Original Article

Histological and Histochemical Study on the Effect of Tamoxifen on Liver of Adult Female Albino Rats and Possible Ameliorating Role of Coenzyme Q10

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Abstract

BACKGROUND: Tamoxifen is one type of hormonal therapy that is used in the treatment of breast carcinoma , as well as in the treatment of infertility and hormonal depended conditions as gynecomastia . However, long term use of Tamoxifen may be associated with many side effects but the most serious one is the hepatic injury or even sometime hepatocellular carcinoma. AIM OF STUDY: The purpose of this work is to examine the biochemical, oxidative/ antioxidant state as well as histological and histochemical changes in the rat liver structure after treatment with Tamoxifen. In addition to evaluate the possible protective role of CoenzymeQ10.

Materials and Methods: A total of 40 female albino rats were classified in 4 different groups of ten animales in each group . Group I had no treatment and regard as control whereas group II (coenzymeq10 treated group) received 10 mg/ kg orally for three month . Group III (tamoxifen treated group) treated with tamoxifen in a dose of 20 mg/kg orally for three month, finally group IV was treated with the same previous dose of tamoxifen, in addition to the coenzymeq10 . At the end of the experiment, blood samples were drawn from the retro oorbital vein for biochemical tests then rats were killed, liver were exited and processed for light microscopic examination. Results: The present work found that tamxifen therapy produced significant elevation of liver enzyme associated with disturbances of oxidant/ antioxidant status of liver these liver dysfunction were confirmed by histopathological finding which revealed central and sinasoidal veins congestion, vacuolar degeneration of hepatocyte with infiltration of inflammatory cells. Rats received coenzymeq10 after being treated with tamoxifen , on the other hand, revealed biochemical and histological improvement. CONCLUSION: Coenzyme Q10 seemed to have some defense against tamoxifen induced liver injury by decreasing earlier degenerative changes.

Keywords: Tamoxifen, liver injury, hormonal therapy, CoenzymeQ10.

1. INTRODUCTION

Tamoxifen citrate is a type of hormonal therapy known as a selective estrogen receptor modulator (SERM)1. It acts as an estrogen antagonist so it constrains binding of estrogen to its receptor and increases apoptosis in estrogen receptor positive cells2. On the other hand, tamoxifen has an estrogen agonist action on some tissues as the brain and liver. It has been used to treat infertility, male gynecomastia and hormonal depended breast carcinoma particularly those with estrogen receptors +ve cells3. However, tamoxifen enhances lipid peroxidation and oxidative stress by suppressing mitochondrial respiration and reducing cytochrome enzyme4. Therefore, long- term use of this drug may be associated with many side effects 5, 6. It affects the liver more than other organs and this may because the affinity of this drug for liver tissue is higher than that for other tissues 7. Cases of hepatitis, fatty liver disease, fibrosis or even sometime hepatocellular carcinoma have been reported among patient using this drug8. Some authors tried to use different agent which has antioxidant activity to study their ability to reduce the adverse effects and to improve the antioxidant value of tamoxifen drug9. Coenzyme Q10 (ubiquinone) is an oil soluble, vitamin like agent existing in most of the cells in the body10. It presents in high concentration in organs with high energy requirement as the liver. It acts as an antioxidant against lipid peroxidation which disturb the integrity of basement membrane of the cells11. Today, CoenzymeQ10 (Coq10) is a well-known dietary supplement that play an important role against toxic agent12. It promotes the action of enzymes , protein and protects DNA from harmful effects of free radicles. The effect of Coq10 on some age related diseases such as hypertension and diabetes has been indicated by several clinical studies13,14. Recently, some researchers investigate the hepatoprotective action of CoQ10 against toxicity of certain drugs15. It has an anti-adipogenic action that provides the energy required for liver cell restoration and growth therefore, the chances of developing liver disease are decreased16. So the aim of this study is to investigate the role of CoQ10 on liver of tamoxifen treated rats.

