IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. VIII (Jul – Aug 2017), PP 67-76
www.iosrjournals.org

Histological and Biochemical Study of Different Doses of Tamoxifen Drug on Liver Of Albino Male Rat

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***Abstract**: Tamoxifen is an effective anticancer drug. It's a hormonal treatment against estrogen which is necessary for the growth of cancer cells. This study was conducted to evaluate the negative effects of Tamoxifen on some physiological and histological. Use for this purpose 32 rats of the strain Sprague dawley albino rat and divided into 4 equal groups. 3 groups were given different doses (30, 40, 50)mg/kg body weight of TAM 3 times a week for 8 weeks as well as control group that was injected with physiological solution (normal saline 0.9%). At the end of dosage duration, the blood was collected for the purpose of studying some Biochemical parameters (Liver Functions). The results of Biochemical tests showed significant differences in the treatment groups were the three groups showed a significant increase in the levels of (TSB, GOT, GPT, ALP). Histopathological examination of liver tissues in TAM groups showed histological changes that increased with the increased dose compared to control group such as inflammatory cells infiltration and blood congestion. As well as the occurrence of necrosis in the cells and colleting Neutrophils and Monocytes and Aggregation of mononuclear scatter in the liver parenchyma. The results clearly indicated that Tamoxifen had an adverse effect on liver and blood and chemical parameters that cause hepatotoxicity.*

Date of Submission: 18-08-2017 Date of acceptance: 05-09-2017

I. Introduction

Breast cancer is the most prevalent cancer in women around the world. It is the second most common cancer worldwide and the leading cause of cancer death in women. regrettably, the incidence rates of breast cancer are increasing**.**[1,2]

Breast cancer cells demand estrogen to continue growing and about >70% of infiltrating breast carcinoma are estrogen receptor alpha (ERα) positive, thus presentation clinicians the opportunity for hormonal therapies (HTs) in adjuvant and/or metastatic situations. Modulation of estrogen signaling pathways using anti estrogens.[3]

Tamoxifen (TAM) has been widely used for many decades as the adjuvant treatment for patients with breast cancers.[4,5]

Tamoxifen, a selective estrogen receptor modulator (SERM), acts as an estrogen receptor (ER) antagonist in breast tissue, and decreases breast cancer repetition and mortality in women with ER-positive breast cancer. TAM is also effective in primary prevention of breast cancer in high-risk women. However, the use of TAM for prevention is limited due to its side effect profile. TAM commonly causes a range of side effects such as hot flashes and occasionally causes more serious adverse events such endometrial hyperplasia or endometrial cancer and venous thromboembolic disease. Other side effects impute to TAM are night sweats, gynecologic symptoms (vaginal dryness, vaginal discharge), depression, forgetfulness, sleep alterations, weight gain, diminished sexual functioning and hepatic injury such as hepatocarcinoma, hepatic steatosis and hepatotoxicity.[6,7] Due to oxidation that leads to liver cirrhosis after TAM therapy.[8]

TAM is the most recommended breast anticancer oral medication for prevention and treatment of breast cancer for both men and women. TAM is a hormonal selective estrogen modulator and its Pharma-cological effects are based on its binding to estrogen receptors and suppressing epithelial generation of different cell types that lack estrogen receptor.[9]

This study was aimed to investigation the effect of different doses of TAM as histological changes and functional parameters of liver and blood in male albino rats throw the following:

- Study the histological changes in liver.
- Study the liver function parameters (GOT, GPT, ALP and total bilirubin).
- Determination the toxicity of tamoxifen with long-term dose and damage to the liver at the tissue level.

II. Materials and Methods

Preparation of TAM Drug

Tamoxifen citrate (Nolvadex-D) produced by AstraZeneca Oak Limited. In this study, animals were administration orally by a feeding tube 6 cm^2 after grinding discs with a clean mortar. Add 2 ml of regular salt water and mix very well to form a suspension solution.[10]

Tamoxifen tablets are weighed after grinding in a sensitive balance to obtain the three doses to study their effect, which is: (30 mg, 40 mg and 50 mg). And according to the weight of the rat and its metabolism compared to the weight of the human.

