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The possible protective effect of captopril on liver and bone marrow after cyclophosphamide-induced toxicity in adult albino rats

Samah AboZaid*, Abdelhamid Abobakr, Abil Hassan, Merry Malak
Anatomy department, Minia medical college.
* Correspondence:samah.abuzaid@ucm.edu.sa.com; Tel:+020862347460; Fax: +20862347460

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Abstract

Cyclophosphamide (CP) is commonly used in regimen of treatment of cancers and autoimmune diseases as systemic lupus. Hepatotoxicity and bone marrow depression are acute side effects of CP through inducing oxidative stress. The concomitant use of captopril ameliorates these side effects. This study aimed to evaluate the possible protective effects of captopril against hepatotoxicity and bone marrow depression induced by CP in adult albino rats. In this experimental study, 60 rats were divided into 4 groups; the control group, the captopril treated group, CP treated group and the captopril-CP treated group. The liver tissues and bone marrow of rats in all groups were subjected to light and electron microscopic studies. The serum levels of liver function markers were assessed. The results showed that the injection of CP elevate serum ALT and AST compared to the control group. Concomitant treatment of captopril and CP decreases serum levels of ALT and AST. CP treated group showed diffuse lesion of liver tissue and bone marrow. The captopril-CP treated group showed minimal hepatic congestion and decreased adipose tissue in bone marrow. The current study suggested that captopril has protective effect against cyclophosphamide induced hepatotoxicity and myelotoxicity.

Introduction

Cyclophosphamide (CP) is an alkylating agent nitrogen mustard, commonly used to treat various types of cancer as chemotherapy regimen. It is also used in the treatment of autoimmune diseases as systemic lupus, vasculitis, lupus nephritis and scleroderma (Emadi et al, 2009) also as an adjunct to surgery and radiation in a variety of cancers such as retinoblastoma, Wilms' tumor, rhabdomyosarcoma and Ewing sarcoma (Akay et al, 2006). It is believed to work by interfering with the duplication of DNA and the creation of RNA, inhibiting the protein synthesis, it has cytotoxic effect on cancer cells. Besides antimitotic and ant replicative effects, CP has immunosuppressive as well as immunomodulatory properties. It can be used in high dose therapy for complete eradication of hematopoietic cells also increase the number of myeloid-derived suppressor cells causing bone marrow depression. Hepatotoxicity is a common side effect that occurs with CP as a result of many mechanisms, including production of free radicals (Pacini et al, 2009).

Captopril was the first angiotensin-converting enzyme (ACE) inhibitor for oral administration and it has been widely studied in the treatment of patients with hypertension and patients with chronic congestive heart failure (Bakris et al, 2000). It provides beneficial effects on cardiovascular system and renal protection (Higashi et al, 2000). Captopril is also believed to function as an antioxidant by increasing the activities of antioxidant enzymes such as super oxide dismutase and glutathione peroxidase. It is also found to protect both bone marrow and hepatocytes as it acts a free radical scavenger because of its terminal sulfhydryl group (Gurer et al, 1999).

The current study aimed to assess the possible protective effects of captopril in CP-induced hepatotoxicity and myelotoxicity in rats.

Materials and Methods

In this study 60 adult males albino rats weighing 200-220 grams were used. They were housed in clean plastic cages (as 5 rats /cage) in the laboratory room of the anatomy department in the faculty of medicine, Minia University under standard laboratory conditions. All animals were given free access to food and water. The experiment was approved by the ethical committee for animal handling for research work in Minia University.

The rats were subdivided into 4 groups 15 rat in each group as following:

- Group I (control): received only food and distilled water .
- Group II (captopril): received captopril tablet (50 mg/kg), daily for 5 days orally (Gandhi et al, 2004 .(
- Group III (CP): received CP(150 mg/kg), a single dose intraperitoneal administration (Massafra et al, 2000.(
- Group IV (captopril-CP): receiving captopril (50mg/kg, daily for 5 days orally) followed by CP (150 mg/kg, a single dose intraperitoneal administration .(

All rats in all groups were sacrificed by decapitation under light halothane; the liver tissues and bone marrow of rats were removed for further analysis.

The tissues were divided into small pieces for light and electron microscopic studies :

1-Histopathological assay: The liver and bone marrow will be dissected from control and the experimentally treated animals.

Tissue samples are fixed in 10% formaldehyde and proceed to routine histological process Sections of 5 µm are prepared then stained with hematoxylin and eosin (H & E) to evaluate the histological structure of the liver and bone marrow (Jones et al, 2008). Electron microscopic examination: The liver specimens were immersed in 2.5% glutaraldehyde (pH 7.4). Ultrathin sections were double stained with 4% uranyl acetate and 0.1% lead citrate. It was performed in the Regional Centre for Mycology and Biotechnology (RCMB), Assiut University using Joel 100S transmission electron microscope at 60 KV (Hayat, 1986.(

2-Serum biochemical assay: The liver enzymes as ALT and AST were determined using the quantitative detection kits expressed as U/L. Complete blood count for all groups were assessed .

