DOI Number: 10.37506/v14/i1/2020/ijfmt/193048

Comparative Molecular Study between Some Plant Extract and Tinidazole of Hydatid cysts in Basrah, Southern Iraq

Jalal Y. Mustafa[1], Ghazi Y. Azzal[1], Suzan A. Al-Azizz[1], Fatin A. Muatafa[2], Arwa R. Lazim[2]

[1]Veterinary Medicine colleges / Basrah university, Iraq,
[2]Education colleges of Biological science / Basrah University, Iraq

Abstract

Hydatid disease or Echinoccosis is one of the serious public health problems. This study was designed as a comparative molecular study between some plant extract and tinidazole of sheep hydatid cysts in Basrah province. In this study, 213 sheep were examined and the number of those infected with hydatid cysts was 75 (35.2 %). The results showed that hydatid cyst were detected successfully by performing PCR technique. In addition, *Quercus aegilops* has been shown the best plant extract for destroying all genes, the second plant extract which is *Capparis spinosa* showed destroying of two genes (*G6-7, COI*) and failed to destroy (*sh4-1*). Furthermore *Prosopis fracta* has showen to destroy only (*G6-7*) and tinidazole failed to destroy any of these genes used in this study.

Key words: *Comparative Molecular Study. Plant Extract with Tinidazole.*

Introduction

Hydatidosis is one of the very important parasitic defect of domestic animals that cause economic losses and public health disease worldwide [1] . It is caused by adult or larval stages of cestodes (metacestodes), which is belonged to the genus *Echinococcus* and the family *Taeniidae*, also known as Hydatid Disease, is an infection caused by the larval stage of the flat worm *Echinococcus granulosus*. It has a worldly distribution and is one of the most general parasitic zoonosis [2]. The metacestodes progress are fluid - filled hydatid cysts, in which protoscoleces are construct as the following generation of tapeworms. In liver and lung, cyst develop. Their growth cause acute defect and death in the intermediate host. Protoscoleces inside entire hydatid cysts in organs of perished or slaughtered animals stay infectious for up to 2 weeks, according to the temperature and other conditions [3]. It has been observed that tinidazole has the ability to rupture the helix structure of DNA and prevent building of nucleic acid which leads to destroy the cells and the parasite [4]. Many types of medical plants have been used to treat some parasitic infection. Arab people have traditionally used *Quercus sp.* to treat dysentery (which is caused by *Entamoeba histolytica*), stomach cancer and bloody vomiting [5], as well as many disorder like eczema, cancer heeling and vomiting. This is due to the presence of active substance (Phenols and Tannins) in the plant [6] . Acorns serves an paramount role in early human history and it were a source of food for many cultures around the world. In the past, the poorer would origin eat acorns in their food [7]. According to Edible and Useful Plants of California, by Charlotte Clark, "people who use acorns today concur that they resemble other nuts in oiliness and taste". In general, acorn flour contains significant quantities of calcium, magnesium, phosphorus, potassium, sulfur, fat and protein [8]. Many species of *Capparis spinosa* extract, many of these species are reported from Iraq, northern to southern of the country [9]. The plant is well known for the edible buds and fruits (caper berry), which are consumed pickled [10]. *Prosopis fracta* is belonged to Memosaseae family. It is a grapple shrub with 30-100cm height, grows extravagantly in Iraq ([11,12,13]).

Materials and Method

In this study, A total 213 organs (lung and liver) of sheep **infected with hydatid disease were examined to determine the effect of some plant extract comparing with drug used for treatment (**tinidazole**) from the period between October 2018 to March 2019**. The hydatid cysts were taken from the infected organs and

put in a clean container with 70% ethyl alcohol and transported to the laboratory of Veterinary Parasitology at College of Veterinary Medicine / University of Basrah. The samples were divided to five groups, each group has 10 samples as follows: group one was a control, represented by cyst without any treatment, group two was treated by tinidazole, group was three treated by *Capparis spinosa*, group four was treated by *Prosopis fracta* and group five was treated by *Quercus aegilops*. The extract or tinidazole after prepared to the plain tube were added to each group, followed by adding part of the germinal layer of the cyst for 24 hours. A part of cyst of each group was processed for DNA extraction.

