Erythrocytes' Detoxifying and Anti-oxidant capabilities in Human Fascioliasis: Effect of Egaten

Ebied SA, Farag HF**, Youssef AI*, Ossman MM**,*
*EL-Samak MY*** and Hassnian NA**
Departments of Applied Medical Chemistry*, Parasitology** and Chemical Pathology*** Medical Research Institute, Alexandria University

ABSTRACT

The present study was carried out on 21 fasciolia patients (6 with acute fascioliasis and 15 with chronic fascioliasis), and 10 age matched controls. Specific activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), glutathione reductase (GR), glutathione S-transferase (GST), glucose 6-phosphate dehydrogenase (G6-PD) as well as glutathione content (GSH) were measured in red blood cells as it offers a number of advantage for studying the effects of oxidants. Egaten (the human form of the fasciolocidal drug Triclabendazole) was supplemented to all patients in a dose of 10 mg/kg body weight for 2 successive days. Before treatment specific activities of SOD, GP_X, GR, G6-PD and GSH content were significantly reduced in the erythrocytes of all patients. The only exception was CAT enzyme that didn't show any significant difference from controls and GST that showed significant elevation. Liver function tests were significantly elevated in plasma of fasicola patients. After treatment the level of all studied parameters except CAT were significantly elevated than that before treatment and became more or less round the figures of controls. Liver function tests became normal again, and anemia was recovered. Conclusively, Egaten has cured all cases of fascioliasis under study and it has abolished the effect of the parasite's oxidants on the antioxidant capabilities of erythrocytes.

INTRODUCTION

Since more than two decades, fascioliasis has emerged as a human infection. That parasite affects the liver particularly during the acute stage, when the juvenile flukes migrate through the liver parenchyma, causing lysis of the liver tissue[(1)]. Severe immunological and inflammatory reactions were observed during that stage, as the excretory, secretory products of the worms were in direct contact with the tissues[(2)]. During the chronic stage, the worms were present in the bile ducts and gall bladder, the epithelium of the biliary system shows inflammation and hyperplasia ending in an obstructive phase[(3)].

Infection with that parasite leads to an intimate contact between the parasite and the host immune system. Neutrophils, eosinophils and macrophages play an important role in defense against the parasite[(2,4)]. The initial reaction of these cells is the generation of reactive oxygen species (ROS)[(5)]. ROS generation, through

normal cellular metabolism and by means of exogenous insult is a constant problem, for which cells have developed multiple protective mechanisms[6]. Central to that defense are the anti-oxidant enzymes of the blood. These include SOD, GP_X, CAT, the detoxifying enzyme GST, as well as the enzymes involved in recycling the oxidized GSH such as GR and G6-PD[7]. Red blood cells could be regarded as circulating anti-oxidant carriers; they offer a number of advantages for studying the effects of oxidants [8].

TCBZ (6-chloro-5 [2, 3 dichlorophenoxy]-2- methylene-benzimidazole) has been extensively used in the treatment of animal fasciolasis[9], Egaten, a form of TCBZ, specially prepared for human use proved safe against fasciolasis. That drug has the advantage of treating both acute and chronic fasciolasis.

The aim of the present work was to study the effect of fasciola infection, acute and chronic, on the activities of some erthrocytes' detoxifying and anti-oxidant enzymes. Besides, the effect of Egaten on these enzymes was assessed after three months from treatment.

MATERIALS & METHODS

The present study was carried on 21 patients 15-30 years old classified into the following groups:

Group I: Six patients with acute fasciolasis diagnosed clinically by fever, abdominal pain and enlarged liver, haematologically by eosenophilia and serologically by a positive indirect haemagglutination test (titre > 1/640).

Group II: Fifteen patients with chronic fascioliasis passing eggs in the stools and diagnosed by the kato katz technique[10]. Control group of ten healthy persons; age matched were chosen.

None of the patients and controls had received blood transfusion, antibiotics or medication at least three months before the study. None gave a history of jaundice, diabetes mellitus or chronic diseases.