3-Statistical analysis: Quantitative results were statistically described in terms of mean and standard deviation (mean ± SD) and were analyzed using statistical package SPSS version 24 by one-way ANOVA and Tukey tests. P value < 0.05 was considered statistically significant.

Results:

Assessment of Liver enzymes:

The serum level of hepatic markers such ALT and AST were measured in blood samples of each group as indicators of liver toxicity. The results showed that the injection of CP increased serum ALT and AST activities compared to the control group. Concomitant treatment of CP and captopril significantly decrease ALT and AST values as shown in table 1& histogram 1.

Table 1: Serum ALT&AST Levels in the different Studied groups (IU/L).

		Mean ± SD of ALT	F	P value
Experimental groups	Control (15)	31.8 ± 4.7	265.727	0.0001 *
	Captopril (15)	35.5 ± 3		
	CP (15)	63 ± 2.6		
	Captopril and CP (15)	46.6 ± 2.5		
		Mean ± SD of AST	F	P value
Experimental groups	Control (15)	29.5 ± 2.8	707.718	**0.0001 ***
	Captopril (15)	31.9 ± 2.5		
	CP (15)	71 ± 3		
	Captopril and CP (15)	50.5 ± 2.9		

Assessment of blood elements:

Blood samples of each group are examined for complete blood count as indicators of bone marrow toxicity. The results showed that the injection of CP decreased hemoglobin level p value<0.05 (table 2). Compared to the control group also CP injection decreased red blood cells and white blood cells p value 0.0001 (table 3&4). Concerning platelets, the group treated with CP revealed significant decrease in platelet count p value<0.05 (table 5). On the other hand, administration of captopril prior to CP, improve these parameters p value<0.05

Table 2: Effect of CP and captopril on hemoglobin(Hb%) level(g/dl)

		Mean ± SD of Hb	F	P value
Experimental groups	Control (15)	15.9 ± 1.2 (14.5-18)	59.387	0.0001*
	Captopril (15)	16.3 ± 1.1 (14.5-18)		
	CP (15)	11.4 ± 0.6 (10.5-12.3)		
	Captopril and CP (15)	15.5 ± 1.5 (10.5-18)		

Table 3: Effect of CP and captopril on red blood cells count (RBCs) (cell/ mm^3)× 10^6

		Mean ± SD of RBCs	F	P value
Experimental groups	Control (15)	7.9 ± 0.5 (7-8.5)	197.983	**0.0001***
	Captopril (15)	7.9 ± 0.4 (7.2-8.5)		
	CP (15)	5.1 ± 0.4 (4-5.5)		
	Captopril and CP (15)	6.2 ± 0.2 (6-6.4)		

Table 4: White blood cells (WBCs) (cell/ mm^3)× 10^6 in the different Studied groups

		Mean ± SD of platlets	F	P value
Experimental groups	Control (15)	570.2 ± 91.8 (420-673)	63.893	**0.0001***
	Captopril (15)	511.3 ± 62.2 (400-600)		
	CP (15)	283.3 ± 63.5 (200-390)		
	Captopril and CP (15)	569.3 ± 33.3 (500-600)		

Histological study:

Light microscopic examination of liver tissue

1- Control group:

The liver sections of control group revealed a normal architecture of hepatic lobules; the hepatocytes radiated like spokes of a wheal from the central vein, forming anastomosing plates of liver cells, separated from each other by hepatic sinusoids (Fig.1).

The hepatocytes appeared polyhedral with acidophilic cytoplasm and large rounded nuclei. The nuclei of hepatocytes were vesicular with prominent nucleoli. Hepatocytes may be binucleated. Portal tract contains many structures including branches of portal vein with large diameter and thin wall, branches of hepatic artery with small diameter and thick wall and branches of bile duct that is lined by simple cuboidal epithelium (Fig.2).

2- Captopril-treated group:

The histological sections of this group were similar to control group without apparent difference (Figs.3&4).

3- CP-treated group:

Liver sections showed marked distortion of hepatic architecture and significant dilated sinusoids especially in the central areas of the liver with marked congestion of the central vein (Fig.5). In the periportal region, there is congestion of the portal vein and liver sinusoids are markedly dilated (Figs.6&7).

The hepatocytes were swollen and ballooned with vacuolated cytoplasm and pyknotic enlarged nuclei were observed, also inflammatory cell infiltration appeared (Fig.6). Apoptotic cells were also scattered around the central vein. Apoptotic hepatocytes are characterized by having hyper eosinophilic cytoplasm and condensed darkly stained nuclei (Fig.8).

4- Captopril-CP treated group:

Improvement of hepatic congestion and reduction of inflammatory infiltration, hepatic sinusoids were mildly dilated (Fig.12). Also, there were few apoptotic hepatocytes around the central vein compared with CP-treated group (Fig.13).

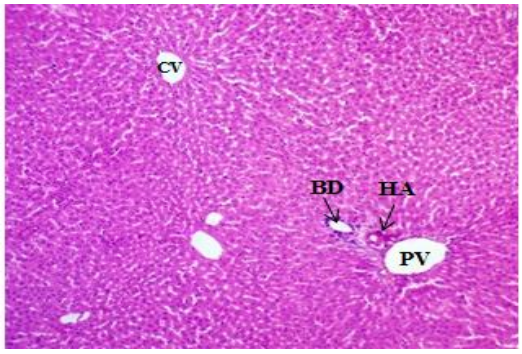

Figure 1: A photomicrograph of rat liver tissue of control group showing normal lobular architecture. Notice the central vein (CV) (Hx&E X 100).