Experimental Animal

The experimental animals used in this study were male albino rats *Rattus norvegicus* weighing (225 – 250 g), and at the age of (14 – 16) weeks. The animals were purchased from Pharmaceutical control of the Ministry of Health in Baghdad. Animals were given food and water *ad libitum*. Rats were maintained in a friendly environment with a 12 h/12 h light-dark cycle at room temperature (22 °C – 25 °C). Rats were acclimatized to laboratory conditions for 7 days before commencement of the experiment.[11]

Animal Handling

At the end of the experiment rats were killing under anesthesia there blood sample were collected and centrifuged at (3000 r.p.m. 15 minutes) serum was stored at (-20ºc) until used for biochemical assays (GOT, GPT, ALP and TSB).

Experimental Design

A total 32 of male rats were divided at random into 4 groups of 8 animals each group and treated as following: (figure 1)

- **Group I (control):** Animals were injected orally with normal saline (0.9 %) 4 times/week for 8 weeks.
- **Group II (treated):** Animals were injected orally with TAM (30mg/kg) 4 times/week for 8 weeks.
- **Group III (treated):** Animals were injected orally with TAM (40mg/kg) 4 times/week for 8 weeks.
- **Group IV (treated):** Animals were injected orally with TAM (50mg/kg) 4 times/week for 8 weeks.

Histological Examination

The liver was fixed in 10% neutral buffered formalin solution and embedded paraffin wax blocks. Section of (5µm) thickness were stained with hematoxylin and eosin then examined under light microscope for determination of pathological changes.

Statistical Analysis

In order to comparison between parameters in each (Liver function),Using analysis of variance, F-test, t-test, in complete randomized design.Different between means have analyzed by least significant differences (LSD) at ($p \leq 0.05$) and expressed as (Mean ± SEM) ,Small letters indicate significant differences between means in columns. Using spss program 2010 and excel application to find the result and draw the figures with some effects

III. Results

Liver Function test

The results in the present study in the table (1) showed a significant increase ($P<0.05$) in the level of GOT, GPT in all treatment groups with TAM (30, 40, 50) mg/kg body weight respectively compared with control group as in the. While ALP, TSB also significant increase ($P<0.05$) in all treatment groups with no big significant differences between groups (40, 50) mg/kg. (fig. 1)

Histopathological Examination of liver

Control group animals

The control animals showed no clear lesions in the liver (Fig. 2). The normal microscopic architecture of the liver showed radially arranged hepatocyte with well demarcated regularly sized nuclei intervening sinusoids, normal control vein, hepatocyte and portal vein.

Animals treatment with TAM (30 mg/kg):

Microscopic section in the liver of animals treated with TAM (30 mg /kg body weight) showed congested of Blood vessels (Fig. 3)

Animals treatment with TAM (40 mg/kg):

The animals treated with dose TAM (40mg /kg body weight)expressed few mononuclear cells infiltration around central vein (Fig.4), while in (Fig.5) showed severe mononuclear cells infiltration around blood vessels. As well as there is small Aggregation of mononuclear scatter in the liver parenchyma with proliferation of Kupffer cells (Fig.6).

In (Fig.7) it is observed that proliferation of Kupffer and mononuclear cells in liver parenchyma with congest central vein and macrophage cells.

Animals treatment with TAM (50 mg/kg):

Histological section in the liver of animal treatment with dose TAM (50mg/kg body weight) revealed large granulomatous lesion With necrotic cells and dilated sinusoids (Fig.8). In other animals it was found aggregation of neutrophil and mononuclear cells in Portal area and scatter of Kupffer & mononuclear cells in liver parenchyma with Macrophage cells and necrotic cells(Fig.9).In addition to macrophages with Mononuclear and neutrophils cells aggregation in portal area(Fig.10). The liver of animals treated with TAM(50mg /kg body weight) showed granulomatous lesion with focal necrotic area in the liver parenchyma and small aggregation of mononuclear cells with ballooning de-generation (Fig.11)**.** Other animals showed hyperplasia of epithelial cells of bile duct inflammatory cells infiltration neutrophils and mononuclear cells in portal area and congested blood vessels(Fig.12). In other microscopic section in the liver showed inflammatory cells particularly neutrophils infiltrated in necrotic area of liver parenchyma with of vacuolation hepatocytes (Fig.13).

