Journal of the Medical Research Institute
Vol. 23 No. 3 (2002)

THE ANTIOXIDANT DEFENCE STATUS IN FASCIOLA HEPATICA AND SCHISTOSOMA MANSONI INFECTION

El-Dardiry SA*, Abulnaja KO **, Boghdadi IM *, Salman TA****, Nosseir M* and Kassem S*.**

** Biochemistry, Faculty of Medicine, Tanta University, ** Biochemistry, Faculty of Science, King Abdel-Aziz University, *** Internal Medicine, Faculty of Medicine, Menoufia University, ****Tropical Medicine, National Liver Institute, Menoufia University.*

Summary

Medical and surgical problems especially in tropical and subtropical areas arise frequently from helminthic invasion affecting the liver and/or biliary tract. The antioxidant enzymes have a potential role in evasion of the oxidative burst killing mechanisms by the immune cells. The aim of the study was to evaluate the influence on hepatobiliary system posed by Fasciola hepatica with or without S. mansoni co-infection respective to the antioxidant status, total bile acids, liver function tests, ultrasonography and endoscopic examination. Thirty subjects infected with Fasciola hepatica (mean age 38.93±4.6) were categorized into those with S.mansoni infection (GI) or without (GII) compared to thirty age matched subjects free of Fasciola hepatica but with S. mansoni infection (GIII) or its absence represented as controls (G IV). Ultrasonography, computed tomography (CT), magnetic resonance insonnation (MRI), and endoscopic retrograde cholangeo-pancreatography (ERCP) are used for diagnosis of early and late stages of F. hepatica and antifasciola antibody titer. Biochemical assessments of the antioxidant defense status by measuring serum level of enzymatic activities of superoxide dismutase, catalase, and total glutathione peroxidase, and vitamins A, C, and E were done as well as total serum bile acids and liver function tests. Results exhibited reduced levels of superoxide dismutase (SOD), catalase and glutathione peroxidase oxidase as well as the antioxidant vitamins A, C, E in GI>GII>GIII>GIV and they were statistically significant P< 0.01. The serum total bile acids was statistically significant increased (P< 0.01), and data of liver function tests were within normal range in the diagnosis of F. hepatica of the liver and biliary tract. In conclusion the serum antioxidant enzymes superoxide dismutase, catalase, and glutathione oxidase as well as the antioxidant vitamins A, C and E were statistically significantly decreased in patients with Fasciola hepatica, S. mansoni or both than in healthy control versus increased levels of total bile acids. This potentates fibrogenic mechanisms and necessitates the supplementation of antioxidants with traditional therapy.

Introduction

On a global outlook, medical and surgical problems specially in tropical and subtropical areas arise from helminthic invasion affecting the liver and/or the biliary tract either during passage of worms through these structures or because of the natural habitats of these organs. Prominent examples are Schistosoma mansoni and Fasciola hepatica (1,2). Although the Schistosomes invade the liver parenchyma, yet they are not associated with major biliary outcome (3). In contrast, once the metacercaria of F. hepatica are ingested with contaminated leafy vegetables, they can exist in duodenum and thereafter migrate through duodenal wall into the peritoneal cavity through which they penetrate the liver. Then, via the Glisson capsule the flukes traverse the parenchyma and lodge in bile ducts (4).

Cholestasis is common sequelae of hepatic fascioliasis owing to alterations in: hepatic bile acid synthesis, excretion and intestinal absorption reflecting derangement in plasma bile acid levels (5). Bile acids have been always considered as a sensitive monitor of cholestasis (6). In hepatic fascioliasis, the flukes and their metabolites irritate the biliary passages resulting in inflammation and hyperplasia, then fibrosis and even obstruction may occur (7,8).

Reports indicated that the redox cascade involved in the maintenance of cell homeostasis, as well as in the parasite protection against reactive oxygen species produced by the host is known to influence the pro-oxidant/antioxidant balance in both host and parasite (9). Also, it has been indicated that the antioxidant enzyme superoxide dismutase (SOD), isolated from soluble somatic and excretion–secretion (E-S) preparations from adult F. hepatica, showed some similarity with S. mansoni cytoplasmic SOD. These enzymes may have a potential role in the evasion of the oxidative burst killing mechanisms of immune cells (10). The host reaction involving reactive oxygen intermediates influences the ultimate pathophysiological effects of oxidative processes (11).

