

Effects of Fascioliasis and Schistosomiasis Mansoni on the Anti-Oxidant Defense Mechanisms

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ABSTRACT:

Background/Aim: 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 this study was to evaluate the influence on hepato-biliary system posed by *Fasciola* species with or without *Schistosoma mansoni* co-infection respective to the antioxidant status, total bile acids and liver function tests.

Subjects & Methods: Thirty subjects infected with *Fasciola hepatica* were categorized into those with *S. mansoni* infection (GI) and without *S. mansoni* infection (GII) compared to thirty age matched subjects free of *Fasciola hepatica* but with *S. mansoni* infection (GIII) and a fourth group including healthy subjects as controls (GIV). Ultrasonography, computed tomography (CT), and endoscopic retrograde cholangio pancreatography (ERCP) as well as antifasciola antibody titre were used for diagnosis of early and late stages of fascioliasis. Diagnosis of schistosomiasis mansoni was done by stool examination for eggs, sigmoidoscopy and rectal snips for negative stool. In addition, abdominal ultrasonography was done for diagnosis of periportal fibrosis. Biochemical assessments of the antioxidant defense status by measuring serum level of enzymatic activity 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: Significant reduced levels of superoxide dismutase, catalase and glutathione peroxidase as well as the antioxidant vitamins A, C, E in groups I, II, III as compared to GIV and the decrease was highly significant with mixed parasitic infection (GI) ($P < 0.01$). The serum total bile acids were significantly increased in GI and GII as compared to GIII and GIV ($p < 0.05$), meanwhile the liver function tests were significantly higher in patients with *Fasciola* (GI, GII) than those with *S. mansoni* alone or in healthy control (GIII, GIV).

Conclusion: The serum antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase as well as the antioxidant vitamins A, C and E were statistically significantly decreased in patients with fascioliasis, schistosomiasis mansoni and with co-infection than in healthy control versus increased levels of total bile acids. These findings potentiate the fibrogenic mechanisms and necessitate the supplementation of antioxidants in combination with traditional therapy.

Key words: Fascioliasis, Schistosomiasis Mansoni, Anti-Oxidant Defense Mechanisms.

INTRODUCTION

Medical and surgical problems especially 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 in these organs. *Schistosoma mansoni* and *Fasciola* species are the most prominent examples of Platy-helminthes inhabiting the liver. Millions of people in subtropical countries are infected with *S. mansoni*, the most common human Schistosoma.⁽¹⁾ Whereas, fascioliasis affects over 17 million people worldwide causing significant morbidity. ⁽²⁾ In Egypt, it has been reported that not only *Fasciola gigantica* is responsible for fascioliasis, but also *F. hepatica*, which has even become more common. As many animals that act as reservoir hosts for *F. hepatica* have been imported from Europe and other countries where *F. hepatica* is common ^(3, 4). Although adult schistosomes mature inside the liver, yet they are not associated with major biliary outcome ⁽⁵⁾. Hepatic involvement in schistosomiasis mansoni results from the host immune response to disseminated eggs that are laid in the portal venous system and become trapped in hepatic sinusoids producing granulomatous lesions that are followed by fibrosis ⁽⁶⁾. Hepatic fibrosis is considered as the main morbid sequela of schistosomiasis mansoni, which precipitates portal hypertension and bleeding oesophageal varices ⁽⁷⁾. In contrast, once the meta-cercariae of *F. hepatica* are ingested with contaminated leafy vegetables, they ex-cyst in duodenum. Thereafter, they migrate through duodenal wall into the peritoneal cavity through which they penetrate the liver via the Glisson capsule and lodge in bile ducts ⁽⁸⁾.

Cholestasis is a common sequela of hepatic fascioliasis owing to alterations in hepatic bile acid synthesis, excretion and intestinal absorption reflecting derangement in plasma bile acid levels ⁽⁹⁾. Bile acids have been always considered as a sensitive monitor of cholestasis⁽¹⁰⁾. In hepatic fascioliasis, the flukes and their metabolites irritate the biliary passages resulting in inflammation and

hyperplasia, and then fibrosis and even obstruction may occur ^(11,12).