Table (1): Effect of TAM (30, 40, 50 mg/Kg) on serum activates of GOT, GPT, ALP and TSB in male rats. small letter s indicate to comparison in column, similar letters are non-significantly differences between means at ($p \leq 0.05$), Using (LSD test).

Liver function / Dose	GOT	GPT	ALP	TSB
Control	22.103 d ± 0.586	16.480 d ± 0.601	138.875 c ± 1.342	0.346 c ± 0.019
30 mg	32.466 c ± 2.095	23.455 c ± 0.296	206.125 b ± 11.226	0.418 b ± 0.014
40 mg	43.150 b ± 1.425	29.088 b ± 0.315	248.625 ab ± 26.140	0.480 a ± 0.013
50 mg	49.784 a ± 1.523	33.263 a ± 2.420	296.875 a ± 19.054	0.513 a ± 0.014
LSD P ≤ 0.05	4.365	3.666	49.632	0.043

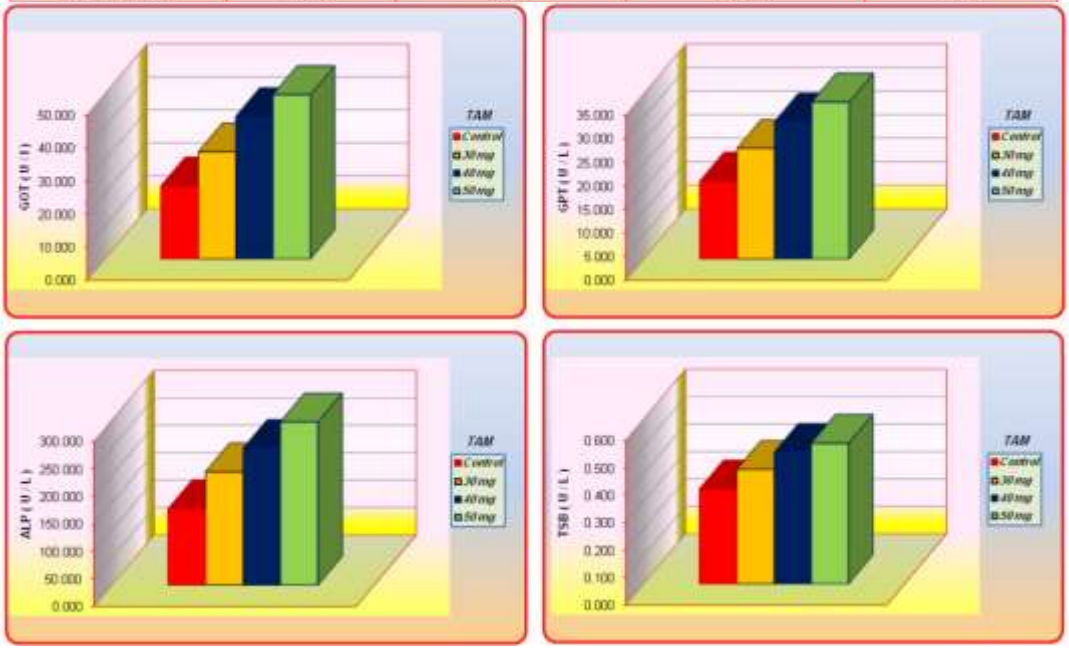

figure (1) : Effect of TAM (30, 40, 50 mg/kg) on serum GOT, GPT, ALP and TSB concentration compared with control group in male rats.