Previous studies showed that schistosomules of S. mansoni elaborate an anti-inflammatory, immunomodulatory factor, which may help the parasite to evade host immune response (12). Like many pathogens that undergo an intravascular stage of development, larvae of the parasite S. mansoni migrate through the blood vessels, where they are in close contact with endothelial cells (13,14). Chronic Schistosomiasis is associated with impaired cell-mediated immune responsiveness (15). The H2O2 / peroxidase system, which is the cornerstone of the antimicrobial defense associated with inflammation, is activated in close contact with parasite eggs. Moreover, hepatocytes undergo oxidative stress in the entire organ, which induces decrease of liver antioxidant defenses (16). Therefore the present study aims to determine the relationship between disorders of hepatobiliary system and antioxidant defense strategies respective to total serum bile acids and liver function tests in cases of F. hepatica with or without S. mansoni co-infection.

Subjects and Methods:

The present study involved sixty selected subjects with age range= 22- 58 (38.93±4.6) years classified into four groups:

Group (I): 15 patients (9 males and 6 females) with mixed infection with Fasciola hepatica and Schistosoma mansoni, mean age = 41.2±6.7 years.

Group (II): 15 patients (10 males and 5 females) with Fasciola hepatica, mean age = 39.7±8.1 years.

Group (III): 15 patients (8 males and 7 females) with Schistosoma mansoni infection, mean age = 43±5.9 years.

Group (IV): 15 apparently healthy subjects (9 males and 6 females) serving as control, mean age = 42.1±7.5 years.

All subjects were subjected to full clinical assessment to exclude other parasitic diseases. Neither patient nor control had evidence of neither any chronic diseases nor any being treated with drugs known to affect hepatic metabolism at the time of the study. Cigarette smoking subjects were excluded. None of the patients had evidence of liver cell failure or signs of portal hypertension. Diagnosis of F. hepatica was achieved by absolute eosinophilia (17) and high antifasciola antibody titer by using indirect hemagglutination test using (Fumouze kits). Ultrasonography and computed tomography (CT) were done to diagnose early Fascioliasis (18) and endoscopic retrograde cholangeo-pancreatography (ERCP) for late stages of F. hepatica by defining changes in the bile ducts as well as demonstrating the parasites directly, their location and movements (19). Schistosoma mansoni infection detected by positive stool analysis using the direct smear technique, heamagglutination test, sigmoidoscopy and rectal snips for negative stool, urine for Schistosoma eggs, abdominal ultrasonography; hyperechoic periportal areas, indicating periportal fibrosis (20). The laboratory investigations for liver function tests, which include determination of total serum bilirubin by colorimetric technique of Bartles and Bohmer (21), the determination of the activity of alkaline phosphatase (ALP) by the method of Kind and King (22), alanine transaminase (ALT) and aspartate transaminase (AST) according to the method of Reitman and Frankel (23) and Gamma glutamyl transpeptidase (GGT) using the method of Naftalin et al (24). Total bile acids were determined (25). The antioxidant defense status was investigated by measuring serum levels of superoxide dismutase (SOD) activity according to the method of Misra and Fridovich (26), catalase activity by the spectrophotometric technique of Beers and Sizer (27) and total glutathione peroxidase (GSHPx) activity using reduced substrates as described by Hafeman et al. (28). Evaluation of vitamin A was performed by high performance liquid chromatography method (29). Determination of vitamin C level was detected by liquid chromatography method (30). Assessment of vitamin E was done by spectrophotometry (31).

Results

Symptoms encountered in early fascioliasis (11cases) were: right hypochondrial and epigastric pain and dyspepsia, while in late fascioliasis (19

cases) there was obstructive jaundice accompanied by itching. Hepatosplenomegaly was found clinically in 60% of schistosomal cases. Ultrasonographic examination revealed: hepatomegaly in 83.3% of schistosomal cases, splenomegaly in 60% of schistosomal cases, periportal thickening with its different grades in 86.7% of schistosomal cases, dilated common bile duct (C.B.D.) and intrahepatic biliary radicals in 63.3% of Fasciola cases. Another sonographic finding in fascioliasis was the presence of a moving echogenic body (without a posterior shadow), either in gall bladder or in C.B.D . CT diagnostic criteria of early fascioliasis were: small pseudotumour or Olympic game sign (small subcapsular hypodense lesions), while in late cases there were masses in gall bladder and dilated intrahepatic radicals. E.R.C.P. was done in cases with obstructive jaundice revealing C.B.D. stricture, crack-earth appearance and/or leaflet like flukes in gall bladder or C.B.D.

