

Histo Toxic and Pathological Study of Tamoxifen Citrate on The Kidney of Female Rats

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Abstract: we took 32 laboratory female rats (*Rattus norvegicus*) sexually mature 16 weeks age and 200±50 gm in weight, were used, divided to four groups control, low dose, intermediate dose and high dose, later administration by oral gavage tamoxifen citrate for three months 50mg/kg low dose, 100mg/kg intermediate dose and 200mg/kg high dose. after that the animals killed, tissue were taken fixed in 10% formalin, section prepares and stained with hematoxylin and eosin in addition special stains for connective tissue by Mallory was done. Histopathological result showed as the kidney with moderate vacuolated subcapsular cortical tubules, dilated tubules in inner cortex and positive Mallory blue stain collagen fibers around congested blood vessels were seen. Finally it appears that subcapsular cortical tubules (proximal convoluted tubules) as the target toxic effect of tamoxifen citrate in the kidney.

Keywords: histopathology, toxicity, tamoxifen, in rats

I Introduction

Tamoxifen is a drug that has been in worldwide use for the treatment of anti estrogen receptor (ER)-positive breast cancer for over 30 years; it has been used in both the metastatic and adjuvant setting (Matteo et al., 2012). At present, tamoxifen is the only proven oral agent for the adjuvant hormonal treatment of hormone receptor positive breast cancer in premenopausal women (Colleoni et al., 2006), and it can be used in both pre- and postmenopausal women who are at increased risk of breast cancer (Fisher et al., 2005). The concentrations of TAM were detected 2-9-Fold higher in kidneys, than the serum levels. Similar results have been reported following long term TAM administration both in human and animals, Tissue accumulation of TAM may be related to the presence of the anti estrogenic binding-sites (AEBS) in the tissue (Fronson et al., 1973). In the Tamoxifen treated the kidney was extensively degenerated with severe vasocongestion and edema in the renal parenchyma and also wide dilation in the Bowman's capsule. In most areas the Bowman's capsule lost its normal morphology (Sanjeev et al., 2014). The tamoxifen increased incidence of hypernephromas in DEN-initiated rats (Wolf and Jordan, 1992).

II Materials and methods

The experiment was conducted at the laboratory animal house of the Veterinary Medicine College – University of Basra, where 32 white laboratory female rats (*Rattus norvegicus*) sexually mature 16 weeks age and 200±50 gm weighing, were used. The animals were accommodated in the same laboratory condition by keeping them in special cages (acrylic cages) as (4 animals per cage) for about 10 days for acclimation. The experimental conditions were unified for all animals, where the room temperature was set between 20-25 °C by the use of air conditioner, and the light period was 12 hours daily, by the use of two fluorescent lamps, and the humidity rate was about 50%. Food and water were provided daily (ad libitum). The experiment was for 90 days (3 months) by daily dosing. The study consisted of four groups high dose of 40 mg for body weight, 20 mg body weight as intermediate dose and 10 mg body weight low dose and untreated controls.

2-1. Procedure of Tissue Processing

In brief the routine sequence of events according to (Luna, 1968 and Bancroft *et al.*, 1990) is as follows:-

1	The tissue was obtained .
2	The tissue was fixed for 24 hours or more in an appropriate fixative buffered formalin 10%.
3	Tissue was dehydrated through ascending alcohol (increasingly higher concentration) alcohols overnight (12 hrs).
4	Alcohol was replaced with xylol or chloroform for clearance.
5	The specimen was infiltrated with paraffin.
6	Also it was embedded in a block of paraffin.
7	Thin sections were cut on the microtome (5µm- thick).
8	The sections were mounted on glass slides.
9	The embedding medium was removed (dissolved) by putting the slides on hot plate overnight.
10	The sections were rehydrated in descending alcohols.
11	The sections were stained with an appropriate staining sequence haematoxylin and eosin (H&E).

