

The Effect of Clove Extracts on the Vitality of the Primary Heads of the Granulomatous Echinococcus of Sheep Origin Inside the Body of the Organism¹

*Abeer M. Hatem, **Fouad S. I. Al-Rubaye

*College of Pharmacy

**College of Education for Girls

University of Mosul, Mosul, Iraq

*Corresponding Authors: Abeer.21gep11@student.uomosul.edu.iq

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ABSTRACT

We sought to identify the effect of clove extracts (*Eugenia caryophyllus*) on the vitality of the primary heads of *Echinococcus granulosus* of sheep origin was injected in vivo, where the primary heads treated with plant extracts, whose vitality ranged between 30-50% in laboratory, were injected into mice of the BALB/C strain of the *Mus musculus* type for both ethanol extract at a concentration of 0.2 mg/ml for 15 minutes and extract Ethanol 0.2 mg/ml for 30 minutes, hot water extract at 75 mg/ml for 45 minutes and hot water extract at 100 mg/ml for 15 minutes to demonstrate the effect of extracts in reducing the growth and development of primary heads of secondary hydatid cysts. The mice explained after four months. The results showed that aqueous clove extract and ethanolic extract showed significant differences compared to the control group at $P \leq 0.05$. The results showed a decrease in the number, diameters and weights of hydatid cysts, as well as an increase in the percentage reduction in the number of cysts in the current study, as it reached 100% and no hydatid cyst grew, and the direct use of aqueous clove extract and ethanolic extract and four doses injected into the peritoneum two days after the injection of the initial heads in laboratory mice when dissected after four months to a clear significant decrease in the rate of the numbers, diameters and weights of the hydatid cysts from the control group and after Four months of treatment, and the percentage reduction of the number of hydatid cysts has also increased. In the current study after four months of treatment to 96% and 93% for treatment after two days respectively and may be the result of the effect of the extract on the cells generating the hydatid cyst that caused calcification of some cysts.

INTRODUCTION

Cystic echinococcus or Hydatidosis is one of the most serious and widespread human-animal diseases worldwide caused by the metacestoda of *Echinococcus granulosus* (1), the seriousness of the disease lies in the fact that it is a silent disease, as it is discovered by chance when examining the abdominal cavity for other diseases, liver injuries are often without obvious symptoms, but some complications can occur in 21% of patients, including rupture of cysts causing anaphylactic shock or secondary infection, as well as the mechanical effect when the cyst is compressed on neighboring tissues, causing bile obstruction and portal hypertension, usually the right lobe. More affected by infection due to hepatic portal circulation (2). Treatment of echinococcosis remains a complex and significant problem both locally and globally that has not been definitively solved (3-5) according to the WHO-IWGE (WHO Informal Working Group on Echinococcosis), treatment classifications include surgical treatment, chemotherapy, percutaneous aspiration injection and reaspiration (PAIR) and non-invasive monitoring and waiting (2,6).

In the case of small and inactive cysts, chemotherapy with benzimidazole derivatives (mebendazole and albendazole) is the treatment of choice (7), according to previous studies that have indicated side effects of chemotherapy such as hepatotoxicity, leukopenia, thrombocytopenia, osteoporosis and hair loss (7,8), surgery is also the best treatment for removing large and active hydatid cysts, but it is difficult to perform in cases of cyst proliferation in a number of

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organs or cyst formation in risky locations (9). Healthcare providers inject chemical agents that kill primary heads such as hypertonic saline, silver nitrate, formalin, povidone iodine, ethanol, and cetrimide (10) to avoid the risks and complications of surgery if the cyst ruptures or leaks its contents causing secondary cystic echinococcosis or anaphylactic shock and even death in a number of patients (11).

The clove plant is a fascinating herbaceous annual. With multiple branches that gently sway, its flower buds start off pale in color, gradually transitioning to a vibrant green, and finally maturing into a radiant red hue, indicating they are ready for harvest. These buds measure anywhere between 2 to 1.5 centimeters in length and boast an elegant long cup that ends with four flat cup leaves. Nestled in the center, four petite petal leaves form a charming little ball (10,11). When it comes to reproduction, clove employ two distinct methods. The first is the vegetative method, accomplished through cuttings. This approach allows for the preservation of the genetic characteristics of the parent plant's variety. On the other hand, the sexual method involves using seeds. However, this method is less desirable if one wishes to maintain the specific genetic traits. As a result, the vegetative method takes precedence as the primary means of reproduction, ensuring similarity between the parent plant and its offspring (12).