The aqueous extract was prepared by distal water and plant extract according to [19] with some modification. Twenty five gram of plant powder were added to 100ml distal water, and left for 24 hours on magnetic stirrer (IKA-combimag) type RCT, the extract was then filter by vacuum pump using special filter paper type Whatman No-1. The extracts were concentrated and streams by rotary vacuum evaporator in type Rota Vapor-RE, Buchi, at Biochemistry Laboratory colleges of science / University of Basrah. After drying them, they were kept in dark bottle until used.

DNA Extraction:

DNA was extracted from liquid (Protoscolices) and germinal layer using Wizard® Genomic DNA Purification Kit (Promega/USA) following the manufacturer's instructions. The concentration of extracted DNA was determent by NanoDrop spectrophotometer at 260 nm and 280 nm and stored at -20°C.

Polymerase Chain Reaction:

DNA of hydatid cyst was amplified by performing PCR technique using (GoTaq Hot Start Green Master Mix, Promega) following the manufactures instructions. Three pairs of primers were used for the detection of *Echinococcus* were used in this study: *COI*: F: 5' TTGAATTTGCCACGTTTGAATGC 3', R: 5' GAACCTAACGACATAACATAATGA 3' with product size 792bp [16] ; *sh1-4*: F: 5' GTTATAAGAGGCCTCTCCGTGTTGTGG 3', R: 5' CGTACGATTAGTTTCACAATATACATAT 3' with product size 295bp [14] and *G6-7*: F: 5' TGGGGTAGTTACAATAGTTATTC 3', R: 5' CATAATCAAATGGAGTACGATTA 3' with product size 234bp [14]. The annealing temperature for all PCR are 56ºC

The PCR conditions are as follows: initial denaturation for 5min at 95°C. followed by 35 cycles of 95°C for 30sec, 56°C for 30sec, 72°C for 30sec. The reaction was then held at 72°C for 10min, and then cooled down 4°C for 5min (table 2). The PCR product was then detected on agarose gel stained with ethidium bromide, used 100-1000bp (KAPA BIOSYSTEM) and 1kb DNA ladder (Promega) [20] .

DNA Sequencing and Sequences analysis

PCR product was sent to Macrogene (Korea) company, for sequencing. The sequences were edited and aligned using Parbi-Doua and NCBI BLAST programs. Contrasted the results with data gained from Gene Bank published ExPASY program which is available at the NCBI online. Phylogenetic analysis was performed by using the NCBI program.

Results and Discussion

This study showed that the total number of the examined sheep was 213 and the total number of infected sheep was 75. The total percentage of infection was (35.2%) which is agreed [21,22] and disagreed with [23,24] who showed that the prevalence of infection was 1.5%, 5.9% in north and middle of Iraq, respectively. The result of infected liver and lung in sheep by hydatid cyst is -shown in figure (1, A and B). This difference could be accounted on the basis of differential management practices, natural resistance, drug treatment and nutrition. The live and dead protoscolices were stained with green and red color, respectively (Figure 1, C).

The results of this study showed that the percentage of infection is high in sheep in Basrah province. Greater endeavor is wanted to control the transmission of hydatid cysts from slaughter house by the suitable disposal of infected residue, especially in sheep. This will reduce the transmission of cysts from slaughter house to potential hosts in this region. Now, veterinary authority put rules to control this disease through the creation good standardization abattoirs system to raise knowledge of farmers about hydatid cysts and removal stray dogs from Basrah province [25] .

The results of the present study are compatible with other studies in different parts of the world which showed that the sheep strain is the most important pattern accountable for human injury and a broad range of intermediate hosts [26,27,28].

No effective chemotherapy is presently accessible for the medical treatment of cystic and alveolar hydatid disease in human [29] . However recently several anthelmintic drugs (which have shown encouraging results in the reduction to the larval cystic mass) [30] , there is an evidence effect of the drug tinidazole and praziquantel on lethal the protoscolices ([31,32]).