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 ⁽¹³⁾. 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 cell ⁽¹⁴⁾. The host reaction involving reactive oxygen intermediates influences the ultimate pathophysiological effects of oxidative processes ⁽¹⁵⁾.

As regards to schistosomiasis mansoni, previous studies showed that the schistosomes elaborate an anti-inflammatory, immunomodulatory factor, which may help the parasite to evade host immune response ^(16, 17). In addition, chronic schistosomiasis is associated with impaired cell-mediated immune responsiveness⁽¹⁸⁾. 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⁽¹⁹⁾. 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 fascioliasis with or without *S. mansoni* co-infection.

SUBJECTS AND METHODS

The present study involved sixty selected subjects 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 the patients nor the control groups had evidence of any other 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 fascioliasis was achieved by stool examination for eggs of *Fasciola*, absolute eosinophilia⁽²⁰⁾ and high antifasciola antibody titre by using indirect hemagglutination test (Fumouze kits). Ultrasonography and computed tomography (CT) were done to diagnose early fascioliasis⁽²¹⁾ and endoscopic retrograde cholangio-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⁽²²⁾. *Schistosoma mansoni* infection was detected by positive stool analysis using the direct smear technique and Kato thick smear⁽²³⁾, sigmoidoscopy and rectal snips for negative stool. Abdominal ultrasonography was done for diagnosis of periportal fibrosis⁽²⁴⁾. The laboratory investigations for liver function tests were done which include determination of total serum bilirubin by colorimetric technique of Bartles and Bohmer⁽²⁵⁾, the determination of the activity of alkaline phosphatase (ALP) by the method of Kind and King⁽²⁶⁾, alanine transaminase (ALT) and aspartate transaminase (AST) according to the method of Reitman and Frankel⁽²⁷⁾ and Gamma glutamyl transpeptidase (GGT) using the method of Naflalin et al.⁽²⁸⁾.

Total bile acids were determined⁽²⁹⁾, the antioxidant defense status was investigated by measuring serum levels of superoxide dismutase (SOD) activity according to the method of Misra and Fridovich⁽³⁰⁾. Catalase activity by the spectrophotometric technique of Beers and Sizemore⁽³¹⁾ and total glutathione peroxidase (GSH-Px) activity using reduced substrates as described by Hafeman et al.⁽³²⁾ Evaluation of vitamin A was performed by high performance liquid chromatography method⁽³³⁾. Determination of vitamin C level was detected by liquid chromatography method⁽³⁴⁾. Assessment of vitamin E was done by spectrophotometry⁽³⁵⁾.

STATISTICAL ANALYSIS

The obtained values were statistically analyzed using SPSS software program where F= analysis of variance by ANOVA test. P (probability value) less than 0.05 were considered significant.

RESULTS

Symptoms encountered in early fascioliasis were: fever, dyspepsia, right hypochondrial and epigastric pain while in late fascioliasis, 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 (CB.D) and intrahepatic biliary radicals in 20% 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 CB.D. CT diagnostic criteria of early fascioliasis were: small pseudotumour or Olympic game sign (small sub-capsular hypodense lesions), while in late cases there were masses in gall bladder and dilated intrahepatic radicals. ER.C.P. was done in cases with obstructive jaundice revealing CB.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 serum bile acid in the studied groups.

The serum bilirubin, alkaline phosphatase and liver enzymes (AST and ALT) were significantly higher in patients affected by *F. hepatica* with or without *S. mansoni* affection (GI and GII) than those with *S. mansoni* alone or in healthy control (GIII and GIV), ($P < 0.01$, 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 GII 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 GIV) 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 decreased significantly in those with *F. hepatica* and *S. mansoni* affection than in normal control and the decrease was highly significant with mixed affection (GI) ($p < 0.01$). Also catalase activity was significantly decreased in those with *F. hepatica* and those affected with *S. mansoni* than in healthy control, $P < 0.01$. The glutathione peroxidase activity was significantly decreased in those affected with *F. hepatica* and in those affected with *S. mansoni* or in those with mixed infection than in healthy control, ($P < 0.01$).