MATERIALS AND METHODS

Collection and Test Hydatid Cysts of sheep was obtained from butchers' shops in the center of Mosul and transported to the laboratory to ensure the fertility of the hydatid cysts by containing live primary heads.

Protoscolices Isolation Primary Head Isolation: To isolate the primary heads, the method of (13) was used, by sterilizing the outer surface of the hydatid cysts twice with a medical cotton moistened with ethanol at a concentration of 70%, then part of the hydatid cyst fluid is withdrawn by a medical syringe with a capacity of 10 ml and a needle measuring G21, in order to reduce the pressure inside the cysts, then the cyst is opened by surgical scissors and sterile forceps, and the liquid is emptied by a Pasteur pipette and the cyst is washed from the inside by a saline phosphate shield solution with a pH of 7.2 pH in a large petri dish. The heads were combined with the hydatid fluid in a baker capacity of 500 ml and the heads were placed with the phosphate buffered solution in test tubes and the washing process was carried out by the centrifuge three times at a speed of 3000 cycles / minute and for 10 minutes per wash, where the first wash used saline phosphate buffer and in the second wash the phosphate buffered solution was used in addition to 20,000 international units (IU) of penicillin and 1 g of streptomycin per liter of buffer solution, and the third wash was with phosphate buffer solution only, then the floater was withdrawn from the liquid and sterile PBS was added to the precipitate containing the protoscolices.

ESTIMATION OF PROTOSCOLICES VIABILITY

Using the method of (Smyth, 1985), the vitality of the protoscolices was estimated by taking 20 μ liters of the protoscolices suspension and adding the same volume of aqueous eosin dye at a concentration of 0.1% on a glass slide and examined under a microscope at the power of X10. Live heads on the total number of protoscolices calculated \times 100, the process was repeated three times and in the current study used primroses with vitality of 95% -100%.

Statistical Analysis: The SPSS statistical analysis system was used to analyze the results of the study statistically, as it used the Complete Randomized Design system with the Two Way analysis of Variance system and one way analysis of variance, and extracted the Standard error, as well as the Duncan's Multiple Rang Test to measure the significance of the difference between the averages of the study experiments at a level of significance $p \leq 0.05$.

RESULTS AND DISCUSSION

The results showed that the aqueous clove extract and the ethanolic extract showed significant ($P \leq 0.05$) differences compared to the control group, as primary heads with vitality ranged between 30-50% were injected into laboratory animals. To demonstrate their ability to develop secondary hydatid cysts in laboratory mice, the results showed a decrease in the number, diameters, and weights of hydatid cysts, as well as an increase in the percentage of reduction of the number of cysts in the current study, reaching 100% and no hydatid cyst was developed at the time of treatment. Aqueous clove plant extract and ethanolic extract. Thus, it was similar to the results of Ramadan and Ali, (2020), which obtained the highest reduction of 100% hydatid cyst counts in mice injected with 15% primroses exposed for 15 seconds to ultrasound after four months of treatment.

Table (1) shows the percentage reduction of the numbers of hydatid cysts as it reached 96% and 93% when treated with aqueous clove extract and ethanolic extract respectively, which is congruent with the study (14) that dealt with the effect of methanolic extract of garlic plant and albendazole on hydatid cysts in fertilized mice 1500 primary heads, and after four months of infection was treated for thirty days and they noticed a decrease in the total number of hydatid cysts, their diameters and weights compared to the control group at $p < 0.05$.

Table 1. The percentage reduction of the numbers of hydatid cysts in treated versus control group

Groups	Hepatic cysts			Spleen cysts			Cysts in other organs			% reduction
	No.	Diameter (mm)	Weight (mg)	No.	Diameter (mm)	Weight (mg)	No.	Diameter (mm)	Weight (mg)	
Control	8.35±6.7 A	2.38±0.14 A	11.13±5.66 A	7.45±1.57 a	2.14±0.12 a	4.23±0.55 a	3.75±1.50 A	1.34±0.28 a	2.84±0.02 a	
ethanolic extract (15 min)	0±0 C	0±0 C	0±0 C	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 b	%100
ethanolic extract (30 min)	0±0 B	0±0 B	0±0 B	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 b	%100
Aq.extra cts (75g.)	5.25±3.75 C	1.96±0.96 C	5.29±2.65 C	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 b	%96
Aq.extra cts (100g.)	2.±4.90 B	0±2.13 B	2.1±4.50 B	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 b	%93

The direct use of aqueous clove extract and ethanolic extract in four doses injected into the peritoneum two days after the injection of the initial heads in laboratory mice led to a significant decrease in the average number, diameters and weights of hydatid cysts from the control group and after four months of treatment, this finding is in the line with the result of Labsi et al. (2016) with a decrease in their diameters and weights after three months of infection of laboratory mice when using aqueous extract of pomegranate plant peels, and the percentage of reduction increased (15).