2. MATERIAL AND METHODS

2.1.Chemicals : Both Tamoxifen citrate (Nolvadex) and Coenzyme Q10 (CoQ10) is obtained from the local pharmacy in Iraq as 20 mg and30 mg tablets respectively.

2.3. Animals and Experimental protocol

The experiment was done according to the animal ethics and national institutes of health instruction for the usage of laboratory animals (Ethical N:004548/2).

Forty adult female wistar albino rats weighing about (200- 225 g) and aged about three months were purchased from the animal house of veterinary collage, university of Mosul. The animals were kept in well-ventilated polypropylene cages, under standard environments, with free access to the standard diet and water. All animals had been examined carefully for general health status. The animals were divided into four groups (ten rats in each group) as following: Group I (control group) received nothing. Group II rats were given CoQ10 in 10 mg/kg b.w orally for three month 17. Group III (tamoxifen treated group) the animals in this group received tamoxifen orally by gasric tube in a dose of 20 mg/ b.w once daily for three month 18. Group IV (tamoxifen + Co Q10 treated group) rats were given CoQ10(10 mg /kg) orally followed by tamoxifen (20 mg/ kg) with 2 hour interval for three month. Towards the end of the experiment blood samples were collected from the retro-orbital venous plexuses. Then all animals were anesthetized using chloroform and killed by cervical decapitation, the liver was dissected out, washed with saline to remove excess blood .

2.4 Assessment of liver function

All blood samples were aspirated in the morning about 10-11 AM. The blood centrifuged at 400 rpm for 15 mint then serum was separated and stored at −20°C. liver alkaline phosphate (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and total bilirubin (TB) levels were determined by using the minividis technique (Compact multiparametric immunoanalyzer) which based on the Enzyme Linked Fluorescent assay (ELFA) principles.

2.5. Estimation of liver lipid peroxidation (MDA) and antioxidant level

From each animal, part of liver tissue (not less than 1 gm) was cut and preserved at aluminum foil to be stored in refrigerator. Later on, the tissue was homogenized and used for estimation of

1- Malonaldehyde (MDA) level which is the end product of lipid peroxidation and regard as oxidative stress marker. The liver tissues were mixed with trichloroacetic acid and thiobarbutyric acid reactive substances reagent and incubated at 100 C for 60 min then the samples were centrifuged for 20 min at 3000 rpm, and the absorbance of the supernatant was read at 535 nm. MDA levels were estimated from the standard calibration curve as nmol/g19 .

2- Tissue reduced glutathione (GSH) and glutathione peroxidase (Gpx) which have antioxidant activity , they were measured by the method of Beutler 20 and the method of Tamura et al21 respectively .

2.6. Histopatological and histochemical examination of the liver

For histopatholgical assessment, the pieces of liver were fixed in neutral buffered formalin (10%) solution, processed through a series of ascending grades of alcohol and embedded in paraffin wax to get sections of 5µm thickness which was then stained with haematoxylin-eosin (H&E) and masson trichrom method to assess the hepatocyte and stromal structure of the liver tissues. All sections were observed under a light microscope and a scoring scheme was used to evaluate the severity of hepatic injury according to the degree of a- inflammatory cell infiltration b-sinusoidal dilatation c- hydropic degeneration of hepatocyte d-edema and congestion of space of diss , these parameters were scored as 0 (normal), 1 (mild), 2 (moderate), or 3 (severe). The summation of scores given to previous criteria was done in order to calculate the score for each tissue.

For histochemical study Periodic acid Schiff's (PAS) was used for demonstration of glycogen and mucin deposition in the cytoplasm of the hepatocytes. The stained sections were examined using olympus light micoscope.

2.4. Statistical analysis : All data were reported as mean±SE (standar error). Statistical significant of data was performed by SPSS version 20 for windows software using one-way ANOVA. P-value ≤ 0.05 was considered significant.

