Global Journal of Pharmacology 6 (3): 199-207, 2012
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2012.6.3.64222

Changes in Hepatic Phase-I and -II Drug-metabolizing Enzymes and Antioxidant Capacity in Egyptian Ovine Fascioliasis Treated with Triclabendazole

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Abstract: Sheep experimentally infected with the Egyptian strain of *Fasciola* were used to study the influence of infection on the activities of some drug-metabolizing enzymes and antioxidant capacity in the liver. Twenty-two sheep were randomly divided into a group of 12 animals (all subsequently infected with 150 metacercariae of *Fasciola*) and a group of ten (left uninfected, as normal controls). Three months post-infection, seven of the infected sheep were treated with triclabendazole (TCBZ) as a single oral dose of 10 mg/kg b.wt. and the other five were left untreated. The uninfected sheep were, similarly, treated with TCBZ (n=5) or left untreated (n=5). Four weeks after the end of treatment, the animals were ethically slaughtered. Significant decreases (40-53%) were observed in the hepatic microsomal contents of cytochrome P450 (CYP450; $P < 0.001$), cytochrome b5 (Cyt b5; $P < 0.001$) and aminopyrine N-demethylase, aniline 4-hydroxylase ($P < 0.01$) and glutathione S-transferase (GST; $P < 0.05$) activities when compared with the uninfected untreated sheep. Moreover, reduced glutathione (GSH) content; glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were significantly lowered by 33.25%, 40% and 53.35% respectively. Meanwhile, glutathione reductase (GR) activity and malonaldehyde (MDA) level were significantly elevated by 66.92% ($P < 0.05$) and 65.79% ($P < 0.001$) respectively. Treatment with TCBZ restored all previously measured parameters to their normal levels. In conclusion, Egyptian fascioliasis impaired oxidative, conjugative drug metabolism and induced oxidative stress. This study also confirms that TCBZ is the ideal fasciocidal drug for recovery of all the disease-related pathogenic factors.

Key words: *Fasciola Spp* · CYP450 · CYP450-Dependent Monooxygenases · Oxidative Stress · MDA · Triclabendazole

INTRODUCTION

The trematode liver fluke, *Fasciola hepatica* (*F. hepatica*), along with *Fasciola gigantica* (*F. gigantica*) are the causative agent of fasciolosis, a food borne zoonotic disease affecting grazing animals and humans worldwide [1]. There has been a change in the pattern and incidence of disease in recent years, which has been attributed to changes in climate [2] and this trend is predicted to continue well into the future [3]. Due to an increase in the number of cases of this fluke infection in humans all over the world, fasciolosis has been classified by the World Health Organization (WHO) as a serious threat to public health [4]. In domestic ruminants, adverse effects of acute or chronic fascioliasis include annual losses of more than US$3000 million to livestock production worldwide through livestock mortality and by decreased meat and milk productions, decreased female fertility and increased veterinary costs [5]. At present, there is no vaccine available for the prevention of fascioliasis [6] and hence chemotherapy is the current mainstay of control. Triclabendazole (TCBZ) is the current drug of choice in human and veterinary medicine due to its high efficacy against both juvenile and adult *F. hepatica* and its excellent safety profile [7].

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In fascioliasis, in particular the immature parasite is highly pathogenic, causing fibrosis, hemorrhaging and anemia [7]. The hepatic pathology due to fascioliasis, even when only small liver areas are damaged, are associated with chemical alterations in the cell, such as enhancement of membrane lipid peroxidation, marked suppression of the microsomal drug-metabolizing monooxygenase system and declined hepatic microsomal cytochrome P-450 (CYP450) concentration [5, 8-11]. The GSTs, phase-II drug-metabolizing enzymes, are a family of multi-functional proteins, which catalyze the conjugation and the detoxification of a wide range of hydrophobic electrophiles with glutathione [12]. Fascioliasis has been described to decrease significantly hepatic mixed function oxidase and transferase systems, which are involved in the detoxification of xenobiotics and endogenous compounds prior to excretion from the body [13, 14].

A balance between oxidants and antioxidants is known to exist under physiological conditions. However, even small changes in oxidant and/or antioxidant levels may disturb this balance. Many parasitic infections, whether single or combined, enhance reactive oxygen species (ROS) generation including super oxide radical, nitric oxide and hydrogen peroxide, produced especially by leukocytes [15]. In the course of fascioliasis, enhanced generation of ROS is observed accompanied by disturbances in antioxidant mechanisms, which lead to ineffectiveness in ROS scavenging [16]. Lipid peroxidation is a general mechanism whereby free radicals induce tissue damages and implicated under several diverse pathological conditions [17].

