Biochemical assessment of oxidative status versus liver enzymes in patients with chronic fascioliasis

Hanan H. Kamel · Rania M. Sarhan ·
Ghada A. Saad

Received: 13 October 2013 / Accepted: 22 January 2014 / Published online: 5 February 2014
© Indian Society for Parasitology 2014

Abstract The aim of this study was to examine the oxidative status in Egyptian patients suffering chronic fascioliasis. The relationship between serum malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities was investigated in relation to the level of liver enzymes; ALT and AST compared to healthy controls. Twenty patients versus ten controls were included in the study. Among cases the MDA, CAT, AST and ALT were higher than controls, while SOD and GPX higher values were present among controls. There was a highly significant difference between cases and controls as regard MDA, CAT, SOD, GPX, and AST, and a significant difference regarding ALT. The findings of increased serum lipid peroxidation and decreased antioxidant enzymes in erythrocytes of chronic fascioliasis patients indicated the presence of persistent inflammation and oxidative stress which confirms the underlying pathogenesis and reflected the stage of infection providing a baseline data for comparison between normal and infected patients guided by the level of liver enzymes in relation to oxidative status.

Keywords *Fasciola gigantica* ·
Reactive oxygen species · Lipid peroxidation ·
Liver enzymes

Introduction

The liver flukes, *Fasciola hepatica* and *Fasciola gigantica* are the causative agents of fascioliasis, a food-borne

zoonotic disease affecting grazing animals and humans (Boray 1997). It is a re-emerging human disease with estimates of between 2.4 and 17 million people infected worldwide (Mas-Coma et al. 1999). Fascioliasis is recognized as an important infectious condition by the World Health Organization which has added it to the preventative chemotherapy concept (WHO 2006). It is becoming an increasingly important clinical and epidemiological health problem, with prevalence of the suitable snail intermediate host and the presence of large varieties of reservoir hosts that remain as a continuous source of infection in Egypt (Abou-Basha et al. 1983; Hassan et al. 1995). *F. gigantica* was considered the endogenous species found in the Nile Delta, while *F. hepatica* was thought to be present only in imported animals (Abd-Rabo and Abou-Rawash 1998; Halawani and Gindy 1957). Human in the chronic stage, suffer vague or non-specific gastrointestinal symptoms (MacLean et al. 1999). Some of them are complicated and, as a result, are diagnosed in operating rooms, where adult parasites are found obstructing the bile ducts or causing hepatic dysfunction. The diagnostic aspects include parasitological, radiological, histopathological and serological tests (Hadden and Pascarelli 1967; Jimenez et al. 2001).

Migration of juvenile forms of *Fasciola* into the host's liver is accompanied by an inflammatory reaction followed by fibrosis and cirrhosis (Dawes 1963; Kolodziejczyk et al. 2005; WHO 1995). The high chemical reactivity leads to reactions with almost all constituents of the cell, including proteins, lipids, and DNA. This destructive process is invariably associated with chemical alterations in the cell, such as enhancement of membrane lipid peroxidation (LPO) (Maffei Facino et al. 1993). LPO is an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and are decomposed to form a series of compounds, including

H. H. Kamel · R. M. Sarhan (✉) · G. A. Saad
Department of Parasitology, Faculty of Medicine, Ain Shams
University, Cairo, Egypt
e-mail: raniasarhan@yahoo.com

malondialdehyde (MDA) which is widely used as an indicator of LPO (Singh et al. 1999).

Production of reactive oxygen species (ROS) and LPO occurs in clinical settings such as hepatic surgery, hemorrhagic shock, and parasitic infections (Bilzer and Gerbes 2000; Jaeschke 2000). Normal cellular metabolism involves the production of ROS (McCord 1993). ROS including superoxide anion, hydrogen peroxide, and hydroxyl radical act as subcellular messengers in complex processes such as mitogenic signal transduction, gene expression, and regulation of cell proliferation when they are generated excessively or when enzymatic and non-enzymatic defense systems are impaired (Michiels et al. 1994). Low levels of ROS are vital for proper cell functioning, while excessive in vivo generation of these products can adversely affect cell functioning (Fattman et al. 2003; Gregorevic et al. 2001). Attacks by ROS cause alterations in molecular structure and biological properties which may be detrimental to the cell when antioxidant mechanisms are compromised or ROS scavenging is ineffective (Fujita 2002).