Number of hydatid cysts: after four months of treatment with the extract the number of hydatid cysts reduced to 96% and 93% for treatment after two successive days (Table 1). It may be the result of the effect of the extract on the cells generating the hydatid cyst, which caused calcification of some cysts, and thus converged with the result of Al-Matioti (2017), who obtained a reduction rate of the number of hydatid cysts 90.16% when injected with eggplant plant extract three days after injecting mice with primary heads, explaining the reason that the closer the treatment date is to the date of injection of the primary heads, the more extract effect (16).

The shorter the duration, the less the effect, because the primary heads are able to install themselves in the host and form hydatid cysts, unlike the current study, which obtained a reduction rate after two days higher than the injection of the primary heads in addition to not showing any significant difference between them in relation to the average number of hydatid cysts, their diameters and weights in the liver, spleen and other organs of the two treatments, and between Al-Khashab (2014) that he obtained a reduction rate of 100% when injected aqueous extracts of Hawthorn, Ruta and Green tea plants after three days from initial head injection (17).

The results of the AL-Quraishi, (2015) study (18) showed the effect of phenols and alkaloids extracted from the leaves of Artemisia Herba-Alba and its flowers on the vitality of primordial heads in vivo. There are statistically significant differences in the diameters and weights of the scalar cysts, as phenols led to a clear reduction in the diameter of the hydatid cysts compared to the Control group. Phenols and alkaloids also showed therapeutic efficacy of 55.17% and 48.27%, as reported Torabi et al. (2018) that he obtained a significant reduction in the number and size of secondary

hydatid cysts when treated with bendazole alone or when mixed with nanoparticles with degeneration of hydatid cysts as a result of treatment(19).

Table (2) shows the changes in the numbers, diameters, weights and percentage reduction of their numbers when treated with aqueous clove extract and ethanolic extract, all treatments showed significant differences when compared with the control group in which the cysts appeared in the form of clustered masses or in the form of prominent lobes or partially implanted within the liver tissue, spleen and other organs, and their appearance was concentrated in the liver in the first place, in which the average number of cysts in the liver was 8, diameters of 3.08 mm and weights amounted to 11.13 mg. In the spleen, the average number was 5 and the average diameters were 0.25 mm, the average weight was 1.18 mg, while the number of cysts in other organs was 5, with diameters of 0.37 mm and weights of 2.84 mg.

Table 2. The changes in the numbers, diameters in treated versus control group

Groups	Hepatic cysts			Spleen cysts			Cysts in other organs			% reduction
	No.	Diameter (mm)	Weight (mg)	No.	Diameter (mm)	Weight (mg)	No.	Diameter (mm)	Weight (mg)	
Control	6.±8.40 80 A	2.38 ±0.14 A	11.13±5 .66 A	7.30±1. 45 a	2.14±0. 12 a	4.23±0. 55 a	4.75±1. 70 A	1.34±0. 09 a	2.84±0. 02 a	
ethanolic extract (15 min)	0±0 C	0±0 C	0±0 C	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 b	%100
ethanolic extract (30 min)	0±0 B	0±0 B	0±0 B	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 B	%100
Aq. extracts (75g.)	4.25±2. 75 C	1.68±0. 235 C	2.93±1. 90 C	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 B	%96
Aq. extracts (100g.)	3.90±± 2.3 b	0.75±0. 10 B	1.40±0. 30 B	0±0 b	0±0 b	0±0 b	0±0 B	0±0 b	0±0 B	%93

As for the treatments, the aqueous clove extract and the ethanolic extract showed significant differences, as no injury was recorded, and the latter did not show any injury in the spleen and other organs and its appearance was limited to the liver only, and no significant differences appeared in the numbers, diameters and weights of the cysts, as their average numbers reached 1.65, 1.75 for the treatments for the aqueous clove extract and the ethanolic extract respectively, and the averages of their diameters amounted to 1.65, 1.75 mm, respectively, while the weights amounted to 5.29, 4.36 mg, respectively, and amounted to the highest A percentage of reducing the number of hydatid cysts by 100% when treated with aqueous clove extract and ethanolic extract respectively, while when treated with aqueous clove extract and ethanolic extract, the reduction rate was 92% and 90% respectively.