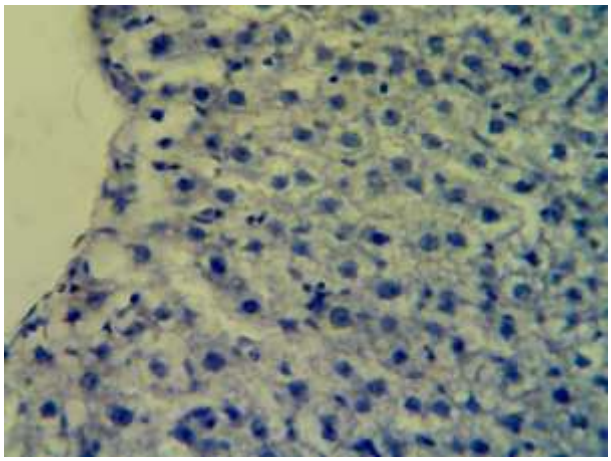

Figure (2): Cross section in the liver of control animal shows no clear lesions the architecture of the liver showed radially arranged hepatocyte with well demarcated regularly sized nuclei intervening sinusoids, normal control vein, hepatocyte and portal vein.

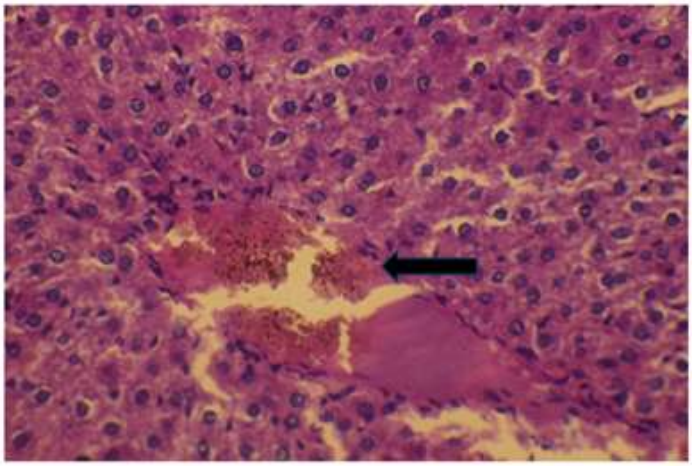

Figure (3) Cross section in the liver of animal treated with tamoxifen(30mg/kg)shows congested of Blood vessels ⟵

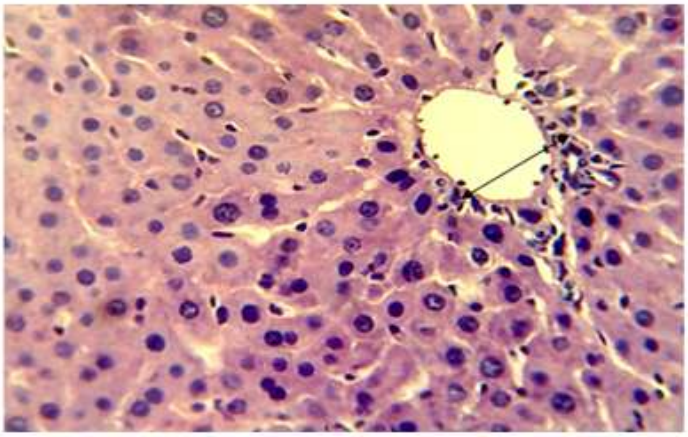

Figure (4) Cross section in the liver of animal treated with tamoxifen (40mg/kg) (H&E X40) shows few mononuclear cells infiltration around central vein ⟷

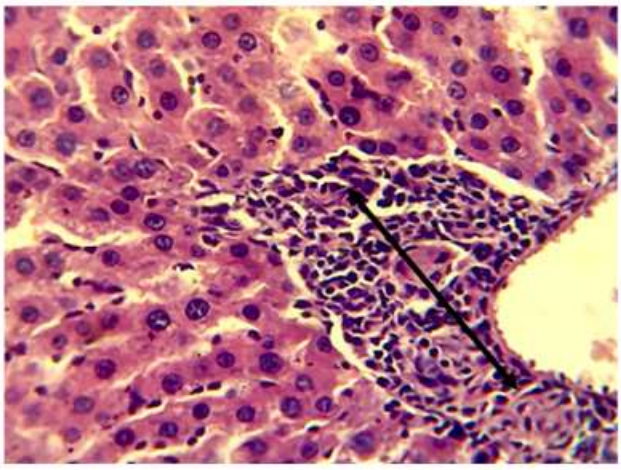

Figure (5) Cross section in the liver of animal treated with tamoxifen (40mg/kg) (H&E X40) shows severe mononuclear cells infiltration around blood vessels ⟷