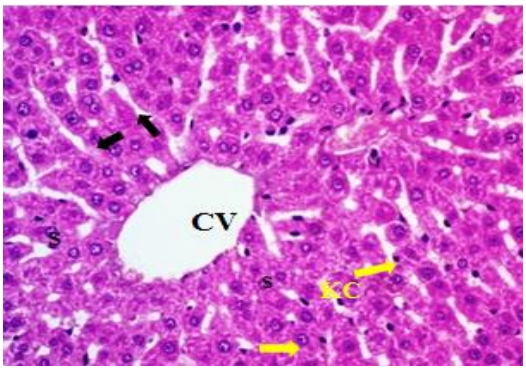

Figure 2: A photomicrograph of rat liver tissue of controlGroup showing the classic hepatic lobule with the central vein at its center.the hepatocytes radiated from centralvein separated by blood sinusoids (S),the nuclei of hepatocytes are vesicular with prominent nucleoli.it also Showed binucleated hepatocytes (black arrows) and Kupffer cells (yellow arrows).(Hx&E X400).

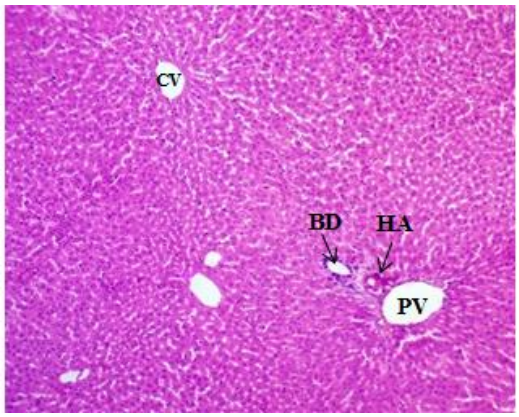

Figure 3: A photomicrograph of rat liver tissue of group 2(captopril) showing preserved lobular architecture with central vein(CV)and portal tract (portal vein PV,hepatic artery HA and bile duct). (Hx&E X 100).

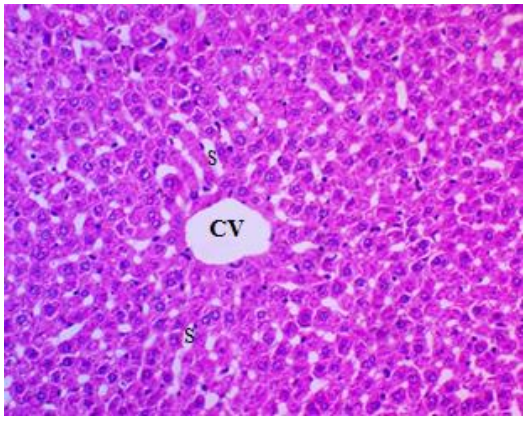

Figure 4: A photomicrograph of rat liver tissue of group 2(captopril) showing preserved lobular architecture. (Hx&E X 400)

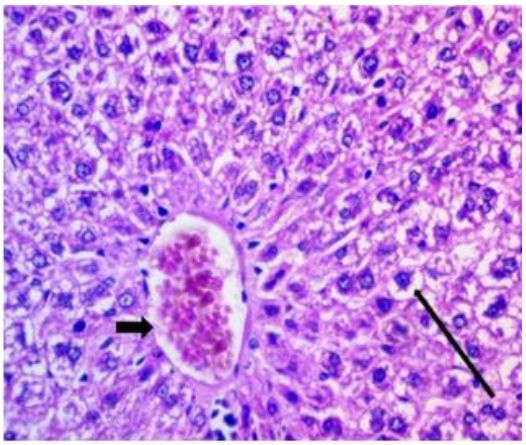

Figure 5: A photomicrograph of rat liver tissue

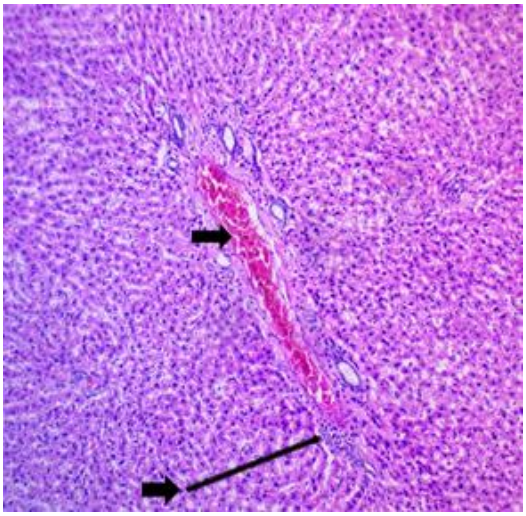

Figure 6: A photomicrograph of liver tissue of group 3showing congested central vein of group 3(CP) showing (short thick arrow) with disturbed architecture. congestion of the portal vein(short thick a Hepatocytes are swollen with vaculated arrow) and inflammatory cell infiltrationcytoplasm (long thin arrow). (Hx&E X 400 (long thin arrow).(Hx&E X100).

