Original Article

The Effect of Triclabendazole on ALT Enzyme Activity in *Fasciola hepatica* Helminths and Parasitized Sheep Liver Tissues

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Introduction: To determine an indicator for Triclabendazole (TCBZ) efficacy, alanine aminotransferase (ALT) activity in *Fasciola hepatica* (Iranian isolate) parasite in presence and absence of TCBZ was evaluated by an in vitro cultivation method. Also, ALT enzyme activity between none and parasitized-infected sheep liver tissues was assessed. **Method:** The sheep livers were collected and transferred immediately to the Department of Parasitology. Adult living parasites were recovered, washed and divided into two groups, treatment and control groups with 10 parasites in each. We added 15 µg TCBZ to the treatment group; then incubated both groups for 4 h at 37ºC. The parasites, infected and parasite free liver tissues were ground and homogenized by a mortar and pestle, centrifuged, and supernatants were recovered. Protein concentration and ALT enzyme activity were measured in the supenatants. **Results:** The results of ALT enzyme activity assay showed 0.03 U/ml/mg protein for treated *F. hepatica* and 0.01 U/ml/mg protein for untreated samples, the mean values of difference was not significant ($p>0.05$). The difference between ALT activity in none and parasitized-infected liver was not significant ($p>0.05$). However, two-sample T-test analysis showed higher ALT activity in treated and untreated parasite in comparison with none and parasitized-infected liver specimens ($p<0.05$). In addition to ALT protein band for parasite and liver tissue, Cathepsin enzyme (proteases) was detected for parasite by SDS-PAGE analysis. **Conclusion:** ALT activity cannot be considered as a useful marker for TCBZ efficacy in *F. hepatica* treatment. However, ALT enzyme showed comparable activities in parasite and its host liver tissue. *J Med Microbiol Infec Dis, 2015, 3 (1-2): 1-5.*

Keywords: Alanine Aminotransferase, *Fasciola hepatica*, Egaten®, Triclabendazole, Liver.

INTRODUCTION

Fascioliasis is a common parasitic worm infection which is significantly important in human and animals that live in temperate climates around the world [1]. *Fasciola* parasites infect humans and ruminant livestock worldwide; approximately, 2.4 million people are infected with different *Fasciola* species, and 180 million are at risk [2]. The presence of *Fasciola hepatica* in the biliary ducts or gallbladder can create inflammation, portal infection, hepatopathies and malfunction of liver, which is the result of both mechanical and toxic effects of the helminths that may finally lead to hyperplasia of the epithelium and relative or full obstruction of the biliary ducts [3].

Alanine aminotransferase (ALT) is one of the aminotransferases (transaminases) enzymes, which catalyzes the transfer of the amino group (NH2) from L-alanine to α-ketoglutarate. It plays a key role in carbohydrate and protein metabolism especially in the liver, where the higher density of the enzyme exists. This enzyme is released into the blood as the result of liver damages, so its measurement is an index in the evaluation of hepatocellular injury [4].

Triclabendazole (TCBZ), a member of the Benzimidazole family, is a drug of choice with high efficacy with the dosage 10-12 mg/kg of body weight in Human Fascioliasis [5]. This medication can cause the destruction of helminths by disturbing tubulin organization and also preventing the processes of energy production by inhibition of the fumarate reductase [6, 7, 8]. On the other hand, prescribing the TCBZ for a long time in the most countries for controlling and treatment of *Fasciola*-infected animals has led to increased drug resistance in parasites [9].

Two transaminase systems, ALT and Aspartate aminotransaminase (AST), have been measured in various species of parasites including two species of nematodes (*Ascaris lumbricoides* and *Ascaridia galli*), five species of trematodes (*Clonorchis sinensis, F. hepatica, Eurytrema pancreaticum, Paramphistomum cervi* and *Paragonimus westermani*) and five species of cestodes (*Diphyllobothrium*

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mansoni, *Dipylidium caninum*, *Taenia pisiformis*, *Cysticercus cellulosae* and *Cysticercus pisiformis*) [10, 11].

