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DNA extraction by different methods from hydatid cyst from Iraqi's patients

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Abstract :

During the period from January 2018 until March 2018 were collected 30 samples from different regions of Iraq to study the DNA extraction by different methods from hydatid cyst Where was a comparison between the four methods of preparations for the extraction (sonication , Mechanical grinder method , Freezing-thawing method and Boiling method) , Mechanical grinder method has best result then Freezing-thawing method and Boiling method respectively while sonication didn't give successful results in germinal layer whereas in protoscoleces result was accepted.

Keyword : DNA extraction , Hydatid cyst . Mechanical grinder, Boiling, Freezing-thawing

INTRODUCTION :

The causative agent of cystic echinococcosis (CE) is the larval stage of tape worm *Echinococcus granulosus* has a worldwide distribution and is one of the most considerable zoonosis at the most regions of the world (1). Mans obtained infection by coming in approach with infected dogs accommodating adult *Echinococcus granulosus* in their digestive tract caused in excretion of ova in their feces. Consequently , the most way of infected the intermediate host and humans is by swallowing eggs that contaminate water, food or the environment mostly (2) .In Iraq , *Echinococcus granulosus* comprise one of the essential endemic diseases and its represent seriousness in both animals and humans (3,4).To date ten featured genotypes (from G1 to G10) was described in all around the world according to nucleotide sequences analysis of the (CO1gene), (ND1gene) and intra transcribed spacer 1 (ITS1) ,these various genotypes has been correlating with discrete ,intermediate hosts : cattle ,sheep , camels, pigs , goats , horses and cervides (5).

Cystic echinococcosis is diagnosed by various ways such as CT scan, X- ray, other serological and immunological tests including modern technique (PCR) which has elevated specificity and sensitivity in detection of hydatid cyst infection in addition to that applied in genotyping of Echinococcus granulosus to simplify vaccination and treatment. Also, by applied (PCR) purification protein from entire parasite body that's afford 100 % protection (6).

The aim of study is to compare between four different DNA extraction methods in order to ascertain their relative effectiveness for extraction.

MATERIALS AND METHODS

1- Extraction of DNA (disruption of cells and DNA release)

Four methods (sonicator, mechanical grinder, freezing – thawing and boiling) were used for crude DNA extraction from germinal layer and protoscoleces of hydatid cyst.

All 30 hydatid cyst that used in this study were given a good result in DNA extraction from germinal layer and protoscoleces even those of rupture cyst. Samples of solid tissue need to be disrupted prior to initiating DNA isolation procedures (7). This is accomplished by subjecting such samples to mechanical forces that yield dissociated individual cells. This disruption was achieved by using tissue mortar to prepare an emulsified tissue material ready for extraction mtDNA (8).

The results showed that all samples had a distinct band for DNA extracted from protoscoleces on agarose gel (Fig.1). However, the size of DNA was apparently equal in the first two extraction method (1, 2, 3 and 4 lanes) while boiling method showed thick scattered bands (5, 6 and 7 lanes) then sonicating was with faint and pale bands (8, 9 lanes).

On the other hand, germinal layer yielded a little amount of DNA extraction in comparison to protoscoleces DNA (Fig.2). However, the DNA extracted by sonication (1, 2, 3, 4 and 5 lanes) showed an absence DNA bands in comparison with other three methods.

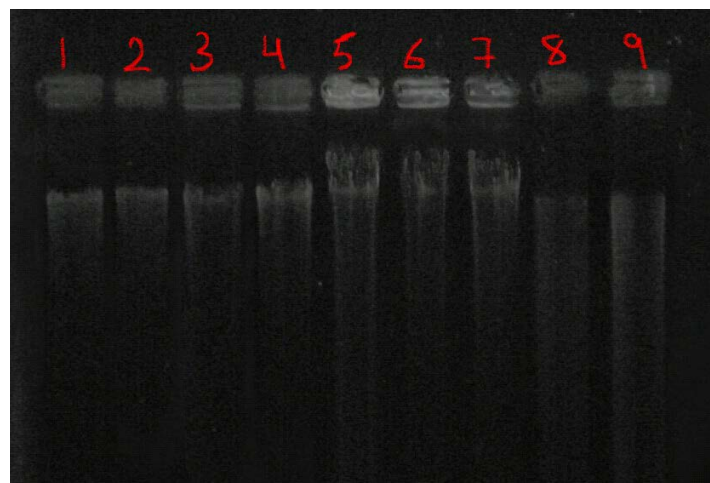

Figure (1): Agarose gel electrophoresis of extracted DNA from protoscoleces by using 4 preparation methods (mechanical grinder lanes 1 and 2, freezing – thawing lanes 3 and 4, boiling lanes 5, 6 and 7 and sonicating lanes 8 and 9). (0.8 % agarose, 80 V, 70 Am, 1 hrs).

