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The Effect of Aqueous and Alcoholic Extract of Nerium Oleander on Biochemical Parameters of Rats Infected with Echinococcus Granulosus

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ABSTRACT

In view of the importance of the biochemical aspect and its direct and indirect relationship with the various damage that occurs in different tissues, it has been necessary to study chemical changes in natural and pathological conditions.

The present study was conducted to evaluate the effect of active substances in the aqueous and alcoholic extracts of Nerium oleander on the Protoscolies viability of the echinococcus in vivo. The protoscolies were collected from livers of sheep naturally infected with Hydatid Cysts at Tikrit slaughterhouse. The viability of these Protoscolies was measured using the eosin aqueous stain. The results showed that there are significant differences in the concentrations of Nerium oleander for aqueous and alcoholic extracts. The alcoholic extract at a concentration of (270) mg / ml achieved good results, as the values of protein, glucose, triglycerides, urea, creatine, magnesium, sodium, potassium and zinc increased, while the values of liver enzymes GOT, GPT, and ALP decreased in the treated animals.

Keywords: Biochemical, oleander, hydatid cyst

Introduction

Hydatid cyst disease is one of the serious epidemiological health problems in most parts of the world (Eckert & Deplazes, 2004). It has several names: Echinococcosis, Cystic echinococcosis and Hydatidosis (Roberts & Janovy, 2000).

It is a Zoonotic disease, and the countries of the Middle East, North Africa, Sudan, the Caspian Sea Basin and some countries of South America are highly endemic to this disease (Wen et al., 1993). The disease in humans and other intermediate hosts (sheep, cows, buffaloes, camels, horses and other animals) results

from the larval stage of the tapeworm parasites of Echinococcus, which includes many species, the most important of which is E. granulosus and the E. multilocularis and this stage can attack any organ in the body of the intermediate host. The disease is more common in rural areas where there are frequent breeding of farm animals and carnivores, which helps to complete the life cycle of this parasite that needs the middle host and the final host (dogs, wolves, hyenas, leopards and other wild animals) (Marquardt et al., 2).

Disease or epidemic in humans and other intermediate hosts is caused by ingestion of food and water contaminated with tapeworm eggs produced by adult parasitic worms in dogs. Currently, granulosus is the only type of echinococcosis in the Mediterranean region, where the pet dog acts as the only reservoir for the adult tapeworm and thus plays a major role in the infection. It was found that cystic echinococcosis more prevalence in temperate regions, including South and Central Asia, China, Australia and parts of Africa. In general, disease distribution is usually related to underdeveloped countries, especially rural societies where humans and other domestic animals are in close contact with dogs. Granulosus E is the only cause of hydatid cyst in Iraq

Biochemical studies are necessary to know or distinguish the genetic differences of E. granulosus in different countries. Biochemical analyses also show quantitative differences in the parasite metabolism, and the variation in the biochemical components of the cyst fluid reflects the difference of strains in different intermediate hosts, and this diversity in hosts can cause metabolism mutations which are important for the survival of the parasite in different environments, and biochemical analyses provide information in diagnosing and distinguishing strains of the parasite that are related to human infections, these analyses assist in the diagnosis and treatment.

Human infection with E. granulosus provokes or causes the cellular and humoral immunity, which is generally represented by elevated levels of immunoglobulins in the blood serum.

Medicinal plants are better than medicines that are used for two reasons. The first is that plants are a repository of medicines that can be tried on humans without side effects, and second, the availability of plants in large quantities in most countries

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of the world, as it was found that medicinal plants contain a number of effective compounds that are attributed to the anti-disease effect including Alkaloids, phenolic and Terpenes and other compounds (Chan-Bacab & Pena (Cowan, 1999)

The present study aimed to investigate the effect of the active substances isolated from Nerium oleander extract (alkaloids and phenols) on the vitality of the Protoscolies in vivo.

2-3: Methods of Preparing the Solutions Used in the Current Study

1-2-3: Normal Saline

This solution was prepared according to the method of (Collee et al., 1996), in washing the wall of the hydatid cyst to collect the suspended Protoscolies.