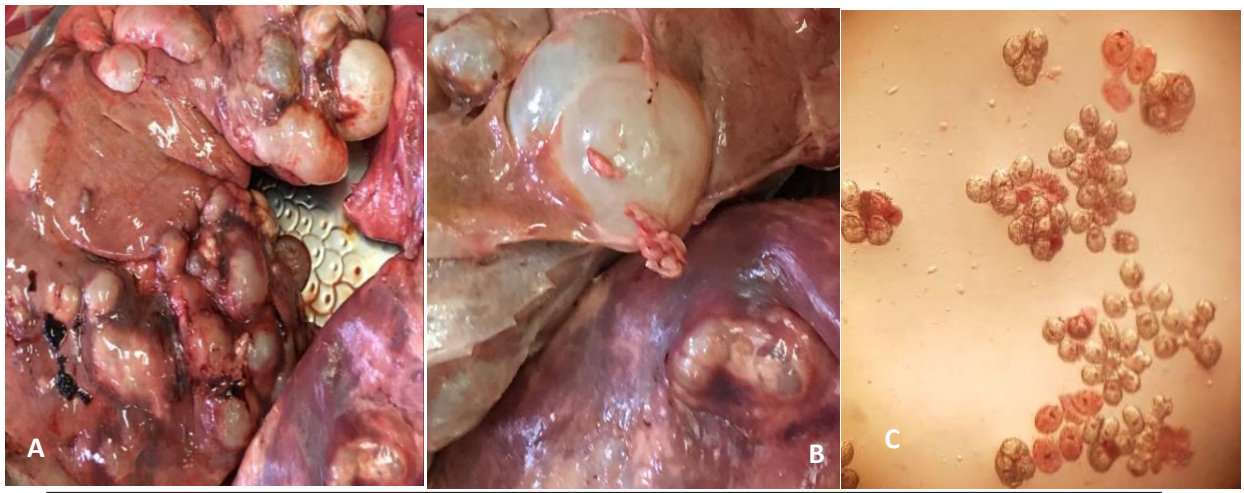

Fig. (1): Sheep infected by hydatid cyst (A) in liver (B) in lung.

(C) Live and dead protoscolices isolated from sheep.

Molecular study

In this study, the effect of plant extract or drug against *Echionococcus* was evaluated through the determining of specific genes of three groups: control, extract and treated by tinidazole.

Three genes, including strain *G6-7* (234bp), strain *sh4-1* (295bp) and strain *COI* (792pb) were detect in this study.

Fig.(2) showed the results of PCR (*G6-7, sh4-1, COI*) in group one (control group) and group two (treated by tinidazole), the result showed PCR amplification for *G6-7* 237bp, *sh4-1* 295bp and *COI* 792bp. While fig. (3) showed group four (treated by *Prosopis fracta*), the results of PCR the amplification for *sh4-1* 295bp and *COI* 792bp and absent for *G6-7* because the effect of plant extract. Fig.(4) appeared the results of PCR (*G6-7, sh4-1, COI*) in group three (treated by *Capparis spinosa*), the result showed that *sh4-1* 295bp was amplified but absent in *G6-7, COI* because effect of plant extract. The same figure showed the last group (group five) appeared treated by *Quercus aegilops*, the result showed absent of any amplification because effect of plant extract. The high efficacy of *Quercus aegilops* come from phenol and tannins properties [33] , Tayler study pointed out because tannins polar properties give the inhibition effect many type of microorganism, as well as limited the inhibition effect of protease enzyme [33]. For all that, this study reported the *Quercus aegilops* is the best extract against three genes used in current study, table (4) appeared all the result of genes in all groups.