The antioxidant vitamins (A, C and E) were significantly decreased in the infected groups (GI, GII, GIII) than the control group (GIV) $P < 0.01$, $P < 0.05$ and $P < 0.05$ respectively (table 4) and the decrease was more severe with mixed coinfection (GI).

Table (1): Liver function tests and total bile acids

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

Table (2) Eosinophilic count and anti-Fasciola antibody titer

Cases under study	Eosinophils count/cmm		anti-Fasciola Ab titer	
	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 (3) Antioxidant enzyme activities

PARAMETER		Cases with F, hepatica		Cases without F. hepatica		F&p
		+ve	-ve	+ve	-v	
		S. mansoni (GI)	S. mansoni (GI)	S. mansoni (GII)	S. mansoni (GIV)	
SOD(Mg/dl)	X±SD	30.4±10.1	42.6±11.7	54.3±12.7	72.8±14.9	8.99, <0.01*
Catalase (mg/Hb)	X±SD	224.1±38	249.8±31	278.3±29	346.5±524	9.23, <0.01*
GSHPx (mg/Hb)	X±SD	10.1±3.9	12.4±4.6	13.7±4.2	19.04±6.9	6.53, <0.05*

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

Table (4) Antioxidant vitamins

Parameter		Cases with F, hepatica		Cases without F. hepatica		F&P
		+ve	-ve	+ve	-v	
		S. mansoni (GI)	S. mansoni (GI)	S. mansoni (GII)	S. mansoni (GIV)	
Vit. A (mg/dl)	X±SD	21.3±6.7	26.8±7.4	33.9±9.2	59.7±10.9	11.32, <0.01*
Vit. C (umol/L)	X±SD	20.4±7.6	24.8±8.1	39.6±9.3	49.6±11.3	5.69, 0.05*
Vit. E (umol/L)	X±SD	2.66±0.64	2.98±0.78	3.84±0.86	4.62±1.44	4.25, 0.05*

(Vit. = Vitamin, X±SD = mean ± standard deviation, * = significant).

DISCUSSION

It is evident from reported data that newly encysted juvenile (NEJ) *Fasciola* flukes are relatively resistant to killing in vitro by free radicals and this resistance could in part be due to the activity of oxidant scavenger enzymes of NEJ worms⁽³⁶⁾. On the other hand, plasma ascorbic acid levels in sheep experimentally infected with *F. hepatica* was found to become decreased owing to impaired synthesis as a result of the hepatic damage caused by the flukes⁽³⁷⁾. This was in confirmation with the assessed reduction of vitamin C level herein, in the selected cases of *F. hepatica* (GI, GII) aligned with the hepatic damage monitored by liver function. It agrees with other reports correlating a decreased plasma ascorbic acid and elevated plasma iron concentrations as the iron-binding capacity was noted to rise during *Fasciola* infection especially between weeks 13 to 19-post infection⁽³⁸⁾. Such an inter-relationship would reflect an aspect of oxidant defenses that are not completely efficient. Hence, increased free radical formation in the body is likely to increase damage, necrosis or apoptosis⁽³⁹⁾ as noted elsewhere.

Confirming the impact of free radical insult to hepatic cellular membranes noted by Ismail et al.⁽⁴⁰⁾, the present data illustrates the reduced levels of the antioxidant vitamins: A, C and E, verifying their consumption by the high magnitude of oxidative stress. Consistently, this influences the pathologic outcome of *F. hepatica* and/or *S. mansoni* in cases under study owing to the diminished antioxidant defense mechanisms, hence the free radical scavenging role of vitamin C in the extracellular fluid is known to coordinate with the influence of the chain breaking antioxidant capacity and singlet oxygen quencher potency of vitamin A and E, protection polyunsaturated fatty acids in cell membranes from per-oxidation⁽¹⁵⁾. Moreover, the previous studies implemented a reduced protective role of vitamin E to EDE oxidation, besides an inadequate immunomodulatory potential that is known to enhance cell mediated and humoral immunity⁽⁴¹⁾. It would also influence the capacity of vitamin E to suppress the lipopolysaccharide-mediated production of

tumor necrosis factor alpha (TNF α) and by Kupffer cells in rat liver⁽⁴²⁾.