In Egypt, fascioliasis is an enzootic disease in farm animals and is considered as an emerging public health problem [18]. Since *Fasciola Spp*. is not properly identified in Egypt and by comparing the electrophoretic results with the morphologic and morphometric findings, the results were matching, so that the presence of both the *Fasciola* species in Egyptian animals could be confirmed [19]. By tracing the available literature, there is, to our knowledge, no studies regarding the activities of hepatic drug-metabolizing enzymes and antioxidant enzymes status of host liver during Egyptian ovine fascioliasis and the impact of TCBZ treatment. Because fascioliasis mainly affects the liver, which is the major site of drug biotransformation and antioxidant defense system. Accordingly, it was thought of interest to determine the effects of an Egyptian strain of *Fasciola*, with/without TCBZ treatment, on the activities of hepatic phase-I and -II drug-metabolizing enzymes and on antioxidant capacity by assessing of liver glutathione antioxidant system-related enzymes and lipid peroxidation. Such information will give additional insight regarding the pathogenesis of the disease in Egypt.

MATERIAL AND METHODS

Selection and Maintenance of Sheep: For the study, 22 healthy sheep, each aged 5-6 months old and weighing about 35 kg, were purchased from the Egyptian Ministry of Agriculture and transferred to the animal house of Theodor Bilharz Research Institute (TBRI), in Giza. Each sheep was checked for helminth infection, by coproscopy on three consecutive days and found negative. All the sheep were ear-tagged, numbered, weighed and kept under environmentally-controlled conditions, under the supervision of a veterinarian, with free access to food and water.

Infection of Sheep: Fresh metacercariae of an Egyptian strain of *Fasciola* (*F. hepatica* or *F. gigantica*), supplied by the Schistosome Biological Supply Centre (SBSC) of TBRI, were distributed into sterile Eppendorf tubes so that each tube contained 150 metacercariae in 1 ml sterile water. Each sheep to be infected was given the contents of one tube, by oral intubation [20].

Drug and Dosage: Triclabendazole (Fasinex®; Novartis Animal Health, Frimley, U.K.) was administered as a single oral dose of 10 mg/kg b.wt., after an overnight fast [21, 22]. Treatment with TCBZ was started 3 months post-infection.

Animal Groups: A total of 22 sheep were randomly divided into a group of 12 animals (all subsequently infected with *Fasciola*) and a group of ten (left uninfected, as normal controls). Three months post-infection, seven of the infected sheep were treated with TCBZ and the other five infected animals were left untreated. The uninfected sheep were, similarly, treated with TCBZ (n=5) or left untreated (n=5). All experimental procedures with animals were in accordance with the international ethical guidelines for the care and use of animals for research purposes.

Preparation of Tissue Samples: Four weeks after the end of the treatment, the animals were ethically slaughtered. Each slaughtered sheep was eviscerated immediately and its liver, with intact bile ducts and gall bladder, was separated out and freed of extra tissue and excess moisture was absorbed before organs were weighed.

The flukes were removed by dissection of liver parenchyma and bile ducts. Small samples, weighing each about 5 g, were collected from the left and right hepatic lobes of each sheep.

Assessment of Hepatic Phase-I and -II Drug-Metabolizing Enzymes: The activities of CYP450, Cyt b5, aminopyrine N-demethylase, aniline 4-hydroxylase and GST were determined in the hepatic microsomes of normal and infected sheep with the Egyptian strain of *Fasciola* with/without TCBZ treatment. Microsomal fractions were prepared by homogenization of randomized samples (5.0 g) of liver in five volumes (w/v) of ice-cold 0.1 M potassium phosphate buffer (pH of 7.4) followed by sequential centrifugation in a Christ cooling centrifuge at 4°C and 600 g for 10 min and then at 3,000 g for another 10 min. The supernatant fraction was further centrifuged at 105,000 g for 60 min at 4°C in a Sorval ultracentrifuge to yield the microsomal pellet, which was resuspended in 0.1 M potassium phosphate buffer (pH 7.4) and stored at-80°C until assay [23]. CYP450 and Cyt b5 activities were determined spectrophotometrically [24], using a double-beam spectrophotometer (Lambda 3B, Perkin Elmer, USA.). The specific activities of CYP450 and Cyt b5 enzymes were expressed as units of the enzyme content (nmole/mg microsomal protein). Microsomal protein concentrations were assayed according to the method described by Lowry *et al.* [25]. The activities of aminopyrine N-demethylase and aniline 4-hydroxylase were estimated spectrophotometrically by the determination of formaldehyde produced by oxidative N-demethylation [26] and p-aminophenol produced by the hydroxylation of aniline [27], respectively. GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate [28]. GST catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The reaction mixture contained suitable amount of the enzyme, potassium phosphate buffer, ethylene diamine tetraacetic acid (EDTA), CDNB and reduced glutathione (GSH). The reaction was carried out at 37°C and monitored spectrophotometrically by the increase in absorbance of the conjugate of GSH and CDNB at 340 nm. A blank was run in parallel in absence of the enzyme. One unit of GST activity is defined as one µmole product formation/min/g liver.