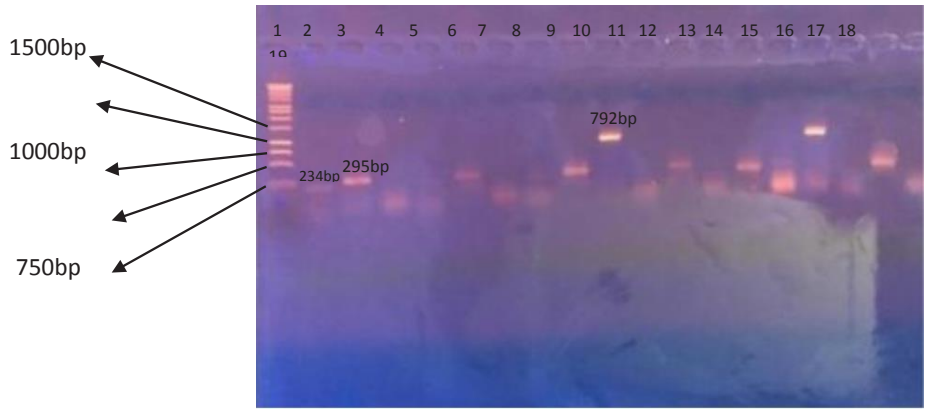

Fig. (2): Results of PCR of control and tinidazole groups. lane (1) is the ladder 1kb DNA ladder (Promega), lanes (2,8) for *G6-7*, lanes (3,6,9) for *sh4-1*, lane (10) for *COI.* Samples treated v
G6-7, lanes (12,14,18)

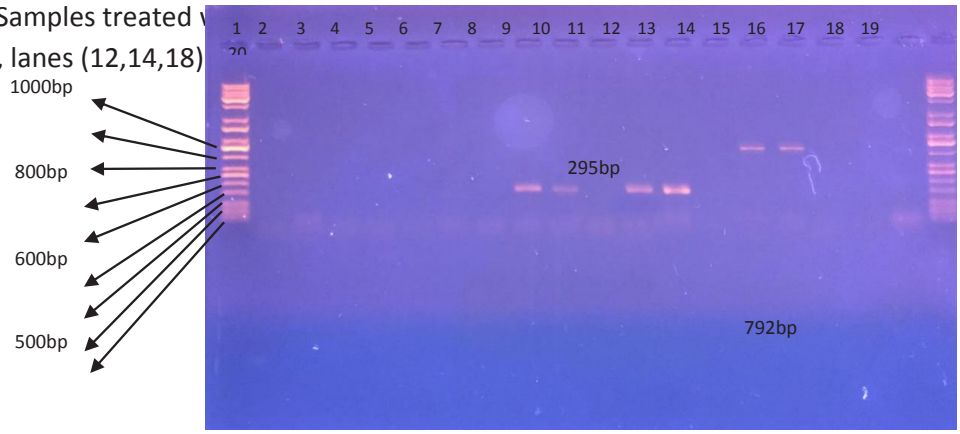

Fig. (3): Results of PCR for the fourth group (treated by *Prosopis fracta*), lanes (1,20) is the ladders 100-10000bp (KAPA BIOSYSTEM), lanes (9,10,12,13) for *sh4-1*, lanes (15,16) for *COI*.

