Some Biochemical and Histopathological Effects of *Cynodiplostomum Azimi* (Trematode, Digenea) Parasite on Hepatoxic Rats

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Abstract: The aim of the present study was to assess some biochemical and histopathological changes in the hepatotixic rats infected with the parasite *Cynodiplostomum azimi*. In this study twenty four adult male rats were used, each weighting about 100-120 g. These animals were divided into four groups. Group (A) used as control (uninfected rats), group (B) contains infected rats with parasite (*Cynodiplostomum azimi*), animals of group (C) received Carbontetrachloride (CCl_4 99%) to induce liver injury, and animals of group (D) were infected with the parasite followed by CCl_4 treatment. The results of the biochemical analysis revealed significant decrease in the activity of the AST enzyme in group (D), in total protein in groups (B, C and D), albumin in groups (C and D), globulin in groups (B and C) and total bilirubin in groups (B and D) compared to control. On the other hand, a significant decrease was observed in malondialdehyde (MDA) in group (D), SOD in group (B) and in GPx in group (B, C and D) compared to control. From the histopathological point of view liver sections taken from the liver of animals infected with the *Cynodiplostomum azimi* group (B) was manifested vacuolation of the hepatocytes together with nuclear pyknosis in addition to sinusoidal cells activation. Mononuclear cells aggregations specially around blood vessels were also observed. Moreover, mild focal hepatic cells necrosis was also observed. On the other hand the histopathological changes were manifested in the liver of animals treated with CCl_4 (group C) showed apoptosis and necrosis of hepatic cells, but the liver belonged of animals infected with the parasite followed CCl_4 treatment (group D) showed congestion of the blood vessels. In some cases hemorrhages in addition to obvious increase in the apoptotic figures. Mononuclear cells infiltrations specially around the blood vessels and bile ducts have been obviously detected.

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Keywords: antioxidant enzymes (MDA - SOD- CAT-GPX) -Total protein- Albumin -Globulin- Total bilirubin- Aspartate and alanine aminotransferases AST and ALT *Cynodiplostomum azimi*– hepatotoxic rats.

1. Introduction

Trematode, also called flukes, infections occur worldwide causing various clinical infections in humans. Trematodes normally enter the body through the act of eating uncooked fish or plants and fish from fluke-infested water. The trematode parasite causes histopathological changes in the intestinal architecture (Gomaah, 1988). Since, the liver regulates many important metabolic functions, so the hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999). Oxidative stress and enhanced lipid peroxidation have been associated with several models of liver injury (Panozzo *et al*.1995).

The generation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, hydroxyl radical, and singlet oxygen, in biological systems are dependent on oxygen consumption and can cause cellular damage by lipid peroxidation (Sanchez-Campos *et al* 1999). Murray, *et al*. (1993) showed that products of lipid peroxidation formed in various biochemical reactions are normally scavenged by antioxidants.

In a number of studies, it has been demonstrated that in the cells of hosts infected with different species of parasites, the amount of reactive oxygen radicals which cause lipid peroxidation are increased, thereby causing cell and tissue damage (Sarin *et al*.1993 and Santiard, *et al*. 1995).

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. The antioxidative defense system (AOS) is necessary for the maintenance of redox homeostasis in organisms. Oxygen free radicals and other reactive oxygen species (ROS) can react with the main cellular components, causing oxidative stress, which includes oxidation of proteins, DNA, as well as peroxidation of unsaturated lipids in cell membranes. Antioxidant defenses system are widely distributed and include both enzymatic and nonenzymatic components that neutralize ROS that are continuously produced during aerobic metabolism. The major enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). Reduced glutathione, vitamin C, vitamin E, β-

carotene, ceruloplasmin and bilirubin are some of the nonenzymatic factors that may function as antioxidants (Murray, *et al.* 1993 and Niki *et al.* 1994). These enzymes have been proposed as biomarkers of contaminant or seasonally mediated oxidative stress in a variety of marine and freshwater organisms and their induction reflects a specific response to pollutants Borković, *et al*. (2005).

Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplements are vital to combat oxidative damage. Yeter, *el al.* (2008) found that the concentration of malondialdehyde (MDA), activity of glutathione peroxidase (GPX), ALT and AST serum activities were higher in infective sheep liver with natural *distomatosis* than in the control group, in contrast superoxide dismutase (SOD), catalase (CAT)) were significantly lower than in the control group. Rodrigo, *el al.* (2011) suggested that liver diseases are often associated with hyperglycemia, inflammation, and oxidative stress where administrated glycolaldehyde might play an important role in liver diseases through impairment of the antioxidant defences and generation of advanced glycation end products. In children with chronic B or C hepatitis, low catalase (CAT) and superoxide dismutase (SOD) activity evidence of increased lipid peroxidation indicated inadequate antioxidant defence (Chrobot *et al.* 2000). Detection of an increase in malondialdehyde (MDA) levels, which is a product of lipid peroxidation in hepatitis B virus (HBV), indicates that oxidative stress is increased in HBV infection (Acar *et al*. 2009).

