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Assessment of Albendazole and Mebendazole Effects on the Excretory/Secretory Proteome of Gastrointestinal Strain of *Echinococcus granulosus* Protoscoleces Using Two-Dimensional Gel Electrophoresis

Mohsen Eshraghi[1], Seyed Jafar Adnani Sadati[2,3*], Ali Farahank[4], Hakimeh Zali[5], Babak Aghili[3]

Abstract

Objectives: The larval stage of *Echinococcus granulosus* in the internal organs of a human causes cystic hydatidosis. Thus, the identification of excretory/secretory (E/S) proteins from *E. granulosus* protoscoleces may help to discover new candidates for drug targets, along with immunodiagnostic and vaccine products. The purpose of this study was to assess the efficacy of albendazole and mebendazole on protein spots of E/S products of hydatid cyst protoscolices which can be helpful for detecting some target proteins for therapeutic purposes.

Materials and Methods: In this experimental study, protoscoleces were divided into three groups in order to assess the effects of the drug. The first and the second groups were treated with albendazole/mebendazole, respectively, and the third group was considered as control. To determine the proteome spots, E/S proteins were precipitated with trichloroacetic acid/acetone and loaded on the isoelectric focusing gel, resulting in gel differentiation on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the 20 mA constant current. In addition, the gels were stained with Coomassie Brilliant Blue. Finally, the protein spots resulted from 2-DE gels were analyzed using the Progenesis SameSpots software.

Results: The comparison between the proteome gels of the treated groups and the control group showed that 35 protein spots are paired among which, 11 spots had significant differences in their expressions ($P<0.05$).

Conclusions: In general, the comparison between the expression levels of protein gel spots indicated the increasing expression of some protein spots while the suppression of the others. This suppression can be considered as a specific effect of the drugs on the E/S product of the protoscoleces of hydatid cyst.

Keywords: Protoscoleces, Albendazole, Mebendazole, Proteome, Two-dimensional gel electrophoresis

Introduction

Echinococcus granulosus adult worms live in small intestine dogs and other carnivores as a definitive host. The metacestode larval stage develops chiefly in the liver and lungs of different intermediate hosts such as domestic ungulates and causes cystic hydatid disease in humans by incidental exposure (1,2).

As a zoonotic disease, hydatidosis is widely distributed with the global geographical spread. Iran is known as one of the endemic regions of *E. granulosus* with high rates of infection remarkably in rural areas and the hydatidosis prevalence rate is reported to be 0.61-2 in 100 000 populations (3,4). Dogs play a prominent role in disseminating the disease in Iran. The *E. granulosus* infection rate was reported to range from 2% to 63% in dogs (5,6) whereas it was from 1.5% to 70% in the intermediate hosts in clouding camels, goats, cattle, and sheep (7,8).

In addition, hydatidosis appears after the ingestion of eggs and the growth of the metacestode in various organs. Cystic echinococcosis is mostly in the liver followed by the lungs while other organs are rarely affected (9).

The clinical symptoms mainly rely on the involved organ, the size and location of the cyst, pressure to different organs, and complications including the rupture and spread of the larval tissue with the development of secondary cysts and potential sepsis. If the cyst ruptures, it may result in anaphylaxis (10,11).

Albendazole and Mebendazole are two drugs that are mostly used to treat hydatidosis. Several studies have shown that Albendazole is superior to mebendazole in terms of efficacy (12).

Hydatid cyst contains the protoscoleces excretory/secretory (E/S) products, which can maintain itself through scape mechanisms such as cytotoxic and modulation of host immune responses regardless of being able to cause

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[1]Department of Surgery, Shahid Beheshti Hospital, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran. [2]Cellular and Molecular Research Center, Qom University of Medical Sciences, Qom, Iran. [3]Department of Medical Microbiology and Immunology, Faculty of Medicine, Qom University of Medical Sciences, Qom, Iran. [4]Department of Parasitology and Mycology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran. [5]School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran.
*Corresponding Author: Seyed Jafar Adnani Sadati, Email: Jafaradnani@yahoo.com

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severe host humoral and cellular reactions (13).

The recognition and description of E/S proteins from *E. granulosus* protoscoleces may help to discover new candidates for production immunodiagnostic tools and vaccines. Two-dimensional electrophoresis (2-DE) is a tool for survey proteins from parasite, even those which lack a genome project (14).

In the present study, the available protein spots in the E/S products of *E. granulosus* protoscoleces treated with albendazole and mebendazole were compared with those of the control group using the 2-DE technique in order to detect the target proteins for the drug design.

