Research article

Investigations into Nolvadex toxicity on the kidneys of albino male rats: Physiological and histopathological study

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ABSTRACT

Introduction and Aim: Breast cancer is the most prevalent sex-based malignancy worldwide. Nolvadex is commonly used as first-line therapy for patients with breast cancer. However, studies have indicated that long-term usage of this medicine can result in nephrotoxicity, which can damage kidney tissues. So, the aim of this research was to examine what happened to the serum levels of urea, uric acid, and creatinine when the drug was given to rats in a range of different doses.

Materials and Methods: Male Wistar rats (n=40) were randomly divided into four groups (n=10 each). The physiological solution (normal saline, 0.9%) was given to the control group (G1). Rats in Groups 2, 3, and 4 received Nolvadex at dosages of 30, 40, and 50 mg/kg of body weight four times weekly for a total of 10 weeks. At the end of the experimental period blood drawn from these animals were checked for serum concentrations of urea, uric acid, and creatinine. The animals were sacrificed and their kidneys subjected to histopathological examination. Data obtained was subjected to statistical analysis.

Results: The levels of urea, uric acid, and creatinine that were discovered in the experimental groups were all determined to be significantly higher than the levels that were discovered in the control group. In the experimental group that was given Nolvadex, an infiltration of inflammatory cells, blood congestion, pyknic and necrotic nuclei in the glomerulus, substantial epithelial deterioration in the renal tubules, and an altered renal tissue architecture were detected.

Conclusion: Serum levels of urea, uric acid and creatinine levels increased when experimental rats were administered with varying concentration of the drug Nolvadex. Its administration caused alteration to the architecture of renal tissue.

Keywords: Breast cancer; Nolvadex; Kidney; Urea; Uric acid; Creatinine.

INTRODUCTION

Although breast cancer in men is uncommon, it is the most common form of cancer in women and is a concern for public health everywhere in the world. Breast cancer is a type of cancer that develops when abnormal cells in the breast tissue begin to grow in an uncontrolled manner. It is the most common form of cancer in women and is the leading cause of cancer deaths among women worldwide (1). According to projections provided by the World Health Organization, breast cancer is one of the most frequent types of cancer found around the globe, and it is anticipated that there will be 2.26 million new cases of breast cancer diagnosed across the globe in the year 2020. It is anticipated that there will be 685,000 deaths worldwide caused by breast cancer in the year 2020. This makes breast cancer one of the major causes of mortality from cancer among women. The significance of early identification and the availability of effective treatment options for breast cancer is shown by these data. Screening for breast cancer on a regular basis and raising knowledge about the condition can aid in the disease's early diagnosis and care, which can lead to improved results for patients (1, 2). Nolvadex is a medication that has been used for several years to treat breast cancer and, in certain people, to lessen the risk of breast cancer returning. It is also known by its generic name, tamoxifen. It is advised as an anticancer medicine that can be used orally for the purpose of lowering the risk of breast cancer development in both men and women. Although Nolvadex has the potential to be a helpful treatment, just like any other prescription, it comes with the risk of experiencing negative effects (3). On the other hand, there are things that both men and women can do to lessen their chances of acquiring breast cancer (4). An anti-estrogen medication with the brand name Nolvadex is referred to as tamoxifen. Tamoxifen is the generic name for Nolvadex. It accomplishes this by affixing itself selectively to estrogen receptors, which, in turn, lessens the effect that estrogen has on the breast cells. It is possible that using Nolvadex will have unfavorable side effects, such as damage to the liver (5), congestion of the blood vessels, and edema in the renal architecture (6). An increase in the number of hypernephromas in mice

that have been started on DEN (7, 8) is one of the gynecological adverse consequences of using Nolvadex. Drug-induced liver injury and non-alcoholic fatty liver disease are other gynecological adverse effects of using Nolvadex (9,10). Rats were administered varied doses of Nolvadex, and their serum levels of urea, uric acid, and creatinine were measured to determine how the drug affected those parameters. The purpose of the study was to investigate the effects of Nolvadex on the renal system. Alterations in the histology of the kidneys were also investigated. Because the findings from trials conducted on animals may not necessarily be applicable to those conducted on humans, additional research is required before determining the effects of Nolvadex on renal function in people.