Carbon tetrachloride (CCl_4) is one of the oldest and most widely used toxins for experimental induction of liver fibrosis and cirrhosis in laboratory animals (Tsukamoto, *et al*.1990 and Parola, *et al.* 1992).

Many reports indicate that CCl4 causes necrosis, fibrosis, mononuclear infiltration, steatosis and foamy degeneration of hepatocytes and cirrhosis in the liver (Al-Shabanah *et al*., 2000; Teocharis, *et al*., 2001; Kus, *et al*., 2005). CCl4 has also been reported to cause apoptosis in liver cells (Noyan *et al*., 2006; Wu, *et al*., 2007).

The aim of this investigation was undertaken to assess some biochemical and histopathological effects on hepatotoxic rats infected with *Cynodiplostomum azimi*.

2. Materials and Methods

Twenty four adults male rats were used in the present study, each weighing about 100-120 g. The experimental animals were fed on diet consisted of 10% casein, corn oil, 4% salt mixture, 0.2% choline chloride, 0.3% methionine, 1% vitamin mixture, 5% cellulose and 69% corn starch (Muller, 1964).

For experimentation, rats were divided into four groups. The first group containd unifected rats as control (A). Animals of the second group (B) were infected with *Cynodiplostomum azimi* (100 ± 5) cyst /rat). Animals of the third group (C) received carbon tetrachloride (CCl_4) to produce hepatotoxic effect. Animals of the fourth group (D) were infected with *Cynodiplostomum azimi*, then given CCl_4.

The used rats (group B) were individually infected through oral inoculation with 100 ± 5 encysted metacercaria of the parasite *Cynodiplostomum azimi* which were obtained mechanically from the skeletal muscles of naturally infected *Clarias gariepinus* obtained from the Nile Delta (Khalil, 1987). Infection with the parasite was repeated weakly for six constitutive weeks. All groups were fed on the basal diet for six months.

Animals of group (C) were injected with 3ml carbontetrachloride (CCl_4 (99%) /kg/ bw to produce necrosis and steatosis in the liver of the experimental rats according to (Wensing *et al*., 1990).

Biochemical analysis:

Forbiochemical analysis, liver tissue samples were accurately weighed and homogenized in a 10-fold volume of ice-cold distilled water. The following parameters were determined.

The level of total protein, serum albumin, globulin and total bilriubin were estimated using the methods of Henry (1964), Bartholomev and Delaney (1966), and Jendrassik (1938), respectively. The level of aspartate aminotransferases (AST) and alanine aminotransferases (ALT) activeties were measured according to the method of (Bohnert *et al*., 2000). Liver lipid peroxidation product, malondialdehyde (MDA), content, catalase, (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were determined according to the methods of Esterbauer and Cheeseman (1990), Xu *et al.* (1997), Nishikimi *et al.* (1972) and Pagila *et al.* (1967), respectively.

Histopathological study:

For this study, liver specimens of all groups were carefully taken from the animals and fixed in 10% neutral buffer formalin for 24 hours and washed in running water for 12 hours to remove excess of formalin. Specimens were then dehydrated in an ascending series of ethanol, cleared in xylol and embedded in paraffin wax to prepare block and then section of 5-6 um thick were prepared. The sections were then stained with haematoxylin and eosin (Bancroft *et al.*, 1996) and examined microscopically for histopathological alteration.

Statistical Analysis:

Data of the biochemical analysis were computed by comparing values of group (B), (C) and (D) with the values of the control group (A). Results from independent observations were expressed as mean ± SD. The significance among values was analyzed by using Student's *T-test*.

3. Results

The results of the liver function of all groups are presented in table (1). A significant decrease in the activity of the enzyme aspartate amino-transferases (AST) in group (D) was at $p < 0.01$ compared with the control, also the decrease in the activity of total protein in group (B) was at $p<0.01$, in group (C) was at $p<0.001$ and in group (D) was at $p<0.0001$ compared with the control. The decrease in the albumin content was significant in group (C) was at $p<0.01$ and in group (D) was at $p<0.0001$ compared with the control. Concerning the total globulin, a significant decrease was observed in group (B) and in group (C) at $p<0.0001$ compared to the control group. Also significant increase in total bilirubin in group (B) and in group (D) at $p<0.0001$ compared to the control group.