Materials and Methods

Collection of *Echinococcus granulosus* Protoscoleces

Livers with the hydatid cyst of infected sheep were gathered from a slaughterhouse in Rey located near Tehran. The cold box and ice were used to reduce the environmental effects on the liver tissue and hydatid cyst. Then, the surface of the cyst was sterilized by using alcoholic cotton 70%. A sterile syringe was used to extract the cyst liquid containing *E. granulosus* protoscoleces from the cysts. The protoscoleces were settled in a Falcon tube and then washed several times by the phosphate-buffered saline (pH: 7.2). The viability of the protoscoleces was detected by eosin 0.01%. In this kind of coloring, live protoscoleces are unable to absorb the eosin color thus they are observed colorless or transparent while the dead protoscoleces which absorb the eosin color are detected in red color. Protoscoleces with the viability of more than 95% were chosen for the collection of E/S products (2,15). Some of the separated protoscoleces from hydatid cyst were stored in 70% ethanol until their DNA was extracted.

Genotyping

DNA Extraction

Frist, protoscoleces extracts were removed from ethanol and then washed twice with distilled water. In addition, the initial digestion was performed in 20 µL proteinase K and 180 µL lysis buffer for 4 hours at 56°C. Further, protoscoleces genomic DNA was extracted using a commercial DNA extraction kit (Roche, Mannheim, Germany Cat No: 11796828001) according to manufacturers' instructions. Finally, the extracted DNA was eluted with 50 µL elution buffer and stored at -20°C until use.

Polymerase Chain Reaction

The nucleotide sequence of the primers producing a specific Cox1 product of 444 bp was for forward and reverse primers of JB3 (5′-TTT TTT GGG CAT CCT GAG GTT TAT-3′) and JB4.5 (5′ TAAAGAAAGAACATAATGAAAATG-3′), respectively (16).

Culture of Protoscoleces

The RPMI 1640 culture medium (Gibco, CET. No: K4111-500) was used to culture protoscoleces, which was supplemented by 100 µg/mL of streptomycin and 100 U/mL of penicillin. Albendazole and Mebendazole were prepared in dimethyl sulphoxide (DMSO) as the stock solution 1 mg/mL and the protoscoleces were divided into three groups of five. The first and second groups were considered as treatment groups containing 500 µL protoscoleces and 1 µg/mL albendazole and mebendazole, respectively. The third group was regarded as the control without DMSO. To collect E/S products, culture media were incubated at 37°C in 5% CO_2 (17,18).

Excretory/Secretory Protein Collection

To collect the E/S products of the parasite, culture supernatants were removed every 12 hours during 72 hours, followed by adding the same volume of the fresh medium after removing culture supernatants. Then, the medium supernatant was centrifuged at 10 000×g at 4°C. The precipitate was discarded and the aliquoted samples were kept at -20°C until further use (2,19,20).

To prepare the protein, 600 mL of the E/S product of the protoscoleces was removed then the same volume of the acid/acetone solution (acetone 9 mL, Trichloroacetic acid 1 mL, and 2 ME 7 µL) was poured into a microtube and frozen at 20°C for 1 hour. After freezing, the liquid was centrifuged (15 minutes, 15 000 × g at 4°C), the supernatant was discarded, and the precipitate was washed twice with ice-cold acetone. To remove the remaining acetone from the prepared protein precipitate, the vacuum pump was used or frozen until the complete removal of its acetone. Then, the lysate buffer including 7 M urea, 1% (w/v) dithiothreitol, 4% (w/v) 3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propane sulfonate, 0.2% (v/v) ampholytes (pH: 3-10), and 0.8% (v/v) ampholytes (pH: 5-7, (Fluka, Germany) was added to each sample to prepare it for gel injection in isoelectric focusing (IEF) tubes.

Two-dimensional Gel Electrophoresis

In the first dimension, 40 µL of the samples were run on the IEF gel including urea 165 mg, acrylamide 11.4 mg, bisacrylamide 0.6 mg, triton X-100 6 µL, ampholytes (pH: 3-10) 3 µL, ampholytes (pH: 5-7) 12 µL, deionized distilled water 170 µL, AMPS 0.5 µL, and TEMED 0.6 µL with 4000 Vh. Following IEF, the gel was reduced in the equilibrium buffer (6 M urea, 30% glycerol, 2% sodium dodecyl sulfate (SDS), and 0.375 M Tris, pH of 8.8, and Bromophenol blue 25 mg) containing 1% DTT for 30 minutes. In the second dimension, the strips were run onto 15×15-cm 12% SDS-PAGE gels (acrylamide 1.6 gr, bisacrylamide 43 mg, tris base 366 mg, HCl 1M 1 mL, SDS 20 mg, deionized distilled water up to 16 mL, TEMED 5 µL, and AMPS 80 µL). The gels were then stained with Coomassie blue and scanned with an image scanner (Biorad, Gs-800). Three analytical replicates were made for control and treated groups (21-23).