Regimen of treatment

Egaten (Novartis, Pharma AG, Bale, Switzerland) was given to all patients in a dose of 10 mg/kg body weight for two successive days after intake of a fatty meal (as recommended by the manufacturer)[11].

After the end of therapy, patients were followed up every fortnight for a period of 3 months to check the presence of eggs in stools; IHAT was repeated for patients with acute fascioliasis after 3 months.

Blood sampling

Ten mls. venous blood, were withdrawn from all patients before and 3 months after treatment, and from the control group. 200 µl were immediately used for assay of GSH. One ml was dispatched into a heparinized tube for studying the blood picture[12]. The remaining blood sample was pipetted into tubes containing 10 mM (EDTA). Within 4 hours, the blood was centrifuged at 3000 rpm for 20 minutes in a cooling centrifuge to separate the plasma for assessment of liver function tests (ALT, AST[13], ALP[14], Bilirubin[15]. The Buffy coat was removed and the remaining erythrocytes were drawn and washed 3 times in cold sterile saline and used for assaying the activities of the different enzymes. SOD[16], CAT[17], GSH[18], GPx[19], GR[20], GST[21], G6-PD[22], protein

content by Lowery OH et al.[23] and Hb[24].

Statistical analysis was done using SPSS8/ windows. Student "t" test, paired "t" test and ANOVA test were used to compare means of different variance. Difference was considered significant at $p < 0.05$.

RESULTS

I- Parasitological results

All cases of acute fascioliasis revealed a drop in eosinophilia and in the IHA titres after treatment confirming their cure. Chronic cases ceased to pass ova in the stools and the cure rate was 100%

II- Biochemical results

Results of the present study revealed that the specific activities of Cu/ Zn-SOD, GP_x, GR, G6-PD as well as GSH content were significantly reduced when compared with controls in the erythrocytes of patients with acute and chronic fascioliasis before treatment, (P = 0.045, P = 0.001, P=0.032, P = 0.009, P = 0.043) for acute fasciolasis and (P = 0.000, P = 0.001, P = 0.001, P = 0.000, P = 0.019) for chronic fasciloiasis respectively (Tables I-V). The only exception of these was GST specific activity which showed significant increase in acute and chronic fascioliasis when compared with controls before treatment (P = 0.001, P = 0.000) respectively (Table VI).

Catalase specific activity in both acute and chronic fascioliasis didn't show any significant difference before treatment when compared with controls (P=0.105, P=0.300) respectively (Table VII).

Enzyme activities of GP_X, GR, GST and GSH level didn't show significant difference in acute stage of fascioliasis when compared with chronic stage (P>0.05).

On the other hand, SOD, CAT and G_x6-PD activities were significantly higher in acute stage of the disease when compared with the chronic stage (P=0.01, P=0.001, P=0.001) respectively.

After 3 months of Egaten treatment, specific activities of SOD, GP_x, GR, G6-PD as well as GSH level were significantly elevated in both acute fascioliasis (P=0.001, P=0.000, P=0.006, P=0.022, P=0.015) and chronic fascioliasis (P=0.000, P=0.001, P=0.000, P=0.000, P=0.000) respectively, when compared with that before treatment and all became around the figures of normal controls (Tables I-V). Erythrocyte GST mean activity in both studied groups was significantly lower than that before treatment (P=0.022, P=0.000) respectively and regained its normal value (Table VI). Regarding catalase specific activity after 3 months of Egaten treatment showed significant elevation when compared with that before treatment (P=0.003, P=0.000) respectively and, also, with that of control (P=0.000, P=0.004) respectively (Table VII).

III- Haematological results

In comparison with control group, Hb content in acute and chronic fascioliasis was significantly lower (P = 0.000 and P = 0.000). After 3 months of Egaten treatment, Hb content was significantly elevated than that before treatment (P = 0.000, P = 0.000) and became within the normal range of control. Heamatocruit

values was significantly lower in the two studied groups when compared with controls (P = 0.002, P = 0.000) but after treatment with Egaten it regained its normal value. The same result was detected in red blood cells count which showed significant decrease in both patients with acute and chronic fascioliasis (P = 0.000, P = 0.000). After 3 months of Egaten treatment the count increased to show significant elevation than that of before treatment (P= 0.000, P = 0.000) respectively and to become within the normal range of control.