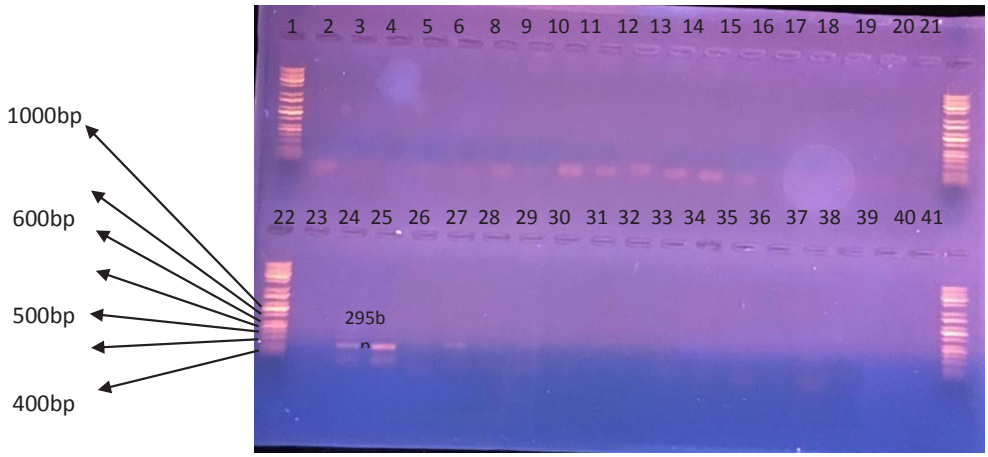

Fig. (4): Results of PCR for the third and fifth groups (treated by *Quercus aegilops*). lanes (1,21) is the ladders, lanes (2-20) show no PCR product. Group three (treated by *Capparis spinosa*), lanes (22,41) is the ladders, lanes (24,25,27) for *sh4-1*.

The results of this study agree with Hosseinzadeh in sheep genes strain (*G6-7, sh4-1, COI*) [34] , these authors extracted DNA and used *G7-6* and *sh4-1* genes in order to detect *E. granulosus* in sheep. *COI* gene aligned (792bp) sequence matrix of partial of gene *cox1* which contained 124 variable sites (15). *COI* gene was used for the determination and identification of *Echinococcus* [16] .

The results showed that the effect of *Quercus aegilops* extract on hydatid cyst was big in the absence of all genes in PCR product. On the other hand, the effect of *Capparis spinosa* extract was observed on only two genes (G6-7 and COI), whereas the effect of *Prosopis fracta* extract was observed only on G6-7 gene (Table 1). *Prosopis spp.* were also known to have medicinal effects. It was demonstrated to have cytotoxic effects on its fruits, which has a significant activity against lung carcinoma [35] . The very important point was in the tinidazole group which gave positive amplification results for all genes which means the frailer to brake any of the gene.

Table (1) Effect of tinidazole, *Capparis spinosa, Prosopis fracta, Quercus aegilops* aginst three genes with control group.

Substance	Control	Tinidazole	*Capparis spinosa*	*Prosopis fracta*	*Quercus aegilops*
G6-7	+	+/-	-	-	-
sh4-1	+	+	+	+	-
COI	+	+	-	+	-
Percentage	100 %	33 %	66 %	66 %	0 %

Sequencing

The results of sequencing determined the identity of (*COI, G6-7, sh4-1*) genes. Sequence similarity of *G6-7* was 97% with Austria isolation, while the *sh4-1* was 99% with Oman isolation, 79% with Estonia isolation which was recorded in GenBank in accession number (KX039965.1) and the partial gene recorded by [36] and *COI* was 99% identical with Australia isolation.

Phylogenetic Analysis

The phylogenetic trees were constructed using NCBI website according to the compartment between sequences published in a recent study and other sequences of the other *E. granulosus* which were published in GenBank. Figure 5 show the phylogenetic analysis of gene *COI* for *E. granulosus*, rooted neighbor joining phylogenetic tree. This tree shows the distribution and phylogenetic relationships between *E. granulosus* in Iraq and other countries.

Conclusion

The results showed that hydatid cyst were detected successfully by performing PCR technique. In addition, *Quercus aegilops* has been shown the best plant extract for destroying all genes, the second plant extract which is *Capparis spinosa* showed destroying of two genes (*G6-7, COI*) and failed to destroy (*sh4-1*). Furthermore *Prosopis fracta* has showen to destroy only (*G6-7*) and tinidazole failed to destroy any of these genes used in this study.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Veterinary Medicine colleges / Basrah university, Iraq and all experiments were carried out in accordance with approved guidelines.

References

1. Eckert J , Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. See comment in PubMed Commons below. Clin Microbiol Rev. 2004; 17: 107-135.
2. Jones O , Kebede N, Kassa T, Tilahun G, Macias C. Occurrence of bovine hydatidosis and evaluation of its risk to humans in traditional communities of Southern Region of Ethiopia. Ethiop. J. Health

Dev. 2012; 26: 43-48.