The result presented in table (2) showed a significant decrease in catalase enzyme (CAT) in group (D) was at $p < 0.01$ compared with the control, also a significant decrease in the activity of superoxide dismutase (SOD) in group (B) was at $p < 0.0001$ compared with the control, On the other hand, in the activity of glutathione peroxidase (GPx) the result showed that a significant decrease in groups (B) was at $p < 0.001$, in group (C) was at $p < 0.01$ and in group (D) was at $p < 0.001$ compared with the control.

Microscopical examination of a section in liver of normal rat (group A) showed normal hepatic lobules which is made up of radiating strands of hepatocytes forming a net work around a mildy dilated central vein. Blood sinusoids of normal diameters and hepatocytes with granular cytoplasm and centrally located nuclei each with one or two nuclei. Binucleate cells are also seen (Fig.3).

Examination of a liver section of a rat from group (B) infected with the parasite *Cynodiplostomum azimi* showed vacuolation of the hepatocytes (Fig.4) together with nuclear pyknosis in addition to sinusoidal cells activation. Mononuclear cells aggregations (Fig.5) especially around blood vessels were also observed. Moreover, mild focal hepatic cells necrosis (Fig.6) was also observed. Histopathological changes (Fig.7) were manifested in the liver of animals treated with CCl_4 (group C) apoptosis and necrosis of hepatic cells.

On the other hand examination of a liver section of a rat from group (D) showed congestion of the blood vessels and in some cases hemorrhages (Fig.8) in addition to obvious increase in the apoptotic figures (Fig.9) as well as mononuclear cells infiltrations especially around the blood vessels and bile ducts (Fig.10).

Table (1) Liver function results of all groups include AST, ALT, Total protein, Albumin, Globulin and total Bilirubin.

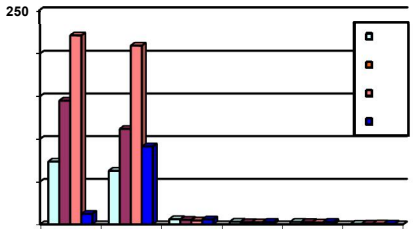
Parameter / **Group sample**	**AST** Mean ± SD	**ALT** Mean ± SD	**Total protein** Mean ± SD	**Albumin** Mean ± SD	**Globulin** Mean ± SD	**Total Bilirubin** Mean ± SD
Control (A)	73.5 ±9.98	62.75 ±5.91	6 ±0.82	3.1 ±0.29	2.9 ±0.83	0.63 ±0.07
Parasite Infection (B)	144.8 ±47.8	111.75 ±87.4	* 5.25 ±0.96	2.5 ±0.1	*** 2.68 ±0.85	*** 0.86 ±0.78
Hepato toxic rat (C)	221.25 ±18.50	209.25 ±4.65	** 4.5 ±0.58	* 2.38 ±0.15	*** 2.13 ±0.68	0.94 ±0.053
Parasite + Hepato toxic (D)	* 12.3 ±18.28	91.25 ± 5.91	*** 5.75 ±0.96	*** 2.92 ±0.17	2.82 ±0.99	*** 0.83 ±0.089

Values represent mean ± SD.
* Significant at $p < 0.01$
** Significant at $p < 0.001$
*** Significant at $p < 0.0001$

Table (2): Antioxidants enzyme Catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPX), and Malonaldehyde (MDA) in liver tissue of all experimental groups

Parameter / Groups	CAT mean± SD	SOD mean± SD	GPX mean± SD	MDA mean± SD
Control (A)	4.45 ±0.31	16.48 ±0.26	4.53 ±0.27	5.42 ±0.17
Parasite Infection (B)	3.24 ±1.14	*** 14.87 ±3.19	** 4.125 ± 0.537	7.13 ±0.084
Hepatotoxic rat (C)	0.92 ±0.02	5.63 ±0.48	* 2.55 ±0.28	21.1 ±0.85
Parasite + Hepatotoxic (D)	* 2.25 ±0.47	8.72 ±1.13	** 2.94 ±0.73	18.55 ±1.019

Values represent mean ± SD. * Significant at $p < 0.01$; ** Significant at $p < 0.001$ *** Significant at $p < 0.0001$



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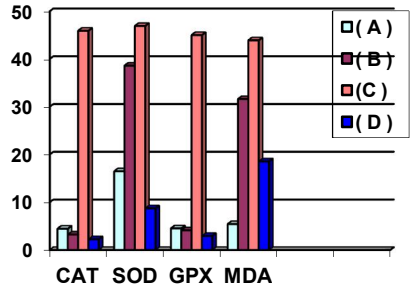

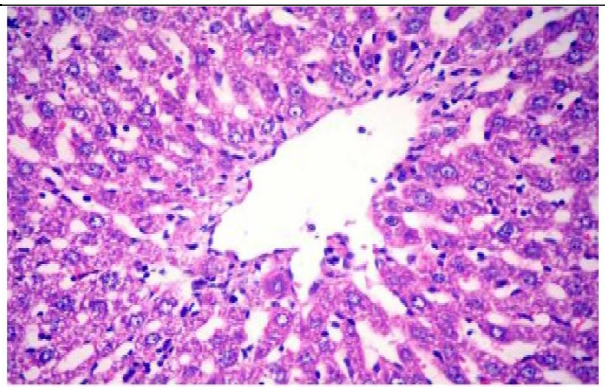

Fig. (3): liver of rat from group (A) showing the normal histological structure of hepatic lobules (X 20).