Assessment of Hepatic Antioxidant Enzymes: Liver tissue was homogenized in four volumes (w/v) of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM EDTA and centrifuged at 10,000 g for one hour at 4°C. The supernatant was collected and kept at -80°C for subsequent analysis for determination of liver content of GSH, glutathione-related antioxidant enzymes and lipid peroxidation.

GSH Assay: The level of GSH was determined in liver homogenate according to the method of Ellman [29]. Briefly: 0.5 ml homogenate was added to a tube with 0.5 ml of 10% trichloroacetic acid (TCA). The tubes were centrifuged at 3,000 g for 10 min. A 0.2 ml aliquot of the resulting supernatant was added to a tube containing 5 ml of 0.1 M potassium phosphate buffer and 0.1 ml of 5, 5'-dithio-bis-2-nitro benzoic acid solution (DTNB; Ellman's reagent) and the absorbance was measured at 412 nm. With the help of the standard curve drawn using gradual concentrations of a standard GSH, content of GSH in the liver homogenates of the experimental animals were calculated.

Glutathione Peroxidase (GPx) Assay: GPx catalyzes the oxidation of glutathione and its activity was measured based on the method described by Paglia and Valentine [30]. The enzyme reaction mixture contains nicotinamide adenine dinucleotide phosphate reduced (NADPH), reduced glutathione and glutathione reductase, was initiated by addition of hydrogen peroxide (H_2O_2) and the change in absorbance at 340 nm was monitored by a spectrophotometer. One unit of GPx activity is defined as the amount of the enzyme that catalyzes the formation of one µmole of NADPH/min/g liver.

Glutathione Reductase (GR) Assay: GR activity was assayed using oxidized glutathione (GSSG) as a substrate according to the method described by Zanetti [31]. GR activity was measured by monitoring the oxidation of NADPH at 340 nm. One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of one µmole of NADPH/min/g liver.

Superoxide Dismutase (SOD) Assay: SOD activity was assayed spectrophotometrically at 560 nm by the procedure of Winterbourn *et al.* [32]. The activity of SOD depends on its ability to inhibit phenazine methosulphate (PMT)-mediated reaction of nitroblue tetrazolium (NBT) dye. One unit of enzyme activity is defined as the amount of enzyme that causes half-maximal inhibition of NBT reduction. Activity was expressed in µmole/min/g liver.

Catalase Assay: Catalase activity was determined according to Aebi's [33]. The test is based on the determination of the H_2O_2 decomposition rate constant (k) at 240 nm. Results were expressed as k/g liver.

Assessment of Hepatic Lipid Peroxidation Products: Degree of lipid peroxidation in the liver tissue homogenates of sheep was determined in terms of thiobarbituric acid reactive substances (TBARS) formation [34]. One ml of enzyme supernatant was mixed with one ml of TCA (10% w/v) in a centrifuge tube and centrifuged at 1,850 g for 15 min at room temperature. One milliliter of TBA solution (0.67 % w/v) was added to 1 ml of supernatant and kept in a boiling water bath for 45 min. Absorbance was read after cooling at 530 nm against a blank containing all the reagents except the tissue homogenate. As 99% of the TBARS is malondialdehyde (MDA), TBARS concentrations of the samples were calculated using the extinction coefficient of MDA, which is $1.56 \times 10^5\ M^{-1}cm^{-1}$.

Statistical Analysis: Data were analyzed using version 9.0 of the SPSS software package (SPSS Inc., Chicago, IL) and the results were expressed as means ± SEM. Group means were compared using unpaired Student's *t*-tests. A *p* value less than 0.05 was considered as statistically significant.

RESULTS

Infected control and infected TCBZ-treated mean values of phase-I and -II drug-metabolizing enzyme activities are presented in (Table 1) and the percent inhibition from normal untreated group are presented in (Fig. 1). In *Fasciola*-infected sheep, statistical analysis clearly demonstrated a 48.74% and 53.19% significant inhibition ($P < 0.001$) in the total CYP450 and Cyt b5 activities respectively when compared with the normal untreated sheep. Moreover, significant inhibition ($P < 0.01$) in the activities of aminopyrine N-demethylase and aniline 4-hydroxylase by 41.54% and 42.17% respectively was also observed. This was concomitant with significant decrease in GST activity ($P < 0.05$) by 40%.

Liver GSH content, GPx, GR, catalase and SOD activities as well as MDA level in normal, *Fasciola*-infected and TCBZ-treated sheep were shown in (Fig. 2). Data showed that GSH content, Gpx and SOD activities were significantly lowered in *Fasciola*-infected sheep by 33.25%, 40% ($P < 0.01$) and 53.35% ($P < 0.001$) respectively in comparison with the normal untreated group (Fig. 2). Meanwhile, GR activity and MDA level were significantly elevated by 66.92% ($P < 0.05$) and 65.79% ($P < 0.001$) respectively. On the other hand, catalase activity was not significantly changed.