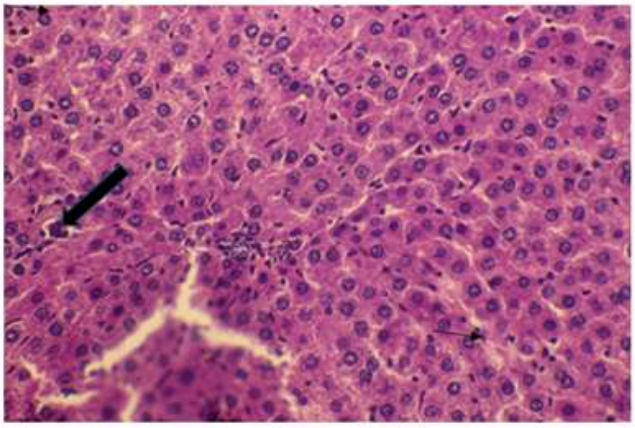

Figure (6) Cross section in the liver of animal treated with tamoxifen (40mg/kg) (H&E X40) shows small Aggregation of mononuclear scatter in the liver parenchyma ⟵ with proliferation of Kupffer cells ↘

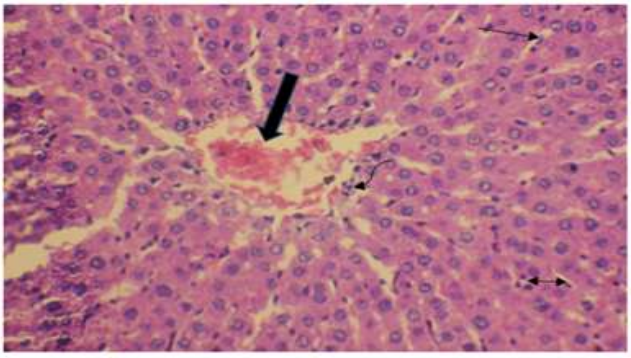

Figure (7) Cross section in the liver of animal treated with tamoxifen (40mg/kg) (H&E X40) shows proliferation of Kupffer → &mononuclear cells ↔ in liver parenchyma with ⟵ congest central vein ↙ and macrophage cells

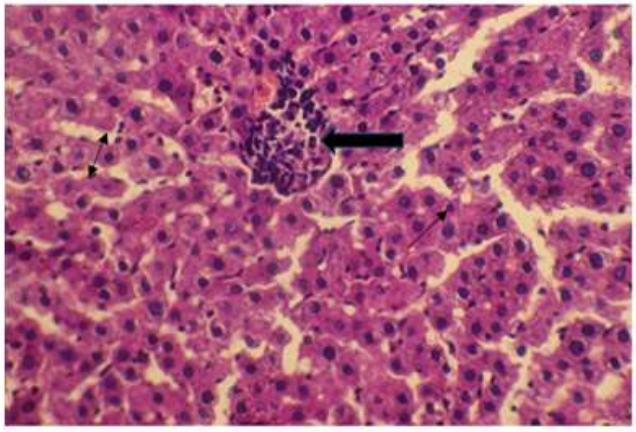

Figure (8) Cross section in the liver of animal treated with tamoxifen (50mg/kg) (H&E X40) shows large granulomatous lesion ⬅ With necrotic cells ⟶ and dilated sinusoids ⟷

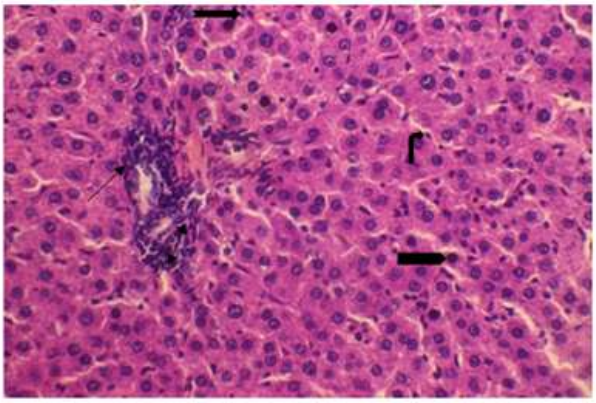

Figure (9) Cross section in the liver of animal treated with tamoxifen (50mg/kg) (H&E X40) shows aggregation of neutrophil ⟶ &mononuclear cells ⟷ in Portal area and scatter of Kupffer & mononuclear cells ↱ in liver parenchyma with Macrophage cells and necrotic cells ⟶