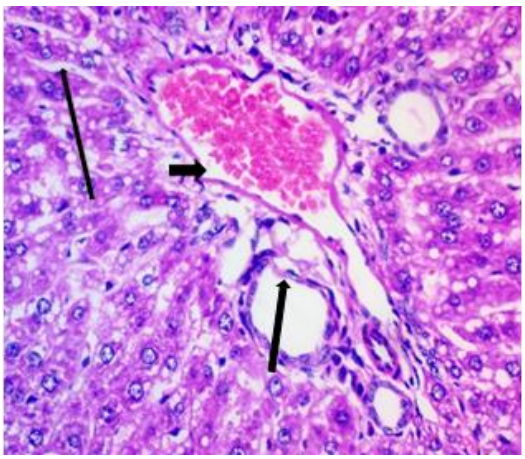

Figure 7: A photomicrograph of rat liver tissue of group 3

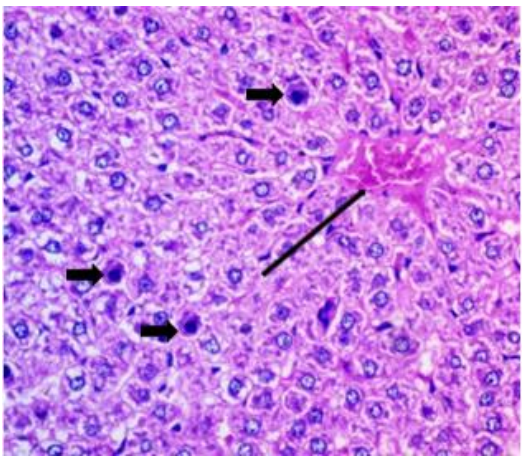

Figure 8: A photomicrograph of rat liver of showing the portal area with congested portal vein group 3 showing disturbed architecture with (short thick arrow). Sinusoids are dilated and congested(long thin arrows). (Hx&E X 400). congestion of the central vein(long thin arrow) Apoptotic cells appearwith hypereosinophilic cytoplasm and darkly stained condensed nuclei (short thick arrows) (Hx&E X400).

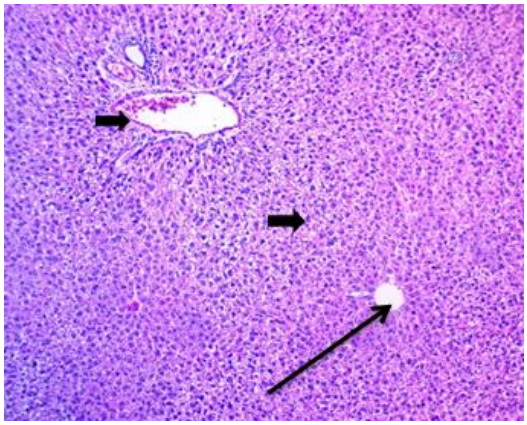

Figure 9: A photomicrograph of rat liver tissue of group 4(captopril + CP) showing slightly disturbed lobular architecture with mildly congested portal vein (short thick arrow) and mildly dilated blood sinusoids (long thin arrow). (Hx&E X 100).

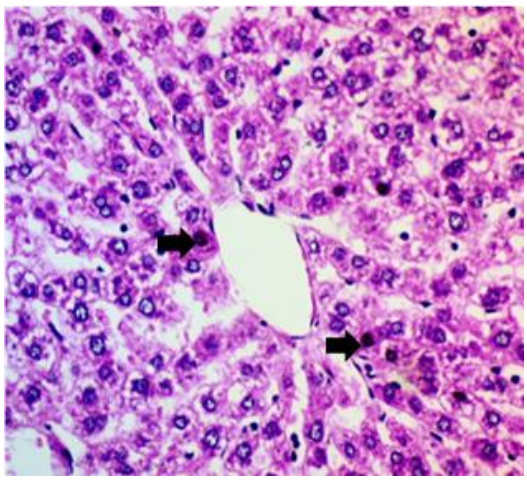

Figure 10: A photomicrograph of rat liver tissue of group 4 at the pericentral region showing few apoptotic hepatocytes (short thick arrow) with hyper eosinophilic cytoplasm and densely stained nucleus, pyknotic and less vaculation (Hx&E X 400).

Light microscopic examination of bone marrow:

Sections of bone marrow tissues from control group showed normal histological picture, the stromal elements form an extensive, closely packed network in which the hematopoietic cells are embedded. Maturated myeloid cells and megakaryocytes were present in intertrabecular spaces. The normal bone marrow of adult albino rats contains about 80% or more hematopoietic cells with the remaining cells composed of adipocytes (figure11&12)

Slides of bone marrow tissue in captopril-treated group are similar to control group (Figure 13)

In the CP-treated group, bone marrow revealed empty spaces. Bone marrow was markedly hypo-cellular, with the distortion of the myeloid and erythroid tissues. In addition, megakaryocytes couldn't be identified in the slides of this group; there is an increase in adipose tissue more than cellular tissue compared with control group (Figs. 14, 15).