The protein database (https://www.expasy.org/proteomics) was used for the identified protein spots of

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the gels. Then, the isoelectric point and molecular weight of the selected spots were described by using prediction tools (i.e., TagIdent ExPASy server).

Statistical Analysis

By using Progenesis SameSpots Software, the protein spots were revealed from 2-DE gels and compared precisely and those with significant differences ($P \leq 0.05$) and more than 2-fold expression changes were identified as candidate spots. Eventually, multivariate analysis such as clustering and principal component analysis (PCA) was used to get more information among the groups.

Bioinformatics Analysis

UniProt database (https://www.uniprot.org/) was used to find the ID of the protein for *Echinococcus granulosus*. The human orthologous gene ID was used since there was no gene ontology (GO) enrichment for this specious.

Further, the DAVID database (https://david-d.ncifcrf.gov) was used to identify the GO analysis related to gene expression in the cells exposed to the drug. In the DAVID algorism, molecular function, significant biological process, and cellular component enrichment were determined by applying different databases such as GO.

Results

Dimensional Gel Electrophoresis

The mean protein concentrations in E/S products in protoscoleces exposed to albendazole and mebendazole were obtained 3.552 and 4.582 µg/mL, respectively. The mean protein concentration was 6.064 µg/mL in the control group. The gels of the treated groups with albendazole and mebendazole were compared with that of the control group (Figure 1A-C). Most protein spots were placed between a pH of 3 and 10 and their molecular weights were between 30 and 100 kDa.

The recognized proteins in this study showed protoscoleces E/S products during their first 72 hours of culture. Furthermore, 35 protein spots were separated by the 2-DE and 12% SDS-polyacrylamide gel and duplicate profiles from the same samples demonstrated 90-95% matching. Therefore, the mentioned protein spots represented significant differences ($P \leq 0.05$). In other words, they had more than 2-fold expression changes and identified as candidate spots. The results indicated that the expression of the proteins changes in response to the used drugs. Moreover, the results of the coupling gel in the treatment of the gel pattern obtained from the control group showed that 35 protein spots are together. Additionally, the comparison between the expression levels of proteins represented the increased expression of some proteins whereas the suppression of the others. In general, 11 protein spots showed significant differences by comparing the proteome gels of albendazole and mebendazole treated groups with the control group. Protein spots 1, 4, 5, 13, 19, 21, 22, and 26, as well as 1, 3, 6, 9, 11, 14, 16, and 21 were down-regulated in albendazole and mebendazole groups, respectively. Two protein spots (27 and 32) in the control group were expressed in none of the treated groups.

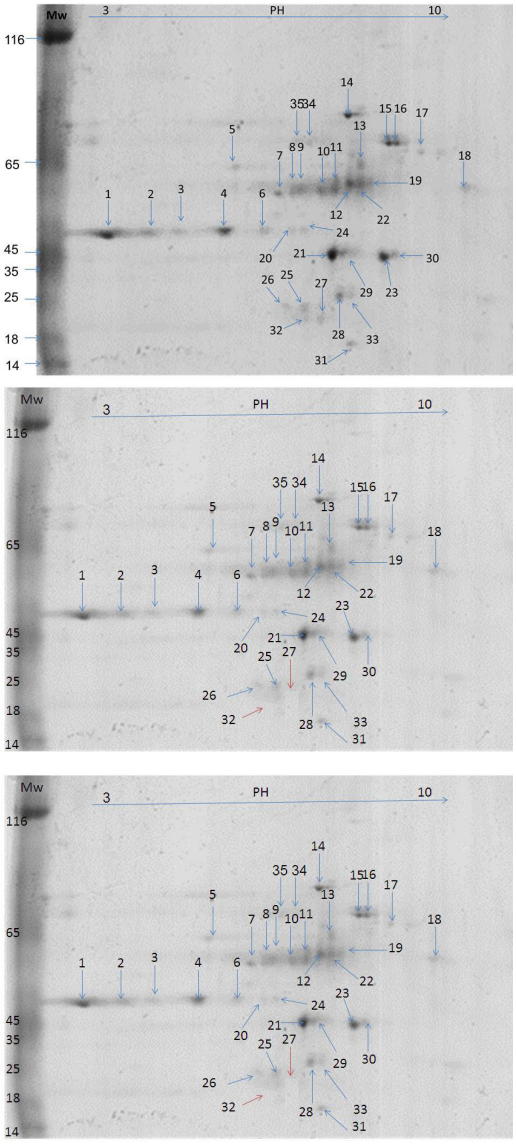

Figure 1. (A) Comparison of protein spots between Albendazole and control groups. (B) Comparison of protein spots between Mebendazole and control groups. (C) Comparison of protein spots between albendazole and mebendazole groups