MATERIALS AND METHODS

Animals

The male albino rats (n=40) of the Wistar strain, numbering forty in all, were obtained from the pharmaceutical control section of the Ministry of Health in Baghdad. The rats ranged in weight from approximately 250 - 270 grams and had an age range of 16 - 18 weeks. For the course of the investigation, the rats were housed in a facility that maintained a light-dark cycle of 24 hours on and 12 hours off and a constant room temperature of 25 °C. Ten days of acclimatization to this setting were performed on the rats before the start of the test. During the experiment, the rats were fed with a standard rodent diet and filtered water ad libitum.

Experimental animal groups

The rats used in the experiment were split up into four groups according to a random selection, with ten rats in each group: Group 1:(control group), in which rats received four doses per week of normal saline (0.9%) orally for ten weeks. Rats in Groups 2, 3, and 4 were given Nolvadex orally 30 mg/kg, 40 mg/kg, and 50 mg/kg of their body weight once a week for 10 weeks.

The drug Nolvadex (Nolvadex citrate, AstraZeneca Oak Limited, India) was procured in tablet form. The tablets were weighed (30 mg, 40 mg and 50 mg respectively) using a sensitive balance. The weighed Nolvadex drug was ground using mortar and pestle, to which 2 ml of normal saline (0.9% NaCl) was added and mixed well to form a suspension solution (11). Rats in the experimental test groups were given the suspension orally using a 0.6 mm in diameter feeding tube depending on their body weight.

Each group's rats were euthanized under general anesthesia when the experiment was complete. Blood was collected from these animals before they were sacrificed, centrifuged at 3000 rpm for 20 minutes, and the resulting serum was frozen at -20 ºC for later use. The urea, uric acid, and creatinine concentrations in serum were measured spectrophotometrically using kits (Agappe, India), following the manufacturer's instructions.

Histopathological study

We took kidney tissue sections (0.5cm3) from each animal in the test group, fixed them in formalin at a concentration of 10%, and then dehydrated them in ethanol at a progression of concentrations ranging from 50% all the way up to 100%. Tissue was required for whatever reason. Following the preparation of 5-6 mm sections using a conventional microtome, the sections were stained with hematoxylin and eosin (H & E) and visualized using a compound light microscope (12,13).

Statistical analysis

The statistical software SPSS 2010 was used to do the analysis on the data. The acquired data is shown as the Mean+ Standard Error, and the difference between the means was examined using Least Significant Differences (LSD) with a significance level of less than 0.05. Each parameter of the renal test function was examined and compared using one way analysis of variance, F-test, and t-test.

RESULTS

Urea

Among According to the findings, each of the three experimental groups that were given Nolvadex exhibited substantially different levels of serum urea in comparison to the group that served as the control ($p<0.05$). However, there was not a significant difference in the levels of serum urea between Groups 2 and 3, which were the two groups that were given greater doses of Nolvadex (40 and 50 mg/kg of body weight, respectively) (Table 1; Fig.1).

Uric acid

When compared to the control group, all treatments demonstrated significant differences in the serum level of uric acid ($p<0.05$), although Groups 3 and 4 did not show any significant differences (3.350±0.200, 3.475±0.249) respectively. However, these two doses showed significant differences compared with the control group (2.700±0.050). No significant difference was seen between Group 2 (3.050±0.180) and control group (Table 1; Fig. 2). It's worth noting that the passage reports that no significant difference was seen between Group 2 and the control group, indicating that the dose of 30 mg/kg of body weight may not have a significant impact on serum uric acid levels in rats.