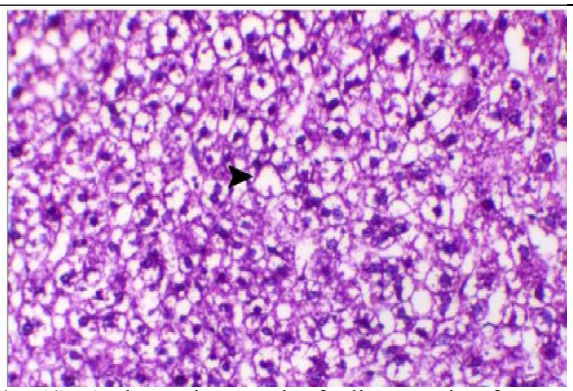

Fig. (4): A photomicrograph of a liver section from rat infected with *Cynodiplostomum azimi* (group B) showing hepatocytic cell vacuolation (head arrow) and nuclear pyknosis. (X 20).

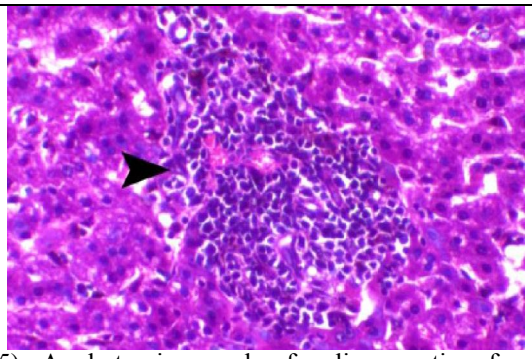

Fig.(5): A photomicrograph of a liver section from rat infected with *Cynodiplostomum azimi* (group B) showing mononuclear cells aggregations (head arrow) in addition to mild sinusoidal cells activation. (X 20).

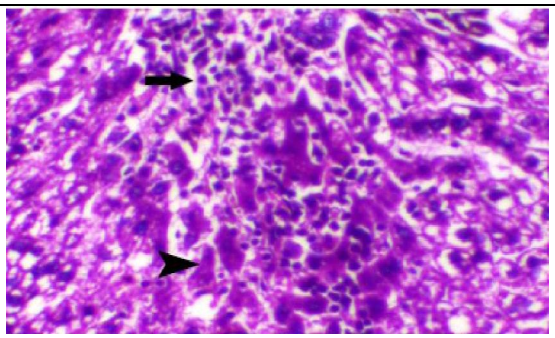

Fig.(6): A photomicrograph of a liver section from rat infected with *Cynodiplostomum azimi* (group B) showing focal hepatic cells necrosis where the necrosed cells (head arrow) were surrounded and infiltrated with inflammatory cells (arrow). (X 20)

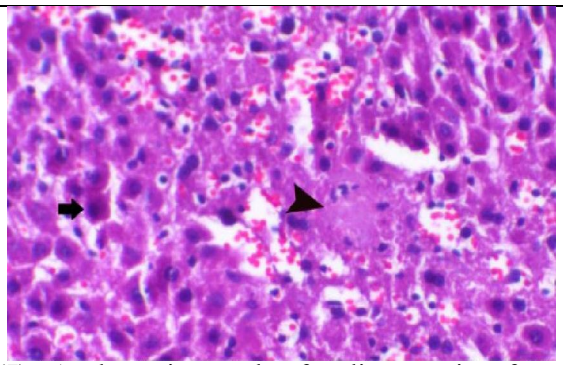

Fig.(7): A photomicrograph of a liver section from rat treated with CCl_4 group (C) showing apoptosis (arrow) and necrosis of hepatic cells (head arrow). (X 20).