Based on the results of the coupling gel model from the gel pattern of albendazole and Mebendazole groups, 33 protein spots were paired in repeatability among which, 3 spots (19, 22, and 26) had significant differences and were down-regulated.

Nonlinear Progenesis SameSpots software was used to analyze the protein spots. The arithmetic cluster analysis was done on these 35 proteins and demonstrated the correlation between protein levels across various samples. Close correlations among protein levels could reveal their involvement in the same biological pathway. A clear

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cluster analysis (dendrogram) was created with diverse subgroups of proteins (Figures 2B, 3B and 4B).

In addition, total down-regulated protein spots represented two main subgroups (I and II). Subgroup II involved two branches (blue spots in Figures 2B, 3B and 4B) and two main subgroups were shown by the total up-regulated protein spots (subgroups III and IV). Further, subgroup IV involved two branches (red spots in Figures 2B, 3B and 4B).

PCA was done on all protein spots and demonstrated two important up- and down-regulated groups (Figures 2C, 3C and 4C).

The results revealed that 35 protein spots are paired (together repeatability), and 11 of them showed significant differences in expression. In the treated groups, 7 protein spots indicated decreased expression while 1 protein spot had increased expression while 2 protein spots showed no expression. The comparison between albendazole and mebendazole groups demonstrated that 33 protein spots are paired (together repeatability) among which, 3 protein spots showed significant differences in the albendazole group with decreased expression (Figure 1A-C and Table 1).

Hierarchical clustering and PCA analysis (for spots with power >0.9995) are represented in Figures 2-4 and *E. granulosus* and human orthologous gene ID from the UniProt database are shown in Table 2.

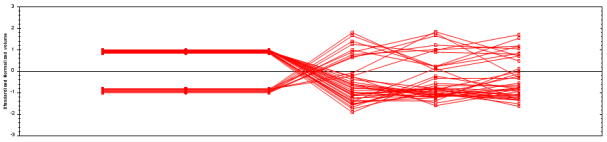
Discussion

So for, 10 separated genotypes have been reported in the world based on biological differences and by using molecular and genetic analysis nuclei and mitochondrial genes, the most common of which is G1 (25,26). Based on taxonomic characteristics and mitochondrial genetic studies, *Echinococcus* is present in four species of *E. granulosus* sensu stricto (G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), and *E. Canadensis* (G6-G10). Some studies reported three distinct genotypes of *E. granulosus* in Iran (25,27).

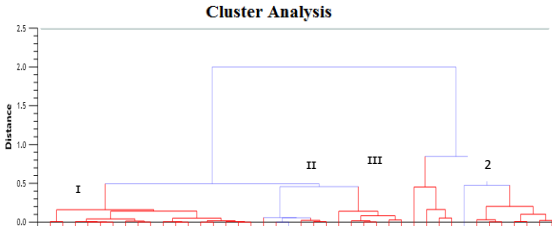
Three mitochondrial genes mostly used for the genotyping of *E. granulosus* (i.e., cox1, nad I, and atp 6) were chosen for the genotyping of the protoscoleces (15). The amplified region of cox1 is 444 base pairs and has 100% BLASTN similarity with the G1 genotype of *E. Granulosus* according to GenBank (accession No: AB893250). Our finding showed that the genotype of analyzed *E. granulosus* protoscoleces was sheep G1.

Infection caused by *E. granulosus* and their permanence in the host is because of proteins which can be found in E/S products and the tegument of the parasite (2).