Table (1) shows the liver function tests and S. bile acid in the studied groups. The serum bilirubin, alkaline phosphatase, liver

Table (I): Liver Function Tests and Total Bile Acids

PARAMETER		Cases with F. hepatica		Cases without F. hepatica	
		+ve S.mansoni (GI)	-ve S.mansoni (GII)	+ve S.mansoni (GIII)	-ve S.mansoni (GIII)
Bil.	X±SD	$0.92^{a} \pm 0.21$	$0.78^{a} \pm 0.19$	$0.42^{b} \pm 0.13$	$0.3^{b} \pm 0.12$
(mg/dl)	F, p		8.69, <0.01*		
AP	X±SD	$18.4^{a} \pm 4.9$	$16.2^{a} \pm 3.97$	$6.1^{b} \pm 1.2$	$4.9^{b} \pm 1.87$
(U/dl)	F, p		61.95, <0.01*		
AST	X±SD	$44.2^{a} \pm 14.8$	$37.9^{a} \pm 11.6$	$29.8^{b} \pm 6.1$	$20.8^{b} \pm 5.2$
(U/ml)	F, p		6.98, <0.05*		
ALT	X±SD	$42.9^{a} \pm 12.1$	$37.2^{a} \pm 9.4$	$25.7^{b} \pm 8.4$	$18.7^{b} \pm 6.1$
(U/ml)	F, p		8.98, <0.01*		
GGT	X±SD	$19.8^{a} \pm 5.4$	$13.7^{a} \pm 4.8$	$11.1^{b} \pm 3.4$	$8.9^{b} \pm 2.8$
(U/ml)	F, p		6.55, <0.05*		
T.B.A.	X±SD	$8.67^{a} \pm 2.98$	$6.89^{a} \pm 1.94$	$4.27^{b} \pm 0.53$	$3.19^{b} \pm 0.52$
(mg/dl)	F, p		2.95, <0.05*		

Bil.=Bilirubin, AP=Alkaline phosphatase, AST=Aspartate transaminase, ALT=Alanine transaminase, GGT= Gamma glutamyl transpeptidase, T.B.A.=total bile acids, X±SD=mean±standard deviation, *=significant
Means indexed by the same superscript are not significantly different.

enzymes (AST and ALT) were significantly higher in patients affected by F. hepatica with or without S. mansoni affection (GI and G II) than those with S. mansoni alone or in healthy control (GIII and G IV), p<0.01, <0.01, <0.05 and <0.01 respectively. GGT was significantly higher in GI compared to other groups, p<0.05. Total bile acids was significantly increased in GI and II compared to GIII and IV, p<0.05.

The eosinophilic count was significantly higher in those affected by F. hepatica either with or without S. mansoni affection (GI and GII) than those without F. hepatica affection (GIII and G IV) respectively, p<0.001. The anti-Fasciola antibody titer was detected only in those affected by F. hepatica (GI & GII) (table 2).

Table (3) shows the antioxidant enzyme activity in cases under study. The superoxide dismutase decreases significantly in those with F. hepatica and S. mansoni affection than in normal control and the decrease is highly significant with mixed affection p<0.01. Also catalase activity was significantly decreased in those with F. hepatica and those affected with S.

Table (II): Eosinophilic count and anti-Fasciola antibody titer

	Eosinophils count/cmm	Anti-Fasciola Ab Titer	
Cases under study	X±SD	X±SD	P
GI	318±912	2119±648	<0.0001**
GII	261.9±804	1694±472	<0.0001**
GIII	148±47	0	-
GIV	121±31	0	-

(X±SD=mean±standard deviation, **=highly significant)

Table (III): Antioxidant enzyme activities

PARAMETER		Cases with F.hepatica		Cases without F.hepatica	
		+ve S.mansoni (GI)	-ve S.mansoni (GII)	+ve S.mansoni (GIII)	-ve S.mansoni (GIII)
SOD	X±SD	$30.4^{a} \pm 10.1$	$42.6a^{b} \pm 11.7$	$54.3^{b} \pm 12.7$	$72.8^{c} \pm 14.9$
(ug/dl)	F, p	8.99, <0.01*			
Catalase	X±SD	$224.1^{a} \pm 38$	$249.8^{a} \pm 31$	$278.3^{a} \pm 29$	$346.5^{b} \pm 52.4$
(ug/Hb)	F, p	9.23, <0.01*			
GSHPx	X±SD	$10.1^{a} \pm 3.9$	$12.4^{a} \pm 4.6$	$13.7^{a} \pm 4.2$	$19.04^{b} \pm 6.9$
(ug/Hb)	F, p	6.53, <0.05*			

(SOD=Superoxide dismutase, GSHPx=Glutathione peroxidase, X±SD=mean±standard deviation, *=significant)

Means indexed by the same superscript are not significantly different.

the tripeptide glutathione into its oxidized form GSSG (47,48,49).