In vitro proteomics support the release of several major protein superfamilies from liver flukes. These products include enzymes with important antioxidant functions (Jefferies et al. 2001). Superoxide dismutase (SOD) catalyses the dismutation of superoxide radical to hydrogen peroxide and water, Catalase (CAT) catalyses the breakdown of hydrogen peroxide to oxygen and water, and Glutathione peroxidase (GPX) facilitates the destruction of both hydrogen peroxide and organic peroxides (Kidd 1997). SOD is the first line of defense against ROS and is active in catalyzing detoxification of superoxide radical (Gonzales et al. 1984). The hydrogen peroxide generated in this reaction is restored to water in the presence of CAT and GPX. Polyunsaturated fatty acids in membrane phospholipids are the main target substrates for oxygen radical activity (Romero et al. 1998).

The aim of this study was to assess the oxidative status in Egyptian patients suffering chronic fascioliasis reflected by MDA, SOD, GPX, and CAT activities and to measure the coinciding levels of liver enzymes; ALT and AST as markers reflecting liver damage with comparison of results to healthy controls.

Patients and methods

Collection of test and control samples

Blood samples required for the present case control study were collected from cases referred to the Diagnostic and Research Unit, Parasitology Department, Faculty of Medicine, Ain Shams University. The study protocol was approved by the Scientific-Ethics Committee at Faculty of medicine, Ain Shams University. Informed consent was

obtained from the study subjects using standard guidelines. Venous blood was taken, inoculated into both non-anticoagulated and anticoagulated tubes (containing sodium EDTA). Samples were categorized into; Group I; 20 patients suffering chronic fascioliasis negative for other parasitic infections by stool examination. Group II; 10 healthy subjects (control). All patients were suffering symptoms for more than 4 months and were passing eggs. Diagnosis was confirmed by Indirect Haemagglutination Test (IHAT). The control groups were sero-negative. Patients with liver dysfunction, sero-positive for hepatitis B and C viruses, suffering other parasitic infections affecting the liver, diabetics, cardiac, suffering renal failure, and those taking antioxidant or lipid-lowering therapy within the previous six months were excluded from the study.

Determination of oxidative stress parameters

One ml of anticoagulated blood was used for hematologic analysis. Hemoglobin values were determined using an automated blood counter. The remaining anticoagulated blood was separated into plasma and erythrocytes. After centrifugation at $760\times g$ for 15 min, plasma was separated and erythrocyte lysate was prepared. After triple washing of erythrocyte mass with physiological solution, 0.5 ml of cell suspension was dissolved in 2 ml cold water for lysis of erythrocytes. Hemoglobin was then precipitated by adding 1.8 ml water and 0.2 ml ethanol/chloroform (3:5 v:v) to 0.2 ml lysate. The tubes were shaken for 5 min and centrifuged at $760\times g$ for 20 min. The supernatant was used for the determination of enzyme activities. Samples were stored at $-20\text{ }^{\circ}\text{C}$ for less than 3 months pending measurement of enzymatic activity (Assady et al. 2011).

SOD activity

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined. The test is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged at $4,000\times g$. One unit of SOD was defined as the enzyme amount causing 50 % inhibition in the NBT reduction rate. Activity was expressed as units per gram of hemoglobin (Kaya et al. 2007).

GPX activity

GPX (EC 1.6.4.2) activity was measured. The enzyme reaction in the tube, which contains NADPH, reduced glutathione, sodium azide, and glutathione reductase, was initiated by addition of hydrogen peroxide, and the change

in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given as units per gram of hemoglobin in erythrocyte samples (Patterson and Leacke 1998).

CAT activity

CAT (EC 1.11.1.6) activity was determined. The test is based on the determination of the rate constant (k) or the H_2O_2 decomposition rate at 240 nm. Results were expressed as k (s^{-1}) per gram hemoglobin. Assays were performed at room temperature (25 °C) (Aebi 1984).