Table 1: Phase-I and -II drug-metabolizing enzyme activities in liver microsomes of normal and Egyptian *Fasciola*-infected sheep and the effect of triclabendazole treatment

	Phase-I drug-metabolizing enzymes				Phase-II drug-metabolizing enzymes
Animal groups	CYP450 (nmole/mg protein)	Cyt b5 (nmole/mg protein)	Aminopyrine N-demethylase (nmole/min/mg protein)	Aniline hydroxylase (nmole/min/mg protein)	GST (µmole/min/g liver)
Normal (n=5)	1.19±0.18	1.88±0.19	5.32±0.43	0.83±0.08	12.25±1.73
Normal-TCBZ treated (n=5)	1.13±0.11(-5.04%)	1.72±0.08(-8.51%)	4.98±0.36(-6.39%)	0.81±0.05(-2.41%)	11.87±1.86(-3.10%)
Fasciola-infected (n=5)	0.61±0.07 *** (-48.74%)	0.88±0.04*** (-53.19%)	3.11±0.11** (-41.54%)	0.48±0.01** (-42.17%)	7.34±0.98 * (-40.08%)
Fasciola-TCBZ treated (n=7)	1.09±0.06 $$$ (-8.40%)	1.48±0.06 $$$ (-21.28%)	5.11±0.36 $$$ (-3.95%)	0.75±0.03 $$$ (-9.64%)	11.88±1.73 $ (-3.02%)

Values are presented as Mean ± SEM. n= Number of sheep.

Number between parentheses represents the percent reduction from normal untreated sheep.

* Significant difference from normal untreated sheep at $p < 0.05$, ** at $p < 0.01$ and *** at $p < 0.001$.

$ Significant difference from infected untreated sheep at $p < 0.05$ and $$$ at $p < 0.001$.

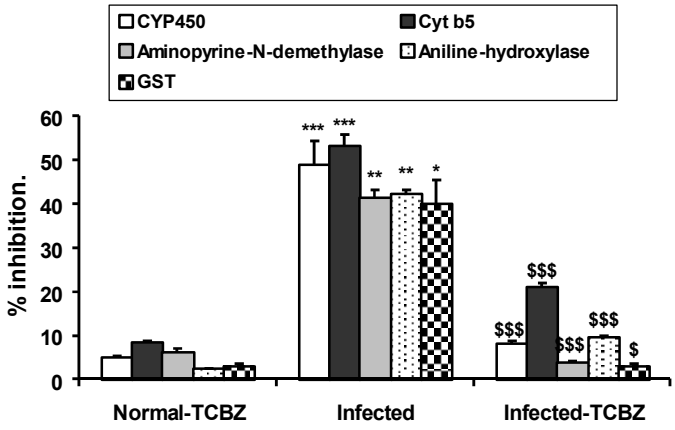

Fig. 1: The percent inhibition in CYP450, Cyt b5, CYP450-depedent monooxygenase enzymes and GST activities in the liver of normal-TCBZ treated, infected and infected-TCBZ treated groups versus normal untreated group. Values are presented as mean ± SEM.
* Significant difference from normal untreated sheep at $p < 0.05$, ** at $p < 0.01$ and *** at $p < 0.001$.
$ Significant difference from infected untreated sheep at $p < 0.05$ and $$$ at $p < 0.001$.