2-2.Staining Procedure of haematoxylin and eosin

In the staining procedures used haematoxylin and eosin stains according to (Drury, 1967) for paraffin sections as following:

1. The sections were de waxed in xylol, then rehydrated through graded alcohol to water.
2. The sections were stained in hematoxyline for a suitable time.
3. The sections were washed well in running tap water until sections "blue 5-minutes or less.
4. Then all sections were stained in 1 percent eosin for minute .
5. The sections were washed in running tap water for 1-5 min.
6. All sections dehydrated through alcohol, cleared in xylol, and finally mounted in Distyrene Plasticizer Xylene (DPX).

2-3.Staining Procedure of Mallory

A stain composed of aniline blue, acid fuchsin, and orange G, It is a good stain to use for distinguishing cellular from extracellular components, Collagen fibers stain an intense blue, Mucus and ground substance take on varying shades of blue, Cytoplasm and neuroglia stain red, Elastic fibrils, red blood cells and nucleoli stain pink or yellow (Mallory, 1961) .

- Carbolfuchsin solution.
- Acid differentiating buffer.
- Phosphomolibdic acid solution.
- Polychrome solution according to Mallory.

III Results

The microscopic examination of the kidneys (especially cortical tubules and glomeruli) in the control group was within the normal histological limits in group (A) as in Figures (1,2).

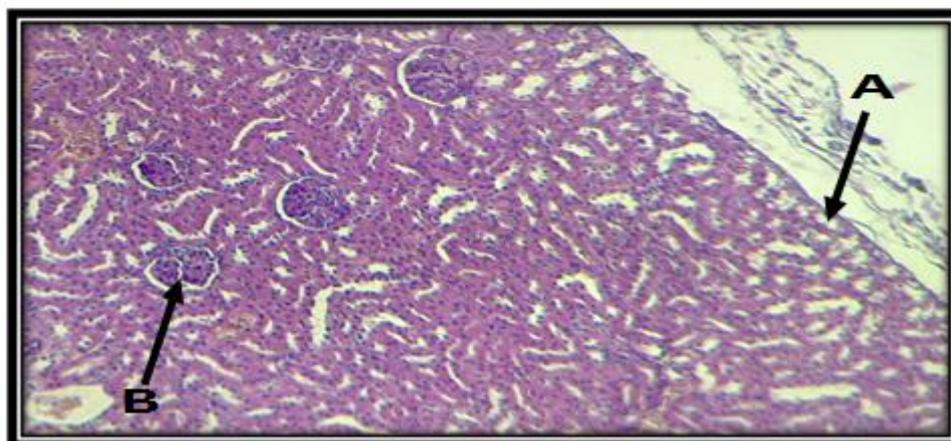


Fig.(1) Kidney Note: Normal Cortical Tubules(A) , Normal Glomeruli (B) Of Rat Treated With Normal Saline Within Normal Limits. (H&E) Stain.100x.

