protoscolices *in vitro*. The detailed procedure involved is as follows: Protoscolices of sheep origin were treated with the sap of the *Hyphaene thebaica* plant at (100 mg/ml) for 10 minutes and then injected in four rats in the peritoneum (2000 protoscolices/rat) and Protoscolices not treated with the *Hyphaene thebaica* extracts injected in four rats in the peritoneum (2000 protoscolices/rat). This was used as a control group.

Anatomy of rats

The rats that were injected with the protoscolices of sheep origin were treated with the sap of plant 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.

Statistical analysis

The appropriate statistical method used to analyze the results obtained from the experiments in this study are Genestat 5 program (1995).

RESULTS

Effect of sap of plant on the protoscolices *in vitro* study

The table (1) shows the effect of 100%, 75% and 50% concentrations of *Hyphaene thebaica* on the vitality of the protoscolices from sheep origin *in vitro* in comparison with the control-group.

The comparative analysis table shows significant differences among the concentration rates used in this study at $p < 0.05$. The sap of the *Hyphaene thebaica* plant led to a decrease in the vitality of the protoscolices at different concentrations and different periods of exposure. The concentration of 100% exceeds the other concentrations at the period of 10 minutes, leading to complete death of protoscolices (100%) compared with control group which was 96% live. After that comes the 75% concentration which lead to 91.33% death. The least mortality rate was 77% at the concentration of 50% at the period of 5 minutes in comparison with control group which was 94.33%. The table also showed significant differences among the periods of exposure at the probability level of $p < 0.05$. The exposure period of 60 minutes exceeds the other exposure periods as the mortality was 100% at all concentrations. This indicates that the greater the concentration and the longer the exposure time, the lower the vitality of the protoscolices to zero.

Effect of sap of plant on the protoscolices *in vivo* study

After performing the above- mentioned experiments related with effect of sap of 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 sap of plant 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*. Three months later the rats were sacrificed and dissected to investigate the presence and growth of secondary hydatid cysts. (Fig. 3). 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 shown the (Fig. 4).

Table 1: Effect of *Hyphaene thebaica* sap on protoscolices sheep origin *in vitro*

Concentration	Control	Time / Minute				Means
		5	10	30	60	
		M %	M %	M %	M %	
100%	96.00	93.00	100.00	100.00	100.00	97.80
75%	96.00	83.33	91.33	100.00	100.00	94.13
50%	94.33	77.00	82.33	94.33	100.00	89.60
Mean of Time	95.44	84.44	91.22	98.11	100.00	
LSD 5%	Concentration (C) = 0.629, Time (T) =0.812, C*T=1.406					

*M= Mortality

Fig. 1: The hydatid cysts in liver of sheep

Fig. 2: Vitality of the protoscolices

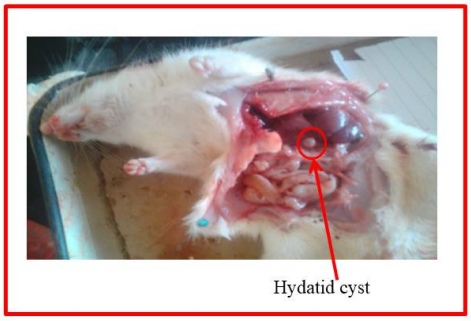

Fig. 3: Rat control

Fig. 4: Rat treated by sap of *Hyphaene thebaica*.

DISCUSSION

Medicinal plants have been used virtually in all cultures as a source of medicine. Assurance of the safety, quality, and efficacy of medicinal plants and herbal products has now become a key issue in industrialized and in developing countries. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Medicinal Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics.[16]

The results of effect of *Hyphaene thebaica* (doum) on the protoscolices formation used in this study, were similar in terms of the exposure period with the results obtained.[17] They used the alcohol extract of the ginger plant over a concentration of 50 mg/ml, which led to the complete death of the protoscolices at time period 10 Minutes. It was also similar to the study.[18] who used the *Zatria multiflora* Boiss (Lamiceae) alcohol extract with concentration of 20 mg/ml they obtained full homicide in 10 minutes. The results of this study were better than the results of the study conducted [19] in terms of the duration of exposure where the use of extract *Elettaria cardamomum* alcohol concentration of 100 mg/ml led to a complete death of the protoscolices at the time of 20 minutes. The results of the present study also exceeded the results obtained [20] when the chloroform extract of garlic plant was used at a concentration of 50 mg/ml with mortality of 98% for the protoscolices after 20 minutes of exposure. We can attribute the cause of the death of the protoscolices when treated with the juice of the plant doum to containing some active substances including alkaloids whose effect is the result of their overlap with the metabolic chain of proteins which is necessary to sustain the vitality of the protoscolices leading to the breakdown of the cellular wall and its protein and fat content until the destruction of the parasite.[21] The phenols may be the cause of the death of the parasite, which may have an effect on the acetylcholinesterase enzyme, which controls the elasticity and permeability of the cell membrane. Phenols have caused the membrane to lose, which resulted in the introduction of various substances without regulating and killing the parasite.[22] 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 .[23]

CONCLUSION

Through the results obtained from the current study, the following can be inferred showed the sap of the plant *Hyphaene thebaica* has a clear effect in the reduction of the intimacy of the protoscolices of the *in vitro* in a short period of time. Lack of growth of secondary hydatid cyst in the rats programmed with the protoscolices treated with the sap of the *Hyphaene thebaica* in this study, suggesting the ability of the plant sap to kill the protoscolices completely.

ACKNOWLEDGEMENTS

We are thankful to Dr. Mazahar Farooqui (Dean of Sciences and Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad) and principal of Dr .Rafiq Zakaria college for women Aurangabad for his constant help and co-operation.

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Cite this article as:

Fadel Y. S. Al-Arabi *et al*. An evaluation of the effect of *Hyphaene thebaica* sap against hydatid cysts protoscoleces In vivo and In vitro. Int. Res. J. Pharm. 2019;10(1):147-150 http://dx.doi.org/10.7897/2230-8407.100124

Source of support: Nil, Conflict of interest: None Declared

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