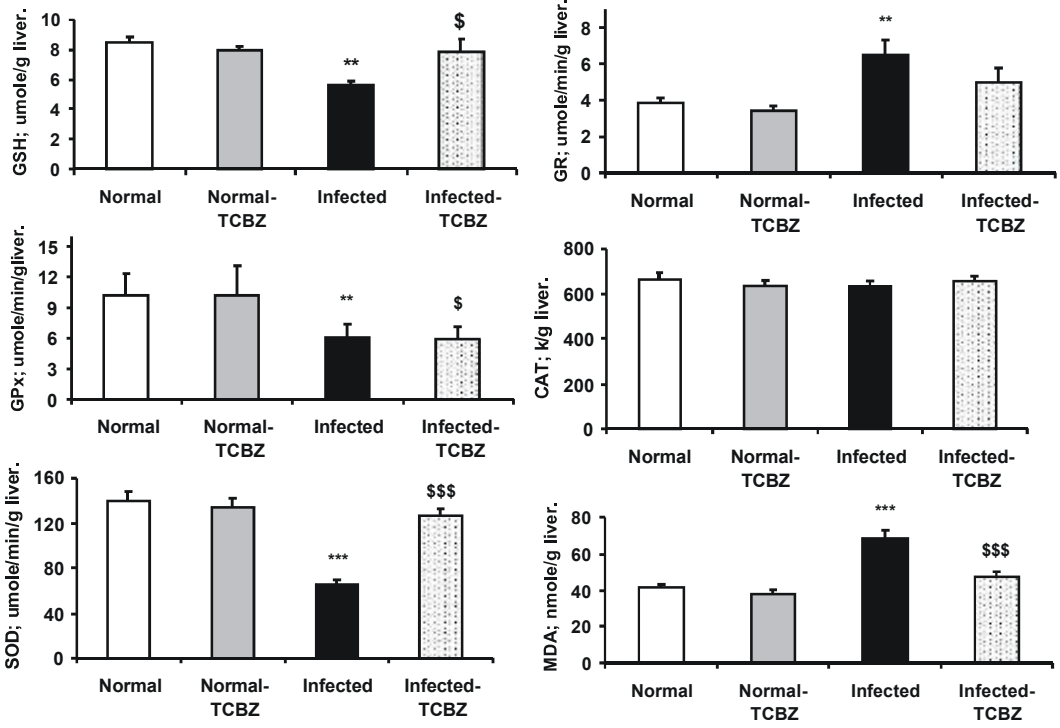

Fig. 2: Hepatic content of glutathione, activities of antioxidant enzymes and lipid peroxidation level in the liver of normal and Egyptian *Fasciola*-infected sheep and the effect of triclabendazole treatment.
Values are presented as mean ± SEM.
** Significant difference from normal untreated sheep at $p < 0.01$ and *** at $p < 0.001$.
$ Significant difference from infected untreated sheep at $p < 0.05$ and $$$ at $p < 0.001$.

Upon treatment of *Fasciola*-infected sheep with TCBZ, activities of total CYP450, Cyt b5, monooxygenase-dependant and conjugative enzyme were significantly ($P < 0.001$) returned to their normal levels (Table 1, Fig.1). Moreover, liver GSH content, GPx and SOD activities, except for GR, as well as MDA level were completely restored their normal levels (Fig. 2).

DISCUSSION

The recovery of adult worms, the biochemical and histopathological signs seen in the *Fasciola*-infected sheep was recorded in our previous study [35]. In our previous study of Botros *et al.* [35], the worms recovered with a mean number of 41.20 ± 6.30 with average hepatic egg load of 105.17 ± 16.52. We also reported presence of chronic hepatitis, cholangitis and cholestasis accompanied with significant elevations in the serum levels of ALT, γ-GT and decreases in total serum proteins and albumin. On this basis, we decided to investigate the levels of both oxidative and conjugative hepatic biotransformation by analyzing the activities of hepatic CYP450, Cyt b5 and some CYP450-dependent monooxygenases including aminopyrine N-demethylase and aniline 4-hydroxylase and GST. Moreover, the level of hepatic MDA as a biomarker of lipid peroxidation and the liver antioxidant capacity status in this experimental model of Egyptian *Fasciola* were also assessed.

In this study, the hepatic CYP450, Cyt b5 contents and aminopyrine N-demethylase, aniline 4-hdyroxylase and GST activities were significantly decreased by (40-53%), 16 weeks post-infection. These findings are in agreement with those of Galtier *et al.* [13], El-Sheikh *et al.* [36] and CalleÂja *et al.* [5], who found significant decrease in the activities of phase-I drug-metabolizing enzymes in the ovine species infected with other *Fasciola* strains. However, there are some differences in the time at which these enzyme activities were decreased. In ovine fascioliasis, data clearly confirmed a 20-23% decrease in CYP450 level in sheep infected for 10 weeks [5] and decreases in Cyt b5 content (10-18%) in the lamb liver at early stages of the disease [13]. Moreover, the authors observed that the decrease in aminopyrine N-demethylase and aniline 4-hydroxylase and GST activities occurred from the 8^{th} until the 16^{th} week post-infection [13]. Meanwhile, El-Sheikh *et al.* [36] reported that the decrease in these enzyme activities occurred 5 weeks post-infection. Such decreases in phase-I and -II drug-metabolizing enzymes in our and other previous studies could be attributed to the parenchymal inflammation associated with the increased secretion of inflammatory factors characteristic of the immune response occurring during ovine fascioliasis. In fact, in sheep, both systemic eosinophilia [37] and the presence of IgG1 as dominant antibody isotype [38] associated with the increase in $CD4^+$ cells in the liver [39] are suggesting a Th2-type immune response during the hepatic stage of fascioliasis. Additionally, it is now well established that Th2 cells secrete interleukins 4, 5 and 6, which regulate humoral immune response by promoting B-cell-dependent IgE production or by activating eosinophils. In this context, highly secreted IL4 and IL6 could reduce the activity of drug-metabolizing enzymes since previous investigations demonstrated that these two cytokines were able to down-regulate hepatic CYP1A, 2C or 3A gene expression [40].

