IJPT
1735-2657/14/131-9-12
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 13: 9-12, 2014

ORIGINAL ARTICLE

Comparative Evaluation of in Vitro Effects of Praziquantel (PZQ) on the Enzyme Activities of the Excretory-Secretory Products (ESP) of *Fasciola hepatica* and *F. gigantica* Parasites

ALI FARAHNAK, TAGHI GOLMOHAMDI, and MOHAMAD REZA ESHRAGHIAN

For author affiliations, see end of text.

Received March 14, 2013; Revised June 8, 2013; Accepted September 21, 2013

This paper is available online at *http://ijpt.tums.ac.ir*

ABSTRACT

Excretory-secretory products (ESP) play an important role in the host biochemical defense by means of activities of detoxifying and antioxidant enzymes. The aim of this study was to evaluate praziquantel (PZQ) effects by detection of glutathione S- transferase (GST) and superoxide dismutase (SOD) enzymes activities in ESP samples of *Fasciola* spp. *Fasciola gigantica* and *Fasciola hepatica* adult parasites were collected and cultured within buffer media for 4 h at 37°C. Treated (with 50, 100 and 150 μg PZQ) and control ESP samples for each species were collected and centrifuged. The supernatants were stored at -20°C. SOD and GST enzymes activities of ESP samples were estimated photometrically. To determine the statistically-significant difference between ESP of treated and control samples, t-test was conducted. ESP protein bands were detected by gel electrophoresis (SDS-PAGE). SOD enzyme activities level in treated *F.hepatica* and *F. gigantica* ESP samples were determined as 0.26, 0.29, 0.76 and 1.73, 1.60, 1.43 U/ml respectively. SOD activities level in control samples were detected 1.31 and 1.35 U/ml. GST activities level in treated *F.hepatica* and *F. gigantica* ESP samples were calculated 9.4, 3.1, 23.96 and 435.4, 333.3, 720.9 U/ml. GST activities levels in control samples were detected 43.8 and 134.4 U/ml respectively. Statistical analysis revealed the significant decrease of GST and SOD enzyme activities in treated ESP samples of *Fasciola hepatica*, and significant increase of GST enzyme activity in treated ESP samples of *F. gigantic*a in comparison to the control samples ($p < 0.05$). There was also no difference between SDS-PAGE results of treated and control samples. Based on the results of present work, PZQ has decreasing effect on the ESP enzymatic activities of *Fasciola hepatica* and increasing effect on *F. gigantica* enzyme activities. In other words, *F. hepatica* has less capability to protect against xenobiotics and free radicals than *F.gigatica* does.

Keywords: *Fasciola gigantica*, *F.hepatica*; Excretory-Secretory Products, Praziquantel, Superoxide dismutase, Glutathione S- transferase

Millions of people are infected with fascioliasis in the world. The disease is caused by trematodes belonging to Fasciola (*F. hepatica* and *F. gigantica*) parasites and treated with triclabendazole [1]. At the present time, praziquantel is not using for treatment of fascioliasis; however, this anthelmintic drug is very effective against other parasitic diseases [2-3].

Various studies have been recorded on the characterization of fasciola parasite ESP [4-5]. Glutathione S-transferase (GST) and superoxide dismutase (SOD) enzymes are common in the ESP of fasciola parasite [6]. The antioxidant enzyme, SOD, is capable of inhibiting the oxidation of other molecules [7]. In the other hand, GST enzymes is detoxifying

Table 1. SOD enzyme activities value of praziquantel (PZQ)-treated excretory- secretory product (ESP) samples of *Fasciola hepatica*; *F. gigantica* and control samples (mean of 10 ESP samples for each)

ESP Samples	SOD* activities of *F. gigantic* (U/ml)**	SOD* activities of *F. hepatica* (U/ml)**
Control ESP samples	1.35	1.31
ESP contains 50 mg PZQ	1.73	0.26
ESP contains 100 mg PZQ	1.60	0.29
ESP contains150 mg PZQ	1.43	0.76

*Superoxide dismutase Enzyme

**One unit enzyme activity is the amount of SOD that inhibits the rate of formazan dye formation by 50%

Table 2. GST enzyme activities value of praziquantel (PZQ) treated excretory- secretory product (ESP) samples of *Fasciola hepatica*; *F. gigantica* and control samples (mean of 10 ESP samples for each)

ESP Samples	GST* activities of *F. gigantic* (U/ml)**	GST* activities of *F. hepatica* (U/ml)**
Control ESP samples	134.4	43.8
ESP contains 50 µg PZQ	435.4	9.4
ESP contains 100 µg PZQ	333.3	3.1
ESP contains 150 µg PZQ	720.9	23.9

*Glutathione S- transferase enzyme

**One unit will conjugate 1.0 µmole of 1-chloro-2, 4-dinitrobenzene with reduced glutathione per minute at pH 6.5 at 25°C

endogenous com pounds as well as breaking down the xenobiotics [8].

