The Effect of Peganum Harmala (Water Extract) on Hydatid Cyst

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Abstract

Hydatid disease is considered as one of the important zoonotic diseases, due to the complications and the difficulties of the diagnosis and treatment. Although there is significant progress in the treatment of this disease, the difficulties of using drugs due to there toxicity and the side effect of the application are still amatter of discussion.

Therefore, lots of the researchers are starting thinking about the alternative procedure of the treatment before going to the surgery. Accordingly, plant extract (Peganum harmala extract) was considered to be one of the alternative procedures , because of their availability and it is usually known plant without side effect and it is poblicaly used for the treatment of different diseases. Therefore it has been decided in this work to apply the seeds extract of this plant in the experimental treaetment of the hydatide disease in vitro and in vivo on experimental animals. The results of this work reveald significant efficacy of these extracts on the activity of protoscolices and also the reduction of numbers of Hydatide cysts in the experimental animals .

Introduction

Hydatid disease is considered to be as an important world wide Problem. This disease is one of the zoonotic diseases in the world which causing the Infection of the different organs of man causing his death in most cases and also infecting the animals causing economical problems [1,2,3].Although there was progress in the medical treatment of man and animals , this disease still considered as abig medical and economical problem , especially in Iraq due to the sanction made by the western countries.

Several research works conducted about the treatment, but it was for alimited succision due to the speciality of the growth of this parasite , as it is surrounded itself with athick layer (

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connective tissue) , during the growth , which prevent the arrival of enough amount of the drugs material [4,5,6] . This of course, improved the idea that the surgical procedure is the best. Therefore, the researchers are seeking cheap, available and easy drug for the treatment. Accordingly, in this work, Peganum harmala (seed extracts) were suggested to be the alternative material. This plant is available and cheap with no toxicity, to be used in the experimental work in vitro and in vivo.

Materials and Methods

Source of the extracts:

Seeds of Peganum harmala were bought from the local market and cleaned well then grinded to produce a powder material. Fifty gms of the powder were diluted in 250 ml of D.w. and filtered after 24 hrs at 37C. The filterate was then lyophilized and used latter on in the proceding experiments

Experimental animals:

Bulb/C white mice were used in the experimental work. These were reared and reproduced in animal house of the medical college, University of Baghdad and used when they were 6-7 weeks.

In vitro efficiency of the seed extracts on the viability of protoscoleces:

The efficiency of (125, 250, and 500) micro-gm of the Peganum harmala extract on the viability of the protoscoleces was estimated in vitro. 3000 protoscoleces were isolated in each of the 12 tubes. The tubes were then divided into 4 groups. The first three groups were treated with 125,250 and 500 microgms respectively. Whereas the fourth group was untreated and considered as acontrol group. Eosin stain was used for the estimation of the viability. During the experimental work, (10) mice in each experiment were used, each (5) of them were isolated in a cage and the experiments were conducted as follow:

Negative control group :Each individual of this group was injected intraperitoneally with 0.2 ml of physiological saline solution.

Positive control group: The individuals of this group were each infected with protoscoleces intraperitoneally and considered as positive control group.

Three months latter , they were killed , dissected and examined for the presence of cysts. The liver and spleen were removed and fixed for preparation of slides to be examined histologically.

Estimation of the development of hydatid cysts from protoscoleces that were attenuated (in vitro) with (500) micro- gms plant extracts in white mice:

Animals of this group were infected with (2500) attenuated protoscoleces intraperitoneally. After three months, they were killed then dissected and the number, size and weight of hydatid cysts were recorded. As in the preveous experiment, the liver and spleen were fixed and left for subsequent work.

The effect of water extract of Peganum harmala, intraperitoneally introduced, on mice after three months of infection with protoscoleces:

In this experiment, the mice were infected with (2500) protoscoleces intraperitoneally. Three months Latter, acourse of treatment with 50 mg/kg of Peganum harmala seed extracts was applied for 7 weeks. The differences in the weight of mice were checked weekly [9]. The treatment of this extract was given intraperitoneally also. The animals were then dissected and examined.

The effect of Peganum harmala seed extract , orally introduced, on the animals infected with protoscoleces:

The same experiment was performed as in (d) except the inroduction of the (50) mg/kg which was through the mouth.

Histological examination:

The method of Bancroft , 1979 [10] , was used for the histological examination.

Results

The in vitro effect of Peganum harmala extract on the viability of protoscoleces :

The result of this experiment revealed the presence of clear and significant difference between the concentrations used and that of the control results within the same period of time and the different periods , table (1) and according to the following:

When the different concentrations used , the viability decreased gradually from the starting of the experiment till the following different periods:

When 125 micro-gms used, the viability was (0.96+ 0.00)becoming (0.00+0.00) on day five .250 micro-gms (0.94+0.063) becoming (0.00+0.00) on day three .