The literature review shows that there is no published study on ALT activity in *F. hepatica* (Iranian isolates) as an indicator for TCBZ efficacy in the treatment of fascioliasis. In this study, ALT enzyme activity in *F. hepatica* parasite in presence or absence of TCBZ was measured. Also, the ALT enzyme activity in none and parasitized-infected sheep liver tissues was assessed.

MATERIAL AND METHODS

Preparation of parasite and liver tissues extracts. Livers of *F. hepatica* infected sheep were collected from a local abattoir (Soleymani, Tehran, Iran). The parasites were isolated, washed three times with PBS buffer pH 7.4 and divided into two groups of ten worms. They were cultivated in a buffer media supplemented with 5% glucose in the presence of 15 µg TCBZ (Egaten®; Swiss, Novartis Pharma AG) or the control group wasn't treated with TCBZ. Both groups (ten parasites per group) incubated for 4 h at 37°C. Recollected parasites and liver tissue were homogenized in three volumes of phosphate buffer (pH 7.2) with a Mortar and pestle, centrifuged at 10000×g for 30 min at 4°C and supernatants were recovered and stored at -20°C until required [12].

ALT activity assay. Enzyme activity was measured by using Pars Azmoon kit (Tehran, Iran). The reaction is evaluated based on transferring of the amino group from alanine to oxoglutarate, producing pyruvate and glutamate. Then, in parallel reaction, pyruvate is converted to lactate by lactate dehydrogenase and NADH changes to NAD^+. The conversion of the NADH to NAD^+ is measured by a photometric method at 340 nm, which is proportional to the level of ALT enzyme in the sample. For the test, solutions A (Tris, L- Alanine and LDH) and B (Oxoglutarate and NADH) were mixed in the ratio of 4:1. One ml of the mixture was transferred to a cuvette, an amount of 100 µl of the sample was added, and the absorbance was read three times at one-minute intervals. The amount of ALT in samples was calculated by multiplying the mean difference of mentioned absorbances in coefficient offered by kit (Pars Azmoon; 92002).

Measurement and identification of Samples Protein. Protein concentration in samples was measured by Bradford technique with BSA standard solutions. The absorbances of parasite and tissues samples were measured at 595 nm. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was utilized to separate the protein components of extracted solutions of the worms and liver tissues. For this purpose, extracted samples were mixed with sample buffer and run on 10% acrylamide gels. Finally, the gels were stained with Coomassie blue R-250. Molecular weights of resolved proteins were compared alongside a protein marker [12]. The proteins were identified by calculation of ratio factor (RF) for molecular weights and using ExPASy protein database (http://www.expasy.org).

Statistical analysis. Independent two samples T-test was used to determine the significant differences of ALT enzyme activities between treated and untreated parasites, none and parasitized-infected liver tissues, and between liver groups and parasite groups (http://www.socscistatistics-.com/ttest).

RESULTS

ALT activity of treated parasite group was higher than untreated ones, but the difference was not significant ($p>0.05$). Two-sample T-test analysis indicated higher ALT activity in the treated and untreated parasite compared to none and parasitized-infected liver tissue ($p<0.05$). Statistical analysis showed that the difference between ALT activity of none and parasitized-infected liver tissue is not significant ($p>0.05$). The results of the enzyme activities of samples are presented in Table 1.

The protein concentration of treated parasite was higher than untreated specimens. Additionally, Protein concentration of non-parasitized-liver tissue was more than the infected; however, in both cases, the t-test was not significant ($p>0.05$). The protein component of parasite and liver tissue samples analyzed by SDS-PAGE electrophoresis are reflected in Figures 1 and 2. In stained 10% gel, two protein bands with MW of 52.9 and 54 kDa appeared for ALT in the parasite and liver samples, respectively. Also, two cathepsin proteases were detected for the parasite. The identified proteins are listed in Tables 2 and 3.

