Effect of different doses of Tamoxifen Drug on kidney of Albino male rats: Histopathological and biochemical study

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ABSTRACT

The Tamoxifen (TAM) is an effective anticancer drug; it is a hormonal treatment against estrogen necessary for the growth of cancer cells. The study was conducted to estimate the effects of TAM on some physiological and histological parameters. We used for 40 rats of the strain albino rat [Rattus norvegicus] divided into four groups. A total of three groups were given different TAM doses (30, 40, 50mg/kg) of the body weight four times a week during 10 weeks period. The control group was injected with physiological solution (Normal Saline 0.9%). At the end of dosage duration, the blood was collected for some biochemical parameters (kidney functions) investigation. The test results showed significant differences in the treated groups as three groups revealed a significant increase in Urea, Uric Acid, Creatinine levels. The kidney tissues histopathological examination of in the TAM groups showed histological changes which increased in accordance with elevated doses administration in comparison with the control group. The histological changes were inflammatory cells infiltration, blood congestion, pyknic and necrotic nucleus in glomerulus with massive degeneration in epithelial of renal tubules, and deformed of architecture of renal tissue. In addition, massive infiltration of mononucleosis adjacent dilated blood vessels with hemorrhage in tubule and necrosis of tubules that exhibited pyknic and necrotic nucleus in glomerulus as well. As well as the exhibited small granulomatous lesion with large dilated of blood vessels and necrotic tubules with great granulomatous trauma containing from accumulation of macrophages and lymphocytes with vacuolar degeneration of tubules and massive necrotic of glomerulus with thickness of Bowman capsule.

INTRODUCTION

The Breast cancer is the greatly diffused cancer in women and the second extreme common cancer worldwide and the prime cause of cancer death in women reference. Unfortunately, the happening rates of breast cancer are growing (Siegel et al., 2012; Weycker et al., 2011). Tamoxifen (TAM) has been abroad employ for many years as an assistance treatment for patients with breast cancer (Zhao et al., 2014). There for TAM has been recommended anticancer of breast by oral medication for both women and men (Shukla et al., 2016).

Tamoxifen reduces the level of estrogen receptor and estrogen without variation in progesterone contents it may be accompanying with hepatic damage (Suddek, 2014) treated with TAM the kidney exposure to degenerated with serious damage such as vascular congesting and edema in the
renal architecture (Pandey et al., 2015) tamoxifen caused increased of hypernephromas in DEN- initiated mice (Wolf and Jordan, 1992) Tamoxifen TAM is one of the drug competent of macrovascular, steatosis and nephrotoxicity which may be refer to the drug ability to spoil mitochondrial respiratory chain causing not only fatty acid oxidation retrogradation and steatosis but also promoted reactive oxygen species (ROS) production (Paschos and Paletas, 2009).

The Steatohepatitis is a rife trait of the metabolic illness and toxic responses to the pharmacological drugs; however, TAM a devilishly used as an anti-breast cancer medicine (Labbe et al., 2008). The current study aimed to investigate the effectiveness of different doses of TAM as histopathological changes and functional parameters of kidney in the males of albino rats as the following:

1. Studying the histological changes in kidney;
2. Testing the kidney function parameters (Urea, Uric Acid, Creatinine);
3. Determining the toxicity of long-term TAM dose with and the damage to the kidney at the tissue level.

MATERIALS AND METHODS

The Preparation of TAM Drug

Tamoxifen citrate (TAM) product is manufactured by AstraZeneca Oak Limited. In this study, a disc of TAM was grinned by clean mortar and 2 ml of regular salt water was added and well mixed to form a suspension solution (Lelliott et al., 2005). Later one, TAM tablets were weighed using sensitive balance to obtain three doses (30 mg, 40 mg and 50 mg) which administrated in accordance to the weight of the rat. The laboratory rates were orally administrated using 6 cm² feeding tube

Experimental Animal

The experimental animals used in this study were 40 Albino rats males; their weight is ranging (250 – 270 g)and at their age of (16 – 18) weeks. The animals were confirmed from Pharmaceutical control of the Ministry of Health in Baghdad. Rats were kept under suitable laboratory conditions with a 24/12 hour light-dark cycle at room temperature (25 °C). Rats were acclimatized with the laboratory conditions for 10 days prior onset of the experiment (Süzme et al., 2001).

Dealing with animals

At the end of experiment rats were killing under anesthesia there blood sample were collected and centrifuged at (3000 r m p 20 minutes), and serum was stored at (-20ºc) until used for Renal Functions test.

Experimental Design

A total of 40 of male rats were randomly divided into four groups (10 rats each) has and treated as following:

GROUP 1: (control): rats were orally administrated with normal saline (0.9 %)
Four times/week for 10 weeks.

GROUP 2: (treated with TAM): rats were orally administrated with(30mg/kg)
Four times/week for 10 weeks.

GROUP 3: (treated with TAM): rats were orally administrated with(40mg/kg)
Four times/week for 10 weeks.

GROUP 4: (treated with TAM): rats were orally administrated with(50mg/kg)
Four times/week for 10 weeks.