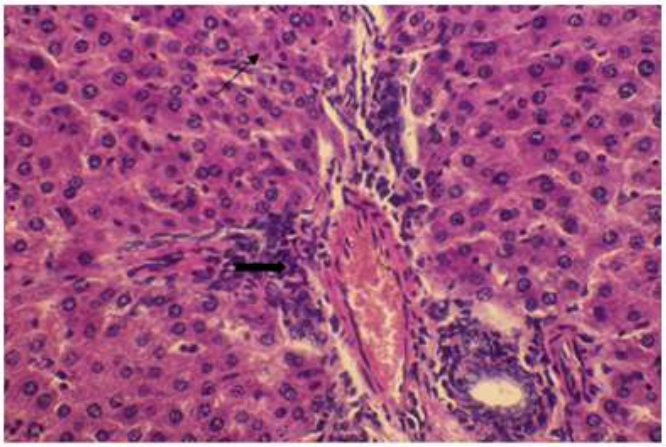

Figure (10) Cross section in the liver of animal treated with tamoxifen (50mg/kg) (H&E X40) shows macrophages ⟶ with Mononuclear and neutrophils cells aggregation in portal area ⟶

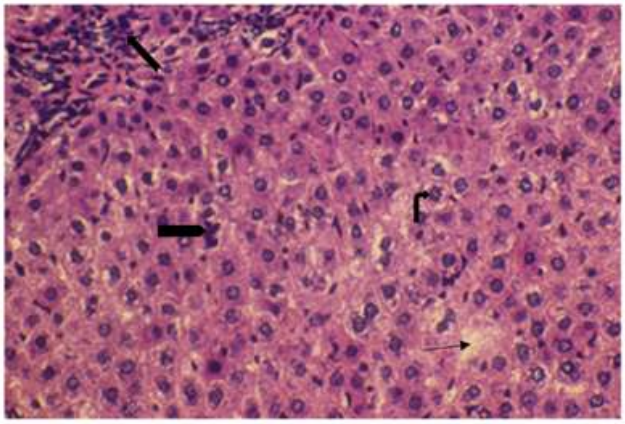

Figure (11) Cross section in the liver of animal treated with tamoxifen (50mg/kg) (H&E X40) shows granulomatous lesion ➡ With focal necrotic area ⟶ in the liver parenchyma and small ↱ aggregation of mononuclear cells ➡ with ballooning de-generation

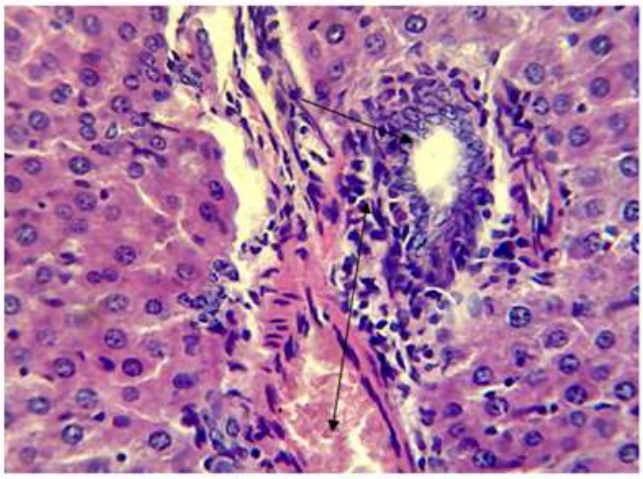

Figure (12) Cross section in the liver of animal treated with tamoxifen (50mg/kg) (H&E X40) shows hyperplasia of epithelial cells of bile duct ⟶ ,inflammatory cells infiltration neutrophils and mononuclear cells in portal area and congested blood vessels ↗↙