Table 1. The mean values of protein amounts and ALTs activities for parasite and liver samples.

EXTRACTION SAMPLES	PROTEIN AMOUNTS (MG/ML)	ALT TOTAL ACTIVITY (U/ML)*	ALT SPECIFIC ACTIVITY (U/ML/MG/PROTEIN)
HEALTHY LIVER	8.47±1.24	0.05±0.017	0.006±0.001
INFECTED LIVER	6.15±1.47	0.03±0.004	0.004±0.006
UNTREATED PARASITE	6.26±0.73	0.12±0.036	0.019±0.004
TREATED PARASITE**	7.44±0.65	0.24±0.09	0.032±0.013

*One unit; is the quantity of enzyme that catalyzes the reaction of 1µmol of substrate (alanine) per minute at 37°C.

** Parasite cultivated in Egaten (15 µg/ml) added medium.

Table 2. Identified proteins from *F. hepatica* by using ExPASy Protein Database

PROTEIN BANDS	MW IN DALTON	IDENTIFIED PROTEINS
1	145628.3	Not adapted expasy protein bank list
2	133853.1	Not adapted expasy protein bank list
3	121313.2	Not adapted expasy protein bank list
4	105409.3	Not adapted expasy protein bank list
5	95534.1	Not adapted expasy protein bank list
6	76297.77	ATP binding cassette protein
7	52946.35	ALT enzyme
8	51479.01	Glucose transporter
9	42883.71	Cytochrome b
10	37789.06	Cathepsin L-like proteinase
11	31040.26	NADH dehydrogenase subunit 2
12	28530.4	Cathepsin B protease
13	26223.4	Beta- tubulin 3
14	22785.64	Putative eggshell protein
15	19522.22	Cytochrome C oxidase subunit 3
16	18197.62	Truncated Ferritin- like protein

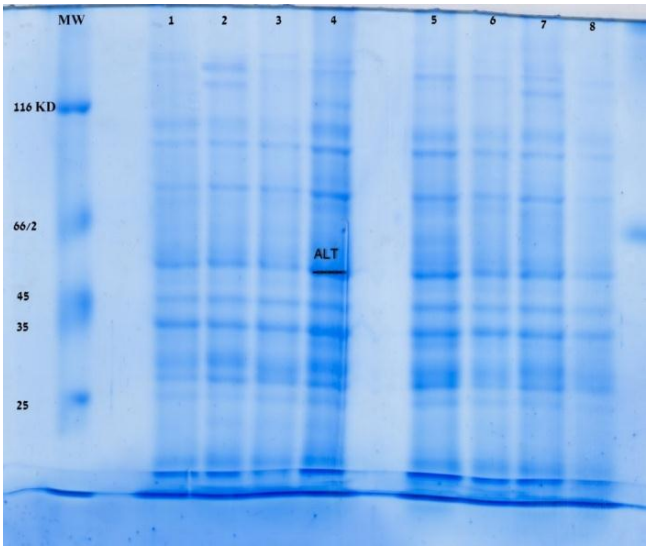

Fig. 1. SDS-PAGE analysis of the proteins in *F. hepatica* helminths. Lanes 1-4, TCBZ-treated worms and lanes 5-8, none-treated specimens.