Determination of lipid peroxidation

Serum was obtained from 2 ml of blood without anticoagulant centrifuged at $760\times g$ at 4 °C for 10 min. Serum samples were used for immediate assay of MDA. MDA level in serum samples was measured using the thiobarbituric acid reaction method. Quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. Values of MDA were expressed as U mol/l (Draper and Hadley 1990).

Determination of liver enzymes

Activities of ALT and AST in serum were determined with the Konelab 30 biochemical analyzer and test kits from Biomerieux.

Data management and analysis

Statistical analysis Continuous variables are expressed as mean and standard deviation. Student t test was used to assess the statistical significance of the difference between two study group mean. Pearson correlation was used to assess the correlation between continuous variables. ROC curve (receiver operating characteristic) was used to

evaluate the sensitivity and specificity of markers. All statistical procedures were carried out using SPSS version 15 for Windows (SPSS Inc, Chicago, IL, USA).

Results

There was a highly significant difference between cases and controls as regard (MDA, CAT, SOD, GPX, and AST), and a significant difference between both study groups regarding ALT. Among cases the MDA, CAT, AST and ALT were higher than controls, while for SOD and GPX higher values were present among controls (Table 1).

The coefficient, r represents the strength and direction of association and correlation between each 2 enzyme values and the P value shows whether the r is significant or not. When the base line is ascending it's a positive correlation while the correlation declines as it goes horizontal or nearly horizontal till it descends and hence will show a negative correlation. Among cases, there was a significant direct correlation between MDA and each of CAT and AST, while a highly significant negative correlation was found between MDA and each of SOD and GPX. SOD was indirectly correlated with CAT and directly correlated with GPX. ALT showed highly significant direct correlation with AST (Fig. 1).

ROC curve showed that MDA, CAT, AST, SOD and GPX level could be used for diagnosis of *Fasciola*. MDA level could be used in diagnosis at a level ≥ 0.495 , with 100 % sensitivity and 100 % specificity, CAT ≥ 24.75 could be used with 85 % sensitivity and 100 % specificity. SOD $\leq 1,259.25$ could be used with 100 % sensitivity and 90 % specificity, GPX ≤ 41.5 could be used with 100 % sensitivity and 100 % specificity (Table 2; Fig. 2).

Discussion

ROS including superoxide radical, hydrogen peroxide and hydroxyl radical have a great impact on the normal

Table 1 Reference values of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and liver enzymes (AST, ALT) in patients with chronic fascioliasis and control

Parameter	Group I Cases (20) Mean $\pm$ SD	Group II Control (10) Mean $\pm$ SD	t	P value
MDA (μ mol/l)	1.09 $\pm$.23	0.21 $\pm$.02	17.290	0.0001**
CAT (K/gHb)	28.45 $\pm$ 3.19	20.77 $\pm$ 1.99	6.930	0.0001**
SOD (U/gHb)	976.87 $\pm$ 187.66	1,345.53 $\pm$ 69.21	−7.790	0.0001**
GPX (U/gHb)	12.96 $\pm$ 1.11	76.35 $\pm$ 5.85	33.940	0.0001**
AST (U/l)	52.85 $\pm$ 20.41	26.90 $\pm$ 8.80	4.855	0.0001**
ALT (U/l)	40.15 $\pm$ 18.19	28.80 $\pm$ 7.41	2.418	0.023*

Student t test; * $P < 0.05$ significant (S); ** $P < 0.001$ highly significant (HS)

Fig. 1 Correlations between levels of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and liver enzymes (AST, ALT) in patients with chronic

fascioliasis. Pearson correlation (r value); Student *t* test; ***P* < 0.001 highly significant (HS); **P* < 0.05 significant (S), *P* > 0.05 non significant (NS)

Table 2 Calculated parameters derived from ROC curve analysis to discriminate between cases and control using tested markers