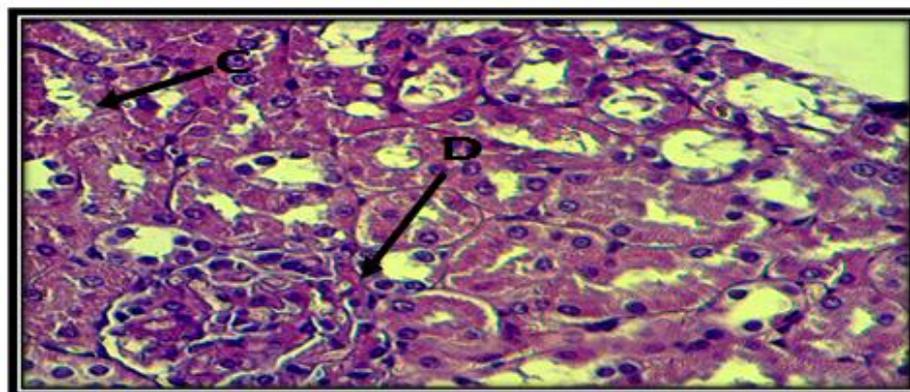
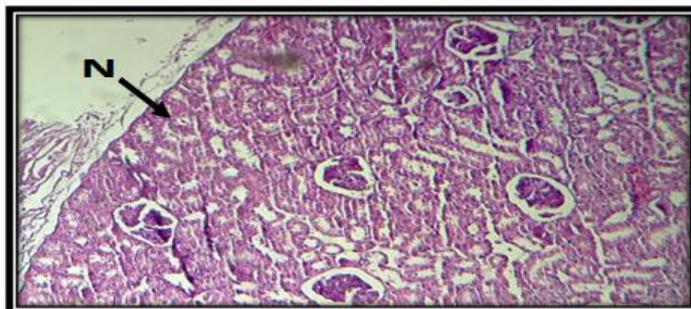
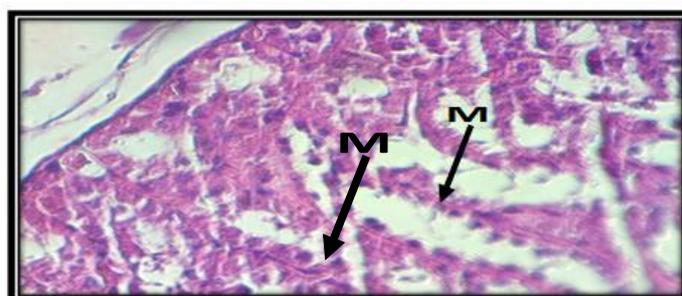


Fig.(2) kidney note: normal cortical tubules(C) and glomeruli (D) of rat treated with normal saline within normal limits. (H&E) Stain.400X.

In group (B) kidney of rat treated with 50 mg/kg b.w. of tamoxifen citrate showed: moderate vacuolated sub capsular cortical tubules and dilated tubules in inner cortex as in figures(3,4).

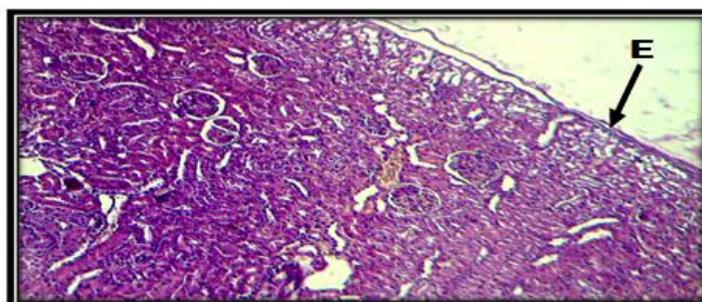


(fig 3.) kidney of rat treated with 50 mg/kg b.w. of tamoxifen citrate showed: vacuolated sub capsular cortical tubules(N). (H&E) Stain.100 X.

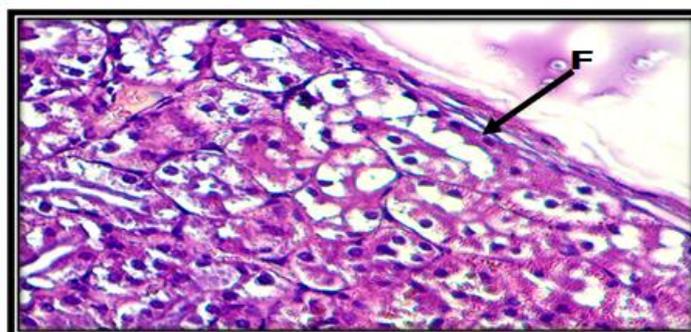


(fig4 .) kidney of rat treated with 50 mg/kg b.w. of tamoxifen citrate showed: dilated tubules in inner cortex(M). (H&E) Stain.400 X.

In group (C) kidney of rat treated with 100 mg/kg b.w. of tamoxifen citrate showed: moderate vacuolated sub capsular cortical tubules and dilated tubules in inner cortex less than high dose as in figures(5,6).