Creatinine

Our findings revealed significant elevated levels (0.800 ± 0.033, 0.850 ± 0.046, 1.171 ± 0.040 respectively) ($p<0.05$) of creatinine in animals administered with t different doses of Nolvadex (30, 40, 50 mg/kg), compared with control group (0.725 ±

0.031), but there was no statistically significant difference between Groups 2 and 3 (Table 1; Fig. 3)

Table 1: The effect of Nolvadex on serum urea, uric acid, and creatinine levels in Albino rats

Nolyadex concentration	Serum concentration		
	Urea	Uric acid	Creatinine
Control	29.250±1.333^{c}	2.700± 0.050^{b}	0.725 ± 0.031^{c}
30 mg/kg	34.000 ± 1.225^{b}	3.050 ± 0.180^{ab}	0.800 ± 0.033^{bc}
40 mg/kg	35.750± 1.830^{b}	3.350 ± 0.200^{a}	0.850 ± 0.046^{b}
50 mg/kg	46.750 ± 1.634^{a}	3.475 ± 0.249^{a}	1.171 ± 0.040^{a}
LSD P ≤ 0.05	4.416	0.536	0.110

^{a,b,c,bc} indicate significant differences between means in columns; LSD: Least significant difference

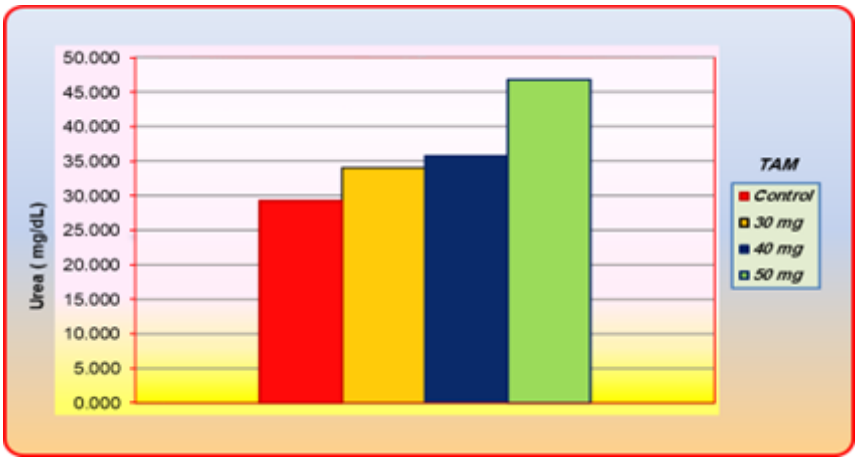

Fig. 1: The effect of Nolvadex (30, 40, 50 mg/kg) on serum urea concentration in male rats

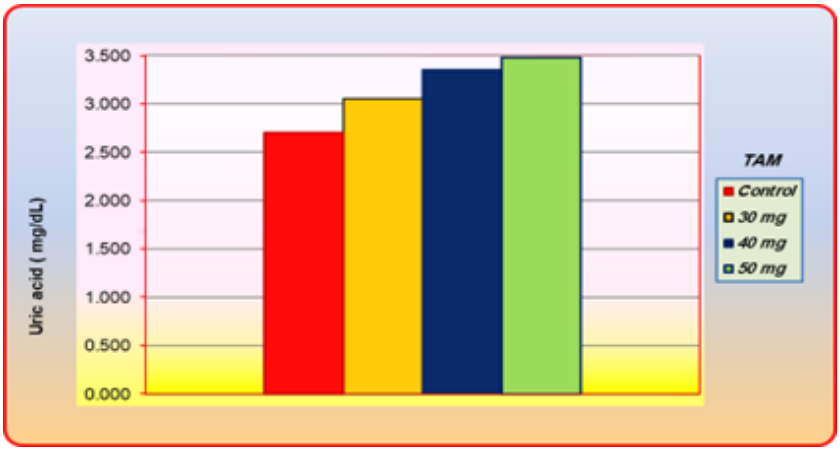

Fig. 2: The effect of Nolvadex (30, 40, 50 mg/kg) on serum uric acid concentration in male rats