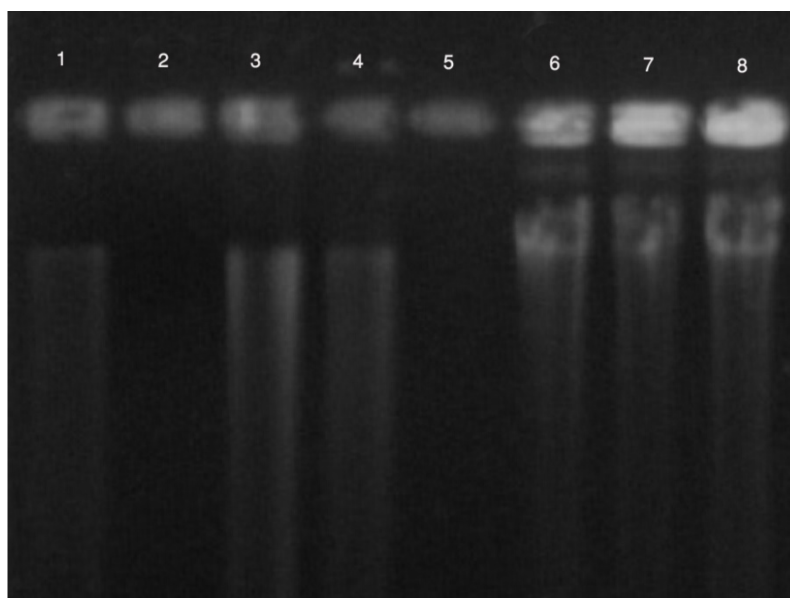

Figure (2): Agarose gel electrophoresis of extracted DNA from germinal layer by using four preparation methods (sonicator lanes 1, 2, 3, 4 and 5, freezing – thawing lane 6, boiling lane 7 and mechanical grinder lane 8). (0.8 % agarose,80 V, 70 Am,1 hrs)

According to these results, the current study adapted protoscoleces DNA as a model for PCR and sequence analysis. Regarding to the four methods used in this study, the mechanical grinder method showed clear and best bands in comparison with other three methods, and this result agreed with previous study was done by (9) and (10) that showed grinder and freezing – thawing have quantitatively better yields of the DNA.

As well as the time requiring for each method was varying, it lasts 5, 10, 10 and 15 mints in mechanical grinder, freezing – thawing , boiling and sonication respectively. In addition, grinder method was faster method contrary to sonication as the most time consuming and labor intensive method.

There are some comparative studies of different DNA extraction methods for organisms such as fungi, bacteria, viruses and protozoa (11 and 12). The current study was in agreement with previous results that showed that grinder is relatively a new method was used on *E. granulosus* (13), which created sharp PCR bands and short time durer in addition to the safety in use. Application of liquid nitrogen was problematic, especially when there are a large number of samples to be examined.

Other disadvantages of this method were difficulties in its handlings and safety hazards in use (10). Although the boiling method did not extract DNA efficiently from all samples even this method took just 10 min, without any additional effort and needs minimal equipments, performed at 95ºC boiling water, and is available in almost all laboratories.

Sonication method resulted in faint and pale bands when protoscoleces used, while in case of germinal layer used there was an absence of DNA bands, because of the increase in duration of sonication may lead to denaturation of DNA. However, a sonication method is not available in all molecular laboratories.

Regarding the cost of each method, freeze – thaw was the most expensive one, followed by grinder and sonication, whereas boiling method was the cheapest.