Although there are many studies on the effects of triclabendazole on the fasciola products, however, there are no reports on PZQ effects on fasciola products [9-11]. In this research, in order to evaluate PZQ effects on GST and SOD enzyme activities, the PZQ-treated ESP of *Fasciola hepatica* and *F.gigantica* were compared with control samples in culture.

MATERIALS AND METHODS

Collection of parasite ESP samples

The adult parasites of *Fasciola gigantica* and *Fasciola hepatica* were collected from local abattoir (Tehran abattoir, Tehran, Iran) and identified, based on morphologic and morphometric characters. Collected alive Fasciola parasites were washed for a minimum of three times in PBS, pH 7.4, to remove host material. Meanwhile, to prepare PZQ dilutions (50, 10, 150 µg/ml), purchased PZQ tablets (Tolid Darou Dami Iran Co.) was homogenized within ethanol as a solvent. The parasites were cultured within the buffer media (treated or control) for 4 h at 37°C (2 parasites in 2 ml of culture media for each sample). The total of 80 ESP samples of *Fasciola gigantica* and *Fasciola hepatica* (30 treated, 10 for each PZQ dilution, and 10 control ESP samples for each species) were collected and centrifuged at 10000 g for 30 min and supernatants were stored at -20°C [12].

SOD enzyme activity assay of ESP samples

SOD activity of ESP samples were determined using RANSOD Kit (Randox Labs, crumlin, UK). The absorbances of samples were measured 30 s after the addition of xanthine oxidase as start reagent at 505 nm on a spectrophotometer (Cecil Instruments Ltd) and 3 min after reaction, in 37°C. A standard curve was plotted using the standard provided in the Kit and the SOD total activity value in U/ml for each ESP sample was read from this curve using the excel software. One unit enzyme activity is the amount of SOD that inhibits the rate of formazan dye formation by 50% [13].

GST enzyme activity assay of ESP samples

For activity assay of GST enzyme in ESP samples, reagent cocktail including, potassium phosphate buffer, reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) substrates were prepared in the cuvette. In each cuvette, from the mentioned mixture, 200 µl sample solution was removed and then the same volume of PZQ-treated *F. hepatica* and *F.gigantica* ESP or control samples was added into cuvette and mixed well. Finally, the cuvette was placed into the barrel of spectrophotometer and absorbances recorded for 5 minutes at 340 nm. Total GST enzyme activity as U/ml of all samples were calculated. One unit GST activity