E/S products are continuously in touch with host immune cells. In addition, parasites mostly secrete the same molecules for penetration to the tissue host, escaping the immune system, for creating oxidative stress, and for nutrient absorption. The identification and analysis of each molecule could be helpful for diagnosing accessibility



A: It shows chart spots marked in figure 1



B: Arithmetic cluster analysis

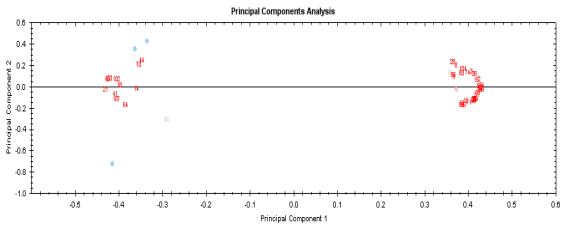
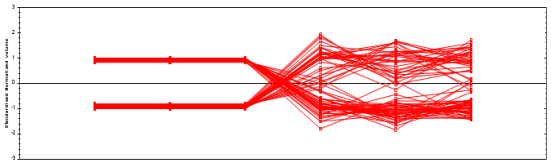
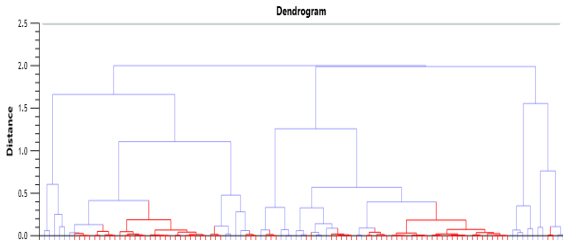


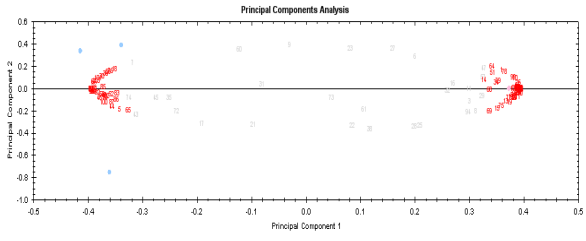
Figure 2. Different protein expression between albendazole and control group (A) that show two different spots groups in expression which represents clustering analysis in B (spots with power >0.9995 are red), C represented the PCA analysis of these spots.



A: It shows chart spots marked in figure 2



B: Arithmetic cluster analysis



C: Principal component analysis was performed on all the spot proteins

Figure 3. Different protein expression between Mebendazole and control group (A) that show two different spots groups in expression which represents clustering analysis in B (spots with power >0.9995 are red), C represented the PCA analysis of these spots.

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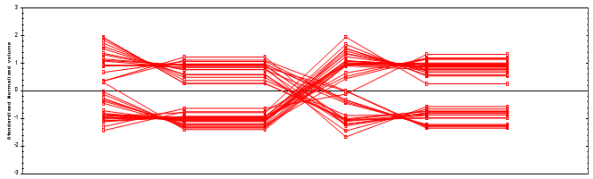
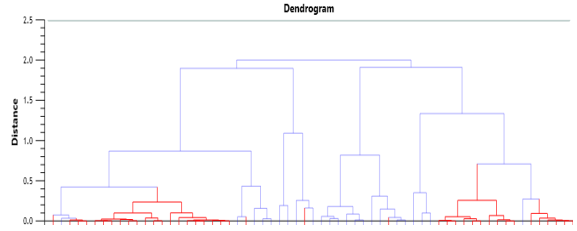
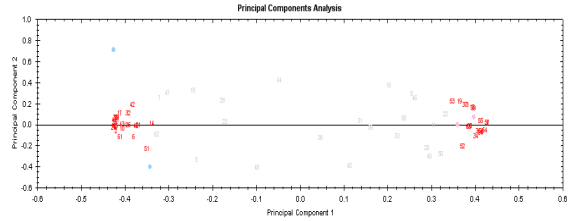


A: It shows chart spots marked in figure 3



B: Arithmetic cluster analysis



C: Principal component analysis was performed on all the spot proteins

Figure 4. Different protein expression between Mebendazole and Albendazol group (A)that show two different spots groups in expression which represents clustering analysis in B (spots with power >0.9995 are red), C represented the PCA analysis of these spots.

to the component of the immune system. It may also lead to a better perception of the correlation between the parasite and the host (28).

In the present study, the 2-DE approach was used to analyze E/S proteins in *E. granulosus* protoscoleces. Further, the proteomic patterns of the E/S products of the treated groups and the control group were investigated to identify the parasite E/S protein changes.

The overall map of protein profiles attained from protoscoleces was similar to those gained from the other worms such as *Fasciola hepatica*, *Angiostrongylus*, and *Ascaris lumbricoides* (28-30).

Furthermore, the proteomic analysis of *E. granulosus* protoscoleces E/S products of the control group allowed identifying 35 proteins.