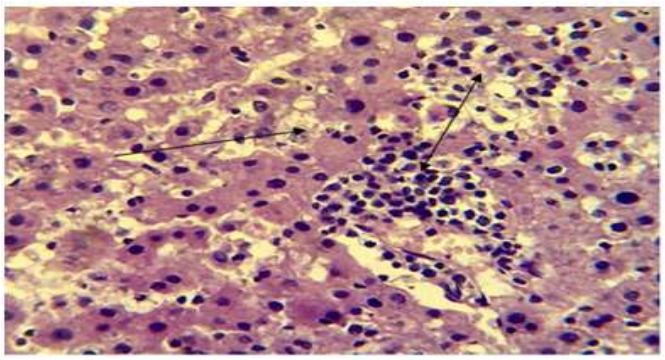

Figure (13) Cross section in the liver of animal treated with tamoxifen (50mg/kg) (H&E X40) shows inflammatory cells particularly neutrophils infiltrated in necrotic area of liver parenchyma ↗↙ with vacuolation of hepatocytes ⟶

IV. Disscution

Estimation of liver enzymes is an important indicator in assessing liver function . these enzymes indicate the extent of liver disease.[12] In the view of the findings of the current study, there was a significant increase of the enzyme GOT in treatment dose of the groups treated with TAM , while the multiplication doses showed a significant increase of the level of GOT and this corresponds to the results.[6,13,14]

This may explain the release of these enzymes from the cytoplasm into the bloodstream after liver cell damage and rupture of the plasma membrane as a result of high oxidative stress.[15]

As for the GPT enzyme, the result of this study showed significant changes in the level of enzyme efficacy of the TAM – treated groups, and this is consistent with the results they have reached.[16,14]

GPT is a cytosolic enzyme of the hepatocyte and an increase in it's level in serum reflects a leakage in plasma membrane permeability, which in turn, is associated with cell death, and is consider to be one of the indicators of liver necrosis.[17]

Also the elevation of enzyme level could be attributed to the damage structural integrity of the liver (possible by oxidative stress and lipid peroxidation).The lipid peroxidation causes distruption of the membrane bilayer and cell integrity and eventually necrosis that leads to leakage of these cytoplasmic enzymes into the blood.[18,19, 20]

This may explain that hepatic damage is still below the level of hepatic impairment and this is consistent with what they considers.[21] that the high efficacy of the GPT enzyme is a good function of chronic liver disease .

As for the efficacy of phosphatase enzyme ALP in the light of the results of the current study, there is a significant increase in the effectiveness of the enzyme in treatment doses treated with TAM and increase the proportion when doubling the dose and this is consistent with the findings of the result.[16,4]

This increase may be explained by membrane permeability changes due to hepatotoxicity under the influence of the drug and by oxidative stress and this is consistent with.[22]

It is also explains high levels of calcium or a shortage in the phosphorus, which are accompanying factors to the effectiveness of enzyme and this is what came flow with the results of the present study for the high levels of calcium and also because of stress and high activity of ROS due to serious chemical changes in the cell components even to the DNA level.[23]

The determination of bilirubin in the serum is of great clinical importance in liver function tests as it is a diagnostic function sensitive to hepatic jaundice as well as it has the characteristics of antioxidants caused by the excess ROS [24] and serves as an important cellular attorney for tissues [25] in the light of the findings of the current study, there are significant changes in bilirubin levels where the treatment groups showed a significant increase in multiplication doses, which is consistent with the result.[13]

This increase may be attributed to hemolytic anemia through a change in cell membrane structure and synthesis with a change in the effective stress of the membranes under the influence of the oxidative stress increased cell size, which results in cellular degradation by macrophages cells in the liver.[26]

The increased release of red cells in the liver and spleen or the formation of blood cells in an in active increases the level of bilirubin in the plasma or may be explained by the occurrence of internal or external liver obstructions in the Hepatocellular ducts.