Accordingly, the paralleled decrement of antioxidant vitamins and of enzymatic antioxidant activities confirms the impact of oxidative stress herein in GI>GII>GIII. Reports identified in vitro effects of F. hepatica on the main functions of polymorphonuclear leucocytes: chemotaxis and free radical generation induced by phagocytosis that was verified by the effects of adult fluke excretion–secretion (ES) which inhibited phagocytosis and/or free radical generation in a dose and time dependent manner (50).

Other reports have indicated that the F. hepatica doesn't express catalase however it expresses little glutathion peroxidase besides the influence of peroxiredoxin antioxidant potency which may be involved in functions such as protection against ROS generated by metabolic processes and/or protection of the parasite against ROS released by immune effector cells (9). Other reports indicated that the catalytic activities of rat cytosolic GSHPx in the course of Fascioliasis was not statistically altered (51). Nonetheless, in F. hepatica reports have identified the characterization of cytochrome C peroxidase (CcP) as an enzyme with potential antioxidant activity in vitro that blocks formation of the highly toxic hydroxyl radical through the removal of H_2O_2 in response to oxidative stress in F. hepatica (52).

On the other hand, in F. hepatica, reports indicated the important role of cytosolic SOD which was found to be similar to that noted in other eukariotic cells and characterized as Cu /Zn SOD (44). The effect of F. hepatica excretory-secretory products was found to inhibit SOD output from human neutrophils (53). Moreover, as no catalase activity was detectable, the possibility that SOD in those trematodes might have the particular importance of removing superoxide radicals (54).

Conceivably, in view of the present findings and those noted in the literature, the molecular regulation of hepatic fibrosis represents an integrated cellular response to tissue injury. Conclusively, the compartmentalized changes in hepatic antioxidant enzyme activity may be crucial determinants of cell survival. Subsequently, it appears that there would be a progressive decline in the level of hepatic reduced glutathione that would event with concomitant increase in serum glutamate pyruvate transaminase (SGOT) activity such as monitored herewith. This verifies that fibrogenic mechanisms are potentiated by the greater tissue damage and impairment of intracellular antioxidant activity, which occurs more profoundly in coinfection of F. hepatica with S. mansoni. Accordingly the therapeutic management would necessitate the administration of antioxidants besides providing a rich diet alongside the traditional therapy for F. hepatica and S. mansoni.

References

1. Makled MKH, Khalil HM and El Sibae EA. Fascioliasis and hepatic affection . J Egyp Soc Parasitol, 1988; 18: 1-19.
2. Arias IM, Boyer JL, Fautso N, Jakoby WB, Schachter D and Shafritz DA. The liver biology and pathobiology. 3rd ed. Raven Press. New York (1994).
3. Sherlock S and Dooly J. Diseases of the liver and biliary system 9th Ed. Oxford Blackwell

Scientific Publications, 1993. London, Edinburgh, Boston, Melbourne, Paris, Berlin, Vienna.