Histopathological Study

The Kidney Specimens were dissected at 0.5cm³ with directed fixed in the neutral buffered 10% formalin and dehydrated in an ascending series of Ethanol (50%–100%), cleared with xylene then embedded in paraffin wax after that sections 5-6μm and stained with Hematoxylin and Eosin (H&E) in accordance with (Chong et al., 2012) method then observed the sections by compound light microscope.

Statistical Analysis

In order to compare between each parameter of the Renal Functions test), we used to utilize analyze of variance, F-test, t-test, in a complete randomized design. The difference between means was analyzed using Least Significant Differences (LSD) at (p<0.05) and expressed as (Mean ± SEM), small letters indicate significant differences between means in columns (Table 1). We used SPSS software 2010 and excel pursuance in order to locate the result and sketch the figures with some effects.

RESULTS AND DISCUSSION

Kidney Function

Urea

The results showed significant differences (p<0.05) in the level of Urea in all treatments with TAM (30, 40, 50 mg/kg) (34.000±1.225, 35.750±1.830, 46.750±1.643) respectively compared with control
Table 1: The effect of TAM (30, 40, 50 mg/kg) on serum activates of Urea, Uric acid and Creatinine in Albino male rats

| Kidney function Concentration | Urea   | Uric acid | Creatinine |
|-------------------------------|--------|-----------|------------|
| Control                       | 29.250 c | 2.700 b  | 0.725 c    |
|                               | ±      | ±         | ±          |
|                               | 1.333 | 0.050     | 0.031      |
| 30 mg                         | 34.000 b | 3.050 ab | 0.800 bc   |
|                               | ±      | ±         | ±          |
|                               | 1.225 | 0.180     | 0.033      |
| 40 mg                         | 35.750 b | 3.350 a  | 0.850 b    |
|                               | ±      | ±         | ±          |
|                               | 1.830 | 0.200     | 0.046      |
| 50 mg                         | 46.750 a | 3.475 a  | 1.171 a    |
|                               | ±      | ±         | ±          |
|                               | 1.634 | 0.249     | 0.040      |
| LSD P ≤ 0.05                  | 4.416 | 0.536     | 0.110      |

Figure 1: The effect of TAM (30, 40, 50 mg/kg) on serum Urea concentration compared with control group in male rats.

Figure 2: The effect of TAM (30, 40, 50 mg/kg) on serum Uric Acid concentration compared with control group in male rats.
Figure 3: The effect of TAM (30, 40, 50 mg/kg) on serum Creatinine concentration compared with control group in male rats.

Figure 4: Cross section in the kidney of control animal shows no clear lesions, the control animals show no clear lesions in kidneys, the normal microscopic architecture of the Kidney showed normal kidneys with well demarcated cortex, medulla, normal Bowman’s capsule and glomerular as well as normal sized renal tubule. (H&E stain 40X)

Figure 5: Cross section in kidney of Animal treated with Tamoxifen 30mg/KG B.W exhibited pyknic and necrotic nucleus in glomerulus with massive degeneration in epithelial of renal tubules and deformed of architecture of renal tissue [H&E stain 40X]
Figure 6: Cross Section in kidney of animal treated with Tamoxifen 30mg/BW Exhibited massive necrotic renal tubules with vacuolation degeneration of tubules [H&E stain 40X]

Figure 7: Cross Section in kidney of animal treated with Tamoxifen 40mg/KG B.W exhibited small granulomatous lesion with large dilated of blood vessels and necrotic tubules also exhibited pyknic and necrosis nucleus in glomerulus [H&E Stain 40X]

Figure 8: Cross Section in kidney of animal treated with 40mg/KG B.W shows moderate granulomatous lesion with vacuolar degeneration of Renal tubules and massive necrotic area also note hemorrhage with tubules [H&E stain 40X]
Figure 9: Cross Section in kidney of Animal Treated with Tamoxifen 50mg/KG B.W shows massive infiltration of mononucleus adjacent dilated blood vessels with hemorrhage in tubule and necrosis of tubules also exhibited pyknic and necrotic nucleus in glomerulus [H&E stain 40X]

Figure 10: Cross Section in kidney of animal treated with Tamoxifen 50mg/KG B.W shows large granulomatous lesion consisting from aggregation of macrophages and lymphocytes with vacuolar degeneration of tubules and massive necrotic of glomerulus with thickness of bowman capsule also exhibited hemorrhage with Renal tubules [H&E stain 40X]

group which was (29.250 ± 1.333) with no significant differences between group (II) and (III) doses (Table 1; Figure 1).

Uric Acid
The results showed significant differences (p<0.05) in the level of Uric Acid in all treatments compared with control group while there were no significant differences between group (III0 and (IV) (3.350 ± 0.200, 3.475 ± 0.249) respectively.