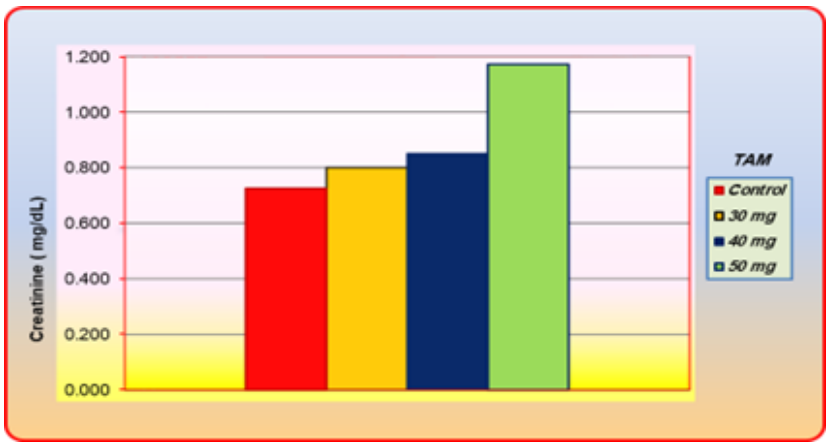

Fig. 3: The effect of Nolvadex (30, 40, and 50 mg/kg) on serum creatinine concentration

Kidney histopathological results

Control group animals

The kidneys of the animals that served as controls did not display any glaring abnormalities or lesions (Fig. 4).

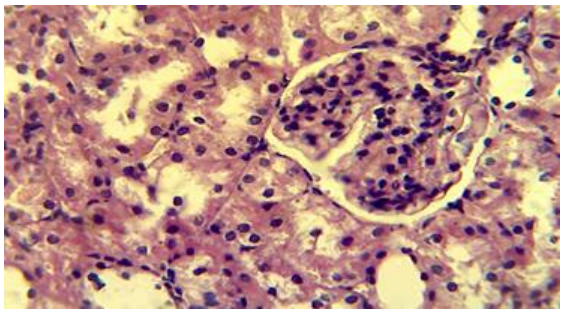

Fig. 4: Cross sections for renal tubules and glomeruli were also normal in size and Bowman's capsule was present in all normal kidneys. The kidneys of the control animals showed no obvious damage, and there were also no obvious lesions in these organs. (40X, H&E stain).

A standard microscopic examination of the kidneys revealed that they were in good health. This was demonstrated by the existence of normal Bowman's capsule and glomerulus, as well as normal-sized renal tubules. Moreover, the cortex and medulla of the kidneys were well-defined.

Animals treated with Nolvadex (30 mg/kg)

When the kidneys of rats were inspected under a microscope after receiving 30 milligrams of Nolvadex, abnormalities such as pyknic and necrotic nuclei in the glomerulus, a wide epithelium of renal tubules, and a deformed architecture of renal tissue were observed (Fig. 5a). There was extensive necrotic tissue present in the kidneys, and vacuolation and tubule deterioration were also present. Also, glomerular filtration was impaired (Fig. 5b).

Animals treated with Nolvadex (40 mg/kg)

In the rats that were treated with Nolvadex, microscopic granulomatous lesions, dilated blood vessels, and necrotic tubules were discovered. In addition to this, they had pyknic and necrosis nuclei in the glomerulus (Fig. 6a), as well as vacuolar degeneration of the renal tubules, an extensive necrotic region, and hemorrhage along with the renal tubules (Fig.6b).