Hepatic oxidative stress is at least a partial factor contributing to the loss of CYP450 in fluke-infected rats [9]. Data described in this study provide a reliable biochemical evidence for the generation of oxidative stress, as detected by reduction of the hepatic antioxidant ability, as evidenced by the depletion of GSH content, GPx, SOD activities accompanied with an increase in GR activity in *Fasciola*-infected sheep in comparison with the healthy group. Higher activities of GR should favor suppression of the reduction of glutathione disulphide. Meanwhile, depletion of GSH, co-substrate of GPx, prevents GPx action, as evident by decreased its activity and this may lead to accumulation of lipid peroxidation products up to toxic levels [41]. It was reported that in hosts infected with *F. hepatica*, the levels of hepatic and blood GSH declined in comparison to healthy controls [9, 42]. In this study, the decreased liver GPx activity has been previously reported in the *F. hepatica* infection [16]. However, Benzer and Temizer [43] showed increased liver GPx activity in this infection. Both catalase and GPx detoxify hydrogen peroxide to water, although the latter appears to have a more potent activity [44]. In this study, while GPx activity was lower in *Fasciola*-infected sheep compared to control, catalase activity was not significantly different between the groups, although the reason for this is unclear. In this study, the decreased liver SOD activity in the infected group is in agreement with the results of Kolodziejczyk *et al.* [16]. This drop in SOD activity could be explained by the superoxide anion dismutation to hydrogen peroxide caused by the overproduction of the superoxide anion linked to oxidative stress [45]. Depression of the protective capability against oxidative stress by SOD may lead to greater tissue damage and initiate a vicious cycle by increasing free radical production, thereby exceeding the antioxidant liver capacity and resulting in further oxidative damage.

The decrease in GSH-dependent antioxidant mechanisms of lipid protection leads to enhanced lipid peroxidation [46]. In this study, the liver MDA activity in the infected group was significantly higher than the healthy control group. In fascioliasis, it was noticed that

the phagocytic response of the rat liver to the parasite invasion and growth leads to free radical-mediated oxidative stress, which is the causative agent in the initiation and development of lipid peroxidation and increased MDA concentrations in the liver and plasma [8, 9, 42]. Moreover, Maffei Facino *et al.* [8] supports the view that lipid peroxidation is the major agent in the loss of hepatic drug-metabolizing enzymes activities in experimental fascioliasis in the rat. Increased MDA concentration during the chronic stage of fascioliasis was also previously reported in serum and livers of rats [16, 41] and in serum of humans [47].

In the present investigation, no modification in the total microsomal CYP450, Cyt b5, CYP450-depedent monooxygenase enzymes and GST activities and in the antioxidant capacity was observed in the uninfected group treated with triclabendazole. In contrast, the recovery of these previously reported parameters to their normal levels upon treatment of *Fasciola*-infected sheep with TCBZ was most evident. In addition, reduced GSH content, SOD and GPx activities and the elevated MDA levels were completely restored to their normal levels. This may be due to complete clearance of the flukes and their eggs with normalization of most of the biochemical markers investigated and marked regression of the hepatitis, cholangitis and cholecystitis; as in our previous study [35]. This is in agreement with the results of Rehim *et al.* [11] who reported improvement in the antioxidant capacity of *F. hepatica* patients after treatment with TCBZ. This study and our previous one [35] confirm that TCBZ is the ideal drug of choice for treatment of the Egyptian strain of *Fasciola* and healing of all disease-related pathogenic factors.

In conclusion, the findings of this study clearly demonstrates that chronic Egyptian fascioliasis as other *Fasciola* strains impaired drug metabolism by lowering the hepatic monooxygenase and transferase activities, which may lead to physiological, pharmacological or toxicological consequences in the parasitized hosts, by affecting their metabolizing capacity for chemotherapeutic and toxic compounds. This pathology could lead to enhanced xenobiotic toxicity, disturbed drug dosage adjustment and increased drug retention in affected animals, potentially compromising withdrawal periods for milk or meat produced by fluke-infected ruminants [48]. Thus, the choice of adequate remedies and drug dosage adjustment become a necessity. In addition, oxidative stress is a significant feature of chronic fascioliasis in sheep as shown by increased lipid peroxidation and decreased antioxidant capacity in liver indicating the presence of an imbalance between ROS production and antioxidant host defenses in inflammatory fascioliasis. This study also showed pronounced improvements in all studied parameters after TCBZ treatment, which confirms the fasciolicidal effect of the drug against all the disease-related pathogenic factors.

ACKNOWLEDGEMENTS

We are thankful to the staff of SBSC for providing us with metacercariae of *Fasciola* and for the veterinary physician who was responsible for supervision and keeping of sheep in the animal unit.