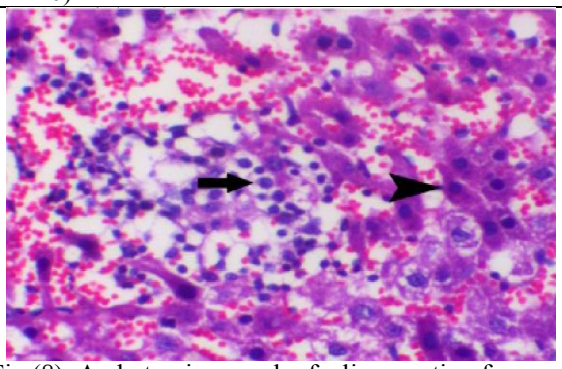

Fig.(8): A photomicrograph of a liver section from rat infected with *Cynodiplostomum azimi* and treated with CCl_4 group (D) showing hemorrhages as well as mononuclear cells infiltrations (arrow) and apoptotic cells (head arrow).(X 20)

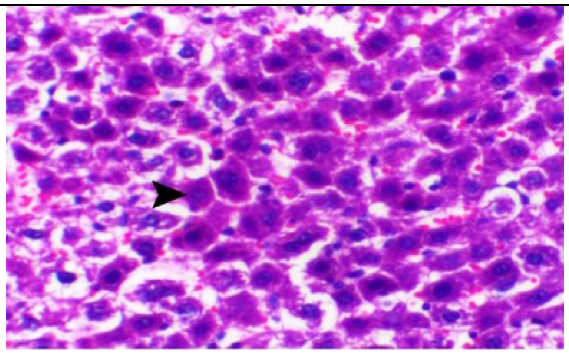

Fig.(9): A photomicrograph of a liver section from rat infected with *Cynodiplostomum azimi* and treated with CCl_4 group (D) showing apoptotic cells (head arrow). (X 20).

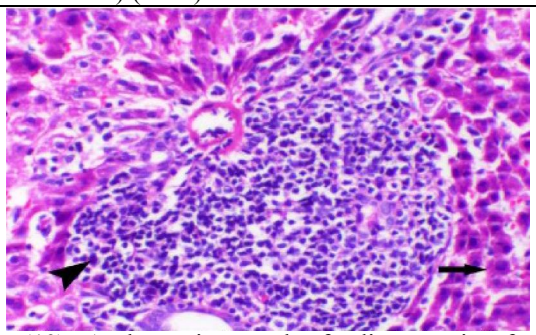

Fig.(10): A photomicrograph of a liver section from rat infected with *Cynodiplostomum azimi* and treated with CCl_4 group (D) showing mononuclear cells infiltrations (head arrow) especially around the bile ducts in addition to apoptosis (arrow). (X 20).

4. Discussion

The results of the current presented in tables (1 and 2) showed that significant variation between the values of SOD, GPx, total protein, globulin and total bilirubin in the infected rats blood with *Cynodiplostomum azimi* group (B) compared with normal healthly rats group (A). Cytoplasmic vacuolation of the hepatocytes together with nuclear

pyknosis have been detected and shown (Fig.4) Meantime sinusoidal cells activation and mononuclear cells aggregations (Fig.5) specially around blood vessels were also observed. Moreover, mild focal hepatic cells necrosis (Fig.6) compared to group (A) in (Fig.3).

These results pointed out a negative effect on the liver activities may be attributed to infection by the *Cynodiplostomum azimi* parasite.

The mentioned parasite exists at the host digestive canal, causing damages due to the mixing up between the excretion toxic material with the digestive matter and absorbed by the walls of intestine. Hence the liver of the host receives it via portal vein. Therefore, the liver activities are negatively affected due to presence of the parasites lead to liver disturbances. Toxic effect of the parasite may be more susceptible to deterioration immune system during the early life stages and in the adult form weakening the host immune system. Parasites are designed by evolution to invade the host and survive in its organism, until they are ready to reproduce then release a variety of molecules that help them to penetrate the defensive barriers and avoid the immune attack of the host Jolanta (2006).

Antioxidant enzymes are essential for parasites to defend themselves against the ROS generated by the macrophages, neutrophils and eosinophils of the host, in addition to their normal functions in aerobic organisms (Sies, 1993).

Aside from superoxide dismutase, catalase and glutathione peroxidase, peroxiredoxins are probably the major H_2O_2-detoxifying enzymes in parasites.

Secretion of antioxidant enzymes is a stage-specific and there are examples of regulation of their expression by the concentration of reactive oxygen species surrounding the parasite. The majority of parasite-secreted molecules are commonly found in free-living organisms, thus parasites have only adapted them to use in their way of life Jolanta (2006).

Bilirubin is one of the nonenzymatic factors that may function as antioxidants which have a cellular protective action against oxidative stress resulting in cell, organ and tissue damage as result of parasitic invasion Murray *et al*. (1993).