Smears of bone marrow tissues in rats treated with both CP and captopril exhibited less deterioration compared to CP group (Figure 16).

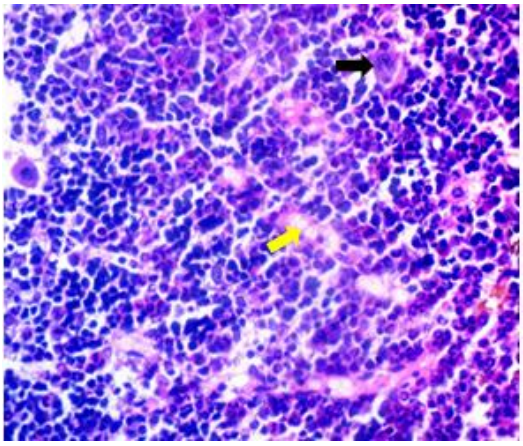

Figure 11: A photomicrograph of rat bone marrow of control group showed normal bone marrow histology with densely packed cellular distribution, presenting normal erythropoiesis and myeloid maturation megakaryocytes (black arrow) & adipose tissue (yellow arrow).(Hx&Ex100).

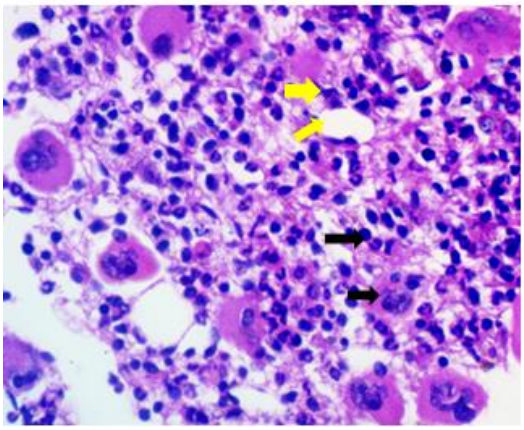

Figure 12: A photomicrograph of rat bone marrow of control group showed normocellular bone marrow with densely packed cellular distribution, presenting Megakaryocytes(black arrow), &Adipocytes (yellow arrow).(Hx&EX 400).

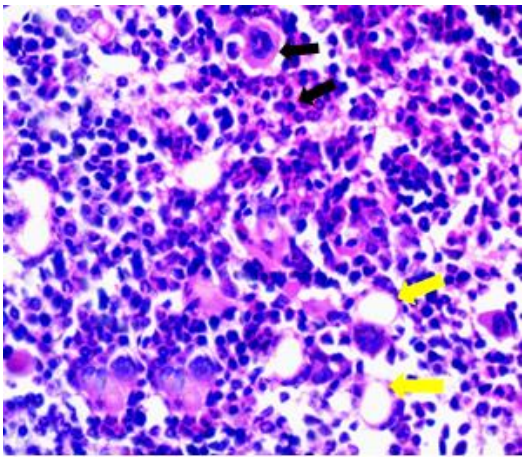

Figure 13: A photomicrograph of rat bone marrow of captopril treated group showed normocellular bonemarrow similar to control group presenting cellular tissue (black arrows) and adipose tissue(yellow arrows).(H&E)

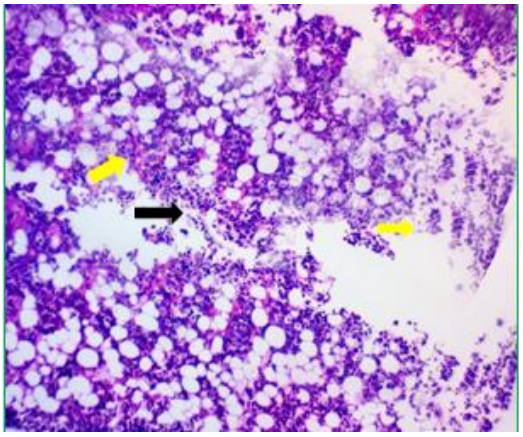

Figure 14: A photomicrograph of rat bone marrow of CP treated group showed aplastic bone marrow exhibiting frequent appearance empty spaces was noted (yellow arrows). Bone marrow was markedly hypo-cellular (black arrow), with the deterioration of the myeloid tissue more than the erythroid tissue, In addition, megakaryocytes couldn't be identified, there is increase adipose tissue more (H&E 100).

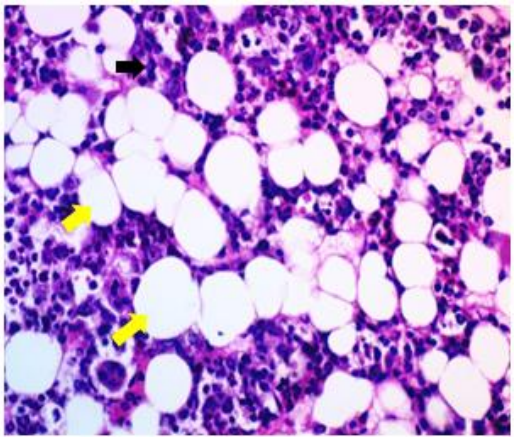

Figure 15: A photomicrograph of rat bone marrow of CP treated group showed increase adipose tissue and decrease cellular tissue (H&E 400).