It is probable that the assessed decrements of the antioxidant vitamins may event from insufficient dietary supply of antioxidants and/or inefficient absorption in parallel to hepatobiliary disorders inflicted by *F. hepatica* when the flukes become lodged in the bile ducts⁽¹²⁾. Accordingly, in correlation with the degree of clinical illness monitored in *F. hepatica* cases and increment of total serum bile acids in GI > GII > GIII was assessed. This aligned with previous reports indicating that serum bile acids act as sensitive indices of hepatocellular uptake of the portal bile acids and the effective hepatic blood flow⁽⁴³⁾. Hence, the monitored elevation in bile acids has been noted to closely depend on hepato-biliary dysfunction than do other parameters that evaluate hepatocellular integrity⁽⁴⁴⁾.

Other reports have identified several mechanisms to alter plasma bile acids in liver disease. Such mechanisms include reduction of functional hepatic mass, alteration in hepatocellular perfusion and porto-systemic shunting of bile acids from homeostatic hepatocytes⁽⁴⁵⁾. As well, the migration of immature flukes in the course of fascioliasis was noted to cause a homeostatic phenomenon⁽⁴⁶⁾. Obstructive liver disease could also develop from *Fasciola* worms, also adult worms may invade the liver parenchyma, producing abscess⁽⁴⁷⁾. The persistence of adult flukes in main bile ducts causes duct wall changes in the form of jagged irregularity and segmental strictures and possibly marked thickening by excessive fibrosis with chronic inflammatory changes⁽²²⁾.

In view of the present data, it is evident that the concomitant infection by *S. mansoni* and *F. hepatica* synergistically induced a reduction in the antioxidant enzymatic levels of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSHPX) that appeared relative to free radical generation from hepatic insult by *S. mansoni* and *F. hepatica*. Hence, these enzymes act altogether in a concert manner to scavenge reactive oxygen species⁽¹⁹⁾. Thus, the

spontaneous dismutation of the superoxide radical to hydrogen peroxide (H_2O_2) and molecular oxygen occurs with superoxide dismutase activity⁽⁴⁸⁾, as well, catalase causes breakdown of H_2O_2 to water and oxygen⁽⁴⁹⁾. Moreover, both enzymes act cooperatively in reducing reactive oxygen species (ROS)⁽⁵⁰⁾. Furthermore, GSHPX removes H_2O_2 generated by SOD in cytosol and mitochondria by oxidizing the reduced glutathione GSH into its oxidized form GSSG^(51, 52, 53). 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 polymorph nuclear leucocytes: chemotaxis and free radical generation induced by phagocytosis that was verified by the effects of adult fluke excretion-secretion (E/S) products which inhibited phagocytosis and /or free radical generation in a dose and time dependent manner⁽⁵⁴⁾.

Other reports have indicated that the *F. hepatica* doesn't express catalase, however it expresses little glutathione peroxidase besides the influence of peroxi-redoxin 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⁽¹³⁾. Other reports indicated that the catalytic activities of rat cytosolic GSHPX in the course of fascioliasis was not statistically altered⁽⁵⁵⁾. Nonetheless, in *F. hepatica* reports have identified the characterization of cytochrome C peroxidase (Cp) 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*⁽⁵⁶⁾.

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 eukaryotic cells and characterized as Cu/Zn SOD⁽⁴⁸⁾. The effect of *F. hepatica* excretory-secretory products was found to inhibit SOD output from human neutrophils⁽⁵⁷⁾. Moreover, as no catalase activity was detectable,

the possibility that SOD in those trematodes might have the particular importance of removing super oxide radicals⁽⁵⁸⁾.

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 even with concomitant increase in serum glutamate pyruvate transaminase (SGPT) 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*.