However, these two doses have a significant differences compared with control group which is (2.700 ± 0.050) and there weren’t significant differences between group (II) (3.050 ± 0.180) and control group (Table 1; Figure 2)

Creatinine
Our results showed significant differences (p<0.05) in the level of Creatinine in all TAM (30, 40, 50mg/kg) (0.800 ± 0.033, 0.850 ± 0.046, 1.171 ± 0.040) doses respectively compared with control group which was (0.725 ± 0.031), while there is not a significant differences between group (II0 and group (III) (Table 1; Figure 3).

Histopathological Examination of Kidney
Group I: Control group animals
The control animals showed no clear lesions in the kidney (Figure 4). The normal microscopic of the Kidney showed normal kidneys with well demarcated cortex, medulla, normal Bowman’s capsule and glomerular as well as normal sized renal tubule.

Group II: animals treated with TAM (30 mg/kg)
Microscopic section in the kidney of animals treated with TAM (30 mg) showed exhibited pyknic and necrotic nucleus in glomerulus massive epithelial of renal tubules and deformed of architecture of renal tissue (Figure 5) with vacuolation degeneration of tubules and Exhibited massive with necrotic renal tubules (Figure 6)

Animals treated with TAM (40 mg/kg)
The animals treated with dose TAM (40mg) exhibited small granulomatous lesion with large dilated of blood vessels and necrotic tubules also exhib-
mented pyknic and necrosis nucleus in glomerulus (Figure 7) with vacuolar degeneration of Renal tubules and massive necrotic area also note hemorrhage with tubules (Figure 8).

Animals treated with TAM (50 mg/kg)

Histological section in the kidney of animal treatment with dose TAM (50mg) shows massive infiltration of mononucleosis adjacent deleted blood vessels with hemorrhage in tubule and necrosis of tubules also exhibited pyknic and necrotic nucleus glomerulus (Figure 9) with considerable granulomatous trauma depend from aggregation of macrophages and lymphocytes with vacuolar degeneration of tubules and massive necrotic of glomerulus with thickness of bowman capsule also exhibited hemorrhage with Renal tubules (Figure 10).

In view of the results of the current study there is a significant increase in Urea, Uric Acid, Creatinine levels of albino rats treated with TAM and when the level of multiple doses (30, 40, 50mg/kg) increased in contrast with the control group. Our results are in agreement with what mention by Saleh et al. (2016); Pandey et al. (2015). In order to explain the potential mechanism of TAM-induced nephrotoxicity made due to Reactive Oxygen Species (ROS) created by TAM which can damage the kidney tissues system and induce free radicals fabrication and production in kidney tissues (reference). The antioxidant is the rule target to lower the nephrotoxicity stimulated by TAM transcending the antioxidant ability so making cell injury and reduces its strength to detoxify (ROS) (Zuhair and Al-amri, 2011). The Oxidative stress is the substantial agent in the expansion of the types of human complications that result from overproduction of (ROS) and weakness in the biological defense system (reference). Regardless, TAM breeds (ROS) realization and reduction in the cellular system; therefore, (ROS) reacts with the renal mitochondrial membranes presenting great quantities of oxygen radicals that cause impairment of the renal tissues (Tabassum et al., 2007; Parlakpinar et al., 2005). The histological sections of the rat kidneys treated with TAM showed clear tissue changes such as necrosis in glomerulus with massive degeneration in epithelail of renel tubules and deformed of architecture of renel tissue exhibited small granulomatous lesion with large dilated of blood vessels and necrotic tubules also exhibited pyknic and necrosis nucleus in glomerulus, moderate granulomatous lesion with vacuolar degeneration of Renal tubules and massive necrotic area also note hemorrhage with tubules, massive infiltration of mononuclear adjacent delited blood vessels with hemorrhage in tubule and necrosis of tubules also exhibited pyknic, large granulomatous trauma consist from ingathering of macrophages and lymphocytes with vacuolar degeneration of tubules and massive necrotic of glomerulus with thickness of bowman capsule also exhibited hemorrhage with Renal tubules and this is consistent with previously reported results (Saleh et al., 2016; Gabri et al., 2004). Many studies on human and animal suggested that kidney injury occur due to oxidative stress, through conversion to electrophiles, nucleophiles and redox-active reactants which has a direct toxic effect and causes cellular dysfunction. Oxidative stress leads to the formation of vasoactive mediators that affect glomerular filtration rate through vasoconstriction which subsequently leads to morphological changes (Ali, 2012; Olufunsho and Akintonwa, 2012).

CONCLUSION

In our current study, we discovered the administration of Tamoxifen (TAM) for four times a week for a 10 weeks period will raise oxidative stress disadvantage in the tissues of albino rats kidney. Oxidative stress generated by TAM could excess the grades of renal function tests (RFT) in addition to nephrotoxicity and the kidney architecture. The suggested mechanism of TAM to make efficient of the kidney function impossibly due to improve the levels of creatinine, urea, and uric acid. Furthermore, it could abolish the poisoning activity of renal free radicals created by TAM through the arrangement of oxidant defense system. According to the present data, TAM induces the change in the kidney tissues; therefore, a greater dose concentration is given, more tissue changes will occur.

Conflict of Interest

Authors declare no Conflict of Interest.

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