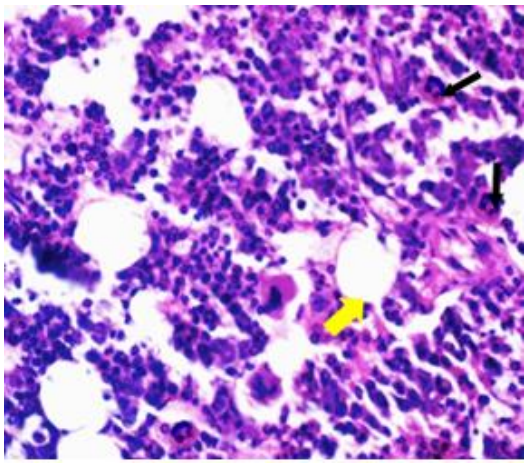

Figure 16: Aphotomicrograph of bone marrow tissue of captopril-CP treated group showed less deterioration compared to CP group, but still some features similar to the group treated with CP cellular tissue begins to increase and megakaryocyte appear(black arrow) and decrease adipose tissue (yellow arrows) (H&E 400).

Electron microscopic study of liver tissue:

Ultrastructural study of hepatocytes in the control group revealed regular shaped nuclei with intact nuclear membrane, rough endoplasmic reticuli, clear mitochondria and few lipid droplets. The majority of nuclei exhibited distinct heterochromatin and thick prominent nucleoli (Figure 17).

Liver tissues in rats treated with captopril alone showed normal picture like control group (Fig. 18).

Hepatocytes of CP-treated group exhibited lipid globules accumulation, distortion of cytoplasmic organelles with irregular nuclear membrane (fig. 19), mitochondrial cristae become vague, vaculated and disorganized (Fig. 21), proliferation of smooth endoplasmic reticulum and a week pyknotic nucleus (Figure 20). Dilatation of intercellular space was also seen (fig. 21). Pretreatment with captopril markedly reduced these changes (Figs. 22&23).

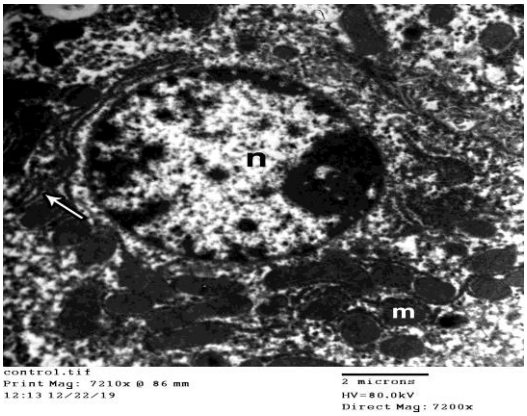

Figure 17: Electron micrograph of liver from a control rat demonstrating normal hepatocyte structure n: Nucleus; m: mitochondria; endoplasmic reticulum (arrow).

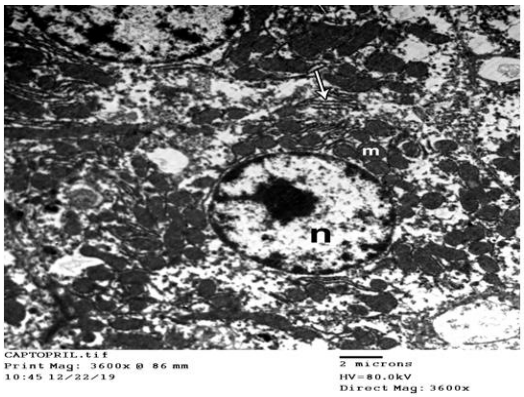

Figure 18: Electron micrograph of liver from group 2(captopril) demonstrating normal hepatocyte structure with few lipid droplets (LD). n: Nucleus; m: mitochondria; endoplasmic reticulum (arrow)

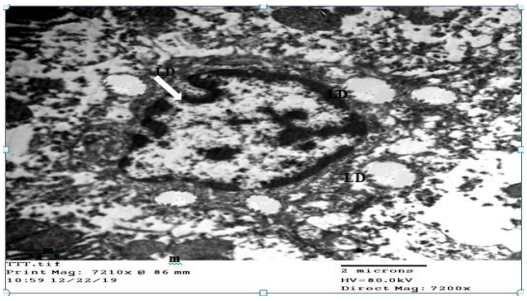

Figure 19: Electron micrograph of liver from CYC-treated rat. Swelling of mitochondria (m), Irregular nuclear membrane (white arrow) and accumulation of lipid droplets (LD) with vacuolated cytoplasm

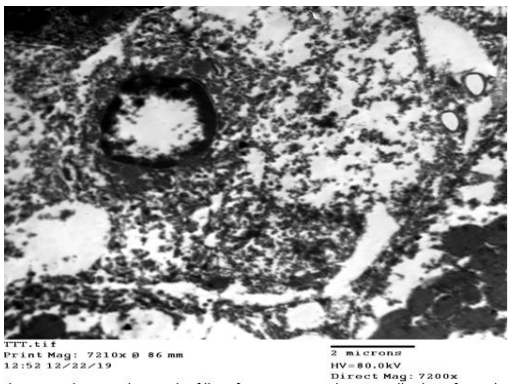

Figure 20: Electron micrograph of liver from CYC treated rat vacuolization of cytoplasm (long arrow) swelling of mitochondria (m),aweak pyknotic nucleus(n) and accumulation of lipid droplets(LD).Besides,dilatation intercellular space was also seen (short arrow).