3. Result

3.1 Analysis of liver function markers

The liver function test of the different groups were measured before the beginning of the experiment however, the statistical analysis showed no significant difference ($p > 0.05$) between the various groups. The mean values of liver enzymes at the end of the study were illustrated in tables (1)

The mean values of ALP, ALT, AST, LDH and TB were significantly higher (P < 0.05) in GIII followed by GIV (tamoxifen and tamoxifen + coq10 groups respectively) compared to other groups , with significant (p≤ 0.05) differences between both groups .

Table 1: Serum concentration of liver enzymes in different groups of female rats.

Groups of animals / parameters	GI Control N= 10	GII Coq10 treated N= 10	GIII Tamoxefin treated N= 10	GIV Tamoxefin+Coq10 N= 10
ALP (U/l)	110.2a±6.6	112.5±7.3 a	386.8± 9.5 b	246.4±7.3 c
ALT (U/l)	20.8±0.2 a	23.2±0.7 a	42.8±0.6 b	30.7±0.6 c
LDH (U/l)	50.3 ± 2.1a	55.1 ± 1.9a	100.6 ± 3.1b	86.0 ± 3.4c
AST (U/l)	35.6±0.5 a	36.7±0.7 a	80.2±1.6 b	67.6±1.5 c
TB (mg/dl)	0.20±0.2 a	0.18±0.1 a	1.8±0.2 b	0.8 ±0.3 c

Data were represented as mean ±SE. ALP,alkaline phosphatase; ALT,alanine aminotransferase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; TB, total serum bilirubin. Different litters within rows mean there is significant difference at p ≤ 0.05.Similar litters with in rows mean there is non- significant difference at p > 0.05. N= number of rats.

3.2. Oxidative stress biomarker and antioxidant level of liver.

The mean concentration of MDA in liver was highest significant in group III followed by group IV in comparison to control and Coq10 groups. The MDA level in Group III significantly increase compared to group IV(p≤0.05). These results were accompanied by a significant decrease in GSH and GPx levels in both groups as compared with control group (table 2).

Table 2: The effect of tamoxefin and Coq10 on oxidative stress biomarkers and antioxidant markers in liver tissues.

Groups / Parameters	GI Control N= 10	GII Coq10 treated N= 10	GIII Tamoxefin treated N= 10	GIV Tamoxefin+Coq10 N= 10
MDA (nmol/g)	19.3±0.21 a	17.7±0.4 a	45.6±1.2 b	30.2 ±1.6 c
GSH (µmol/g)	20.5±0.8 a	22.3±0.8 a	14.1±0.7 b	18.3±0.4 c
GPx (U/g)	153.7±2.9 a	160.6±3.7a	75.3 ±3.2 b	93.2±14.7 c

Values were expressed as mean ±SE . Malondialdehyde ,MDA; glutathione, GSH; glutathione, peroxidase GPx. Different litters within rows indicate significant difference at p ≤ 0.05.Similar litters with in rows mean there is non- significant difference at p > 0.05. N= number of rats.

3.3 .Histopathological changes

Figure 1 represents the histological images of sections stained with heamaxoxylene and eosin (H&E) method for each group. The liver sections of GI(control) and GII(coq10 treated) showed normal liver artichture with normal hepatocytes radiated from central to peripheral region, normal central veins and portal area, no signs of degeneration nor inflammation were observed (A1,A2). On the other hand, liver sections from the GIII (tamoxefin treated) showed disturbance of hepatocyte plate arrangement with severe vacuolar degeneration and coagulative necrosis of hepatocytes associated with focal infiltration of inflammatory cells ,congestion of sinusoids and central vein (B1, B2). GIV (tamoxefin + Coq10 treated) group showing mild vacuolar degeneration of hepatocytes, mild congestion of central vein and sinusoids. The score for different groups were determined from histological sections, the worse score (p = 0.001) were observed in GIII as shown in table 3.