Before treatment eosiphilic % was significantly higher in patients with acute and chronic fascioliasis when compared with control (P = 0.000, P = 0.000) respectively. After Egaten treatment it was significantly decreased than before treatment (P = 0.001, P = 0.000) and regained its normal level only in patients with chronic fascioliasis (P = 0.132), but remained significantly higher than control in patients with acute fascioliasis (P = 0.008) (Table VIII).

IV- Liver function tests

Enzyme activity of both ALT and AST were significantly elevated in acute fascioliasis only but not in chronic fascioliasis when compared with controls. (P = 0.001, P = 0.001) for acute stage, but (P = 0.726, P = 0.524) for chronic stage.

After 3 months of Egaten treatment the activities of the two enzymes in acute fascioliasis were significantly decreased when compared with that before treatment (P = 0.011, P = 0.020, respectively) and regained the normal values. No significant change was noticed in case of chronic fascioliasis.

ALP specific activity was significantly elevated in both patients with acute and chronic fascioliasis when compared with control (P = 0.001, P = 0.011). After treatment the plasma level of the enzyme was statistically decreased in both groups (P = 0.000, P = 0.000) than that before treatment and returned to the normal values.

Bilirubin level in acute and chronic fascioliasis was significantly elevated when compared with control (P = 0.006, P = 0.002). After treatment the level decreased significantly when compared with that before treatment (P = 0.001, P = 0.000) and became within the normal range.

Table (I): Erythrocyte superoxide dismutase specific activity of controls and patients infected with *Fasciola* before and after treatment with Egaten.

	Control group	Acute *Fasciola* patients		Chronic *Fasciola* patients	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	10.91-27.43	6.25-16.83	17.24-21.55	2.32-18.71	9.02-24.24
Mean ± S.E.M	18.14 ± 1.66	12.78±1.43	19.21±0.56	8.55 ± 1.41	19.36 ± 0.87
		P= 0.001 ↑		**P=0.000** ↑	
		P=0.045 ↓			
		P=0.635			
		P=0.000			↓
		P=0.483			

The results are expressed in U/ gHb.

Table (II): Erythrocyte glutathione peroxidase specific activity of controls and patients infected with Fasciola before and after treatment with Egaten.

	Control group	Acute *Fasciola* patients		Chronic *Fasciola* patients	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	0.82-2.65	0.35-0.82	1.04-1.87	0.1-1.31	0.53-2.54
Mean ± S.E.M	1.52 ± 0.16	0.57± 0.06	1.49±0.12	0.72 ± 0.14	1.40 ± 0.15
		P=0.000 ↑		**P=0.001** ↑	
		P=0.001 ↓			
		P=0.899			
		P=0.001		↓	
		P=0. 603			

The results are expressed in U/ gHb

Table (III): Erythrocyte glutathione reductase specific activity of controls and patients infected with Fasciola before and after treatment with Egaten.

	Control group	Acute *Fasciola* patients		Chronic *Fasciola* patients	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	3.10-7.91	2.09-3.20	2.21-6.50	1.49-5.87	2.70-12.43
Mean ± S.E.M	4.29 ± 0.43	2.93±0.17	5.19±0.64	2.61 ± 0.25	5.50 ± 0.69
		P=0.006 ↑		**P=0.000** ↑	
		P=0.032 ↓			
		P=0.247			
		P=0.001		↓	
		P=0. 201			

The results are expressed in U/ gHb.

Table (VI): Erythrocyte glucose 6-phosphate dehydrogenase specific activity of controls and patients infected with Fasciola before and after treatment with Egaten.