The immune system is a sensitive target to the toxic action of chemicals in several organisms (George 1983 and Fournier *et al.* 2002). CCl_4 compound has toxic effect on liver, therefore this toxicity has been recorded significant effects on total protein, globulin and GPx in group (C) causing apoptosis and necrosis of hepatic cells (Fig. 7). The combined parasite and CCl_4 has indicated increasing significant effects in aspartate amino-transferases (AST), biliruin content, and catalase enzyme (CAT) in group (D).That increasing significant effects produced histopathological changes including hemorrhages as well as mononuclear cells infiltrations and apoptotic cells specially around the bile ducts Fig. (8,9,10) which have been agreeded upon by Wu *et al*. (2007) found that treatment of rats with carbon tetrachloride produced severe liver injury, as demonstrated by dramatic elevation of serum ALT, AST levels and typical histopathological changes. including hepatocyte necrosis or apoptosis, haemorrhage, fatty degeneration, In addition, carbon tetrachloride administration caused oxidative stress in rats, as evidenced by increased reactive oxygen species (ROS) production and MDA concentrations in the liver of rats, along with a remarkable reduction in hepatic SOD activity and GSH content.

Changes in the antioxidant abilities of the liver accompanied by activities of CAT, SOD, GPx, AST, total protein, albumin, globulin and total bilirubin, this agreed with the study of Yeter, *et al.* (2008) who found that changes in the antioxidant abilities of the liver and changes in the phospholipid structure of the cell membrane were accompanied by rising activities of ALT and AST as markers of liver damage.

It has been suggested that the lipid peroxidation may be a link between tissue injury and liver fibrosis by modulating collagen gene expression Parola *et al*. (1993). It was reported that CCl_4 is suitable to induce lipid peroxidation in experimental animals within a few minutes after administration, and it is a long-term used results in liver fibrosis and cirrhosis by lipid peroxidation pathway Sherlock (1970). It is generally thought that CCl_4 toxicity is due to reactive free radical (CCl_3.), which is generated by its reductive metabolism by hepatic cytochrome P450. The reactive intermediate is believed to cause lipid peroxidation and breakdown of cellular membranes De Groot and Sies (1989). Mehmet *et al.*(2005) found decreased the antioxidant enzyme levels and liver enzymes in CCl_4 -treated rats.

It was demonstrated that lipid peroxidation led to increase the concentrations of enzymatic and non-enzymatic antioxidants compounds. These results are in agreement with the results of Değer1 *et al.*(2008) found that antioxidant enzyme has a cellular protective role against oxidative stress resulting in liver tissue damage as a result of parasitic invasion.

Our data indicated that the presence of parasite may contribute in suppressing immune system and causing disturbance host environment allowing, or may be facilitating survival any other viruses in the host. It could raise ability of other organisms to be more susceptible to cause damage. This agreed with Bashir *et al.* (2002) found that tendency of many parasitic worms to pacify the host's immune response allows them to mollify some diseases while

worsening others. Also Kamal and Khalifa (2006) indicated that ability to effectively respond to that is less responsive than normal the immunological milieu associated with helminthic infections and its impact on viral infections, mainly hepatitis B virus, hepatitis C virus, and human immunodeficiency virus in humans and experimental animals. Also, (Gorchilova *et al*.,1986 and Kirby *et al*., 1994b) found that functional changes of the parasite may have been caused either indirectly via toxicant altering the structure and metabolism of the host liver tissue or by the pollutant directly affecting *F. hepatica*. Baey *et al.* (2011) found that histological distributions specific to CsPRx2 and CsPRx3 as explained that parasitic worms often weaken the immune system's antioxidant enzymes in *Clonorchis sinensis*, which invades the human hepatobiliary tracts might suggest different physiological functions of the antioxidant enzymes in protecting the worms against oxidative damage. infection with *Cynodiplostomum azimi* cysts may have the ability to certain immune system cells, leading to a gentler immune response and often, such a response is beneficial to parasite that of to activate the virus to be acute through pollution of the host immune system to play a role in its tissues degradation this suggestion was in agreement Hartgers and Yazdanbakhsh (2006) and Correale and Farez (2007).

Conclusion:

The contamination of environment with *Cynodiplostomum azimi* cysts parasite has a negative effect on antioxidant system of host represent by MDA - SOD- CAT-GPX- Total protein- Albumin - Globulin- Total bilirubin- Aspartate and alanine aminotransferases AST and ALT) as biomaker to distribance heapatocyte specily when organisms were exposed to toxic matter.

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References

Acar, A.; Görenek, L.; Aydin, A.; Eyigün,C.P.; Eken, A.; Sayal,A. and Pahsa,A. (2009): Investigation of oxidative stress and antioxidant defense in patients with hepatitis B virus infection and the effect of interferon-alpha plus lamivudine combination therapy on oxidative stress. Mikrobiyol Bul. 43(3):411- 423.