The recognized proteins in this study represented the protoscoleces E/S products during their first 72 hours of culture. The number of obtained protein spots from protoscoleces E/S products was more compared to previous studies. For example, Virginio et al detected 32 protein spots on the E/S protoscoleces of *E. granulosus* (2) while Carmena et al reported 20 protein spots (17).

This difference in the number of protein spots may be due to differences in the method or the parasite strain, culture, as well as animal and the infected tissues from which the parasite is separated (31).

Table 1. Characters of the Expression of Protein Spots in the Gels of Albendazole, Mebendazole, and Control Groups

Spots Number	Point Isoelectric	Molecular Weight	Average Normalized Volumes of Protein Spots			Descripted Proteins
			Control	Albendazole	Mebendazole	
1	4.82	67000	13450	5029↓	6678↓	Serine: Threonine protein phosphatase, pI: 4.80, Mw: 66242
2	4.51	75000	24650	13200	18520	Tfiia large subunit, pI: 4.51, Mw: 76676
3	5.54	72000	9317	5568	4534↓	Heat shock 70 kDa protein 4, pI: 5.53, Mw: 71969
4	5.14	70000	16360	7432↓	12657	78 kDa glucose-regulated protein, pI: 5.12, Mw: 69735
5	5.21	98000	2653	832↓	1483	ParamyosinpI: 5.22, Mw: 98742
6	5.73	76000	8695	5490	3792↓	PeroxidasinpI: 5.72, Mw: 75523
7	5.66	71000	10180	7069	8782	Heat shock cognate 70 kDa protein, pI: 5.67, Mw: 72554
8	6.19	85000	7808	4630	5123	Calpain, pI: 6.18, Mw: 85770
9	6.25	62000	13810	9217	6824↓	Tyrosine kinase, pI: 6.24, Mw: 1677
10	6.77	82000	14380	9436	10160	No identified
11	6.73	86000	14140	8018	6430↓	Uncharacterized protein, pI: 6.63, Mw: 86268
12	6.70	75000	15730	9784	9647	No identified
13	7.14	80000	5488	2490↓	3472	Uncharacterized protein, pI: 7.14, Mw: 80253
14	6.92	95000	10690	7927	4896↓	GA-binding protein alpha chain pI: 6.90, Mw: 94583
15	7.56	97000	6973	4856	6381	No identified
16	7.45	91000	3695	2450	1641↓	Uncharacterized protein, pI: 7.47, Mw: 89920
17	7.96	95000	3270	1781	2444	No identified
18	8.12	78000	10210	6279	8565	Uncharacterized protein, pI: 8.13, Mw: 78002
19	7.21	86000	13800	6152↓	12445	Serine/threonine-protein phosphatase 1, pI: 7.20, Mw: 78364
20	6.32	78000	1828	1413	1644	No identified
21	6.33	50000	18040	7516↓	8590↓	Tubulin epsilon chain, pI: 6.33, Mw: 49468
22	7.15	89000	23996	10907↓	21894	Transducin beta protein 3 Chain, pI: 7.14, Mw: 89952
23	7.43	72000	14730	35406↑	18282↑	Multidrug and toxin extrusion protein, pI: 7.41, Mw: 71891
24	5.84	58000	3300	2721	1696	Thioredoxin glutathione reductase, pI: 5.83, Mw: 58311
25	6.21	59000	3541	2610	1947	No identified
26	6.48	61000	4794	2275↓	4593	Glucose-6-phosphate isomerase, pI: 6.48, Mw: 61706
27	6.72	60000	2166	00000	00000	Alkaline phosphatase, pI: 6.74, Mw: 59591
28	6.83	63000	5920	3614	4110	No identified
29	7.10	72000	8420	4242	6985	No identified
30	7.63	72000	6073	4424	5841	Glutamate receptor, pI: 7.60, Mw: 72178
31	7.20	52000	4169	3273	2490	No identified
32	6.04	56000	2346	00000	00000	Tubulin alpha chain, pI: 6.05, Mw: 55941
33	6.49	63000	7948	3987	4293	LIM/homeobox protein Lhx3, pI: 6.49, Mw: 63585
34	6.11	91000	2187	1740	2119	Glutamate receptor ionotropickainate, pI: 6.11, Mw: 90161
35	6.07	84000	6437	3822	4473	TBC1 domain family, pI: 6.08, Mw: 84040

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Table 2. Uniprot ID of Detected Proteins