Table 3: Comparison of microscopic scores between different groups

Groups / Parameters	GI Control N= 10	GII Coq10 treated N= 10	GIII Tamoxefin treated N= 10	GIV Tamoxefin+Coq10 N= 10
Scores	0.5±3.7a	0.55±3.1a	4. 0±1.2 b	2.0±1.6 c

Values were expressed as mean ±SE . Different litters indicate significant difference at p ≤ 0.05.Similar litters mean there is non- significant difference at p > 0.05. N= number of rats.

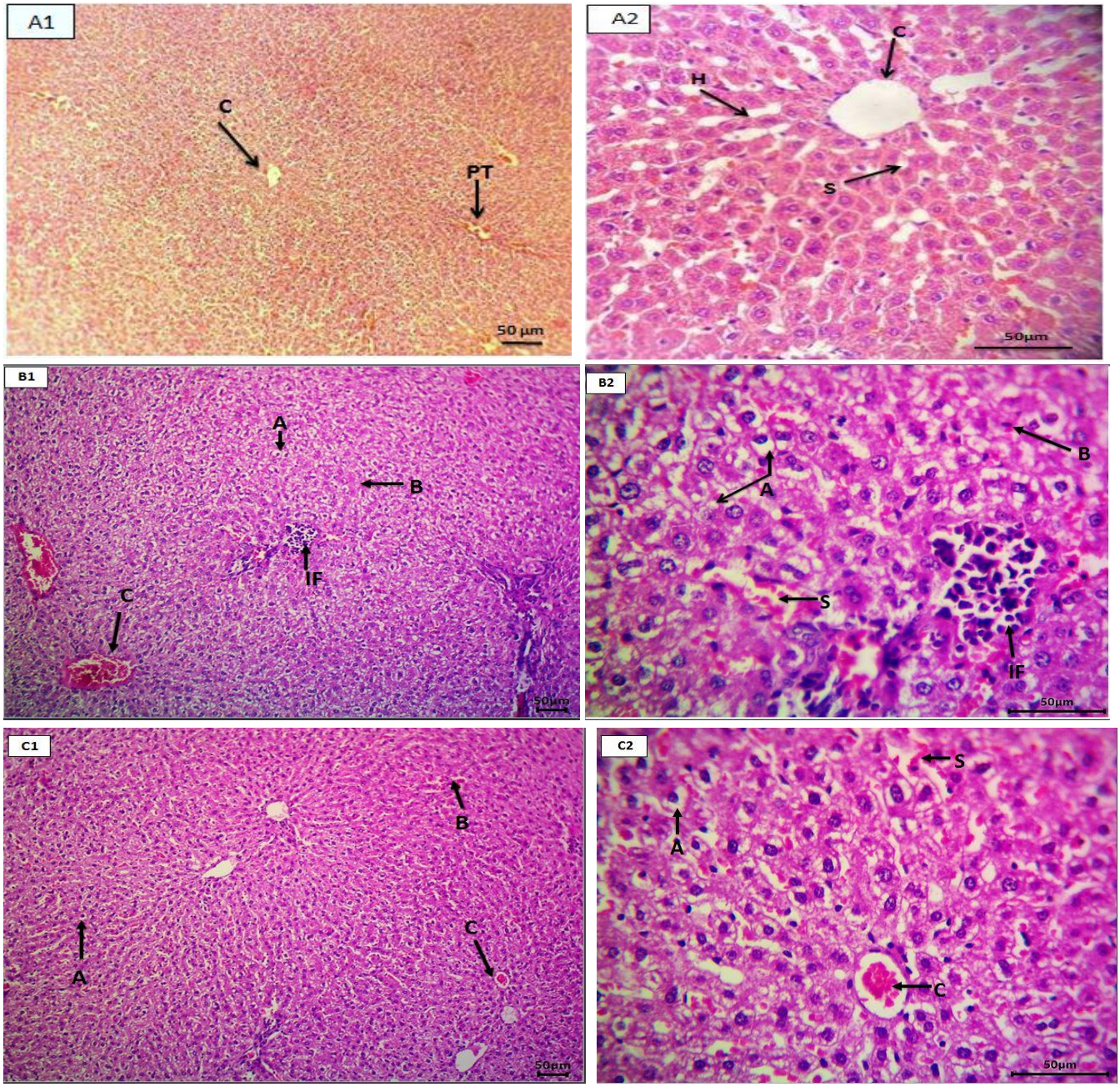

Figure 1: photomicrographs of H&E stain of liver sections from different groups

A1 : Photomicrograph from control group showing normal liver architecture with hepatocyte plate radiating from central vein (C)to the peripheral . portal tract (PT) H&E, 100 X. A2: section of liver from coenzymeq10 treated rat show normal hepatocyte(H) separated by venous sinusoid(S).H&E,400X. B1, B2: photomicrograph of rat liver of Tamoxil group showing severe vacuolar degeneration (A) and coagulative necrosis of hepatocytes (B), focal infiltration of inflammatory cells (IF) and congestion of central vein (C). H&E stain, 100X, 400X. C1,C2: photomicrograph of rat liver of Tamoxifen with co-enzyme group showing mild vacuolar degeneration of hepatocytes (A) and mild congestion of central vein (C) and sinusoids (S). H&E stain, 100X, 400X.

MT staining of control and coenzyme q10 treated groups (figure 2 A1, A2) respectively showed normal distribution of collagen fibers which appear as blue-color around the blood vessels and in portal area. In tamoxifen treated group the MT staining sections showed increasing collagen fiber in the portal area , along the sinusoids between the hepatocyte and around the central vein (B1,B2) . The administration of coenzyme q10 to tamoxifen treated group resulted in reduction of collagen fiber deposition in portal canal and around the central vein (C1,C2) .

The collagen fibers deposition has been quantified by measuring the thickness of fiber in three areas , portal tract (PT),

persinusoid (PS) and pericentral area (PC) of different groups as shown in table 4.