4. Kayabali I, Gokcova IH, Yerdel MA and Dremeci N. Hepatic Fascioliasis and biliary surgery. Int Surg, 1992; 77: 154-7.
5. Burtis CA and Ashwood ER. In: Tietz textbook of clinical chemistry. 2nd Ed WB Saunders Company Philadelphia, London, Toronto, Montreal, Sydeny, Tokyo. 1994.
6. Center SA, Man Warren T, Slater MR and Wilentz E. Evaluation of twelve–hour pre-prandial and two post-prandial serum bile acids concentration for diagnosis of hepatobiliary disease in dogs. J Am Vet Med Assoc, 1991; 199(2): 217-6.
7. Abaza MM, Osman MM, Ismail Y, Amin AA and Badr GA. Pattern of serum bile acids in patients with hepatic Fascioliasis. J Med Res Insti, 1996; 17(3): 157-69.
8. Osman M, Lansten SB, EL-Sefi T, Boghdadi I, Rashed MY and Jensen SL. Biliary parasites (review). Dig Surg, 1998; 15: 287-96.
9. Mc Gonigle S, Curley GP and Dalton JP. Cloning of peroxiredoxin, a novel antioxidant enzyme from the helminth parasite Fasciola hepatica. Parasitology, 1997; 115(1): 101-4.
10. Piacenza L, Radi R, Goni F and Carmona C. Cu/Zn superoxide dismutase activities from Fasciola hepatica. Parasitology, 1998; 117(6): 555-62.
11. Bagchi K and Puri S. Free radicals and antioxidants in health and disease. East Medit Health J, 1998; 4(2): 350-60.
12. Ramaswamy K, Salafsky B and Potheri S, Hey X, Li JW and Shibuya T. Secretion of an anti-inflammatory, immunomodulatory factor by Schistosomules of Schistosoma mansoni. Inflammation, 1995; 46(1): 13-22.
13. Oswald P, Eltoum I, Wynn TA, Schwartz B, Caspart, Poulin D, Sher A and James SL. Endothelial cells are activated by cytokine treatment to kill an intravascular parasite, Schistosoma mansoni, through the production of nitric oxide. Proc Natio Acad Sci USA, 1994; 19 (3): 999-1003.
14. Lejoly-Boissean H, Apprion M, Seignew M and Pruvast A, Tribouley-Duret J and Tribouley J. Schistosoma mansoni: In vitro adhesion of parasite eggs to the vascular endothelium. Subsequent inhibition by a Monoclonal antibody directed to a carbohydrate epitope. Exp Parasitol, 1999; 91(1): 20-9.
15. Zwingenberger K, Richter J, Taupitz S, Vergetti Signeira JG and Correia Dacal AR. Altered generation of Interleukin 1 in chronic human Schistosomiasis mansoni. Scand J Immunol, 1990; 31(6): 729-36.
16. Abdallah OM, Hanna S, De Reggi M and Gharib B. Visualization of oxygen radical production in mouse liver in response to infection with Schistosoma mansoni. Liver, 1999; 19(6): 459-500.
17. Dacie JV and Lewis SM. In: Practical Hematology. 4Th ed. London. Churchill LTD. 1969.
18. Hahn JK, Choi BI, Cho JM, Chung KB , Hahn MC and Kim CW. Radiological findings of human Fascioliasis. Abdom Imag, 1993; 18: 261-4.
19. Rashed MYT, EL-Sefi T and Boghdadi I. Endoscopic Retrograde Cholangiographic patterns of biliary Fascioliasis. Proceedings of European I.H.P.B.A .Congress (Athens 1995) May 25-28 Athen Greece, Manduzzei Editore, PP 259-263.
20. Abdel Wahab MF, Esmat MG, Farrag A and El-Boaraey A. Grading of hepatic Schistosomiasis by the use of ultrasonography. Am J Hyg, 1992; 49: 403-6.
21. Bartles H and Bohmer A. Simple method for bilirubin determination. Clin Chem, 1970; 7, 444-6.
22. Kind PRN and King KS. Estimation of plasma phosphatase by determination of hydrolyzed phenol by amino antipyrine. J Clin Pathol, 1954; 7: 322-5.
23. Reitman S and Frankel S. A colorimetric method for the determination of serum transaminase activity. Am J Clin Pathol, 1957; 28: 56-60.
24. Naftalin L, Sexton M, Whitaker JF and Tracey D. A routine procedure for estimating serum gamma glutamyl transpeptidase activity. Clin Chem Act, 1969; 26: 293-5.
25. Block CA and Watkins JB. Determination of conjugated bile acids in human bile and duodenal fluid by reverse phase high