Detected Proteins	Uniprot ID (*Echinococcus granulosus*)	Uniprot ID (Human)	Gene Symbol
Serine: Threonine protein phosphatase,	U6J7Z4_ECHGR	P60484	PTEN
Tfiia large subunit	A0A068WTY6_ECHGR	P52655	GTF2A1
Heat shock 70 kDa protein 4	-	P11142	HSPA8
78 kDa glucose-regulated protein	Q24798	P11021	HSPA5
Paramyosin	P35417	-	-
Peroxidasin	A0A068WA67	Q92626	PXDN
Heat shock cognate 70 kDa protein	Q24789	P11142	HSPA8
Calpain	W6UGM0	P20807	CAPN3
Tyrosine kinase	A0A068WA91	P04637	TP53
Uncharacterized protein	W6V4B3		EGR_04195
Serine/threonine-protein phosphatase	U6J7Z4	P31749	AKT1
Tubulin epsilon chain	A0A068WKV4	P06734	FCER2
Transducin beta protein 3 Chain	-	Q96EB6	SIRT1
Multidrug and toxin extrusion protein	W6UXV1	Q96FL8	SLC47A1
Thioredoxin glutathione reeducates	F8QQF4	P78417	GSTO1
Glucose-6-phosphate isomerase	Q56JA3	P06744	GPI
Alkaline phosphatase	A0A068W6U9	P05186	ALPL
Glutamate receptor	A0A068WKI3	Q96RI1	NR1H4
Tubulin alpha chain	W6UVP1	Q8IXJ6	SIRT2
LIM/homeobox protein Lhx3	W6UCF0	Q9UBR4	LHX3
Glutamate receptor ionotropickainate	A0A068WHT4	Q96RI1	NR1H4
TBC1 domain family	A0A068WH80	Q9NU19	TBC1D22B

The E/S proteins of protoscoleces which were isolated from the liver hydatid cyst had more variety of protein components compared to the lung hydatid cyst (32). In addition, different environmental pressures motivate parasites to release various products (33). In the present study, the drug-exposed parasite secreted different and specific products such as Multidrug and toxin extrusion protein.

Figure 1A-C depicted images of the 2-DE gel of three groups that were analyzed by software. In general, 35 protein spots were discovered some of which had increased and decreased expressions while the other protein spots had suppressed expression. Further, the comparison between the gel protein of the control and treatment groups showed that 11 proteins have statistically significant differences in their expressions. Albendazole and mebendazole groups expressed 33 proteins and 2 suppressed protein expressions.

Several proteins demonstrated changed expressions in the treatment groups such as the proteins which had a role in the metabolism of parasites including threonine protein phosphatase, glucose-regulated protein, and glucose-6-phosphate isomerase that had decreased expression. However, the multidrug and toxin excretion protein of the treatment groups had increased expression, indicating that these proteins had increased expression when the parasite was exposed to the drug and thus preventing the effects of the drugs.

Totally, 35 proteins were identified out of which (annotated proteins), some were allocated to biological process, molecular function, and cellular component gene ontology (GO) terms. A summary of the GO annotation is represented in Tables 3-5.

Two significant molecular function categories were discovered for protoscoleces E/S products binding and enzyme regulator activities. Other molecular function categories allocated to protoscoleces E/S products included catalytic antioxidant and transporter activities (2), the details of which were provided in Table 4.

Metabolic and cellular processes were the two important

Table 3. GO Analysis Based on Biological Analysis

Biological Process	Gene Name	*P* Value	Benjamini
Response to antibiotic	ALPL, HSPA5, TP53	1.0E-3	2.5E-2
Response to biotic stimulus	ALPL, HSPA5, TP53, NR1H4, SIRT2, AkT1	2.4E-3	4.1E-2
Response to toxic substance	ALPL, HSPA5, TP53	1.4E-2	1.1E-1
Negative regulation of apoptotic process	LHX3, CAPN3, GPI, HSPA5, NR1H4, PTEN, SIRT1, SIRT2, TP53, AKT1	4.4E-8	1.0E-4
Negative regulation of programmed cell death	LHX3, CAPN3, GPI, HSPA5, NR1H4, PTEN, SIRT1, SIRT2, TP53, AKT1	4.8E-8	5.7E-5
Negative regulation of cell death	LHX3, CAPN3, GPI, HSPA5, NR1H4, PTEN, SIRT1, SIRT2, TP53, AKT1	9.1E-8	7.2E-5
Response to nutrient levels	ALPL, HSPA8, HSPA5, NR1H4, PTEN, SIRT1, SIRT2, TP53, AKT1	3.3E-7	1.9E-4
Response to extracellular stimulus	ALPL, HSPA8, HSPA5, NR1H4, PTEN, SIRT1, SIRT2, TP53,AKT1	4.4E-7	2.1E-4

Note. GO: Gene ontology.