Al-Shabanah, O.A.; Alam, K.; Nagi, M.N.; Al-Rikabi, A.C.and Al- Bekairi, A. M. (2000): Protective effect of aminoguanidine a nitric oxide synthase inhibitor, against carbon tetrachloride induced hepatotoxicity in mice. Life Sci. **66**: 265–270.

Baey,A, S.; Kim, H.; Lee, E. G.; Sohn,W. M. and Kong,Y. (2011): Identification and biochemical characterization of two novel peroxiredoxins in a liver fluke, Clonorchis sinensis. Parasito. 138: 1143-1153.

Bancroft, J.D.; Stevens, A. and Turner, D.R. (1996): Theory and practice of histological technique 4th ed. New York, Churchill, Livingstone.

Bartholomev, R.J. and Delaney,A. (1966): Proc Aust. Assoc. Biochemists. 1, 214.

Bashir, M.E.; Anderson, P. and Fuss, I. (2002): "An enteric helminth infection protects against an allergic response to dietary antigen." J Immunol. 169(6): 3284-3292.

Bohnert, J.; Hubner, B. and Botzenhail, H. (2000): Rapid identification of liver function test using a novel 23Sm RNA - targeted oligonudeateoids probe. In. J. Hyg. Eviron. Health., 203(1):77-82.

Borković, S. S., Šaponjić, J. S., Pavlović, S. Z., Blagojević, D. P., Milošević, S. M., Kovačević, T. B., Radojičić, R. M.,Spasić, M. B., Žikić, R. V., and Z. S. Saičić (2005): The activity of antioxidant defence enzymes in the mussel Mytilus galloprovincialis from the Adriatic Sea. Comp. Bioch. Physiol. **141**C, 366 – 374.

Chrobot, A.M.; Szaflarska, S. A.and Drewa, G. (2000): Antioxidant defense in children with chronic viral hepatitis B and C. Med. Sci. Monit. 6(4):713–718.

Correale, J. and Farez, M. (2007). "Association between parasitic infection and immune response in multiple sclerosis." Annals of Neurol. 61(1): 97-108.

Değer1 Y, Ertekin1 A, Değer S, Mert H.(2008): Lipid Peroxidation and antioxidant potential of sheep liver iInfected naturally with Distomatosis. Türkiye Parazitol. Derg. 32 (1): 23-26.

De-Groot, H. and Sies, H. (1989): Cytochrome P-450, reductive metabolism, and cell injury. Drug Metab. Rev; 20: 275-284.

Esterbauer, H. and Cheeseman, K. H. (1990): Determination of aldehydic lipid peroxidation products: malondialdehyde and 4-hydroxynonenal. Methods Enzymol.; 186: 407-421.

Fournier, M.;Pellerin, J.; Lebeuf, M.; Brousseau, P.; Morin, Y.; Cyr, D.(2002) Effects of exposure of Mya arenaria and Mactromeris polynyma to contaminated marine sediments on phagocytic activity of haemocytes. Aquat Toxicol. 59:83–92.

George, S.G. (1983): Heavy metal detoxication in Mytilus edulis kidney Comp Biochem Physiol C. 76:59–65.

Gomaah, M. M. (1988): Studies on experimental Cynodiplostomum Azimi infection in a mammalian host. B.Sc. Thesis, Fac. Of Sci., Tanta Univ., Egypt.

Gorchilova, L.; Polyakova-Krusterva, O. and Vinarova, M. (1986): Electron microscope study of the tegument and intestinal wall of Fasciola hepatica isolated from experimentally infected rots with chronic diethlnitrosamine intoxication. Khelmintologiya. 22: 9-14.

Hartgers, F. C. and Yazdanbakhsh, M. (2006): "Co-infection of 443henobarbi and malaria: modulation of the immune responses to malaria." Parasite Immunol. 28(10): 497-506.

Henry, R. J. (1964): Clinical Chemistry, Harper and Row publishers, New York. P.181.

Mehmet, K.; Omer, C.; Mustafa, B. (2005): Hepatoprotective effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, ntioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats World J. Gastroenterol 14; 11(42): 6684-6688.

Muller, A. (1964): Vitamin mixture. J. Biol. Chem., 20: 150 – 305.

Murray, R.K.; Mayes, A.A.; Granner, D. K. and Rodwell, V.W. (1993): Harper's Biochemistry. 183.

Niki E, Shimaski H, and Mino M. (1994): Antioxidantism-free radical and biological defense. Gakkai Syuppn Center. Tokyo 3-16.

Nishikimi, M.; Roa, N. A.; and Yogi, K (1972): Biochem. Bioph. Res. Common., 46, 849-854.