2-2-3: Phosphate Buffer Saline Solution (PBS)

This solution was prepared according to the method of Hudson & Hay (1984). It was used to wash generating layer of the Hydatid cyst.

3-2-3: Kreb's - Ringer's Solution (KRS)

This solution is considered one of the best media for preserving Protoscolies alive ex-vivo after isolating them from the cysts (Al Rabie, 1999). This solution was prepared according to (Routunno et al. 1974)

It consists of

Sodium chloride -NaCl	0.480 gm
Potassium chloride -KCl	0.157 gm
Calcium chloride -CaCl2	0.0.137 gm
Acidic sodium carbonate -NaHCo3	0.281 gm
Dihydrogen Sodium Phosphate -Na H2PO4	0.470 gm
Disodium phosphate-Na2HPO4	0.490 gm
Magnesium Sulfate -MgSO4	0.072 gm
Streptomycin -Streptomycin	200 mg
Penicillin	4000 I.u.

The above substances were gradually dissolved in half a liter of distilled water so that no substance was added unless the substance before it dissolved, and then completed the volume to 1 liter, the pH measurement was carried out by a PH meter at 4.7 by adding drops of HCL acid, and then the solution was sterilized by placing it in the autoclave at a temperature of 121 ° C and under a pressure of 15 pounds / inch2

for a period of one third of an hour, then glucose 0.090 / g was added after being sterilized with fine-perforated filter papers 22.0 micrometers, then antibiotics were added to prevent contamination. This solution was used to rinse the generating layer. It was also used with Hydatid cyst fluid in a volume of 1 ml at a ratio of 4: 1 to preserve the Protoscolies.

Collection of Hydatid Cysts

Hydatid Cysts samples were obtained from naturally infected sheep livers at Tikrit Slaughterhouse, Salah El-Din Governorate. The samples were placed in special cork containers cooled with crushed ice to preserve the vitality of the Protoscolies, as they were dealt with directly and transported to the laboratory, and as soon as these affected organs were delivered to the laboratory, the cysts were opened directly to ensure their fertility through the presence of the Protoscolies inside, and the vitality of the Protoscolies was examined using Aqueous eosin dye at a concentration of (0.1%).

Dealing with Hydatid Cysts

Smyth (1985) method was used in the process of separating the Hydatid Cyst parts from each other, by washing the outer surface of the cyst with the physiological saline solution prepared in paragraph 3-3-3, then sterilizing with a piece of cotton moistened with ethyl alcohol, then withdrawing the largest possible amount of the liquid using medical syringes (10 and 5 ml) depending on the size of the Cyst, and the liquid was transferred to sterile test tubes, after which the cyst was opened using forceps and surgical scissors. The layer generated and what was left with it of the Cyst and Protoscolies fluid were withdrawn by forceps, after which a longitudinal incision was made in the Cyst wall, and transferred to a sterile vitreous plate. The generated layer was opened longitudinally by scissors and then the remainder of the liquid was withdrawn by means of a syringe free of Needle and added to the previous liquid. After the completion of the Hydatid withdrawal process, the generated layer containing the largest number of Protoscolies was rinsed with Ranker's solution prepared in paragraph 1-3-3, as they were rinsed 3-5 times or until they are confirmed that they are free of Protoscolies by examining the liquid under the microscope after each rinsing process and placing the liquid in sterile tubes, and then the centrifugation is done by a centrifuge at a speed of 1500 rpm for a period of ten minutes. As for

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Hydatid liquid, it was withdrawn by a Pasteur pipette leaving a little liquid with precipitated Protoscolies, where it was placed in sterile test tubes and chemical tests were performed immediately after calculating the vitality of the Protoscolies. The liquid obtained from the rinsing of the generating layer was withdrawn by a Pasteur pipette leaving a little liquid and precipitated Protoscolies. The Protoscolies were collected with the precipitate, then they were washed three times with Renker solution where they were deposited by a centrifuge for five minutes each time washing throwing the filtrate and taking the precipitate, then the Protoscolies were suspended with a volume of 1 ml of Ranker solution. As for the Cyst wall layers, they were washed with a PBS solution and placed in sterile bottles containing the Baun solution prepared in paragraph 3-4 for a period of 24-18 hours, washed well with running water for 90 minutes to ensure that they were completely free of fixative solution, then preserved with 70% ethanol.