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Table 4. GO Analysis Based on Molecular Function

Molecular Function	Count	*P* Value	Benjamini
Ion binding	13	4.9E-3	1.4E-1
Enzyme binding	8	9.4E-4	8.5E-2
Protein kinase binding	4	1.7E-2	3.3E-1
Macromolecular complex binding	7	2.2E-3	1.4E-1
Transcription factor binding	6	1.5E-4	4.2E-2
RNA polymerase II transcription factor binding	3	5.2E-3	1.4E-1
Nucleic acid binding	6	3.9E-1	9.3E-1
Macromolecular complex binding	7	2.2E-3	1.4E-1
Ubiquitin protein ligase binding	4	2.2E-3	1.2E-1
Heterocyclic compound binding	10	1.1E-1	6.8E-1
Adenyl ribonucleotide binding	4	2.0E-1	8.0E-1
Adenyl nucleotide binding	4	2.0E-1	7.9E-1
Purine ribonucleoside triphosphate binding	4	2.8E-1	8.7E-1
Pyrophosphatase activity	3	1.9E-1	8.2E-1

Note. GO: Gene ontology.

Table 5. GO Analysis Based on Cellular Component

Cellular Component	Count	*P* Value	Benjamini
Nuclear chromatin	4	2.2E-3	1.1E-1
Non-membrane-bounded organelle	7	1.0E-1	4.2E-1
Intracellular non-membrane-bounded organelle	7	1.0E-1	4.2E-1
PML body	3	3.3E-3	1.3E-1
Mitochondrion	5	5.5E-2	3.1E-1
Membrane-bounded organelle	17	1.6E-3	1.1E-1
Nuclear part	11	4.4E-4	4.6E-2
Vesicle	9	8.7E-3	1.2E-1
Membrane-bounded vesicle	8	2.7E-2	2.3E-1
Extracellular exosome	7	2.8E-2	2.3E-1

Note. GO: Gene ontology; PML: Promyelocytic leukaemia.

biological process categories. Several other processes also well represented the E/S products of protoscoleces such as biological regulation, localization, multicellular organismal, and developmental processes.

Higher-level ontology represented that most metabolic and cellular processes were connected to the production of precursor metabolites and energy in addition to carbohydrate and protein metabolic processes.

The most frequently used GO term about protoscoleces E/S components are subcellular organelles and cells and to a lesser extent macromolecular complexes and membrane-enclosed lumens. Moreover, higher-level ontology revealed that identified proteins were mostly intracellular.

Albendazole induced degenerative changes in the intestinal wall of the worm, resulting in a reduction in energy production in the parasite and death. This was done by binding to the colchicine-sensitive part of the tubulin, consequently restraining its polymerization into microtubules. The destruction of cytoplasmic microtubules results in the faulty absorption of the glucose by the larval and adult stages of vulnerable parasites and thus empties their glycogen stores, also mebendazole by sticking to and preventing vital intracellular microtubule-dependent transport processes in the parasite (34,35).

Disruption in glucose uptake causes the reduced expression of proteins that are involved in energy production (36). The results also showed reduced expression. Parasites produce neutralizing proteins when they are exposed to the drug or toxic substances (37). In our study, multidrug proteins demonstrated a significant increased expression by considering that the parasites were exposed to the drug.

Additionally, a series of proteins formally known as antigens was identified and these E/S products were involved in the survival of the parasite at the time of infection (19,38,39)

Conclusions

To identify the molecular mechanisms of the drugs, the types of the obtained proteins should be clarified and this may be obtained by mass spectrometry. The mass spectrometry analysis of protoscoleces E/S products from *E. granulosus* is not available in Iran. It is also difficult to obtain the E/S products of the protoscoleces purely in the culture medium and there is the possibility of the contamination of the culture media to the bacteria.

The result of this study provides a better foundation for more proteomic studies about protoscoleces and the applied methods are useful for further studies about possible pathogenic antigens, protein for drug targets, and vaccine production in the future. The results showed that both drugs have an effect on the expression of the protein spots of hydatid cyst protoscolices strain G1. In addition, the comparison between the levels of the expression of protein gel spots indicated the increased or decreased expressions of some protein spots and the silence of some other spots. Finally, the results revealed that the drugs act on alkaline phosphatase and tubulin alpha chain proteins which were not expressed.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

The study was conducted after obtaining the approval of the Ethics Committee of Tehran University of Medical Sciences (ID: 21267).

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