Figure (3): Agarose gel electrophoresis of extracted DNA by manual method (mechanical grinder lanes 1 and 2, freezing – thawing lanes 3 and 4, boiling lanes 5, 6 and 7 and sonicator lanes 8 and 9). (0.8% agarose, 80 V, 70 Am, 1 hrs)

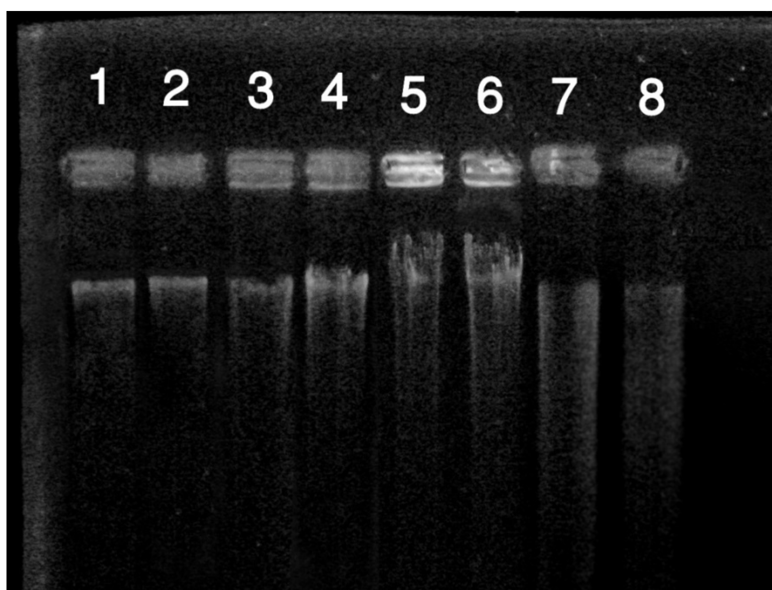

Figure (4): Agarose gel electrophoresis of extracted DNA by promega kit method (mechanical grinder lanes 1 and 2, freezing – thawing lanes 3 and 4, boiling lanes 5 and 6 and sonicator lanes 7 and 8). (0.8 % agarose, 80 V, 70 Am,1 hrs).

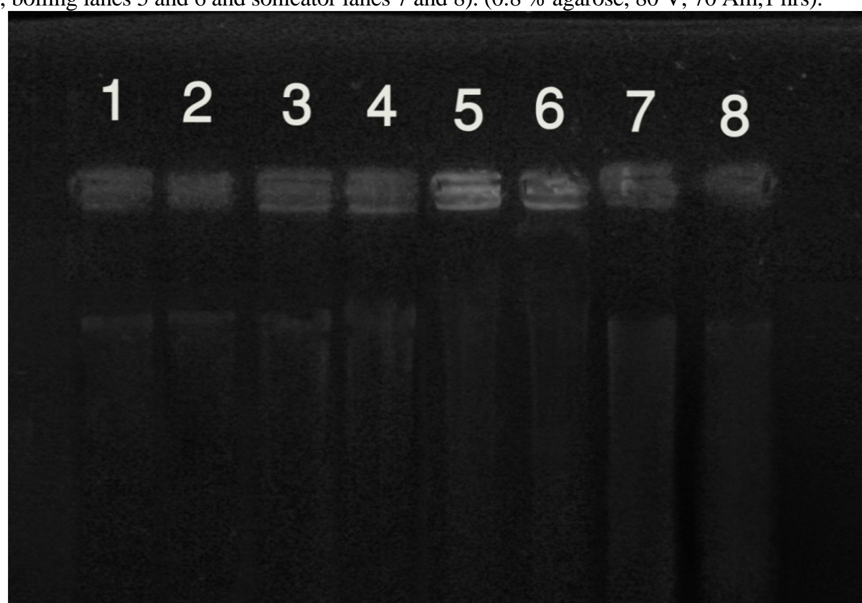

Figure (5): Agarose gel electrophoresis of extracted DNA by genaid kit method (mechanical grinder lanes 1 and 2, freezing – thawing lanes 3 and 4, boiling lanes 5 and 6 and sonicator lanes 7 and 8). (0.8 % agarose ,80 V, 70 Am ,1 hrs).