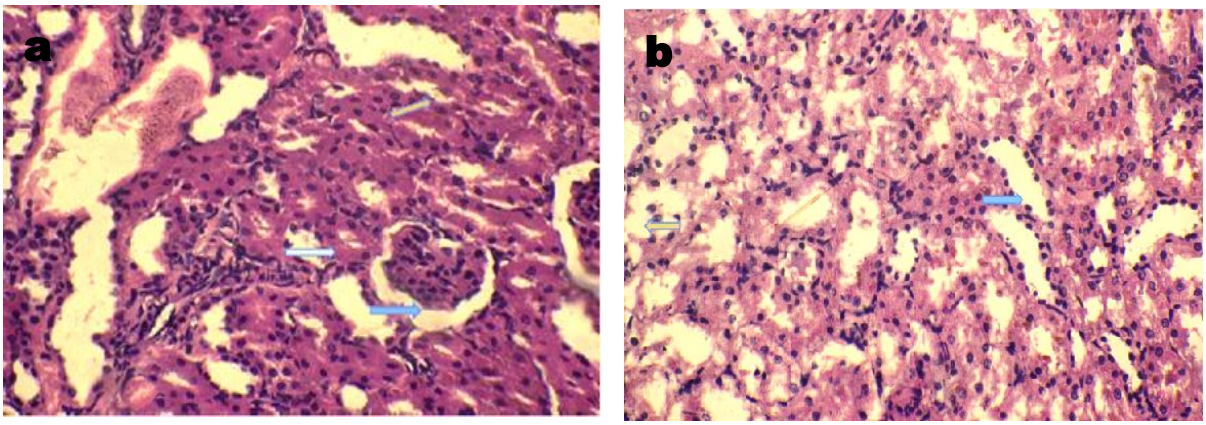

Fig. 5: Tissue cross section of Nolvadex (30 mg)- treated rat kidney showing **a)** pyknic and necrotic nuclei in the glomerulus, as well as severe degeneration in the epithelium of the renal tubules and distorted renal tissue architecture (H&E stain 40X), **b**) massive necrotic renal tubules with vacuolation degeneration of tubules (H&E stain40X)

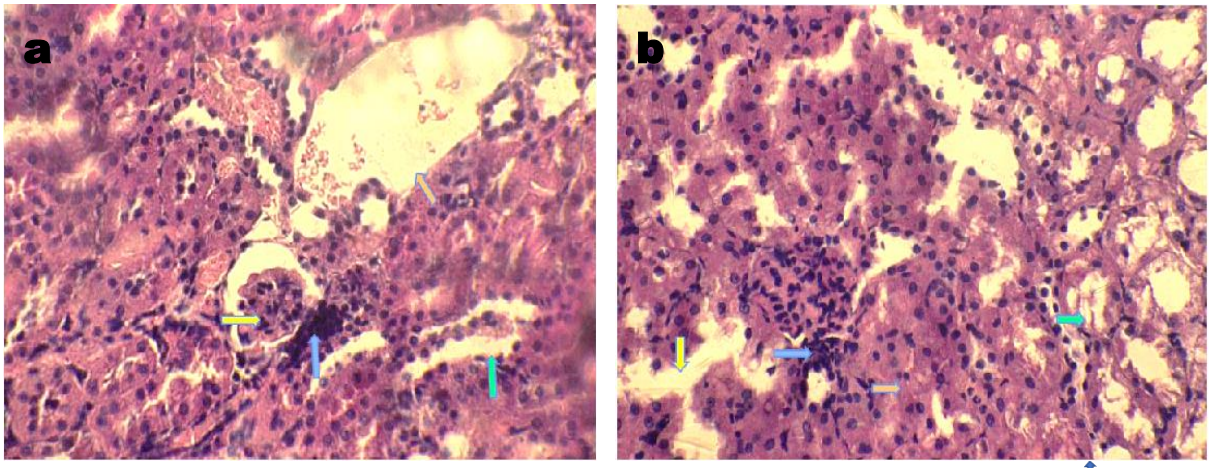

Fig. 6: Tissue cross section of Nolvadex (40 mg)- treated rat kidney showing **a**) tiny granulomatous lesion with big dilated blood vessels and necrotic tubules, as well as pyknic and necrosis nuclei in the glomerulus (H&E stain 40X) and **b**) moderate granulomatous lesion with vacuolar degeneration of Renal tubules and massive necrotic area also note hemorrhage with tubules (H&E stain 40X)