Estimation of Protoscolies Viability

Protoscolies' viability was estimated using the fixed volume transfer method with a micropipette, where 10 micro liters of the suspension prepared in section 3-5 were drawn and mixed with the same amount of eosin stain and placed on a cell counting slide, and examined directly under a light microscope where living Protoscolies appeared with a bright green color to repel the dye compared to the Protoscolies that are pigmented red as the pigments penetrate through their wall. The number of Protoscolies per milliliter was calculated by taking the average number of Protoscolies in 10 microliters for three readings, noting that the number of Protoscolies in the fixed volume depends on Hydatid cyst fertility, as the total number of Protoscolies per milliliter = the number of Protoscolies in constant volume x 100, and the percentage of vitality of Protoscolies was determined according to the following equation (Galindo et al., 2002; Himonas et al., 1994; Jenkins and Rickard. , 1986)

$$\text{Protoscolies vitality (\%)} = \frac{Number\ of\ living\ Protoscolices\ \ calculated\ \ in\ fixed\ volume\ of\ the\ suspen\ sion}{\text{total number } of\ Protoscolices\ \ calcuated\ \ in\ that\ volume} \text{ x } 100$$

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Preparing plant extracts

The aqueous plant extracts of Nerium oleander were prepared according to the method of (Al-Mansour 1995) modified from Harborne (1973). 100 g of dry plant material was placed in 200 ml of distilled water and shaken for half an hour and left for 24 hours, after which it was filtered with two layers of scrap cloth and packed in test tubes and placed in an emitter apparatus for 10 minutes at a speed of 3000 revolutions per minute. After that the sediment was left and the filtrate was collected in glass dishes and put in electric oven at a temperature of 50 ° C until it was completely dry. 5 grams of the dry extract was obtained and kept in glass containers in a refrigerator until use. As for the alcoholic extract, the same method was used, except for replacing water with alcohol when dissolved.

: Determination of the half lethal dose of L.D50 and the pharmacokinetic dose of Nerium oleander in male rats.

The semi-lethal dose and the pharmacokinetic dosage of Nerium oleander were determined by dissolving (10) grams of dry powder of the substance in 10 ml of water. The distillate concentration reached 1000 mg / ml or 1 g / ml. The animals were divided into seven groups, each group containing eight animals, then the animals were dosed orally with 1 ml of this dose, starting with the low doses (1000,900,800,700,600,500,400) and going up to the high doses to find the half-lethal dose and left for 24 hours so that these animals can be monitored noting any side effects such as lethargy or death, and a lethal dose has been determined.

Results:

In this study, the biochemical parameters of the experimental animals were examined before and after infection. The results recorded a decrease in the concentration of protein, glucose, urea, triglycerides, creatine and zinc significantly. The values of magnesium, potassium and sodium of the treated animals were significantly decreased compared with the negative control. In the positive control, there was a significant decrease in these criteria. As for liver enzymes GPT, GOT, ALP, they increased significantly in experimental animals, and they recorded a higher increase in positive control, and the alcoholic extract was better than the aqueous extract, and that the higher concentration of the alcoholic extract recorded better

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results than the rest of the concentrations. The concentration is directly proportional to the responses of the animal groups to the treatment, as the most effective of them is the highest concentration for the aqueous and alcoholic extract.

The table below shows the effect of plant extracts on some biochemical parameters of experimental animals infected with Echinococcus granulosus.