Table (2) shows the changes in the numbers, diameters, weights and percentage reduction of hydatid cysts in the liver, spleen and other organs when treating mice with aqueous clove extract and ethanolic extract two days after injecting them with the primary heads, and after four months of infection, it was found that there were significant differences between the treatments and the control group, which reached the average number of hydatid cysts in the liver, in the spleen 8 and in other organs 5 and their diameters amounted to 3.02, 2.14, 1.34 mm respectively. 8.72, 1.18, 1.66 mg in liver, spleen and organs respectively.

Respectively, for the extract treatments, no infection appeared in the spleen two days after the injection of the primary heads and did not show significant differences in the numbers, diameters and weights of the hydatid cysts in the liver and other organs when treated two days after the injection of the primary heads have averaged 1.35, 0.00, 1.75 and diameters 0.59, 0.00, 1.08 mm either weights 4.26, 0.00, 5.43 mg in the liver, spleen and organs respectively when

treated two days after the injection of the primary heads As for the treatment of injection of heads reached averages The number of cysts is 2.00, 0.00, 0.66 respectively, and their diameters are 0.55, 0.00, 0.72 mm respectively, and their weights are 2.04, 0.00, and 4.64 mg in the liver, spleen and organs, respectively, and the percentage reduction of their numbers was 92%, 90% two days after injection of primary heads compared to the control group.

Table (2) shows the changes in the numbers, diameters and weights of hydatid cysts and the percentage of reduction of their numbers when treated with aqueous clove extract and ethanolic extract after four months of infection The control group showed significant differences at $p \leq 0.05$ for the treatments two days after the injection of the initial heads The number of cysts was 2, 4, 1 and their average diameters were 0.40, 0.68, 0.28 mm and their average weights were 4.50, 3.80, 2.19 mg in the liver, spleen and organs respectively, and for the treatments after Two days of injection, no significant differences were shown in the numbers, diameters and weights of hydatid cysts in the liver, spleen and organs, the average number of cysts amounted to 0.00, 0.00, 0.00 and their average diameters amounted to 0.75, 0.00, 0.00 mm, while the average weights were 3.14, 0.00, 0.00 mg in the liver, spleen and organs respectively.

After two days of injection, it is clear that they did not show any injury in the spleen, and other organs and their appearance was limited to the liver, and for the treatment two days after the injection of the initial heads were averaged 0.75, 0.00, 0.00 in the liver, spleen and organs, and this shows the appearance of infection on the liver only and not appearing in other organs, while the percentage of reducing their numbers in the two treatments two days after the injection of the initial heads amounted to 92% and 90%, respectively, compared to the control group after four months of Infection, macroscopic changes that have also been observed include fibrosis and atrophy of some cysts and their appearance as scar on the surface of the liver in some transactions.

CONCLUSION

1. Clove plant extracts showed lethal and inhibitory effects in the vitality of the primary heads of sheep origin within the vivo, and were directly proportional to increase the concentration and exposure time with the superiority of the ethanolic extract over the rest of the extracts.
2. A decrease in the number, diameters and weights of hydatid cysts and an increase in the percentage of reduction in their numbers when injecting mice with primary heads treated with hot water extract under study.
3. Laboratory mice are free of hydatid cysts when treated with ethanolic extract.
4. Reduction in the thickness of the layers of the wall of the cardinal cyst and lamellar with an increase in the thickness of the germ layer as a result of the injection of primary heads treated with hot aqueous extract with the emergence of some histological pathological changes in the layers of the wall of secondary hydatid cysts