REFERENCES

1. Morphew, M.R., H.A. Wright, E.J. LaCourse, J. Porter, J. Barrett, D.J. Woods and P.M. Brophy, 2011. Towards delineating functions within the Fasciola secreted cathepsin l protease family by integrating *in vivo* based sub-proteomics and phylogenetics. PloS Nglec. Trop. Dis., 5: e937.
2. Van Dijk, J., N.D. Sargison, F. Kenyon and P.J. Skuce, 2010. Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. Animal, 4: 377-392.
3. Fox, N.J., P.C.L. White, C.J. McClean, G. Marion, A. Evans and M.R. Hutchings, 2011. Predicting impacts of climate change on Fasciola hepatica risk. PLoS One, 6: e16126.
4. WHO, 2006. Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control. Geneva: WHO Headquarters, http://www.who.int/neglected_diseases/preventive_chemotherapy/WHO_CDS_NTD_PCT_2007.1.pdf.
5. CalleÂjaa, C., K. Bigota, C. Eeckhouttea, P. Sibilleb, C. Boulardb and P. Galtiera, 2000. Comparison of hepatic and renal drug-metabolising enzyme activities in sheep given single or two-fold challenge infections with Fasciola hepatica. Int. J. Parasitol., 30: 953-958.
6. McManus, D.P. and J.P. Dalton, 2006. Vaccines against the zoonotic trematodes *Schistosoma japonicum*, *Fasciola hepatica* and *Fasciola gigantica*. Parasitology, 133: S43-S61.
7. Fairweather, I., 2005. Triclabendazole: new skills to unravel an old (ish) enigma. J. Helminthol., 79: 227-234.

8. Maffei Facino, R., M. Carini, C. Genchi, O. Tofanetti and I. Casciarri, 1989. Participation of lipid peroxidation in the loss of hepatic drug-metabolizing activities on experimental fascioliasis in the rat. Pharmacol. Res., 21: 549-560.
9. Maffei Facino, R., M. Carini, G. Aldini, R. Ceserani, I. Ceserani, E. Cavaletti and L. Vederio, 1993. Efficacy of glutathione for treatment of fascioliasis, an investigation in the experimentally infested rat. Arzneim-Forsch/Drug Res., 43: 455-460.
10. Behm, C.A. and N.C. Sangster, 1999. Pathology, pathophysiology and clinical aspects: Fasciolosis, In JP Dalton, (Ed.), CABI Publishing, pp: 185-224.
11. Rehim, W.M., I.A. Sharaf, M. Hishmat, M.A. El-Toukhy, N.A. Rawash and H.N. Fouad, 2003. Antioxidant capacity in Fasciola hepatica patients before and after treatment with triclabendazole alone or in combination with ascorbic acid (vitamin C) and tocofersolan (vitamin E). Arzeim-Forsch/Drug Res., 53: 214-220.
12. Tipping, E. and B. Ketterer, 1978. The role of intracellular proteins in the transport and metabolism of lipophilic compounds: In G Blauer, H Sund, Transport by proteins, Walter de Gruyter, Berlin, pp: 369.
13. Galtier, P., G. Larrieu, A.E. Tufenkji and M. Franc, 1986. Incidence of experimental fascioliasis on the activity of drug metabolizing enzymes in lamb liver. Drug Metab. Dispos., 14: 137-141.
14. Galtier, P., Y. Vandenberghe, S. Coecke, C. Eeckhoutte, G. Larrieu and A. Vercruysse, 1991. Differential inhibition of rat hepatic glutathione S-transferase isoenzymes in the course of fascioliasis. Mol. Biochem. Parasitol., 44: 255-260.
15. Sibille, P., O. Tliba and C. Boulard, 2004. Early and transient cytotoxic response of peritoneal cells from Fasciola hepatica-infected rats. Vet. Res., 35: 573-584.
16. Kolodziejczyk, L., E. Siemieniuk and E. Skrzydlewska, 2005. Antioxidant potential of rat liver in experimental infection with Fasciola hepatica. Parasitol. Res., 96: 367-372.
17. Halliwell, B. and S. Chirico, 1993. Lipid peroxidation: its mechanism, measurement and significance. Am. J. Clin. Nutr., 57: 715S-725S.
18. Esteban, J.G., C. Gonzalez, F. Curtale, C. Mun˜oz-Antoli, M.A. Valero, M.D. Bargues, M. el-Sayed, A.A. el-Wakeel, Y. Abdel-Wahab, A. Montresor, D. Engels, L. Savioli and S. Mas-Coma, 2003. Hyperendemic fascioliasis associated with schistosomiasis in villages in the Nile Delta of Egypt. Am. J. Trop. Med. Hyg., 69: 429-437.
19. Lotfy, W.M., H.N. El-Morshedy, M. Abou El-Hoda, M.M. El-Tawila, E.A. Omar and H.F. Farag, 2002. Identification of the Egyptian species of Fasciola. Vet. Parasitol., 103: 323-332.
20. El Ridi, R., M. Salah, A. Wagih, H. William, H. Tallima, M.H. El Shafie, T. Abdel Khalek, A. El Amir, F.F. Abo Ammou and H. Motawi, 2007. *Fasciola gigantica* excretory-secretory products for immunodiagnosis and prevention of sheep fasciolosis. Vet. Parasitol., 149: 219-228.
21. Smeal, M.G. and C.A. Hall, 1983. The activity of triclabendazole against immature and adult *Fasciola hepatica* infections in sheep. Aus. Vet. J., 60:329-331.
22. Wolff, K., J. Eckert, G. Schneiter and H. Lutz, 1983. Efficacy of triclabendazole against *Fasciola hepatica* in sheep and goats. Vet. Parasitol., 13: 145-150.
23. Netter, K.J., 1960. A method for the direct measurement of O-demethylation in liver microsomes and its use in studying microsome inhibition by SKF-525-A. Naunyn Schmiedebergs Arch. Exp. Pathol. Pharmakol., 238: 292-300.
24. Omura, T. and R. Sato, 1964. The carbon monoxide-binding pigment of liver microsomes. I-Evidence for its haemoprotein nature. J. Biol. Chem., 239: 2370-2378.
25. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 193: 265-275.
26. Nash, T., 1953. The colourimetric estimation of formaldehyde by means of Hauntusch reaction. Biochem. J., 55: 416-421.
27. Kato, R. and J.R. 1965. Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. Exp. Ther., 150: 279-284.
28. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferase: the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
29. Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophs., 88: 70-77.
30. Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.
31. Zanetti, G., 1979. Rabbit liver glutathione reductase: Purification and properties. Arch. Biochem. Biophys., 198: 241-246.
32. Winterbourn, C.C., R.E. Hawkins, M. Brian and R.W. Carrell, 1975. The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med., 85: 337-342.

33. Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol., 105: 121-126.
34. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
35. Botros, S.S., N.M. El-Lakkany, A.A. Badawy, S.S. Mahmoud, F.A. Ebeid and A. Fenwick, 2009. Mirazid shows insignificant activity against ovine Fascioliasis. Ann. Trop. Med. Parasitol., 103: 605-616.
36. El-Sheikh, H.A., B.H. Ali, A.M. Homeida, A.A. Lutfi and H.J. Hapke, 1992. The effects of fascioliasis on the activities of some drug-metabolizing enzymes in desert sheep liver. Br. Vet. J., 148: 249-257.
37. Chauvin, A., G. Bouvet and C. Boulard, 1995. Humoral and cellular responses to *Fasciola hepatica* experimental primary and secondary infection in sheep. Int. J. Parasitol., 25: 1227-1241.
38. Movsesijan, M., B. Jovanovic, O. Aalund and P. Nansen, 1975. Immune response of sheep to Fasciola hepatica infection. Res. Vet. Sci., 18: 171-174.
39. Chauvin, A. and C. Boulard, 1996. Local immune responses to experimental Fasciola hepatica infection in sheep. Parasite, 3: 209-215.
40. Abdel-Razzak, Z., P. Loyer, A. Fautrel, J.C. Gauthier, L. Corcos, B. Turlin, P. Beaune and A. Guillouzo, 1993. Cytokines down-regulate expression of major cytochrome P450 enzymes in adult human hepatocytes in primary culture. Mol. Pharmacol., 44: 707-715.
41. Kolodziejczyk, L., E. Siemieniuk and E. Skrzydlewska, 2006. Fasciola hepatica: effects on the antioxidative properties and lipid peroxidation of rat serum Exp. Parasitol., 113: 43-48.
42. Saleh, M.A., 2008. Circulating oxidative stress status in desert sheep naturally infected with *Fasciola hepatica.* Vet. Parasitol., 154: 262-269.
43. Benzer, F. and S. Temizer Ozan, 2003. The statues of lipid peroxidation, antioxidant enzymes and nitric oxide in sheep infected with *Fasciola hepatica*. Turk. J. Vet. Animal Sci., 27: 657-661.
44. Halliwell, B. and J.M. Gutteridge, 1999. Free radicals in biology and medicine. Clarendon Press, Oxford, UK, pp: 1-543
45. Santiard, D., C. Ribiere, R. Nordmann and C. Houee-Levin, 1995. Inactivation of Cu, Zn-superoxide dismutase by free radicals derived from ethanol metabolism: a gamma radiolysis study. Free Radic. Biol. Med., 19: 121-127.
46. Siemieniuk, E., L. Kolodziejczyk and E. Skrzydlewska, 2008. Oxidative Modifications of rat liver cell components during *Fasciola hepatica* infection. Toxicol. Mech. Methods, 18: 519-524.
47. Kaya, S., R. Sutcu, E.S. Cetin, B.C. Arýdogan, N. Delibas and M. Demirci, 2007. Lipid peroxidation level and antioxidant enzyme activities in the blood of patients with acute and chronic fascioliasis. Int. J. Infect. Dis., 11: 251-255.
48. Galtier, P. and M. Alvinerie, 1996. Pharmacological basis for hepatic drug metabolism in sheep. Vet. Res., 27: 363-372.