	T.P	Glocose	Urea	T.G	Creatinin	Mg	Na	K	Zink	GPT	GOT	ALP
Control Negative	8.9 A	106.5 a	56.36 A	114 A	0.56 A	2.93 a	153 A	6.8 a	227 A	41.8 E	82.2 D	121.7 E
Control Positive	4.2 D	48.1 c	24.05 e	63.7 E	0.36 C	1.59 f	129 D	4.3 f	162 F	97.3 D	144.7 C	288.6 Cd
Nerium Oleander **Aqueous** 120	5.8 C	95.2 b	40.50 d	93 D	0.46 B	2.50 e	142 C	4.9 e	190 E	158.2 C	137.2 C	275.2 D
Nerium Oleander **Aqueous**240	5.8 C	96.7 b	40.3 d	97 cd	0.47 B	2.53 d	143 C	5.3 d	197 D	198.7 B	139.4 C	291.5 C
Nerium Oleander **Aqueous** 270	6.0 Bc	96.9 b	40.9 d	101 bc	0.47 B	2.53 d	148 B	5.7 c	201 C	203.5 B	141.5 C	299.7 C
Nerium Oleander **Alcoholic** 120	6.8 Bc	97.5 b	40.37 d	102 bc	0.46 B	2.57 c	148 B	5.8 c	197 D	197.9 B	151.2 B	321.9 B
Nerium Oleander **Alcoholic** 240	7.0 B	98.6 b	45.5 c	104 B	0.47 B	2.49 e	150 Ab	5.8 c	203 C	205.3 B	159.4 B	338.1 B
Nerium Oleander **Alcoholic** 270	7.2 B	103.4 ab	49.32 b	105 B	0.47 B	2.60 b	151 A	6.2 b	216 B	233.7 A	176.3 A	417.5 A

Discussion:

Hydatid cysts represent a global health problem (Karakay, 2007), and surgery is the only and preferred treatment option so far for single-vacuole cysts (Bogitsh et al.,

2005). Therefore, current global trends are moving towards medicinal herbal treatment to get rid of the effects and dangers caused by treatment. The chemist and herbs have been used since ancient times for treatment, as man guided them by virtue of his experiences with them and by virtue of the intellectual ability that God distinguished them over all other creatures

The effective activity of the Nerium oleander extract is due to the chemical content of the active substances such as alkaloids, phenols, flavonoids, tannins and actins (Al-Rabei, 1999). The inhibition of these compounds is explained by the fact that they interfere in the chain of protein metabolism reactions necessary for the continuity of the micro-organism's viability and its ability to break down the cell wall and what it contains of proteins and fats and then the parasite's destruction (Cowan, 1999). It also explained the effectiveness of the components of Nerium oleander of alkaloids on the basis of inhibition of carbohydrate metabolism by affecting the mitochondria and then obstructing the breathing process (Delorenzi et al (2001) that the effect of alkaloids and phenols is due to the union of these active substances by affecting the metabolism of nitrogen and amino acids that are the basis for building the mitochondrial membrane, the nucleus, and the body Colji, which are important in the viability of micro-organisms, or the reason for their ability to combine with proteins, which leads to changes in the chemical properties of the cell wall or a change of the shape of the whole cell and this may result in its death. They act as agents that stimulate cellular and humoral immunity and stimulate the production of IL-2, which increases the efficiency of the immune system and increases the ability of macrophages to attack foreign bodies.

As for phenols, the reason may be due to the fact that phenols have their effect on the enzyme acetylcholine esterase that controls the elasticity and permeability of the cell membrane as the phenols made the membrane lose this permeable property, which led to the entry of various toxic substances without regulation and then the death of the parasite. The phenols cause inhibition process based on their ability in deforming proteins and stopping the action of the enzymes responsible for a series of basic metabolic reactions and thus the microorganism loses its ability to survive.

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As for the flavonoids, they act on reducing the sugars, which leads to an imbalance in the carbohydrate metabolism and thus a decrease in the amount of energy units (ATP) supplied in the vital activities in the parasite (Sarkar et al., 1996).

The results of the current study agree with the findings of it (Al-Hamiri, 2010).

Conclusion

It can be concluded from this study that Nerium oleander is effective against Hydatid Cysts inside the body of infected animals that were treated compared with those infected which were not treated.