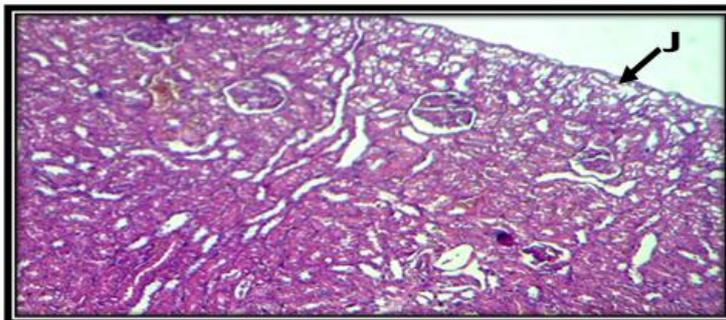


(Fig.5) Kidney of rat treated with 100 mg/kg b.w. of tamoxifen citrate showed:vacuolated sub capsular cortical tubules(E). (H&E) Stain.100 X.

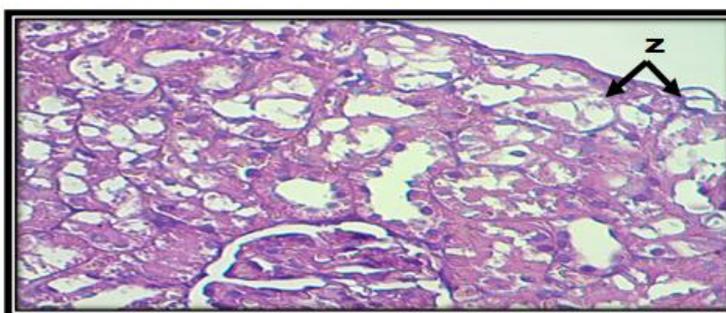


(Fig.6) Kidney of rat treated with 100 mg/kg b.w. of tamoxifen citrate showed:moderate vacuolation sub capsular cortical tubules (F) . (H&E) Stain.400 X.

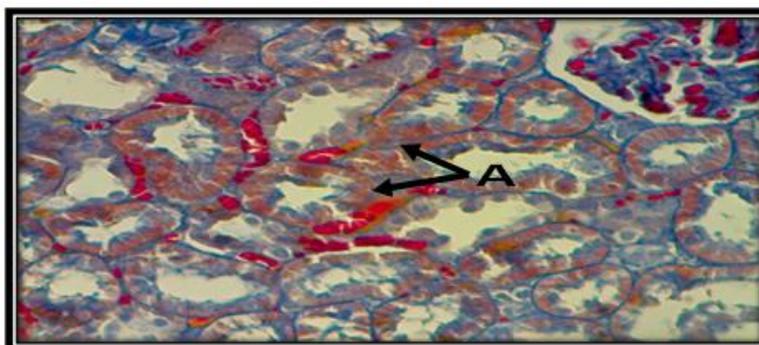
In group (D) kidney of rat treated with 200 mg/kg b.w. of tamoxifen citrate showed: marked vacuolated sub capsular cortical tubules and dilated tubules in inner cortex as in figures(7,8).



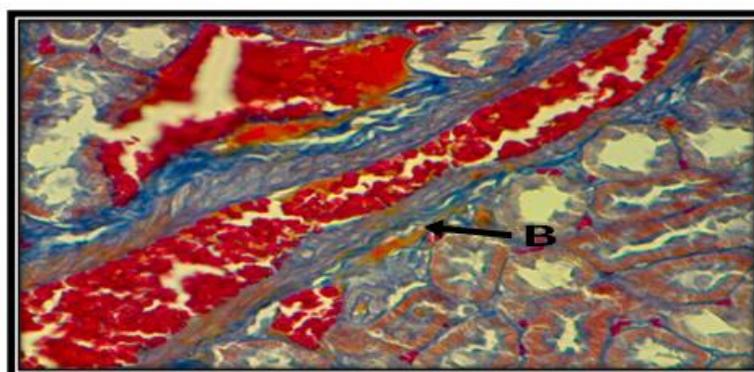
(Fig.7) Kidney of rat treated with 200 mg/kg b.w. of tamoxifen citrate showed :marked vacuolated degenerate sub capsular cortical tubules (proximal convoluted tubules) (J) . (H&E) Stain.100 X.