Table (1):Assessment of purity and concentration of DNA according to different methods used for DNA extraction.

	manual method		promega method		genaid method	
Sample No.	(O.D)	Concentration µg /ml	(O.D)	Concentration µg /ml	(O.D)	Concentration µg /ml
1	1.65	0.34	1.23	0.304	1.17	0.056
2	1.71	0.34	1.21	0.263	1.25	0.061
3	1.62	0.318	1.32	0.406	1.21	0.054
4	1.67	0.298	1.32	0.247	1.18	0.060
5	1.67	0.302	1.32	0.192	1.14	0.059
6	1.70	0.351	1.35	0.300	1.35	0.106
7	1.71	0.345	1.89	0.095	1.25	0.070
8	1.69	0.355	1.21	0.069	1.23	0.060
9	1.74	0.340	1.28	0.088	1.21	0.070
10	1.74	0.349	1.31	0.109	1.26	0.064
Mean	1.69	0.33	1.34	0.20	1.22	0.06
S.D.	±0.03A	±0.02A	±0.19B	±0.11B	±0.05C	0.01C

The different capital letters refer to significant differences between different methods at ($P < 0.05$)

2- Assessment of DNA extraction by using manual and commercial methods

The quality of *E. granulosus* DNA was evaluated with three different methods, including manual method, phenol/chloroform (14). The DNA was precipitated by cold absolute ethanol, the precipitate was formed whitish threads.

Modified promega kit (USA), and Modified genaid kit (Korea). All DNA extraction from each method were electrophoresed on agarose gel to compare their purity and concentration. The present results showed that an obvious differences concerning DNA purity and concentration among these methods (Fig. 3, Fig. 4 and Fig. 5).

The data revealed that manual method recorded a significant ($P < 0.05$) efficient DNA purity (1.69) with concentration of 0.33 μg/ml, in comparison to the second method that done by promega kit with purity of (1.34) and concentration of 0.20 μg/ml. And both method scored significant differences ($P < 0.05$) in comparison to genaid kit that gave a less DNA purity (1.22) and low concentration 0.06 μg/ml Table (1).

DNA based methods are useful for taxonomy at the level of genus, species and subspecies, use of such methods often requires careful attention to prepare of pure DNA in adequate quantities (15 ; 13).

Phenol–chloroform extraction is a liquid-liquid extraction technique in biochemistry and molecular biology for purifying nucleic acids and eliminating proteins. In brief, aqueous samples were mixed with equal volumes of a phenol: chloroform mixture. The proteins will partition into the organic phase while the nucleic acids (as well as other contaminants such as salts, sugars, etc.) remain in the aqueous phase (14).

DNA has important absorption for the ultraviolet range because of the existence of the aromatic bases such as cytosine adenine,thymine, and guanine. This provides a beneficial inspect for DNA template because structural alteration like helix unwinding influence the amplitude of absorption. Moreover, absorption gauge are used as an significance of DNA purity. The main absorption band for clarified Deoxyribonucleic acid (DNA) peaks at around 260 nm. Protein substance, the essential contaminant in DNA, at around 280 nm. The proportion A260/A280 is overwhelmingly used as a proportional gauge of the protein : nucleic acid content of a DNA specimen (16). Phenol–chloroform method is still widely used in many research and diagnostic laboratories. Although the reagent cost is lower compared with the commercial nucleic acid extraction kit, but this procedure requires the use of hazardous chemicals and this method would be expected to reduce the concentration of residual proteins and membrane components potentially inhibiting the enzymatic activity of Taq polymerase (17).

In the current study the highest yield of DNA and purity was obtained by the (phenol/chloroform extraction procedure) , this result was agreed with (17,18).

DNA Purification Kit (promega kit) is based on a four-step process The beginning of purification process lyses the nuclei and the cells for extraction of DNA. Digestion step of RNase may be occur at this period; it is facultative in some implementations, the remaining proteins after that taken away by a salt precipitation stage,then the proteins will become as sediment while DNA remaining in solution. Lastly, the DNA is desalted and concentrated by using isopropanol. Purification of DNA by this method is appropriate for assortment of applications, such as amplification, digestion by using membrane hybridizations and restriction endonucleases (19).

This method was designed to extract DNA with yield and purity similar to those obtained by the phenol/chloroform method, but without using hazardous chemicals.

While geneaid kit was designed specifically for purifying total DNA including (genomic, mitochondrial and viral DNA) from a variety of tissue samples . Proteinase K and chemotropic salt were used to lyse cells and degrade protein, allowing DNA to be easily bound by the glass fiber matrix of the spin column, once any contaminants have been removed, using a wash buffer (containing ethanol), the purified DNA was eluted by a low salt (elution buffer or TE), the entire procedure can be completed within one hrs, although the duration consider shorter but yield and purity of DNA was the lesser in comparison with other two methods, hence this study were used promega kit as method for DNA extraction and processing in PCR due to its sufficient yielded and purity similar to those obtained by the phenol/ chloroform method, but without using hazardous chemicals.

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