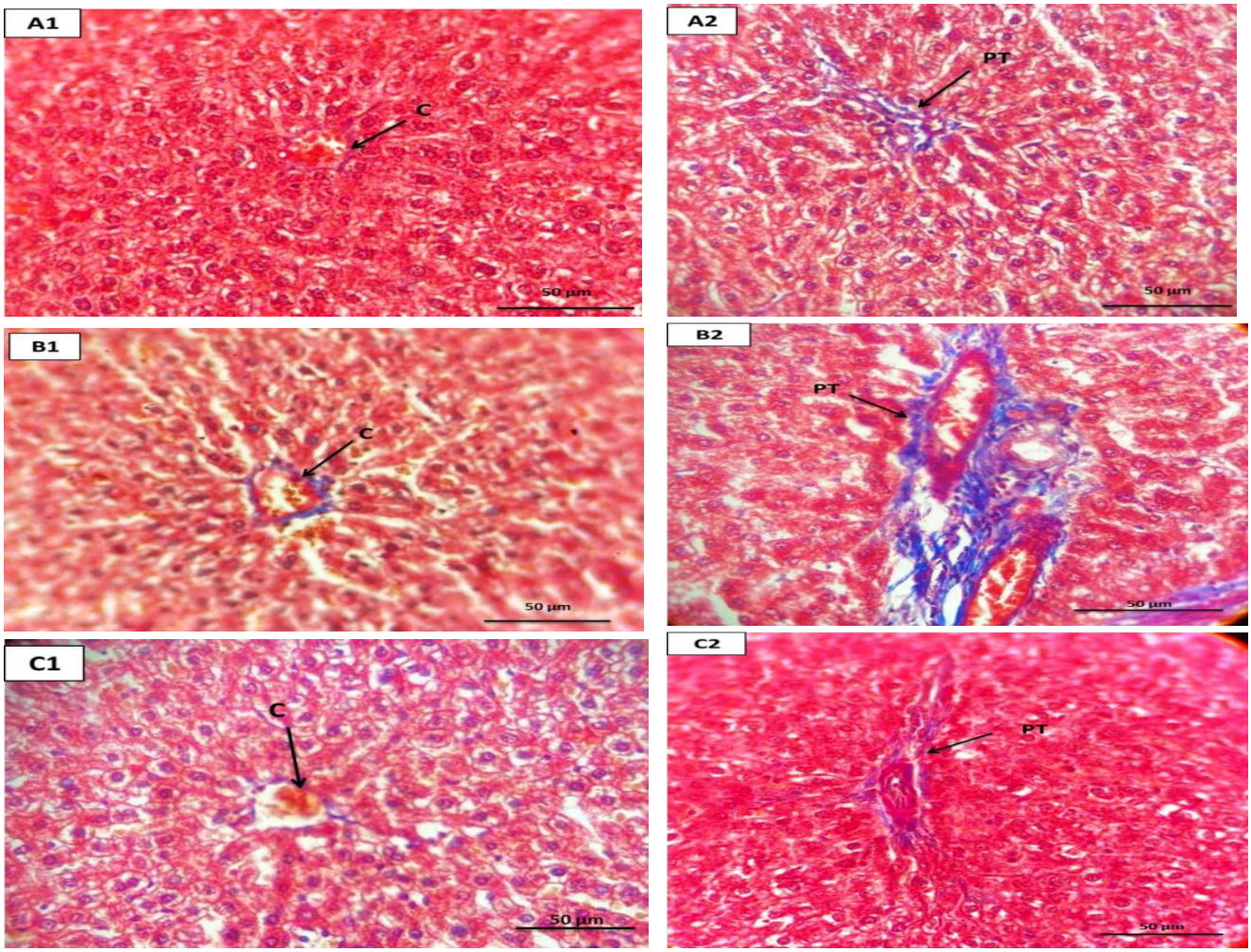

Figure 2: photomicrograph with MT stain of liver sections from different groups .

control group (A1) and coenzyme q10 treated group(A2), showing normal collagen fiber around central vein (C) and portal tract (PT). photomicrograph (B1,B2) of rat liver from Tamoxifen group showing increase collagen fiber deposition around central vein (C) and portal tract (PT) MT stain, 100, 400X. photomicrograph(C1,C2) of rat liver from Tamoxifen with co-enzyme group showing mild collagen fiber deposition around central vein(C) and portal canal (PT) MT stain, 100 , 400 X.

Table 4: Thickness of collagen fiber in different groups

Groups of animals / parameters	GI Control N= 10	GII Coq10 treated N= 10	GIII Tamoxefin treated N= 10	GIV Tamoxefin+Coq10 N= 10
PT(µm)	25.0± 2.1a	27.23±2.4a	60.12± 5.5 b	40.23±2.3 c
PS(µm)	0.8±0.2 a	1.0±0.7 a	3 .0±0.4 b	1.5 ±0.2 a
PC(µm)	2.3 ±1.2	3.0 ± 1.4a	8.6 ± 2.1b	6.0 ± 1.4c

Values were expressed as mean ±SE . (PT) portal tract, (PS) persinusoid and (PC)pericentral area Different litters within rows indicate significant difference at $p \leq 0.05$.Similar litters within rows mean there is non- significant difference at $p > 0.05$. N= number of rats.