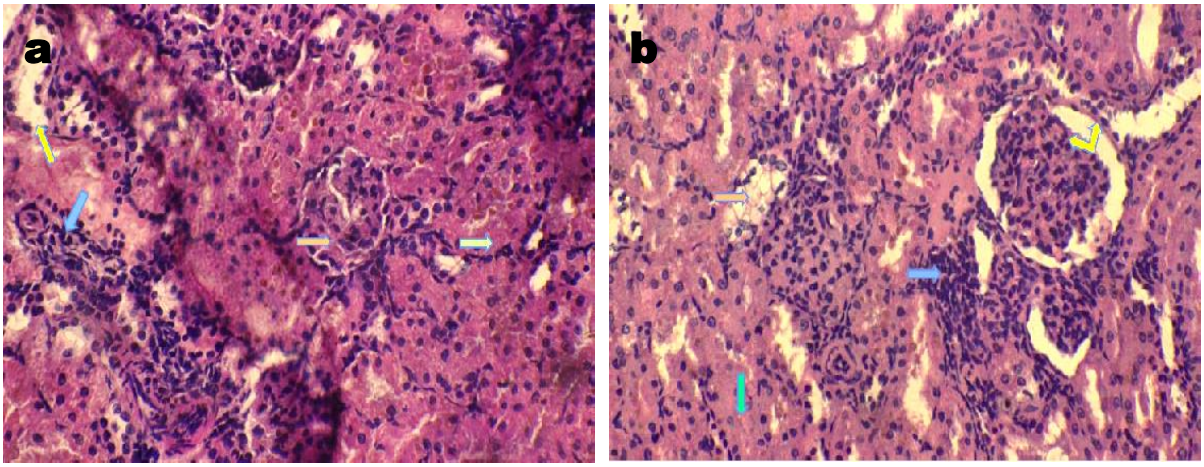

Fig. 7: Tissue cross section of Nolvadex (50 mg)- treated rat kidney showing **a**) massive infiltration of mononucleus adjacent deleted blood vessels with hemorrhage in tubule and necrosis of tubules also exhibited pyknic and necrotic nucleus in glomerulus (H&E stain 40X) and **b**) large granulomatous lesion consisting of 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)

Animals treated wiyh Nolvadex (50 mg/Kg)

Histological examination of the animal kidney treated with Nolvadex (50 mg) revealed significant granulomatous trauma dependent on the aggregation of macrophages and lymphocytes, as well as vacuolar degeneration of tubules and massive necrosis of glomerulus. The thickness of the bowman capsule was also observed (Fig. 7a), along with massive infiltration of mononucleosis adjacent to deleted blood vessels, hemorrhage (Fig. 7b).

DISCUSSION

The current study demonstrated a significant increase in serum urea, uric acid, creatinine levels in albino rats treated with Nolvadex at varying concentrations (30, 40, 50mg/kg body weight) which is in agreement with previous studies (14,15). Administration of Nolvadex at these concentrations was also seen to induce renal tissue damage as evidenced by abnormalities seen in our histopathological studies. Nolvadex -induced renal impairment was demonstrated earlier (16) that showed the accumulation of serum urea and creatinine to cause a decrease in glomerular filtration rate and nephrotoxicity (17). As reported earlier, the drug's nephrotoxic mechanism could be attributed to its ability to activate mitochondrial cells and induce the overproduction of reactive oxygen and nitrogen species, which in turn causes induction of oxidative stress and mitochondrial apoptosis leading to accumulation of urea, uric acid, and creatinine in blood (18,19).

Many studies on both humans and animals have also suggested that kidney damage is caused by oxidative stress, which transforms free radicals into electrophiles, nucleophiles, and redox-active reactants, which directly harm cells and induce cellular malfunction. Vasoactive mediators are produced because of oxidative stress, which causes vasoconstriction, which alters the morphology of the glomerular filtration rate (20, 21).

CONCLUSION

In the most recent investigation that we have conducted, we found that increasing the dosage of Nolvadex to four times per week for a total of ten weeks led to an increase in the serum levels of creatinine, urea, and uric acid. In addition to this, the medicine caused changes in the tissues of the kidneys, which suggests that greater doses of the drug could potentially cause damage to renal tissue.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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