3. Jenkins DJ , Romig T , Thompson RC. Emergence re-emergence of Echinococcus spp., a global update. Int J Parasitol. 2005; 35: 1205-1219.
4. Mustafa FA. Effect of some plant extract and two drugs (Tinidazole, proziquantel) on killing of protoscolices of *Echinococcus granulosus* In vitro and In vivo in the laboratory mice (Balb/c). PhD. Thesis, coll. Educ. Univ. Basrah. 2007; 200.
5. Okuda. Ellagitannins as active constituents of medicine of plants. Planta medica. 1988; 55: 117-122.
6. Alreikaby R L Ch. Antibacterial-anti amoebic activity for some medicinal plants, with signaled to histopathological effect by Entamoeba histolytica in experimental mices Balb/C. Master thesis in Biology/Microbiology, College of Education-University of Basrah. 2003.
7. Harris E , Harris J , James NDG. A British History, 2011;7.
8. Balls EK. Early use of California Plants, University of California.; Press, Berkeley, CA. 1995.
9. Chakararty HL.Plant Wealth of Iraq. 1st edition.; Guha Ray Press, India. 1976.
10. Ozcan M. Mineral composition of different parts of *Capparis spinosa* Desf. Growing in Turkey. J Med Food. 2005; 8: 405-407.
11. Rechinger KH. FIora of Lowland Iraq. Publisher in Weingeim, Germany. 1964; 205-230.
12. Usmanghani K , Saqib QN, Ahmed VD. Occurrence of juliprosopine in Prosopis g/andu/osa Torr. And *Prosopis farcta* (Banks and Sol.) *Macbride. J. Chem. Sco. Pak*. 1982; 4(4): 285-287.
13. Aqeel A ,Khurshecd Ak , Viqaruddin A. Toxicological studies of the antimicrobial alkaloid juliflorine. J. Arzneim. Forsch / Drug Research. 1991; 41(I): 151-154.
14. Hosseinzadeh S , Mehdi F , Arsalan H , Seyed SS. Molecular Characterization of *Echinococcus granulosus* in South of Iran. J. Veterinary Medicine2012.; 2: 201-206.
15. Junying M , Hu W , Gonghua L , Philip SC. Molecular identification of Echinococcus species from eastern and southern Qinghai, China, based on the mitochondrial Cox1 gene. Parasitol. Res. 2012; 111:179-184.
16. Pour AA , Hosseini SH , Shayan P. Comparative genotyping of *Echinococcus granulosus* infecting buffalo in Iran using cox1 gene. Parasitol. Res. 2011;108: 1229-1234.
17. Pezeshki A , Akhlaghi L, Sharbatkhori M . Genotyping of *Echinococcus granulosus* from domestic animals and humans from Ardabil province. Northwest Iran. J. Helminthol. 2012; 1-5.
18. Utuk AE , Simsek S , Koroglu E. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. Acta. Trop. 2008; 107: 192-194.
19. Alade P, Irabi ON. Antimicrobial activities of crude leaf extract of *Acalypha Wilkension*.; J. Ethnopharm. 1993; 39: 171-174.
20. Sambrook J, Fritsh EF , Maniatis T. Molecular cloning. A laboratory manual, 2nd Ed. Cold spring Harbor Laboratory Press. USA. 1989.
21. Umur S. Prevalence and economic importance of cystic echinococcosis in slaughtered ruminants in Burdur, Turkey. Journal of veterinary medicine B, Infectious diseases and veterinary public health. 2003; 50(5): 247-252.
22. Getawa A , Beyenea D , Ayanab D , Megersac B , Abunnac F.. Hydatidosis: Prevalence and its economic importance in ruminants slaughtered at Adama municipal abattoir, Central Oromia, Ethiopia. Acta Tropica. 2010; 133(3): 221-225.
23. Mohamad MM , Al-taie HL , Amin AH. Journal of the Faculty of Medicine, Baghdad. J. Fac. Med. Baghdad. 2008; 2(50):175-183.
24. Thweni MM , Yassen LY. Hepatic Hydatidosis in manand livestock in Nassiriyah, Iraq. Inter. J. Pharm. Tech. Research. 2015;7(2):310-314.