Test markers	AUC	CI		Sensitivity (%)	Specificity (%)	<i>P</i>
MDA (+ve if ≥ 0.495)	1.000	1.000	1.0	100	100	0.0001**
CAT (+ve if ≥ 24.75)	0.963	0.903	1.0	85	100	0.0001**
AST (+ve if ≥ 36)	0.890	0.776	1.0	85	80	0.001**
ALT (+ve if ≥ 29)	0.700	0.495	0.90	75	60	0.078
SOD (+ve if $\leq 1,259.25$)	0.990	0.964	1.0	100	90	0.0001**
GPX (+ve if ≤ 41.5)	1.000	1.00	1.0	100	100	0.0001**

AUC area under curve, SEM standard error of mean, CI confidence interval, *P* probability value, HS highly significant

** *P* < 0.001 highly significant (HS); * *P* < 0.05 significant (S); *P* > 0.05 non significant (NS)

function of biomolecules like nucleic acids, proteins and cell membrane phospholipids. Free radicals are generated during stepwise reduction of molecular oxygen which is an integral feature of normal cellular function (Tang et al. 1994). In contrast, excessive generation and inadequate removal of free radicals results in destructive irreversible damage to the cell (Lopaczyski and Zeisel 2001) with an imbalance in the oxidant/antioxidant system (Khadija et al. 2009). ROS have been indicated in the pathogenesis of various parasitic infections including *Leishmania* (Biswas et al. 1997; Kocyigit et al. 2003; Oliveira and Cechini 2002), *Plasmodium falciparum* (Kumar and Das 1999), *Ascaris lumbricoides* (Kilic et al. 2003), *Toxoplasma gondii* (Yazar et al. 2003), *Trypanosoma cruzi* (Finzi et al. 2004).

GPX represents a major pathway in the cell for metabolizing hydrogen and lipid peroxides. Therefore, its suppression results in growing accumulation of peroxides up to toxic levels (Halliwell and Gutteridge 1999). Our results showing lower activity of GPX in comparison to control group confirms the oxidative stress persisting in the chronic phase of fascioliasis.

SODs have been demonstrated from various helminths of different species such as *Schistosoma mansoni*, *Onchocerca volvulus*, *Dirofilaria immitis*, *Brugia pahangi*, and *F. hepatica* (Callahan et al. 1991; Hong et al. 1992; James et al. 1994; Kim et al. 2000; Thorpe 1965). Infection with *F. hepatica* was accompanied by rising level of the superoxide radicals which opens a way to act as precursor of other ROS (Abo-Shousha et al. 1999; Oberley and Oberley 1997). In our study erythrocyte antioxidant enzyme activities of SOD was lower in chronic patients suffering *F. gigantica* compared to control, this goes with the previous study done by Kolodziejczyk et al. (2005) who confirmed that when the activity of SOD is decreased it leads to elevated content of superoxide anions and indicated a decrease in activities of the main antioxidant enzymes; SOD, GPX and reduction in content of non-enzymatic antioxidants (Kolodziejczyk et al. 2005).

The only exception in this study was the increased activity of CAT. It was earlier shown that oxidants generated in the liver may activate enzyme gene expression through antioxidant responsive elements (Kolodziejczyk et al. 2005); this may explain the increase in CAT activity

Fig. 2 ROC curve for the comparative analysis between MDA, CAT, SOD, GPX, AST and ALT

observed. There was a highly significant difference between cases and controls as regard CAT, SOD, GPX; SOD was indirectly correlated with CAT and directly correlated with GPX. Studies have reported that CAT is a major intracellular antioxidant enzyme (Aslan et al. 2007; Simsek et al. 2006) although, Karsen et al. (2011) reported that CAT activity was found to be unimportant in fascioliasis since no statistically significant difference was available between patients and control groups (Ece et al. 2006).

LPO is an ongoing physiological process, but several lines of evidence have suggested an important role for peroxidation in the pathogenesis of several parasitic diseases (Erel et al. 1997; Karsen et al. 2011; Oliveira and Cechini 2002). LPO caused by ROS results in the disarrangement and ultimately, disruption of cell membranes, which leads to necrotic death (Bagchi et al. 1993). MDA being one of the final products of LPO in human cells increases with increased ROS (Rushmore et al. 1991). In our study Serum MDA levels in patients were higher than in controls with a highly significant difference between both study groups. This increase may be considered an indicator of cell injury caused by *F. gigantica*. This was previously explained by El-Badry (2006) who strongly suggested that one of the main reasons for high MDA levels in patients infected with *F. hepatica* could be decreased activity of the defense system protecting tissues from free radical damage (El-Badry 2006). In our study, there was a significant direct correlation between MDA and CAT among cases, while a highly significant negative correlation was found between MDA and each of SOD and GPX.