References

1. **Al-Hamiri, Ahmad Khudair-Ubys (2010).** Evaluation of the efficacy of Datura stramonium seed extract on the growth and development of Hydatid Cysts of Echinococcus granulosus in White Mice Balb / c (pharmacokinetic, histopathological and immunological study). PhD thesis, College of Science, University of Kufa, p.
2. **Al-Rubaie, Salwa Saber Mohsen (1999).** Effect of some plant extracts in weakening the Protoscolies of Hydatid Cysts outside and inside the body in the white mouse. M.A. Thesis, College of Science, University of Baghdad: 95 pp.
3. Al-Mansour, Nasser Abd Ali (1995). Effect of different extracts of Ibiceila lutea on the life performance of Bemisa tabaci. PhD thesis, College of Science / University of Basrah.
4. **Galindo , M. ; Gonzalez , M. and Galanti , N. (2002).***Echinococcus granulosus* protoscolex fromation in natural infection . Biol. Res. , 35(3-4): 365-371.
5. **Himonas , C. ; Antoniadou- Sotiriadou , K. and Papadopoulos, E. (1994).** Hydatidosis of food animals in Greece : Prevalence of cyst containing viable Protoscolies . J. Helminthol., 68: 311-313.
6. **Jenkins , D.J.and Rickard , M.D.(1986)**. Specific antibody responses in dogs experimentally infected with *Echinococcous granulosus*.Am. J. Trop. Med. Hyg.; 35(2): 345-349.
7. **Smyth , J. D.(1985).** In vitro culture of *Echinococcus* spp. Proc. 13th Int. Cong. Hydatid, Madrid: 84-95
8. **Collee , J. G. ; Frasser , A. G. ; Marmion , B. P. and Simmon, A. S. (1996)** . Parctical medical microbiology. Chunchill living stone. Elsevier.UK: 444- 496 . hydatidosis in Balb/c mice .Dirasat . Agricultural sciences,24(3) .
9. **Cowan , M. M. (1999) .** Plant Products As Antimicrobial Agents . Clin . Micro. Rev. ,12(4) : 564- 582.

10. **Delorenzi, J. C.; Attias, M.; Gattas, C. R.; Andrade, M.; Rezende, C.; Pinto, A. C.; Henriques, A. T.; Bou-Habib, D. C. and Saraiva, E. M. (2001) .**Anti-Leishmanial activity of an indole alkaloid from Peschiera australis. Antimicrob. Agents. Chemother., 45(5):1349-1354.
11. **Harborne, J.B. (1973)** . Phytochemical methods Chapman and Hall. London, 612 pp.
12. **Hudson, L. and Hay, F. C. (1984)** . Practical immunology. 3rd edn.Blackwell. Scientific Publication ,Oxford:86-126.
13. **Landa-Garacia, J. I.;Alonso, E.;Gonzalez- Uriarte, J. and Roderigues-Romano ,D. (1997) .** Evaluation of scolicidalagents in experimental **hydatid** disease model .Eur. Sur.Res.,29:202-208.
14. **Routunno,C. A.;Kammerer, W. S.; Perez-Esandi,M. V. and Cereijido , M.(1974) .** Studies on the permeability to water,sodium and Chloride of the hydatid cyst of *Echinococcus granulosus*. J. Parasitol. 60 (4) : 613-620.
15. **Smyth, J.D. and Barrett, N.J. (1980).**Procedures for testing the viability ofHuman hydatid cyst following surgical removal specially after chemotherapy. Trans. Roy. Soc. Trop. Med. Hyg., 74 : 649-652.
16. **Eckert, J. and Deplazes, P**. (2004).Biological , epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern, Clin. Microbiol. Rev. , 17(1): 107-135.
17. **Roberts, L. S. & Janovy, J. Jr. (2000)**. Schmidt & Roberts' foundations of parasitology, 6th edn. McGraw-Hill Book Co. , New York: 670 pp.
18. **Wen, H. ; New, R.R.C. and Craig, P.S. (1993).** Diagnosis and treatment of human hydatidosis. Br. J. Clin. Pharmacol. , 35: 565-574.
19. **Marquardt, W. C. ; Demaree, R. S. and Grieve, R. B. (2000).** Parasitology and vector biology. Harcourt, Acad. Press. 335-339.
20. **Sarkar, D.; Sharma, A. and Talukder, G. (1996).** Plant extracts as modulators **of** genotoxic effects . Botan.Rev.,62(4):257-300.