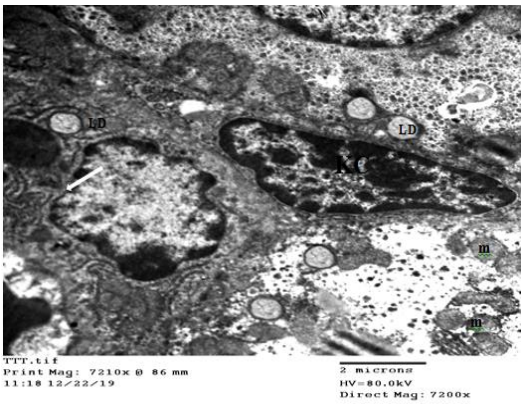

Figure 21: Electron micrograph of liver from CP treated group revealed swelling and vacuolization of mitochondria (m), mitochondrial cristae become vague and disorganized pyknotic nucleus(n), Kuffer cell (KC) Besides, dilatation of endoplasmic reticulum was also seen (white arrow).

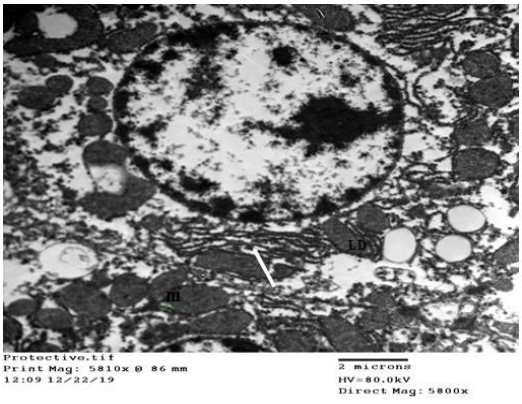

Figure 22: Electron micrograph of liver from (CP+Captopril) treated group showed normal nucleus (n) and mitochondria (m), endoplasmic reticulum (white arrow) and few lipid droplets (LD).

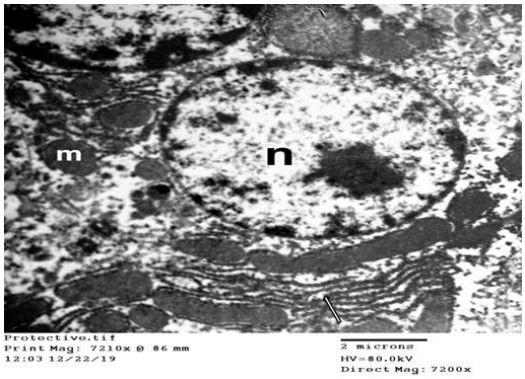

Figure 23: Electron micrograph of liver of (CP+captopril (treated group revealed normal hepatocyte nucleus n, mitochondria m and endoplasmic reticulum (arrow).

Discussion:

The histological patterns seen in the liver and bone marrow tissues in the present study may be attributed to the increase in the oxidative stress of the cell which leads to cytolysis and decrease in the antioxidant agents. CP, as an alkylating drug, has severe toxic effects on normal organs as liver, kidney and bone marrow Senthilkumar S, et al (2006). Phosphoramide mustard and arcolein are main active metabolites of CP that are metabolized in liver and induce oxidative stress in tissues McDonald GB, et al (2003). Previous studies have reported that antioxidants can preserve normal tissues against CP toxicity Ghobadi E, et al (2017). Hence, it is important to give an antioxidant before using CP in patients with malignancies or autoimmune diseases and also assessment of liver function tests in patients under treatment with CP Mukherjee et al, (2013) and Khan et al, (2014).

Captopril has believed to be as a free radical scavenger because of its terminal sulfhydryl group, some in vitro studies indicate that captopril has antioxidant effect by increasing the activities of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase Fasihi et al, (2012) and Efrati et al, (2012).

In the present study, treating of rats with CP (150 mg/kg) single dose i.p, after 5 days captopril administration (50mg/kg) resulted in a significantly improved hepatic markers compared to CP treatment ($p < 0.001$). This is in agreement with Aldahmash and El-Nagar (2016) who studied the protective effect of captopril against hepatic ischemia-reperfusion injury in rats. It was revealed that captopril administration alleviated the ischemia-reperfusion induced liver and renal injury and improved the hepatic structure and function; it seems likely that captopril with its anti-inflammatory and antioxidant properties may be of potential therapeutic value in protecting the liver against oxidative injury due to ischemia-reperfusion Bülbül et al, (2018).