Published online: January 28, 2014

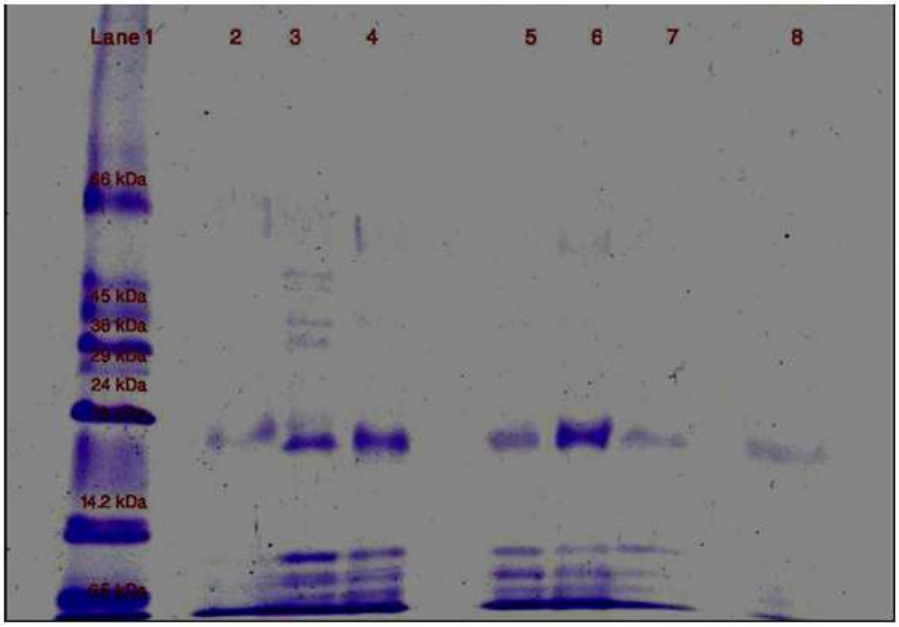

Fig 1. SDS- PAGE analysis of PZQ-treated and control ESP samples from *F. hepatica and F.gigantica*. Each lane represents an ESP sample; the lane 1 corresponds to the protein marker. The lanes 2, 3 and 4 correspond to treated *F. hepatica* and the lanes 5, 6 and 7 correspond to treated *F. gigantica* ESP samples by PZQ dilutions of 50, 100, 150 µg/ml. The lane 8 indicates control ESP sample.

will conjugate 1.0 µmole of 1-chloro-2, 4-dinitrobenzene with reduced glutathione per minute at pH 6.5 at 25°C [14].

SDS-PAGE analysis of ESP samples

Sodium dodecyl sulfate Polyacrylamide gel electrophoresis and coomassie blue staining were used to separate the components of ESP samples protein. Samples were added to each wells of gel, 7.5 %, and were run for 6 hours at 15 mA and finally the gels were stained by comassie blue staining. Molecular weights of sample proteins were detected using the protein marker within the gel [12].

Statistical analysis of ESP samples

To determine the statistical difference between GST and SOD values of enzyme activities in PZQ-treated and control ESP samples, two-sample independent t-tests was used [15].

RESULTS

The results of SOD and GST enzyme activities assay are presented in Tables 1 and 2. In *F. hepatica*, two-sample t-test results reveal that there is significant decrease of SOD and GST activities of treated ESP samples in comparison to the control ESP samples ($p < 0.05$). In F. *gigantic*, t-test results show that there is significant increase of GST activities of treated ESP samples in comparison to the control ESP samples ($p < 0.05$). However, there is no significant difference between SOD activities in treated *F. gigantica* and control ESP samples ($p > 0.05$).

Protein bands of ESP samples were detected using SDS electrophoresis (Fig 1). There is no difference between SDS-PAGE results of treated and control ESP samples.

DISCUSSION

GST and SOD enzyme activities in ES of *Fasciola hepatica* and *F. gigantica* were previously demonstrated by authors [16-17]. Pentoxifylline as adjuvant therapy with praziquantel produced reduction in glutathione-S-transferase (GST) and superoxide dismutase (SOD) in experimental *Schistosomiasis mansoni* [18]. In this study, the significant decrease of GST enzyme activity in PZQ-treated *F.hepatica* ESP may be due to less ability of this species to detoxifies the endogenous compounds as well as breakdown of xenobiotics in comparison to *F.gigantica*. This phenomenon increase parasite attack in the liver tissue of man and animals by the endogenous compounds. On the other hand, the significant increase of GST enzyme activity in treated *F.gigantica* ESP may be due to more ability of this species to detoxify endogenous compounds as well as breakdown of xenobiotics in comparison with the *F.hepatica*. At the present research, the significant decrease of SOD enzyme activity of treated *Fasciola hepatica* ESP increase molecular oxidation and consequently assists the parasite destruction in the liver tissue. Although there is no significant difference for SOD activities of treated *F. gigantica* and control ESP samples, higher activity of this enzyme causes more protection of fasciola gigantic than *F. hepatica* dose against free radical.

In conclusion, the significant decrease of GST and SOD enzyme activities in PZQ-treated *Fasciola hepatica* ESP samples indicate this species has less

capability to protect against xenobiotics and free radicals than *F.gigatica* does.

ACKNOWLEDGEMENTS

The author would like to thank the personnel of Tehran City Abattoir for providing infected liver and M.B Molaei for preparation of gel electrophoresis. This work was supported with funds from Project number # 8429 of Tehran University of Medical Sciences.