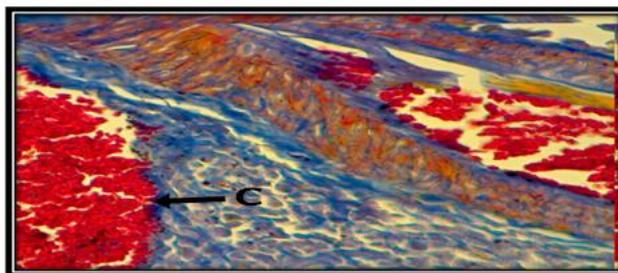
(Fig.8) Kidney of rat treated with 200 mg/kg b.w. of tamoxifen citrate showed :marked vacuolated degenerate sub capsular cortical tubules (proximal convoluted tubules) (J) . (H&E) Stain.400 X.



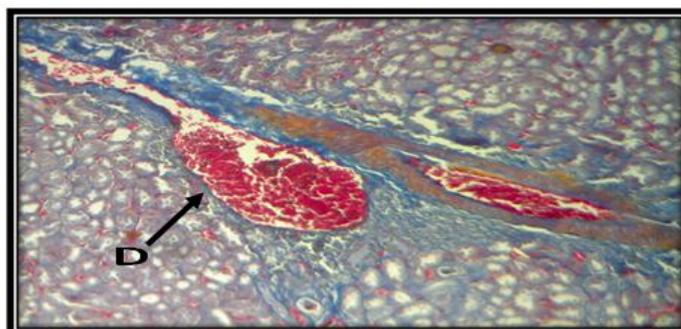
(Fig.9) kidney of rat treated with normal saline hardly any Mallory positive blue collagen fibers between cortical tubules(A). mallory stain 400 X.



(Fig.10) kidney of rat treated with normal saline only few Mallory positive blue collagen fibers in peri vascular regions(B) .mallory stain 400 X.



(Fig.11) kidney of rat treated with 200 mg/kg b.w. of tamoxifen citrate showed: blue stain collagen fibers around congested blood vessels(C). mallory stain 400 X.



(Fig.12) kidney of rat treated with 200 mg/kg b.w. of tamoxifen citrate showed: blue stain collagen fibers around congested blood vessels(D). mallory stain 100 X.

IV. Daisscution

In the present research project of the toxic effect on the toxicologic pathological effects of tamoxifen citrate on the kidney showed varying degrees of histopathological effects in supcapsuler cortical tubules (proximal convoluted tupules) in response to varing dosage levels. The pathological toxic effects on the kidney was accumulative change of the tested compound in the kidney. (Fronson *et al* .,1973) reported to 2-9 fold higher concentration of tamoxifen citrate in the kidney of treated animals, toxicolgic pathological in the study agree with (Fronson *et al* .,1973) that degeneration effects on cortical tubules in treated animals due to accumulative effect of tested compound. (Sanjeev *et al.*,2014) reported changes in the kidney of treated animals characterized by extensive degenerative with sever vasocongestion and edema in the renal parnchymal and wide dilatation of bowman's capsule ,the pathological effect of (Sanjeev *et al.*,2014) agreed with finding in the present paper as there was very clear pathological effects on the supcapsuler cortical tubules (proximal convoluted tubules) characterized by vacuolation and degeneration. (Wolf and Jordan, 1992) stud the effects of tamoxifen citrate and found evidence of hypernephromas in DEN-initiated rats.the apove finding could be due to degenerate effects of the treated rats and that's will agree with finding the present paper as there were very clear evidence of the direct effect tamoxifen citrate in supcapsuler cortical tubules (proximal convoluted tubules).The scientific research on tamoxifen citrate of the present topic strongly indicate that the kidney is the target organ for the toxicolgic pathologic effect of tamoxifen citrate,as the tested compound accumulate in the proximal convoluted tubules resultin degeneration and vacuolation.

Referance

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