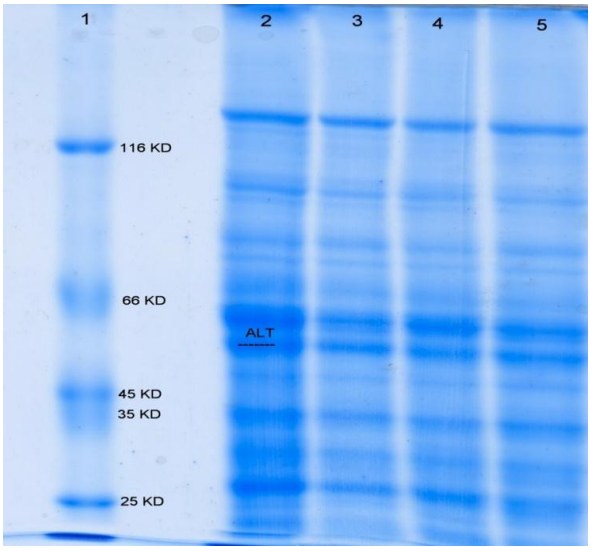

Fig. 2. SDS- PAGE analysis of proteins in liver samples. Lanes 2-3, infected liver sample tissues; lane 4-5, healthy liver samples.

Table 3. Identified proteins from liver samples by using ExPASy Protein Database

PROTEIN BANDS	MW IN DALTON	IDENTIFIED PROTEINS
1	138899	Not adapted expasy protein bank list
2	110814	Not adapted expasy protein bank list
3	104729	Not adapted expasy protein bank list
4	98979	Glycogen phosphorylase, brain form
5	84850	Not adapted expasy protein bank list
6	77958	Not adapted expasy protein bank list
7	68568	Not adapted expasy protein bank list
8	61402	NADH-ubiquinone oxidoreductase chain 5
9	54006	ALT enzyme
10	47623	Corticosteroid-binding globulin
11	40201	Acyl-CoA desaturase
12	35359	Ornithine carbamoyltransferase& mitochondrial
13	29467	Cytochrome c oxidase
14	25587	Beta-carotene oxygenase 2
15	24495	Somatotropin

DISCUSSION

TCBZ is a Benzimidazole derivate that may bind to the β-tubulin molecule and disrupts microtubule-based processes [13]. In this regard, the hepatotoxicity of TCBZ has been reported [14]. The ALT is an enzyme, found primarily in the liver and kidney. It was originally referred to as serum glutamic pyruvic transaminase (SGPT). According to the study performed in Cuba on the value of the liver enzymes in the serum of Cuban patients infected with *F. hepatica*, ALT elevated from days third to seventh following treatment with TCBZ and then decreased to the normal level [15]. The data of a study in Poland in 2008 indicated that ALT in the sera of patients infected with *F. hepatica* increased to high levels [16]. Also another study in Cameroon in 2005 showed no significant difference in serum level of ALT in control and treated goats [17]. A survey performed in 2010 in Argentina to evaluate the resistance of Fasciola against TCBZ in cattle, showed that the enzyme levels decreased at the day 26 [18]. In another study in 1998 on water buffaloes with chronic fasciolosis, the level of transaminases in plasma elevated significantly six weeks after infection [19].

Sun HP *et al*. determined ALT activity in the mammalian and mature *F. hepatica* tissues. Their findings showed that the parasite ALT-specific activity was less than those of mammalian tissues [20], while our results demonstrate that the level of the enzyme in the parasite was significantly more than those of liver tissues. As a result of bile duct infection, ALT activity increases; however, the level of ALT enzyme of healthy and infected liver showed no significant difference in our study [4]. Some of our findings are compatible with others that mentioned above, and the differences between them could be related to some factors such as differences between sampling, measuring methods, instruments, and above all the selected isolates of the parasite. For that, we suggest more surveys on these enzymes in different phases of diseases and more accurate investigation of the pathogenesis of various species of parasite.

Our SDS-PAGE analysis gel showed protein band with molecular weight of 52.9 and 54 KDa for ALT enzyme in the parasite and liver samples, respectively. Detected proteins from SDS-PAGE gel analysis compiled in Table 2 demonstrate two cathepsins in rows 10 and 12, which are absent in Table 3. Cathepsin enzymes are vital in nutrition and migration of the parasites and might be used for diagnosis or development of a vaccine against fascioliasis [21, 22].

ALT activity could not be concerned as an indicator for TCBZ efficiency on the parasite. However, the enzyme has comparable activity in the parasite and its host liver tissue.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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