In an experimental *F. hepatica* infection, Kaya et al. (2007) have investigated the relationship between fascioliasis and MDA, SOD, CAT, and GPX activities. They

reported that the oxidative stress can be one of the underlying factors in the pathogenesis of this chronic disease (Kaya et al. 2007).

Changes in the antioxidant abilities of the liver and in the phospholipid structure of the cell membrane were accompanied by rising activities of ALT and AST as markers of liver damage (Kolodziejczyk et al. 2005). In the present study, there was a highly significant difference between cases and controls as regard AST and a significant difference as regard ALT.

The results of the present study provided a baseline data for comparisons between patients with chronic *F. gigantica* infection, who constitute a respected percentage, versus healthy individuals as regard the fluctuations in the level of antioxidant enzymes in relation to liver enzymes therefore; it can provide a better understanding for biochemical markers of oxidative stress which may add to diagnosis.

References

- Abd-Rabo TMA, Abou-Rawash AA (1998) Some observations on *Fasciola gigantica* in donkeys at Kafr El-Sheikh. In: Proceedings of the fourth veterinarian medical zag congress Hurghada, pp 394–403
- Abo-Shousha S, Khalil SS, Rashwan EA (1999) Oxygen free radical and nitric oxide production in single or combined human schistosomiasis and fascioliasis. *J Egypt Soc Parasitol* 29:149–156
- Abou-Basha LM, El-Magdoub AA, Ebeid SA, Touklhy MA, Micheal AI, Hosny KMF, El-Zoghby SM, Farag HF, El-Sawy MF (1983) Effect of bithionol on B-glucuronidase in serum and liver of rabbits infected with *F. gigantica*. *J Egypt Soc Parasitol* 13:231
- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126
- Aslan M, Kosecik M, Horoz M et al (2007) Assessment of paraoxonase and arylesterase activities in patients with iron deficiency anemia. *Atherosclerosis* 191:397–402
- Assady YM, Farahnak A, Golestani A, Esharghian MR (2011) Superoxide dismutase (SOD) enzyme activity assay in *Fasciola* spp. Parasites and liver tissue extract. *Iran J Parasitol* 6:17–22
- Bagchi M, Mukherjee S, Basu MK (1993) Lipid peroxidation in hepatic microsomal membranes isolated from mice in health and in experimental leishmaniasis. *Indian J Biochem Biophysiol* 30:277–281
- Bilzer M, Gerbes AL (2000) Preservation injury of the liver: mechanisms and novel therapeutic strategies. *J Hepatol* 32:508–515
- Biswas T, Ghosh DK, Mukherjee N, Ghosal J (1997) Lipid peroxidation of erythrocytes in visceral leishmaniasis. *J Parasitol* 83:151–152
- Boray JC (1997) Chemotherapy of infections with fasciolidae. In: Boray JC (ed) Immunology, pathobiology and control of Fasciolosis. MSDAGVET Rahway, New Jersey, pp 83–97
- Callahan HI, Crouch RK, James ER (1991) *Dirofilaria immitis* superoxide dismutase: purification and characterization. *Mol Biochem Parasitol* 49:245–252
- Dawes B (1963) Hyperplasia of the bile duct fascioliasis and its relation to the problem nutrition in the liver fluke. *Parasitology* 53:123–133
- Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 186:421–431