The results showed a microscopic examination of histological sections of the liver treated with TAM . clear tissue changes can be achieved with necrosis with congestion and inflammation (infiltration), with hypertrophy as well as the presence of bleeding and kuppfer cell spread when multiplication doses with the presence of concentrations of neutrophil cells and lymphocytes and this is consistent with [27,14,28,29] results.

Histopathological effects in hepatocytes may be due to the fact that chemical treatments strongly induce hepatotoxic through the increase in generating lipid peroxidation in hepatic tissue [30] which destroys cell nuclei and cellular membranes , and that the generation of ROS by chemical treatments interferes with the anti-toxin defense system , which produces oxidative damage in different tissues , causing dysfunction in liver cells .

The contributory association of chemical agents and toxins with TAM with inter a cellular protein and phospholipids in cell membranes indicate a disturbance in the action of plasma membranes by its effects on effective transport proteins.[31] As the process of decomposition of diabetes and oxidative phosphorylation and then stores ATP depletion and lead to lower energy levels and thus failure work of sodium – potassium pumps , which leads to a malfunction of the ion transport mechanism and the accumulation of water inside the cells , which explains the bluging of hepatic cells.[32] As for the case of bloody congestion , the drug work on the occurrence of acute inflammation leads to changes in the flow of blood inside the vessels or may return to the weakness of blood flow due to blocked liver vein and the disruption of blood flow during the paranchyma cells.[33]

Hepatic cell damage is a companied by weak blood supply of the liver due to arterial blockage or clotting of the hepatic artery , which leads to O_2 deficiency and the release of particle lysosomes enzymes. the most important cause of obstruction of liver function . on the liver as the most important members of toxicity ,

including TAM. It is observed the expansion of blood vessels because of real estate TAM because of the release of fatty acids which permeate the installation of the hepatic cells. this is leading to the activation of certain types of prostaglandins , which cause vasodilation and expansion of bile ducts.[34] As for the necrosis of some liver cells occurs due to the cumulative effect of the drug in the liver cells and the accumulation of fatty droplets in cytoplasm some liver cells the cause of thrombophlebitis due to the accumulation of intense substances in the channels leading to the failure of chalstaeis.[35] The increase in the volume of liver cells is due to the effect of the drug on the drop of K – Na pump which leads to the retention of sodium due to increased intracellular pressure and swelling of the cell.[36]

TAM has an important role in inducing liver cancer, thromboembolic disorders in rats and humans. toxicity of TAM concerning multiple organs and tissues is well-established. This toxicity has been attributed to the reduction of the hexose monophosphate shunt The and the treatment with TAM increasing the incidence of oxidative stress in cells leading to tissue injury.[37]

Although TAM is a drug capable of inducing hepatotoxicity caused by mitochondrial dysfunction. Therefore, the mechanisms underlying TAM-induced liver injury deserve a closer look.[38] It is generally believed that mitochondrial dysfunction is amajor mechanism whereby drugs can promote liver toxicity.[39] Indeed, the evaluation of drug-induced mitochondrial damage has received considerable attention in the last years, as the study of the effects of drugs on mitochondria allows for a better understanding of the pharmacological and toxicological mechanisms underlying the mode of action of drugs. Isolated mitochondria fractions have been shown to predict drug safety, while decreasing the number of laboratory animals and the costs of preclinical studies. More-over, considering the exposure to high concentrations of drugs, the liver is often a target of mitochondrial toxicity.[40] Drugs can damage hepatic mitochondria in some individuals but not in others, and our current knowledge does not allow to predict the idiosyncratic liver injury related with drug-induced mitochondrial dysfunction.[41]

The use of tamoxifen has been shown to induce non-alcoholic steatohepatitis, which is a life-threatening fatty liver disease with a risk of progression to cirrhosis and hepatocellular carcinoma.[42]

V. Conclusion

Increased serum enzymes in the serum due to tamoxifen, liver damage and high toxicity. The dose (50 mg / kg) is the beginning of the high dose and should not be used because of its negative effects on the liver and its functions.

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Aqeel H. Qasim. "Histological and Biochemical Study of Different Doses of Tamoxifen Drug on Liver Of Albino Male Rat." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), vol. 12, no. 4, 2017, pp. 67–76.