	Control group	Acute *Fasciola* patients		Chronic *Fasciola* patients	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	78.56-221.98	65.38-80.97	116.42-149.82	35.79-75.49	69.84-213.14
Mean ± S.E.M	138.75± 15.94	75.35±2.23	135.72±4.56	58.51 ± 2.54	147.04 ± 8.50
		P=0.000 ↑		**P=0.000** ↑	
		P=0.009 ↓			
		P=0.888			
		P=0.000		↓	
		P=0. 622			

The results are expressed in U/ g Hb

Table (V): Erythrocyte glutathione contents of controls and patients infected with Fasciola before and after treatment with Egaten.

	Control group	Acute *Fasciola* patients		Chronic *Fasciola* patients	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	20.49-51.22	25-27.32	27-28	23.59-34.14	23.28-43.46
Mean ± S.E.M	33.47± 2.41	26.40± 0.35	27.50± 0.14	28.18 ± 0.62	32.83± 1.19
		P=0.015 ↑		**P=0.000** ↑	
	P=0.043 ↓				
		P=0.080			
		P=0.019		↓	
		P=0. 795			

The results are expressed in mg%.

Table (VI): Erythrocyte glutathione s-transferase specific activity of controls and patients infected with Fasciola before and after treatment with Egaten.

	Control group	Acute *Fasciola* patients		Chronic *Fasciola* patients	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	28.2-83.17	181.32-572.54	40.41-53.88	119.68-403.04	22.32-98.43
Mean ± S.E.M	63.61 ± 7.72	274.56±68.66	45.56±1.88	233.52 ± 32.23	56.61 ± 5.27
		P=0.022 ↓		**P=0.000** ↓	
	P=0.001 ↑				
		P=0.099			
		P=0.000		↑	
		P=0.445			

The results are expressed in U/ g Hb.

Table (VII): Erythrocyte catalase specific activity of controls and patients infected with Fasciola before and after treatment with Egaten.

	Control group	**Acute *Fasciola* patients**		**Chronic *Fasciola* patients**	
		Before treatment	After treatment	Before treatment	After treatment
n	(10)	(6)	(6)	(15)	(15)
Range	2104.18-6704.42	3613.22-5480.37	4625.56-7692.65	1619.81-4395.81	3699.63-6851.56
Mean ± S.E.M	3434.62 ± 430.70	4466.12± 248.70	6655.73± 449.12	2996.71 ± 180.21	4802.78 ± 206.24
		P=0.003 ↑		**P=0.000** ↑	
	P=0.105				
		P=0.000 ↑			
		P=0.300			
		P=0. 004			↑

The results are expressed in U/ gHb.

Table (VIII): Hemoglobin content, hematocurit values, red blood cell count and eosinophilic (%) in controls and patients with acute and chronic fascioliasis before and after treatment with Egaten.

N	Control (10)	Acute fascioliasis		Chronic fascioliasis	
		Before treatment (6)	After treatment (6)	Before treatment (15)	After treatment (15)
Hemoglobin content (g/dl)					
Range	12.30-15	10-11.90	12.10-13.50	9.10-12.50	12.30-14.50
Mean ± SEM	13.44±0.26	10.99±0.27*	12.76±0.18**	11.35±0.25*	13.29±0.19**
Hematocrit values (%)					
Range	34-45	33-36	38-40	27-38	35-45
Mean ± SEM	39.90±0.99	34.83±0.48*	39.0±0.37	34.20±0.76*	39.73±0.76
RBCs count (M/mm^3)					
Range	4.25-5.17	3.60-3.85	4.38-4.66	3.50-4.51	4.10-5.70
Mean ± SEM	4.69±0.10	3.72±0.03*	4.53±0.04**	4.03±0.06*	4.64±0.10**
Eosinophiliccount (%)					
Range	0-5	17-33	4-10	4-20	2-8
Mean ± SEM	3.20±0.55	24±2.13*	6.17±0.87*	10.27±0.95*	4.27±0.42**

*Significant when compared with control.

**** Significant when compared with that before treatment.**

Table (IX): Liver function tests in controls and patients with acute and chronic fascioliasis before and after treatment with Egaten.