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تأثير الإصابة بالفاشيولا والبلهارسيا المعوية علي الوسائل الدفاعية لمضادة للأكسدة

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إن إصابة الكبد والقنوات المرارية بأنواع الطفيليات المختلفة مثل الفاشيولا والبلهارسيا المعوية تؤدي إلي كثير من المشاكل الطبية ولجراحية وبخاصة في المناطق الحارة.

الهدف من إجراء هذه الدراسة:

هو تقييم تأثير الإصابة المزوجة بكل من الفاشيولا والبلهارسيا المعوية بالمقارنة بالإصابة المفردة لأي منهما علي حدة علي الكبد والجهاز المراري من حيث قدرتهما علي مقاومة الأكسدة ونسبة أملاح لعصارة الصفراوية وكتلك الإنزيمات الدالة علي وظائف الكبد.

تم تشخيص الحالات المصابة بالفاشيولا بقياس نسبة الأجسام المضادة للفاشيولا في أمصال المرضى وعن طرق استخدام الأشعة فوق الصوتية والأشعة المقطعية ولمنظير المراري كما تم تشخيص حالات البلهارسيا المعوية عن طريق فحص وجود بويضات البلهارسيا المعوية في البراز وفي عينة شرجية من المريض باستخدام منظار القولون. هذا بالإضافة لاستخدام الأشعة فوق صوتية في تشخيص تليف الكبد.

احتوت هذه الدراسة علي أربع مجموعات مكونة من:-

- (١) ١٥ مريض بإصابة مزدوجة من كل من الفاشيولا وبلهارسيا المعوية.
- (٢) ١٥ مريض بالفاشيولا فقط.
- (٣) ١٥ مريض بالبلهارسيا المعوية فقط.
- (٤) ١٥ شخص من الأصحاء غير المصابين بأي من الفاشيولا أو البلهارسيا المعوية أو أي من الأمراض الأخرى.

تم تقييم لوسائل الدفاعية لمضادة للأكسدة الموجودة في أمصال الأشخاص في جميع المجموعات التي تضمنتها الدراسة وذلك عن طريق:-

- قياس نسبة الإنزيمات المضدة للأكسدة مثل: سوبر أكسيد ديسميوتيز وكاتاليز وجلوتاثيون بيروكسيديز.
- قياس نسبة الفيتامينات المضدة للأكسدة مثل: فيتامين أ، ج، هـ.
- قياس نسبة أملاح العصارة الصفراوية لكلية كذلك قياس وظائف الكبد.

النتائج الخاصة بهذه الدراسة أوضحت:

أن هناك نقصاً ملحوظاً في نسبة كل من الإنزيمات المضادة للأكسدة والفيتامينات المضادة للأكسدة في مجموعت المرضى (المجموعة ١، ٢، ٣) بالمقارنة بمجموعة الأشخاص الأصحاء (مجموعة ٤). وقد كان هذا لنقص كبيراً في المجموعة الأولى والتي تشمل علي مرضى الإصابة المزوجة بكل من الفاشيولا والبلهارسيا المعوية. أما أملاح العصارة الصفراوية الكلية ووظائف الكبد فقد حدثت زيادة في النسب الخاصة بهم في مجموعات المرضى بالفاشيولا (مجموعة ١، ٢) بالمقارنة بمرضى البلهارسيا المعوية فقط (مجموعة ٣) ومجموعة الأشخاص الأصحاء (مجموعة ٤).

من هذه لنتائج السابقة يمكن استنتاج:

أن إصابة الكبد والجهاز المراري بالفاشيولا أو البلهارسيا المعوية أو كليهما معا يؤدي إلي نقص ملحوظ في الوسائل الدفاعية المضادة للأكسدة والتي تشمل الإنزيمات والفيتامينات المضادة للأكسدة الموجودة في الأمصل بينما وجد ازدياد ملحوظ في أملاح لعصارة الصفراوية ووظائف الكبد في مرضى الفاشيولا وهذه النتائج تؤدي علي تخفيف حدوث عملية التليف الكبدي ومن هنا تؤكد علي ضرورة استعمال مضادات الأكسدة في علاج الفاشيولا والبلهارسيا المعوية مع الأدوية التقليدية لتقليل حدوث لتليف الكبدي.