In the present study, in comparison with the control group, examination of the liver of the rats in group3 which were treated by CP revealed marked distortion of hepatic architecture, congestion of the central vein and the blood sinusoids with inflammatory cell infiltration. Moreover, there were signs of degeneration of hepatocytes in the form of swelling cells up to ballooning, vacuolated cytoplasm. Apoptotic hepatocytes are characterized by having hypereosinophilic cytoplasm and condensed pyknotic nuclei. The present study is in agreement with the studies done by Bhat et at, (2018) and Ali et al, (2014) who detect the damaging effect of CYC on liver in the form of congestion, inflammation and apoptosis El-kussi (2016) and LeBlanc (1990) reported that hepatocyte degeneration was observed to be near the portal region and intensively around sinusoids but in the present study, hepatocyte degenerations were observed intensively around the central vein .

In examination of the liver of the rats in group received CP after captopril revealed marked reduction of pathological changes as agreement with the study done Kelleni et al, (2016) who reported that administration of captopril reduced the histological alterations induced by methotrexate appreciably. This can be attributed to the antioxidant effect of captopril, which significantly reduces the oxidative damage leading to reduction of pathological changes and restoration of normal physiological functions .

Pre-treatment with captopril showed more or less normal lobular pattern with better cord arrangement of well-formed hepatocytes with prominent nucleus, and maintained the central vein. The extensive liver injury induced by CP occurs through its free radical generation mechanism (oxidative stress) which is the same mechanism of many chemotherapeutic agents Demiryilmaz et al, (2012).

Also our histological results in agreement with Cure et al, (2015) who studied the protective effect of captopril on Methotrexate induced hepatotoxicity in rat. The Methotrexate-induced hepatotoxicity in rat is a classical model for studying free radical mediated liver injury and clearly demonstrates that captopril ameliorates the alterations in liver lobular architecture induced by Methotrexate. These results fit well with the data regarding the hepatoprotective effects of other antioxidants Kurtin et al, (2012) and Sontas et al, (2009).

Myelotoxicity, bone marrow suppression, is a condition in which bone marrow activity is decreased or suppressed in one or more of its components of blood cells Feng et al, (2016). Myelotoxicity is one of the adverse effects of systemic anti-neoplastic therapy or radiotherapy to bone marrow. Myeloid pancytopenia is the most frequently seen manifestations of treatment with CP and one of the most common reasons for dose adjustments or discontinuation of therapy, potentially limiting the therapeutic benefit Üstün et al, (2001).

The results of the current experiment showed that CP-induced bone marrow toxicity manifested by severe leukopenia, anemia without significant thrombocytopenia, which was confirmed by the histopathological findings. CP induced myelotoxicity, in our present study, was confirmed by significant histopathological changes seen in smears of bone marrow tissue in the form of marked hypocellular bone marrow, myeloid deterioration more than erythroid tissue and absence of megakaryocytes in comparison to smears of bone marrow tissue of control group which showed normal histological patterns with normocellular bone marrow. The present study is in agreement with the previous studies of Schuurman et al, (2005) who reported bone marrow depression after intraperitoneal injection of 200 mg/kg of CP in mice.

The present study is also in agreement with Von Vietinghoff et al, (2010) who ststed that changes of blood elements with CP administration . Additionally, it was reported that CP caused adverse effects especially in the hematopoietic system, primarily represented by leucopenia, due to its genotoxicity Katsifis et al, (2002). In a retrospective study analyzing the myelotoxicity after 40 adjuvant cycles of CP, granulocytopenia was the most prominent toxicity observed in overall 30% of the cycles. Anemia was observed in 10% of the cycles, and there

was no thrombocytopenia Khazaei et al, (2020). As it is an alkylating agent, CYC was reported to directly and dose-dependently affect myeloid cell proliferation, inducing interstrand cross-linking and double stranded breaks, leading to arrest of cell division and apoptosis of rapidly proliferating cells as haematopoietic cells Abdelmegeed et al, (2012). A clinical study performed by Ramachandran et al, (2011) showed that the risk of myelotoxicity with intravenous CYC in 92 patients with systemic lupus erythematous reported that no patients had platelet counts <50000/mm3 during follow-up

In the present study, administration of CP alone mildly suppressed bone marrow, as evident by the histopathological picture of bone marrow smear and the significant decrease in WBCs and platelet count. As expected, co-administration of captopril with CP did not succeed to ameliorate the effect of CP on peripheral blood elements, despite mildly improving bone marrow smear pattern. This is in agreement with study of Madkour FF & Abdel-Daim MM (2013) who reported that severe bone marrow depression was found only in those recipients of CP with underlying tumors who also received cytotoxic therapy.

The electron microscopy findings in our results were in agreement with Ghosh A & Sil PC (2007) who reported similar hepatic lesions as a result of CP administration. The marked hepatic damage is due to the hepatic oxidative stress induced by CP which stimulates the release of reactive oxygen species

Examination of histological slides of liver treated with captopril prior to CP administration proved that captopril protected the hepatocytes from pathological lesions induced by CP, which showed normal size and shape of liver cells with the normal appearance of nucleolus and nuclear membrane and this comes in agreement with Abd El-aziz et al, (2001).

Conclusion:

Our research elicited that pretreatment of captopril effectively improved hepatotoxicity and myelotoxicity induced by CP through alleviating the oxidative stresses and antioxidant activities of captopril.

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