N	Control (10)	Acute fascioliasis		Chronic fasciloiasis	
		Before treatment (6)	After treatment (6)	Before treatment (15)	After treatment (15)
ALT(U/L)					
Range	10-32	14-133	10-29	7-49	8.42
Mean ± SEM	20.11±1.74	69.37±15.48*	18.0±2.56**	18.80±2.77	18.60±2.11
AST (U/L)					
Range	14-38	19-87	13.19	11-47	10-47
Mean ± SEM	22.88±2.36	45.50±9.33*	16.39±0.80**	24.83±1.91	21.10±2.04
ALP (U/L)					
Range	112-303	153-405	72-186	94-437	63-275
Mean ± SEM	159.60±18.06	296.33±33.44*	129.17±14.72**	244.20±21.77*	160.13±14.18**
Billirubin (mg/dl)					
Range	0.4-0.7	0.6-0.8	0.5-0.6	0.5-1.2	0.4-0.7
Mean ± SEM	0.57±0.03	0.73±0.03*	0.53±0.02**	0.78±0.04*	0.59±0.02**

*Significant when compared with control.
** Significant when compared with that before treatment.

DISCUSSION

The present study indicated that infection with fasciola, was accompanied by changes in the oxidative abilities of erythrocytes, that was manifested by a significant decrease in the activities of the main anti-oxidant enzymes, SOD, GP_X, GR and G6-PD and in the content of the non enzymatic anti oxidant GSH. The only exception from this pattern was increased activity of GST, and unchanged activity of CAT.

Previous studies on rats infected with F.hepatica showed that the antioxidant capabilities of liver enzymes (SOD, GPx, GR) were reduced, GSH content showed significant depletion as a result of infection[25].

Results of the present study support the study of Rehim (2003)[26], which revealed a significant depletion in the erythrocyte GSH content as well as GPx and SOD activities in patients with acute and chronic fascioliasis.

SOD, is the main anti-oxidant enzyme of the cell, responsible for scavenging the super oxide radical. With infection, its level was decreased and the super oxide radicals are thus expected to be elevated. Superoxide anions could cause oxidation of critical SH groups in proteins and possibly induce peroxidation of lipids in the membrane of the RBCs, causing lysis of the red cell membrane.[27-29] Moreover, some of the hemoglobin become oxidized to methemoglobin that does not build or transport O_2. Heme may also be released, it reacts with O_2 and H_2O_2 to produce $OH^{\bullet}$ and ROS.[30]

RBCs CAT is protected from pro-oxidant mediated inactivation by associated NADPH. G6-PD is the main source of NADPH generation. G6-PD was significantly inhibited in patient groups. Accordingly a decrease in catalase activity is expected. Yet, in the present study, the level of CAT in the oxidatively stressed erythrocyte did not show significant difference when compared with the control level. That finding could be explained according to Cooper *et al* (1972)[31] who stated that G6-PD deficiency lead to depletion of NADPH but has a smaller effect on NADH, the latter might prevent inactivation of CAT in the erythrocytes. The unchanged acticity of CAT might also be attributed two its low affinity to H_2O_2.[32]

GSH is believed to function as an important redox buffer. It protects cell membranes from oxidative damage and helps to maintain the SH group of many proteins in the reduced form. In fasciola patients, the erythrocyte level of GSH was found to be low compared to the level in controls. That finding reflects an increase in free radical production at a rate that might exceed the detoxifying ability of GSH.[33]

The decreased level of GSH may be connected with enhanced oxidation of glytathione disulphide (GSSG), catalyzed by free radicals. Moreover, aldehydes generated during lipid peroxidation may form a conjugate with GSH, leading to significant decrease in cellular GSH.[34,35]

GPx a selenium containing protein, reduces H_2O_2 and various hydro peroxides using GSH as a reducing agent.[36,37] That enzyme showed decreased activity in infected patients, a finding which might be explained by the low content of GSH

that acts as a substrate and decreased co-factor of GP_X.[38] Enzyme inactivation may as well be explained according to Kinter and Robert (1996) by the fact that 4-hydroxy non-enal (4 NHE), the aldehyde generated during lipid peroxidation, reacts with selenium in the active site of GP_X and inactivates the enzyme.[35] Moreover, non specific oxidation of the susceptible cysteine SH group adjacent to the selenol group appears to cause irreversible inactivation of the enzyme.[39]