REFERENCES

1. Hizem A, M'rad S, Oudni-M'rad M, Mezhoud H, Ben Jannet H, Flamini G, Ghedira K, Babba H. In vitro scolicidal activity of Thymus capitatus Hoff. et Link. essential oil on Echinococcus granulosus protoscoleces. Journal of essential oil research. 2020 Mar 3;32(2):178-85.
2. Kern P, Da Silva AM, Akhan O, Müllhaupt B, Vizcaychipi KA, Budke C, Vuitton DA. The echinococcoses: diagnosis, clinical management and burden of disease. Advances in parasitology. 2017 Jan 1;96:259-369.
3. Shevchenko YuL, Nazyrov FG. (2016), Surgery of echinococcosis M: Publishing house, Dynasty, 51-62.
4. Nartailakov, M.A. (2016) On the issue of prevention and treatment of recurrence of echinococcosis // Infections in surgery, 14(2). 35-38.
5. Velasco-Tirado V, Romero-Alegría Á, Belhassen-García M, Alonso-Sardón M, Esteban-Velasco C, López-Bernús A, Carpio-Perez A, Jimenez López MF, Muñoz Bellido JL, Muro A, Cordero-Sanchez M. Recurrence of cystic echinococcosis in an endemic area: a retrospective study. BMC infectious diseases. 2017 Dec;17:1-8.
6. Pham H, Kupshik D, King C, Romano P. Cystic echinococcosis. Appl Radiol. 2019 Sep 1;48(5):42-3.
7. Dehkordi AB, Sanei B, Yousefi M, Sharafi SM, Safarnezhad F, Jafari R, Darani HY. Albendazole and treatment of hydatid cyst: review of the literature. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders). 2019 Jun 1;19(2):101-4.
8. Bakan S. Hydatid cysts of the pulmonary artery. Turkish Journal of Thoracic and Cardiovascular Surgery. 2016;24(3).
9. Norouzi R, Ataei A, Hejaz M, Noreddin A, El Zowalaty ME. Scolicidal effects of nanoparticles against hydatid cyst protoscolices in vitro. International journal of nanomedicine. 2020 Feb 17:1095-100.

10. Sharafi SM, Sefiddashti RR, Sanei B, Yousefi M, Darani HY. Scolicidal agents for protoscolices of *Echinococcus granulosus* hydatid cyst: review of literature. *Journal of Research in Medical Sciences: the Official Journal of Isfahan University of Medical Sciences*. 2017;22.
11. Brunetti E, Tamarozzi F, Macpherson C, Filice C, Piontek MS, Kabaalioglu A, Dong Y, Atkinson N, Richter J, Schreiber-Dietrich D, Dietrich CF. Ultrasound and cystic echinococcosis. *Ultrasound international open*. 2018 Sep;4(03):E70-8.
12. Deros MR. *Production and Characterization of Extraction Oil from Natural Spices: A Comparison Study with Functional Group Content of Zea Mays and Elaeis Guineensis Jacq. Oil* (Doctoral dissertation, UMP).
13. Smyth J. In vitro culture of *Echinococcus* spp. *Proceedings of the 13 th International. Congr. Hydatidology. Madrid*. 1985, 84-89.
14. Mohammadi KH, Heidarpour M, Borji H. In vivo therapeutic efficacy of the *Allium sativum* ME in experimentally *Echinococcus granulosus* infected mice. *Comparative immunology, microbiology and infectious diseases*. 2018 Oct 1;60:23-7.
15. Labsi M, Khelifi L, Mezioug D, Soufli I, Touil-Boukoffa C. Antihydatic and immunomodulatory effects of *Punica granatum* peel aqueous extract in a murine model of echinococcosis. *Asian Pacific journal of tropical medicine*. 2016 Mar 1;9(3):211-20.
16. Al-Matioti, Abdullah Hussain Jassim (2017) Effect of aqueous and alcoholic extracts of castor, datura and eggplant leaves on the vitality of primers of granular echinococcus of sheep origin in glass and in vivo. Master's Thesis[Arabic], College of Education, University of Mosul.
17. Al-Khashab, Mohamed Hamed Abdelkader (2014) Effect of aqueous and alcoholic extracts of hawthorn leaves, green tea and solo on the vitality of primary heads of granulocytes of sheep origin in glass and in vivo. Master's Thesis[Arabic], College of Education, University of Mosul.
18. AL-Quraishi MA, Shaalan NN, Almusawi HS. Study the effect of *Artemisia Herba-alba* extracts in adult and larval stages of *Echinococcus granulosus* parasite in vivo and in vitro. *Int. J. Curr. Microbiol. App. Sci*. 2015;4(8):267-82.
19. Torabi N, Dobakhti F, Faghihzadeh S, Haniloo A. In vitro and in vivo effects of chitosan-praziquantel and chitosan-albendazole nanoparticles on *Echinococcus granulosus* Metacestodes. *Parasitology research*. 2018 Jul;117:2015-23.