500 micro-gms (0.92+0.07) becoming (0.00+0.00) on day two whereas the control group was (0.97+0.04) micro-gms becoming (0.075+0.013) on day six.

The effect of Peganum harmala extract on the infectivity of protoscoleces in white mice:

This was assessed by checking the followings:

- The number and distribution of hydatid cysts in the peritone and the internal
- Organs (liver and spleen).
- The weight of the liver and spleen.
- Histological study.

The development of treated protoscoleces with500 micro-gm in white mice:

It has been reveald in this experiment that the mean distribution of the hydatid cysts in the vescera was (3.67+2.5) , in liver (1.7+1.5) and there was no cyst in the spleen . Moreover, the mean weight of the liver was (1.25+0.23)gm and the mean weight of the spleen was (0.11+0.033) gm and the mean weight of the secondary hydatid cysts was (0.004) gm . Through the examination of the discovered cysts, there was no viable and no dead protoscoleces.

Regarding the histological examination , there was blood congestion in the liver with infilteration of inflammatory uninucleated cells. It was the same situation in the spleen as there was also blood congestion and infiltration of inflammatory cells table (2).

ii. The effect of 50 mg/kg of Peganum harmala extract on the secondary cysts, after 3 months of infection through the peritone for 7 weeks (5) days aweek:

After the end of the treatment course , mice were killed and dissected and the results were:

Mean number of the secondary cysts in the vescera was (2.1+ 1.45) compaired to (9.30+4.22) in the control group . Also the mean number in the liver of the control animals was (8.1+5.2). Regarding the mean of the liver weight , it was (1.42+0.02) , whereas the mean liver weight in the control group was (1.76 +0.20) . No significant difference in the weight of spleen of the control and the test groups.

Regarding the histological examination , it has been noticed the presence of blood congestion and the infeltration of the uninucleated inflammatory cells in the liver and the presence of the calcified cysts in the liver tissues Fig. (1). In the spleen there was alymphatic tissue formation with a macrophages.

iii.The effect of 50 micro-gm of Peganum harmala extract , introduced through the mouth , on the secondary cysts after 3 months of infection:

In this experiment and after the dissecting of the infected mice which had the treatment orally , the mean of the hydatid cysts in the liver was (2.4+2.1) contrasted with (8.1+5.2) of the control group . In the spleen it was (0.7+0.40) and in the viscera it was (9.20+4.22) table (2) and Fig. (2).

Regarding the mean weights of the liver and spleen , they were (0.45+0.21) and (0.15+0.025) gm respectively . It has been revealed from the histological study of the organs, that there was a granuloma and necrotic tissues in the liver and the presence of giant cells. Also the presence of cysts and inflammatory response with lymphatic aggregation around the cysts. Moreover, the presence of dead secondary cysts Fig(2).

In the menwhile, the tissues of mice that treated with physiological saline, they were all normals as in the control tissues and also the weights of the tissues were normal table (3. (

Discussion

It was clear from this work that the extract of Peganum harmala had clear effect on the viability of protoscoleces increased proportionally with increase of the concentrations of the used extract table (1). It was the highest when (500) micro-gm used. This might be due to the reason that the extract contains (Alkaloides , Saponins , Harmin , Harmalin and Tannins) materials [7] . The previous studies mentioned that this extract contained some ingridients which were highely efficient against insects and worms and also on their metabolic activities by interferring with their enzymatic activities [11].

It has been illustrated , when different concentrations of the extract were used , that the efficiency on the protoscolecs was proportionally soft during the short periods of applications (2,6,12) hrs. But it was of higher severity during the long periods. This might be interpreated that the efficient compounds in these extracts are not purly concentrated, but it was crude material which needed longer period of time of application to act. Also , it has been noticed as in table (2) , that the application of the different concentrations of the extracts, caused the reduction in numbers and the weights of the secondary cysts during the two ways of treatments (oral and intraperitoneal application) . But for a limited levels , the intraperitoneal procedure of treatment was better than that of the oral . This might be due to the direct absorption of the extract material compaired with oral way.

The results of this work revealed that the extract of Peganum harmala has for the first time aprotective ability against hydatid disease in animals and this might be in man . It could be concluded that the reason could be due to the significant immunosuppresion effects of the metabolic activity of cells in the peritone , as it was clear when 500 micro-gm used , there was infammatory uninucleated cells infeltration with the absence of cysts due to the attachments of these cells to the protoscoleces and secreation of enzymes that analysed them (12). Therefore, it could be concluded that the use of (125,250,500) micro-gm of Peganum harmala extract , could cause the death of the protoscoleces in vitro . And also inhibit the growth of the inplanted protoscoleces in the tissues and converted them to acalcified and fibrinated cysts and cause the reduction in the numbers and weights of these cysts in mice.

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