GSTs play a central role in human detoxification process. They catalyze the conjugation of GSH to toxic products that may be easily excreted. GST activity was elevated in patients with fascioliasis. The elevation is of interest, since mature erythrocytes are incapable of protein synthesis, so, enzyme activation seems not to reside on protein expression but it could be caused by a favourable conformational change following the oxidant insult.[40,41]

G6-PD is involved in the generation of NADPH which is indispensable for biosynthesis of GSH.[42,43] A decrease of G6-PD was encountered in all patient groups and this was expected to participate in causation of chronic hemolysis of erythrocytes.

Three months after Egaten treatment, the level of cell anti-oxidants was restored and became near or within the limits of the normal range.

Elevated levels of ALT and AST in acute phase of fascioliasis could be considered secondary to extensive destruction of the liver parenchyma and to association with some degrees of hepatic necrosis in some areas caused by the penetrating fluke.[25,44]

Elevated level of ALP activity in acute stage may be due to hepatic damage and or increased permeability of the cell due to irritation by toxic metabolites of the worms and eggs. In chronic infection the elevation could be attributed to the mechanical obstruction of the bile duct and toxic action of the parasites leading to hyperplasia of the biliary epithelium with failure to excrete the enzyme in the bile, accordingly it is raised in plasma.[45] These results were confirmed by the elevated level of bilirubin in plasma of patients of both phases of infection.

In both animal and human infection with fascioliasis, anemia was reported as one of the most characteristic symptoms. Anemia could be a result of the fluke which is a blood feeder, hemorrhage that occurs from the erosion of the biliary epithelium due to infection, toxic substances released by the fluke[46,47] that affect the piturity gland leading to reduction in thyrotrophic and adrenocorticoid hormones which in turn leads to reduction in the rate of erythopoiesis,[48] reticulocytes that are increased in the periportal blood and shortens the half life of red blood cells.[49,50]

In addition, the decline in G6-PD activity noticed in the current study leads to lack of reduced GSH necessary to detoxify ROS. All these findings may explain the anemia detected in the present study that was diagnosed by reduction of Hb concentration, red blood cell count and hematocruit value. Also, cross linkages of sulfhydral groups occur

both in hemoglobin and membrane. Heinz bodies that contribute to the rigidity of the red cell membrane resulting in their early removal from the circulation.[51] After 3 months of Egaten treatment, the Hb concentration, red blood cell count and hematocruit percent regained their normal level denoting that Egaten has beneficial value in restoring the Hb level and correcting the anemia induced by fascioliasis.

Increased eosinophilic percent is in consistent with previous study by Hishmat *et al* (1996),[52] it was suggested to be due to the stimulant effect of the parasite antigens specially those of larvae to the Th_2 lymphocytes that secrete IL-4 and IL-5, responsible for eosinophilia.[53,54]

Eosinophilic percent reached normal values by the end of three months post treatment in chronic fascioliasis. In acute fascioliasis, although the eosinophilic percent was statistically decreased than before treatment but was yet higher than control level.

Conclusively, Egaten has cured all cases of fascioliasis whether in the acute or chronic stage; it has thus abolished the effect of the parasite on the different anti-oxidant enzymes. The drug itself was safe and did not induce any insult.