Noyan, T.; Komuroglu, U.; Bayram, I. and Sekeroglu, M.R. (2006): Comparison of the effects of melatonin and pentoxifylline on carbon tetrachloride-induced liver toxicity in mice.Cell Biol.Toxicol. 22: 381–391

Pagila and Valentine, W.N. (1967): Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidae. J. Lab. Clin. Med. 70:158-169.

Panozzo. P.M.; Basso, D.; Balint, L.; Biasin, M.R.; Bonvicini, P.; Metus, P. (1995): Altered lipid peroxidation/glutathione ratio in experimental extrahepatic cholestasis. Clin. Exp. Pharmacol. Physiol. 22: 266-271.

Parola, M. Leonarduzzi, G. Biasi, F. Albano, E. Biocca, M.E. Poli, G. Dianzani, M.U. (1992): Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. Hepatolo. 16: 1014-1021.

Jendrassik, L. (1938): Colorimetric determination of bilirubin. Biochem. 97:72-81.

Jolanta M. D. (2006). Molecules released by helminth parasites involved in host colonization. Acta Biochimica Polonica. 53(1), 33–64.

Kamal, S. M. and Khalifa, K. E. S. (2006): "Immune modulation by helminthic infections: worms and viral infections." Parasite Immunol. 28(10): 483-496.

Khalil, A. I. A. (1987): Biochemical and pathological changes in Clarias lazera (cat fish) naturally infected with metacercariae parasites in Gharbiah province. Ph.D., Thesis, Fac. Of Sci., Tanta Univ., Egypt.

Kirby, G.M.; Pelkonen, P.; Vatanasapt, V.; Camus, A.M.; Wild, C.P.; and Lang, M.A. (1994b): Association of liver fluke (*Opisthorchis viversini*) infection with increased expression of cytochrome P450 and carcinogen metabolism in male hamster liver. Mol. Carcinog. **11:** 81–89.

Kus, I.; Ogeturk, M.; Oner, H.; Sahin, S.; Yekeler, H.and Sarsilmaz, M. (2005): Protective effects of melatonin against carbon tetrachloride-induced hepatotoxicity in rats. Cell Biochem. Funct. 23: 169-174.

Rodrigo, L.; Michael, E.; Rafael, C.; Ryoji, N.; Felipe, D. and José, C. (2011): Oxidative damage in the liver of rats treated with glycolaldehyde. Int. J. Toxicol. 30 (2):253-258

Sarin, K. Kumar, A. Prakash, A. Sharma, A. (1993): Oxidative stress and antioxidant defence mechanism in Plasmodium vivax malaria before and after chloroquin treatment. J Malariol. 30: 127-133.

Sanchez-Campos, S.; Tunon, M.J.; Gonzalez, P.; Gonzalez-Gallego,J. (1999): Oxidative stress and changes in liver antioxidant enzymes induced by experimental dicroceliosis in hamsters. Parasitol. Res. 85: 468-474.

Santiard, D.; Ribiere,C.; Nordmann, R.and Houee-Levin, C. (1995): Inactivation of Cu, Zn-superoxide dismutase by free radicals derived from ethanol metabolism: a gamma radiolysis study. Free Radic. Biol. Med. 19: 121-127.

Sherlock, S. (1970): Drugs and the liver G. Clin. Med. 51: 753-759.

Sies, H. (1993): Strategies of antioxidant defense. Eur. J. Biochem. 215, 213–219.

Teocharis, S.E.; Margeli, A.P.; Skaltsas, S.D.; Spiliopoulou, C.A. and Koutselinis, A.S. (2001): Induction of metallothionein in the liver of carbon tetrachloride intoxicated rats: an immunohistochemical study. Toxicology 161: 129–138.

Tsukamoto, H.; Matsuoka, M. French, SW. (1990): Experimental models of hepatic fibrosis: a review. Seminar in Liver Disease 10: 56-65

Xu, J.B.; Yuan, X.F. and Lang, p.z. (1997): Determination of catalase activity and catalase inhibition by utltaviolet spectrophotometry.Chinase. Envron. Chem.16: 73-6.

Wensing, G.; Sabra,R. and Branch,R.A.(1990): Renal and systemic hemodynamics experimental cirrhosis in rats relation to hepatic function. Hepatology 12: 13-19.

Wolf, P. (1999): Biochemical diagnosis of liver diseases. Ind. J. Clin. Biochem. 14(1), 59-90.

Wu, Y.; Li, L.; Wen, T.and Li, Y.Q. (2007): Protective effects of echinacoside on carbon tetrachloride-induced hepatotoxicity in rats. Toxicolo. 22 (2):50-56.

Yeter, D.; Ali, E.; Serdar, D.; Handan, M.(2008): Lipid peroxidation and antioxidant potential of sheep liver infected naturally with Distomatosis. Turkiye Parazitol. Dergisi. **32**(1) 023-026.

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