performance liquid chromatography. J Lip Res, 1978; 19: 510-513.
26. Misra HP and Fridovich . Superoxide dismutase: a photochemical augmentation assay. Arch Biochem Biphysiol, 1997;18 : 308-10.
27. Beers RFJ and Sizer JW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem, 1952; 195: 33-4.
28. Hafeman DG, Sundae RA and Hoekstra WG. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase. J Biol Chem, 1974; 104: 5580-7.
29. Le BL, Chuo SC, Ong HY and Ong CN. High performance liquid chromatography method for routine determination of vitamin A and vitamin E and â-carotene in plasma. J Chromatog, 1992; 581: 41-7.
30. Esteve MJ, Farre R, Frigola A and Garcia–Cantabellia JM. Determination of ascorbic acid and dehydro-ascorbic acid in blood plasma and serum by liquid chromatography. J Chromato Biomed Sci Appl, 1997; 688: 345-9.
31. Tietz NW. (Ed). Clinical guide to laboratory tests, 1995. Philadelphia. WB Sander Co.
32. Piedrafita D. Juvenile Fasciola hepatica are resistant to killing in vitro by free radicals compared with larvae of Schistosoma mansoni. Parasitol Immunol, 2000; 22(6): 287-95.
33. Gameel AA. Fasciola hepatica: plasma ascorbic acid, plasma iron and iron-binding capacity in experimentally infected sheep with Fascila hepatica. Z Parasitenkd, 1982a; 68(2): 185-9.
34. Gameel AA. Plasma ascorbic acid levels in sheep experimentally infected with Fascila hepatica. Z Parasitenkd, 1982b; 66(3): 321-6.
35. Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet, 1994; 344:721-4.
36. Ismail MM, Bruce JI, Rassmussen SL, Attia M and Slem M. Schistosomiasis and other helminthic infections in Kafr Soliman village–Sharkiya governorate. J Egyp Soc Parasitol, 1995; 18(1): 47.
37. Tengerdy RP. Vitamin E, immune response and disease resistance. Ann Acad Sci, 1989; 570: 335-44.
38. Fox ES, Brower JS, Bellezzo JM and Leingang KA. N-acetylcysteine and á-tocopherol reverse the inflammatory response in activated rat Kupffer cells. J Immunol, 1997; 158: 5418-23.
39. Kuroki S, Okamato S, Naito T, Oda H, Nagase S, Sakai H, Nawata H, Yamashita H, Chijiwa K and Tanka M. Serum 7 a-hydroxycholesterol as a new parameter of liver function in patients with chronic liver disease. Hepatology, 1995; 22: 1182-7.
40. West HJ. Evaluation of total serum bile acids concentration for the diagnosis of hepatobiliary disease cattle. Res Vet Sc, 1991; 5(2): 133-40.
41. Garcia LS and Brackner DA. In: Diagnostic Medical Parasitology. 2nd ed. American Society For Microbiology. Washington DC 1, 1993.
42. Ferri I, Lopez P, Gouzalo-Orden M and Julian MD, Rojevazguez FA and Gouzalez –Gallego J. The effects of subclinical Fascioliasis on hepatic secretory function in sheep. Parasit Res, 1995; 81(2): 127-31.
43. Markell EK, Voge M and John DT. In: Medical parasitology. 7th ed. W.B. Saunders Co, 1992. Philadelphia, London, Toronto, Montreal, Sydney and Tokyo.
44. Fridovich I. Superoxide radical and endogenous toxicant. Annu Rev Pharmacol Toxicol, 1993;23:239.
45. Halliwell B. Antioxidant characterization: methodology and mechanisms. Biochem Pharmacol, 1995; 491: 1341.
46. Bast A, Haenen GR and Doleman CTA. Oxidants and antioxidants: "state of art". Am J Med, 1991; 91(Suppl 3c): 25.
47. Abdel-Rahman SZ, EL-Sharkawy AM, Abou Basha L and Salem AI. Glutathione and related enzymes in Fascioliasis before and after treatment with bithionol. J Trop Med Hyg, 1990; 93(5): 337-40.
48. Halliwel B and Guttreridge TML. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol, 1990:186.
49. Chance B, Sies H and Boveris A. A hydrogen peroxide metabolism in mammalian organs. Physiol Res, 1997; 59: 527.
50. Baeza E, Poitou I and Boulard C. In vitro effects of Fasciola hepatica on the main functions of polymorphonuclear leukocytes: chemotaxis and

free radical generation induced by phagocytosis. Intern J Parasitol, 1993; 23(8): 1077-81.

51. Galtier P, Vandenberghe Y, Coecke S, Larrien G and Vercruysse A. Differential inhibition of rat hepatic glutathione S-transferase isoenzymes in the course of Fascioliasis. Mol Biochem Parasitol, 1991; 44(2): 255-60.
52. Campos EG, Hermes-Lima M, Smith JM and Prichard RK. Characterization of Fasciola hepatica cytochrome c peroxidase as an enzyme with potential antioxidant activity in vitro. Intern J Parasitol, 1999; 29(5): 655-62.
53. Jeffries JR, Turner RJ and Barret J. Effect of Fasciola hepatica excretory-secretory products on the metabolic burst of sheep and human neutrophils. Intern J Parasitol, 1997; 27(9): 1025-9.
54. Sanchez-Moreno M, Leon P, Salas- Peregrin JM, Garcia-Ruiz MA and Monteoliva M. Superoxide dismutase in trematodes. Isoenzymatic characterization and studies of inhibition by a series of benzimidazoles and by pyrimidines of recent synthesis. Arzneimittelforschung, 1987; 37(8): 903-5.