- Ece A, Gürkan F, Kervancioglu M et al (2006) Oxidative stress, inflammation and early cardiovascular damage in children with chronic renal failure. *Pediatr Nephrol* 21:545–552
- El-Badry AA (2006) Serum malondialdehyde levels as a biomarker of cellular injury in human fascioliasis. *J Med Sci* 1(1):57–64
- Erel O, Kocoyigit A, Aktepe N, Avci S (1997) Leukocyte adenosine deaminase, superoxide dismutase activities and lipid peroxidation in cutaneous leishmaniasis. *Acta Parasitol Turc* 21:160–162
- Fattman CL, Schaefer LM, Oury TD (2003) Extracellular superoxide dismutase in biology and medicine. *Free Radic Biol Med* 35:236–256
- Finzi JK, Chiavegatto WMC, Lopez JA, Cabrera OG, Mielniczki-Pereira AA, Colli W, Alves MJM, Gadelha FR (2004) *Trypanosoma cruzi* response to the oxidative stress generated by hydrogen peroxide. *Mol Biochem Parasitol* 133(1):37–43
- Fujita T (2002) Formation and removal of reactive oxygen species, lipid peroxides and free radicals, and their biological effects. *Yakugaku Zasshi* 122:203–218
- Gonzales R, Auclair C, Voisin E, Gautero H, Dhermy D, Boivin P (1984) Superoxide dismutase, catalase and glutathione peroxidase in red blood cells from patients with malignant diseases. *Cancer Res* 44:4137–4139
- Gregorevic P, Lynch GS, Williams DA (2001) Hyperbaric oxygen modulates antioxidant enzyme activity in rat skeletal muscles. *Eur J Appl Physiol* 86:24–27
- Hadden J, Pascarelli E (1967) Diagnosis and treatment of human fascioliasis. *J Am Med Assoc* 202:167–169
- Halawani AA, Gindy MA (1957) Short account on the economic importance of *Fasciola gigantica*, its effect on livestock and snail vector. *J Egypt Med Assoc* 40:547
- Halliwell B, Gutteridge JM (1999) Free radicals in biology and medicine. Clarendon Press, Oxford, pp 1–543
- Hassan MM, Moustafa NE, Mahmoud LA, Abbaza BE, Hegab MHA (1995) Prevalence of *Fasciola* infection among school children in Sharkia Governorate. *J Egypt Soc Parasitol* 25:543
- Hong ZD, Kosman J, Thakur A, Rekosh D, Loverde PT (1992) Identification and purification of a second form of Cu/Zn superoxide dismutase from *Schistosoma mansoni*. *Infect Immun* 60:3641–3651
- Jaeschke H (2000) Reactive oxygen and mechanisms of inflammatory liver injury. *J Gastroenterol Hepatol* 15:718–724
- James ER, McLean DC, Perler F (1994) Molecular cloning of an *Onchocerca volvulus* extracellular Cu–Zn superoxide dismutase. *Infect Immun* 62:713–716
- Jefferies JR, Campbell AM, van Rossum AJ, Barrett J, Brophy PM (2001) Proteomic analysis of *Fasciola hepatica* excretory-secretory products. *Proteomics* 9:1128–1132
- Jimenez J, Loja D, Ruiz E, Maco V, Marcos LA, Aviles R (2001) *Fasciolasis hepatica*: unproblema diagn'ostico. *Rev Gastroenterol Perú* 21:148–152
- Karsen H, Sunnetcioglu M, Ceylan RM, Bayraktar M, Taskin A, Aksoy N, Erten R (2011) Evaluation of oxidative status in patients with *Fasciola hepatica* infection. *Afr Health Sci* S1:S14–S19
- Kaya S, Sütçü R, Cetin ES, Aridogan BC, Deliba N, Demirci M (2007) Lipid peroxidation level and antioxidant enzyme activities in the blood of patients with acute and chronic fascioliasis. *Int J Infect Dis* 11(3):251–255
- Khadija AA, Mohammed S, Saad AM, Mohamed HE (2009) Response of broiler chicks to dietary monosodium glutamate. *Pak Vet J* 29(4):165–168
- Kidd PM (1997) Glutathione: systemic protectant against oxidative and free radical damage. *Altern Med Rev* 2:155–176
- Kilic E, Yazar S, Saraymen R, Ozbilge H (2003) Serum malondialdehyde level in patients infected with *Ascaris lumbricoides*. *World J Gastroenterol* 9(10):2332–2334