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مقدرة مزيلات السمية ومضادات الأكسدة فى كرات الدم الحمراء فى حالات المتورقة الكبدية: تأثير عقار الإيجاتين

سامية عبد المنعم عبيد، هدى فهمى فرج**، أمانى إبراهيم يوسف*،*
*مرفت مصطفى عثمان**، محمد يسرى السماك*** ونيفين عبد المنعم حسنين**
قسم الكيمياء الطبية التطبيقية*، الطفيليات** والكيمياء الباثولوجية***
معهد البحوث الطبية، جامعة الإسكندرية

إن إصابة الإنسان بالمتورقة الكبدية قد تم رصدها فى العديد من البلدان كما تم رصدها أيضاً فى مصر. وقد تم إدراج المتورقة الكبدية من مسببات الفشل الكبدى. ومن ناحية أخرى فإن الإصابة بالطفيل تؤدى إلى زيادة إنتاج الشقائق الحرة والمركبات الأوكسجينية النشطة التى تسبب أضراراً بالغة بالخلايا الحية ومكوناتها، لذلك فالجسم له استراتيجية لتقليل التلف الناتج من التعرض لهذه الشقائق الحرة ومن أهم وسائل الدفاع أنزيمات إزالة السمية والمضادة للأكسدة ومنها سوبر أوكسيد ديزميوتيز . الكاتاليز . الجلوتاثيون بيروكسيديز . والجلوتاثيون ريدتكتيز . الجلوتاثيون إس ترانسفيريز . الجلوكوز –٦– فوسفات ديهدروجينيز.

ومؤخراً تم اعتماد عقار الإيجاتين (التريكلابندازول للاستخدام الآدمى) لعلاج الطور الحاد والمزمن لطفيل المتورقة الكبدية. لقد استهدف هذا البحث دراسة تأثير عقار الإيجاتين على كل من:

١. نشاط الأنزيمات المضادة للأكسدة (سوبر أوكسيد ديزميوتيز . الكاتاليز . الجلوتاثيون بيروكسيديز . الجلوتاثيون ريدكتيز . الجلوتاثيون إس ترانسفيريز . الجلوكوز –٦– فوسفات ديهدروجينيز) فى كرات الدم الحمراء وكذلك محتوى الجلوتاثيون فى الدم للمرضى المصابين بالدودة الكبدية قبل وبعد العلاج بالإضافة إلى الأشخاص الأصحاء.
٢. وظائف الكبد قبل وبعد العلاج.
٣. فقر الدم الناتج عن الإصابة قبل وبعد العلاج.

قد أجريت هذه الدراسة على ٢١ مريضاً بالإضافة إلى ١٠ أشخاص أصحاء ولقد تم جمع عينات الدم الخاصة بالدراسة من المجموعات الآتية:

١- المجموعة الأولى: ستة مرضى بداء المتورقة الكبدية فى المرحلة الحادة.
٢- المجموعة الثانية: خمسة عشرة مريضاً بداء المتورقة الكبدية فى المرحلة المزمنة.

تم إعطاء العقار بجرعة ١٠ مجم/كجم من وزن الجسم لمدة يومين متتاليين. وقد أظهرت الدراسة النتائج الآتية: كل الحالات المصابة بالمتورقة الكبدية. قد شفيت تماماً بعد أسبوع من بدأ العلاج حيث لم تتواجد أى بويضات أثناء شهور المتابعة الثلاثة. لقد أوضحت هذه الدراسة أن الإصابة بالمتورقات الكبدية كانت مصحوبة بتغيرات فى مستوى مضادات الأكسدة بكرات الدم الحمراء. وهذا كان واضحاً من الانخفاض فى نشاط الأنزيمات الرئيسية المضادة للأكسدة مثل سوبر أوكسيد ديزميوتز والجلوتاثيون بيروكسيديز والجلوتاثيون ريدتكتيز والجلوكوز –٦– فوسفات ديهدروجينيز وكذلك الانخفاض فى مستوى الجلوتاثيون. الاستثناء الوحيد من ذلك هو زيادة نشاط إنزيم الجلوتاثيون إس ترانسفيريز. الانخفاض الملحوظ فى نشاط إنزيم السوبر أوكسيد ديزميوتز قد يرجع إلى

الإنتاج الزائد للشقائق الحرة المصاحبة لعملية الالتهاب كرد فعل من العائل للعدوى بالطفيليات. بالنسبة لنشاط إنزيم الكاتاليز فى كرات الدم الحمراء فى جميع المجموعات لم يتغير عن مستواه فى المجموعة الضابطة.