Periodic Acid Schiff's (PAS) stain in the control and coenzyme q10 groups showed a positive (magenda color) reaction particularly in the cells near the central vein Figure 3-(A,B). While in tamoxifen and tamoxifen +coq10 treated groups (C & D respectively) showing weak reaction especially in middle zone of hepatic lobule

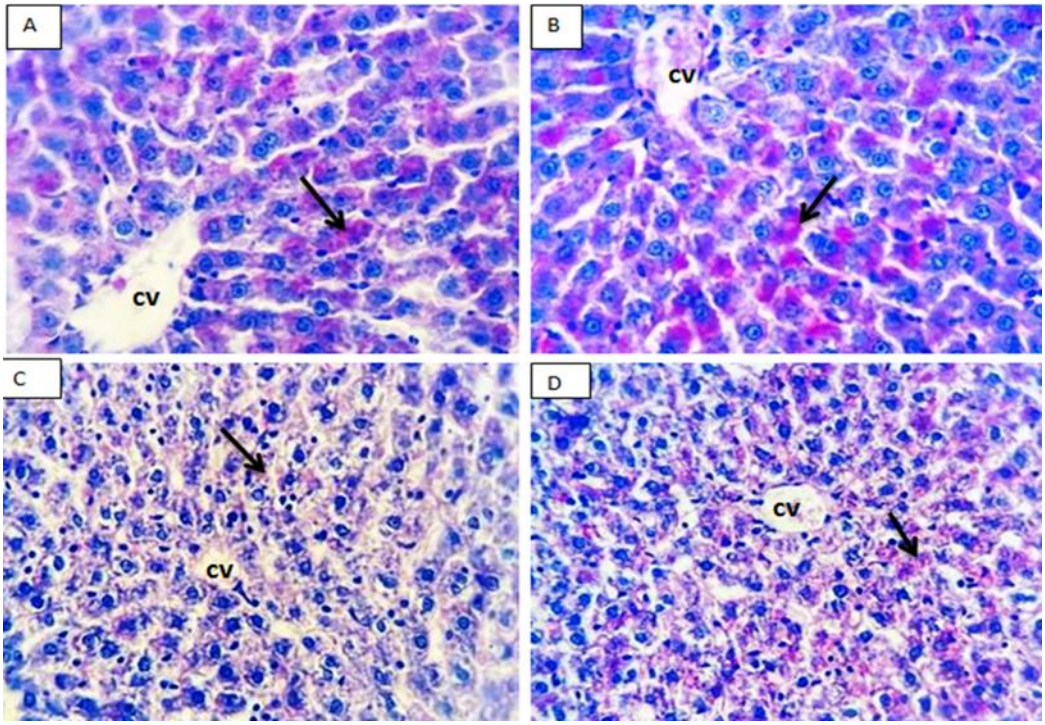

Figure 3: photomicrographs of liver sections from different groups with PAS stain,400 X . control group (A) and coenzyme q10 treated group(B), showing strong positive (magenta color) stain in the cytoplasm of the cells (arrows). photomicrograph (c) of rat liver from Tamoxifen group showing marked reduction of PAS stained material in hepatocyte while , photomicrograph(D) of rat liver from Tamoxifen with co-enzyme group showing improvement of PAS +ve material in hepatocyte. CV: central venule.

4. Discussion

The metabolism and detoxification of many drugs and chemicals occurs in the liver therefore, the liver injury induced by drugs is regarded as the most common cause of liver dysfunction which may be so sever to end with liver failure22. Tamoxifen is one type of endocrinotherapy that is used frequently for treatment of breast carcinoma and other hormones dependent disease 23. It is metabolized in the liver so the long use of this drug may be associated with liver dysfunction and hepatic injury23. The current work was designed to evaluate the probability of protective role of coenzymeq10 against tamoxifen – induced liver injury.