- Kim TS, Jung Y, Na BK, Kim KS, Chung PR (2000) Molecular cloning and expression of Cu/Zn-containing superoxide dismutase from *Fasciola hepatica*. *Infect Immun* 68(7):3941–3948
- Kocoyigit A, Gurel M, Ulukanligil M (2003) Erythrocyte antioxidative enzyme activities and lipid peroxidation levels in patients with cutaneous leishmaniasis. *Parasite* 10:277–281
- Kolodziejczyk L, Siemieniuk E, Skrzydlewska E (2005) Antioxidant potential of rat liver in experimental infection with *Fasciola hepatica*. *Parasitol Res* 96:367–372
- Kumar CA, Das UN (1999) Lipid peroxides, nitric oxide and essential fatty acids in patients with *Plasmodium falciparum* malaria. *Prostaglandins Leukot Essent Fatty Acids* 61(4):255–258
- Lopaczyski W, Zeisel SH (2001) Antioxidants, programmed cell death and cancer. *Nutr Res* 21:295–307
- MacLean L, Guerrant GL, Walker DH, Weller PF (1999) Liver, lung, and intestinal fluke infections. In: Guerrant GL, Walker DH, Weller PF (eds) *Tropical infectious diseases: principles, pathogens and practice*. Harcourt Brace & Co, Orlando, FL, pp 1039–1057
- Maffei Facino R, Carini M, Aldini G, Ceserani R, Ceserani I, Cavaletti E, Vederio L (1993) Efficacy of glutathione for treatment of fascioliasis. An investigation in the experimentally infested rat. *Arzneim-Forsch/Drug Res* 43:455–460
- Mas-Coma MS, Esteban JG, Bargues MD (1999) Epidemiology of human fascioliasis: a review and proposed new classification. *Bull World Health Organ* 77:340–346
- McCord JM (1993) Human disease, free radicals, and the oxidant/antioxidant balance. *Clin Biochem* 26:351–357
- Michiels C, Raes M, Toussaint O, Ramaele J (1994) Importance of glutathione peroxidase, catalase and Cu/Zn SOD for cell survival against oxidative stress. *Free Radic Biol Med* 17:235–248
- Oberley TD, Oberley LW (1997) Antioxidant enzyme levels in cancer. *Histol Histopathol* 12:525–535
- Oliveira FJA, Cechini R (2002) Oxidative stress of liver in hamsters infected with *Leishmania chagasi*. *J Parasitol* 86:1067–107228
- Patterson RA, Leacke DS (1998) Human serum, cysteine and histidine inhibit the oxidation of low density lipoprotein less at acidic pH. *Fed Eur Biochem Soc Lett* 434:317–321
- Romero FJ, Bosch-Morell F, Romero MJ (1998) Lipid peroxidation products and antioxidants in human disease. *Environ Health Perspect* 106:1229–1234
- Rushmore TH, Morton MR, Pickett CB (1991) The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J Biol Chem* 266:11632–11639
- Simsek S, Yuce A, Utuk AE (2006) Determination of serum malondialdehyde levels in sheep naturally infected with *Dicrocoelium dendriticum*. *Firat Univ Saglik Bil Dergisi* 20:217–220
- Singh SK, Dua TD, Tondon A, Kumari S, Ray G, Batra S (1999) Status of lipid peroxidation and antioxidant enzymes in hypoxic ischemic encephalopathy. *Indian Pediatr* 26:659–668
- Tang LX, Ou X, Henkle-Duhrsen K, Selkirk ME (1994) Extracellular and cytoplasmic Cu/Zn superoxide dismutases from *Brugia* lymphatic filarial nematode parasites. *Infect Immun* 62:961–967
- Thorpe EL (1965) The pathology of experimental fascioliasis in the albino rat. *J Comp Pathol* 75:39–44
- WHO (1995) Control of food-borne trematode infections. *World Health Organ Tech Rep Ser* 849:1–157
- WHO (2006) Report of the World Health Organization Informal Meeting on use of triclabendazole in fascioliasis control. WHO Headquarters, Geneva
- Yazar S, Kilic E, Saraymen R, Lahin G (2003) Serum malondialdehyde levels in *Toxoplasma* seropositive patients. *Ann Saudi Med* 3:413–415