إما انخفاض محتوى الجلوتاثيون فى كرات الدم الحمراء للمرضى المصابين بالمتورقة الكبدية فإنه يعكس الزيادة فى إنتاج الشقائق الحرة بمعدل عالى يفوق قدرة الجلوتاثيون على إزالتها.

بالنسبة لنشاط إنزيم الجلوتاثيون بروكسيديز فقد انخفض انخفاضاً ذو دلالة إحصائية فى كل المجموعات تحت الدراسة بالمقارنة إلى المجموعة الضابطة. فى المرضى المصابين بالطفيل الكبدية يعزى انخفاض نشاط الإنزيم إلى انخفاض محتوى الجلوتاثيون الذى يعتبر المادة التى يعمل عليها الإنزيم. أما إرتفاع مستوى نشاط إنزيم الجلوتاثيون اس ترانسفيريز فى المجموعة المصابة بالمتورقة الكبدية قد يرجع إلى تغييرات شكلية فى الإنزيم نفسه. انخفاض مستوى نشاط إنزيم الجلوكوز -٦- فوسفات ديهدروجينز فى المرضى المصابين يؤدى إلى نقص مستوى الـ NADPH والجلوتاثيون فى كرات الدم الحمراء مما يؤدى إلى تدمير جدار كرات الدم الحمراء وبالتالى توقع التكسير المزمن لهذه الخلايا.

بعد ثلاثة أشهر من العلاج بعقار الإيجاتين فى المرضى المصابين بالمتورقة الكبدية فى المرحلتين الحادة والمزمنة فإن معظم الإنزيمات المضادة للأكسدة تحت الدراسة عادت إلى مستواها الطبيعى أو أكثر قليلاً من المستوى الطبيعى.

إرتفع مستوى نشاط الإنزيمات الكبدية الـ ALT&AST فى مرضى المتورقة الكبدية فى المرحلة الحادة للإصابة أما بعد العلاج فكان هناك تحسن ملحوظ فى نشاط هذه الإنزيمات. بالنسبة لمستوى نشاط إنزيم الفوسفاتيز القاعدى فقد ارتفع نشاطه ارتفاعاً ذو دلالة إحصائية فى مصل دم جميع مجموعات المرضى بالمقارنة بمثيله فى المجموعة الضابطة، بعد العلاج بالعقار إستعاد الإنزيم مستواه الطبيعى فى كل المجموعات.

بالنسبة لنتائج صورة الدم: أظهرت الدراسة أن القيم المتوسطة لتركيز الهيموجلوبين وتعداد كرات الدم الحمراء وقيمة الهيماتوكريت كانت منخفضة انخفاضاً ذو دلالة إحصائية فى كل المجموعات تحت الدراسة عند مقارنتها بالمجموعة الضابطة. بعد العلاج بعقار الإيجاتين تحسنت كل القيم مرة أخرى وعادت لمستواها الطبيعى.

أظهرت الدراسة أن القيم المتوسطة لكرات الدم المحبة للإيوسين فى كل المجموعات تحت الدراسة كانت مرتفعة ارتفاعاً ذو دلالة إحصائية بالمقارنة بالمجموعة الضابطة. أما بعد العلاج فقد تراجعت النسبة المئوية لكرات الدم المحبة للإيوسين إلى معدلاتها الطبيعية فى الطور الحاد.

الاستنتاج:

١. أن الإصابة بداء المتورقة الكبدية قد أدى إلى انخفاض نشاط معظم الإنزيمات المضادة للأكسدة تحت الدراسة.
٢. أن عقار الإيجاتين قد أثبت كفاءة عالية فى علاج حالات الإصابة بداء المتورقة الكبدية بمرحلتيها الحادة والمزمنة كما ظهر من متابعة تحليل البراز بعد ثلاثة شهور وكذلك استعادة وظائف الكبد لمستواها الطبيعى واختفاء الأنيميا. وأخيراً استعادة معظم الإنزيمات المضادة للأكسدة تحت الدراسة لمستواها الطبيعى.