The degree of liver injury in the experimental groups of this study was evaluated by estimating serum level of liver enzymes ALT, AST, TB and ALP. Clinically these enzymes are used to determine any abnormality in the liver function. Tamoxifen treated group showed deterioration of liver function as apparent by biochemical analysis of liver enzymes which revealed a significant elevation of these enzymes compared with the untreated group. The same observation were obtained by another investigator 24. High liver enzyme levels often indicate inflammatory state or damage to cell membrane integrity of hepatocyte25 . ALT and AST found in the cytoplasm of liver cell and regard as most sensitive indicators of hepatocyte injury while the alkaline phosphatase present mainly in the cells that is lining the bile canaliculi. Custódio et al stated that tamoxifen affect the cell membrane bilayers which may be responsible for leakage of enzymes to blood stream26. In addition, increased serum bilirubin level indicate the disturbance of biliary system and post hepatic toxic effect of tamoxifen.

Tamoxifen- induced hepatic dysfunction was confirmed by histopatholgical study of liver tissues which showed disturbances of hepatic architecture with vacuolar degeneration of the cells and aggregation of many inflammatory cells . These finding was in an agreement with those observed by previous study 27,28.Wiedemann et al attribute the vaculation to the cellular swelling due to ionic disturbance29 while Higgine stated that vacuolar degeneration may be due to mitochondrial damage and expansion30 . the congestion and dilatation of hepatic sinusoid and central vein observed in this study could be resulted from chemical substances secreted from inflammatory cells causing vasodilatation of blood vessels. Furthermore, depletion of glycogen material in hepatocyte was observed in the present work subsequent to tamoxifen treatment while MT stain revealed thickening of connective tissue deposition in both pericentral and periportal areas. These findings were in agreement with other researcher who attribute this finding to the oxidative stress 31. However, The co-administration of coq10 with tamoxifen in this

study showed improvement of histological structure and improvement of liver function with reduction of serum level of liver enzyme. Faris et al noticed that daily supplementation of coq10 to the patient with non-alcoholic fatty liver disease resulted in the reduction of serum liver enzyme as well as in an inflammatory marker as C-reactive protein32. This shows that coq10 has hepatoprotective activity, may be by restorative hepatic parenchyma and renewing of hepatocytes.

The oxidative stress and increase lipid peroxidation may be responsible for drugs -induced toxicity in different tissues as liver , pancreas and kidney 26, 33,34. In the current study, tamoxifen was shown to significantly change the oxidant/antioxidant balance in treated rats. So, tamoxifen administration resulted in an increase in level of MDA activity which regard as marker for oxidative stress, in addition to the reduction of antioxidant enzyme levels as GSH, GPX in the liver. These results are comparable to those observed by other researchers 35. The increased free radicles can directly rise lipid peroxidation of cell membrane causing destruction of the cells . This results was run in parallel with the current histopatholgical changes. Nagahara eta al reported that tamoxifen metabolite has a suppressive effect on mitochondrial respiratory chain which may impair fatty acid oxidation and causes ATP reduction with production of reactive oxygen particles4. The increased lipid peroxidation led to reduction of intracellular activities of the antioxidant enzyme , since the hydroperoxides had been destructed by the action of GPX so this enzyme has cytoprotective activity36. The inhibition the antioxidant enzyme activity makes the cells sensitized to free radicles. . CoQ10 supplementation led to reduction of MDA and elevated the hepatic antioxidant level which combating free radical and preserve the tissues from oxidative stress. This agrees with a study performed by other researcher who found that administration of coenzymeq10 to rat with experimentally induced hepatocellular injury led to suppression of lipid peroxidation, increased antioxidant activity, and reduced the tumor necrosis factor-α in hepatic tissue of rats with hepatocellular carcinoma. Also, the dysplastic changes in liver were improved by coenzyme Q10 37, 38, 39.

5. Conclusion:

we conclude that coenzymeq10 have ameliorating role in tamoxifen-induced liver toxicity as it improve antioxidant activity and reduce MDA levels and protects liver structure. In light of these study , we can be suppose that coenzymeq10 has therapeutic effects and protective action as it elevates the injury caused by free radicles and increases antioxidant activity so it can be useful in many aspect of clinical practice.

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