REFERENCES

1. World Health Organization [Internet]. Geneva: [WHO 2012]. Neglected tropical diseases; Fascioliasis. [1 screen] Available from: http://www.who.int/neglected_diseases/integrated_media/integrated_media_fascioliasis/en/index.html
2. Geerts S. The efficacy of praziquantel for the treatment of cestode and metacestode infections. *Int J Antimicrob Agents* 1994; 4:321-4.
3. World Health Organization [Internet]. Geneva: [WHO 2012] Media center. Schistosomiasis; [1 screen] Available from: http://www.who.int/mediacentre/factsheets/fs115/en/
4. Morphew RM, Wright HA, LaCourse EJ, Woods DJ, Brophy PM. Comparative Proteomics of Excretory-Secretory Proteins Released by the Liver Fluke Fasciola hepatica in Sheep Host Bile and during in Vitro Culture ex Host. *Mol Cell Proteomics* 2007; 6:963-72.
5. Gourbal BE, Guillou F, Mitta G, Sibille P, Thèron A, Pointier JP, Coustau C. Excretory-secretory products of larval Fasciola hepatica investigated using a two-dimensional proteomic approach. *Mol Biochem Parasitol* 2008; 161:63-6.
6. Jefferies JR, Campbell AM, van Rossum AJ, Barrett J, Brophy PM. Proteomic analysis of excretory-secretory products. *Proteomics* 2001; 1:1128–32.
7. Wikipedia [Internet]. [Last modified on 9 November 2012] Antioxidant [1 screen] Available from: http://en.wikipedia.org/wiki/Antioxidant.
8. Wikipedia [Internet]. [Last modified on 23 August 2012] Glutathione_S-transferase [1screen] Available from: http://en.wikipedia.org/wiki/Glutathione_S-transferase.
9. Chemale G, Perally S, Lacourse EJ, Prescott MC, Jones LM, Ward D, Meaney M, Hoey E, Brennan GP, Fairweather I, Trudgett A, Brophy PM. Comparative Proteomic Analysis of Triclabendazole Response in the Liver Fluke Fasciola hepatica. *J Proteome Res* 2010; 9:4940-51.
10. Shehab AY, Ebeid SM, El-Samak MY, Hussein NM. Detoxifying and anti-oxidant enzymes of Fasciola gigantica worms under triclabendazole sulphoxide (TCBZ-SX): an in vitro study. *J Egypt Soc Parasitol* 2009; 39:73-83.
11. Farahnak A, Golmohamdi T, Eshraghian MR. In vitro Effects of Triclabendazole (TCBZ) on the Excretory-Secretory Products (ESP) of Fasciola spp Parasites. *Acta Medica Iranica* 2012; 50: 164-8.
12. Maizels RM, Blaxter ML, Robertson BD, Selkirk ME. Parasite antigen and parasite genes: *A laboratory manual for molecular parasitology*. 1st ed. Cambridge; 1991.
13. Randox labs. Ransod kit [Internet]; [Randox 2012] Sod assay. [2 screen] Available from; http://www.randoxonlinestore.com/RANSOD-(Superoxide-Dismutase)-p-7904
14. Mnstate. Edu [Internet]; [Updated Spring 2012 KA]. GST Assay Protocol. [1 screen] Available from: http://www.mnstate.edu/provost/GST Assay Protocol.pdf
15. GraphPad Software [Internet]; [2012 GraphPad Software]. t- test calculator. [1 screen] Available from: http://www.graphpad.com/quickcalcs/ttest1.cfm
16. Alirahmi H, Farahnak A, Golmohamadi T, Esharghian MR. Comparative assay of glutathione S-transferase (GSTs) activity of excretory-secretory materials and somatic extract of Fasciola spp parasites. *Acta Medica Iranica* 2010; 48:367-70.
17. Farahnak A, Golestani T, Eshraghian MR. Activity of superoxide dismutase (SOD) enzyme in the excretory-secretory products of *Fasciola hepatica* and *F. gigantica* parasites. *Iranian J Parasitol* 2013; 8:167-70.
18. El-Lakkany N, el-Din SS, Ebeid F. The use of pentoxifylline as adjuvant therapy with praziquantel downregulates profibrogenic cytokines, collagen deposition and oxidative stress in experimental schistosomiasis mansoni. *Exp Parasitol* 2011; 129:152-7.

CURRENT AUTHOR ADDRESSES

Ali Farahnak, Department of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. E-mail: farahnak@tums.ac.ir (Corresponding Author)

Taghi Golmohamdi, Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

Mohamad Reza Eshraghian, Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Published online: January 28, 2014