Physiological and Histological Study of Doxorubicin Effect on Kidney Tissue in Cancer Induced Rats

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Abstract

Doxorubicin (Adriamycin) is one of the chemical drugs commonly used worldwide for the treatment of various types of cancer , and high doses of it cause unwanted toxic side effects on the kidney. This study aims to demonstrate the physiological and histopathological effect of the doxorubicin drug on kidney of rats. For this purpose , twenty adults male were used in this study and were divided equally into four groups (A: control group, B: a group treated with the carcinogen only , C: a group treated with the carcinogen and doxorubicin and D: a group treated with doxorubicin only). Five rats were included in each group and the cancer was developed by injection with azoxymethane once a week for the B and C groups. Compared to the other groups, there was a significant decrease in the weights of animals for the carcinogen-treated group. The concentrations of the hematological parameters of the groups, such as Hb, PCV as well as some tests related to the kidney functions like the concentration of urea , uric acid ,albumin ,globulin, creatinine and total protein were measured and it was found differences between these three groups in term of these testes compared with control group. Regarding to the histological examination of the kidney present study showed specific tissue changes in the treated-carcinogen groups , such as lymphocytes infiltration , glomeruli condense , destroyed of urinary tubules in addition to the presence of bleeding. As for the group treated with doxorubicin , pathological changes were observed include, destroyed of glomerular basement membrane , glomeruli and urinary tubules.

Keywords: *Cancer, Chemotherapy, kidney, Doxorubicin, histopathology*

Introduction

Cancer a major public health problem and the second leading cause of death worldwide. Many chemotherapy drugs are used to many various types of cancer, doxorubicin drug is one of the most highly effective agents against a wide variety of cancer because Quinine-containing anthracycline antibiotic [1]. In spite of its high antitumor efficacy but used in chemotherapy has limited due to side effects and toxicities on kidney, testis, cardiac, lung, liver and blood [2, 3,4]**.** In addition, this chemotherapy causes disorder antioxidant systems [5]. Although, the mechanism of nephrotoxicity that occurs by this drug is unknown , it is believed that this toxicity is through the formation of free radicals and the oxidative damage caused by these free radicals to the tissues [6]. Doxorubicin also causes direct toxic glomerular damage because of its impact on the glomerular filtration barrier composed of glomerular endothelial cells containing glycocalyx, glomerular basement membrane and podocytes, thus affecting membrane permeability, as doxorubicin leads to a decrease in glycocalyx thickness, increasing the size of the holes in the glomerular endothelial cells and decreasing the selectivity of the glomerular charge. All these changes end with passage of large molecules as protein lead to proteinuria and decrease glomerular filtration rate (GFR) [7]. The presence of this protein in the lumen of tubular stimulate to reabsorbed more protein by the proximal tubular cells lead to protein accumulation

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causing tubular obstruction and increased pressure inside the renal tubular and tubular expansion , as well as rupture of the basement membrane and leaking of this protein to the intestitium which trigger the inflammation [8,9]. Furthermore, administration of doxorubicin causes vasoconstriction in vessels and the concentration of the drug in the blood leads to glomerular tuft atrophy and urinary space expansion **[10]**.

Material and Methods

1- Animals experimental design : In this study , twenty adult male Sprague Dawley rats, aged (3-4 months) and (225-240 gm) body weight . The animals were divided into four experimental groups. Five rats for each group. Group A was considered a control group as it was only injected with normal saline , while B and C group were injected with the carcinogen (azoxymethane) (15 mg/kg body weight) once a week for two weeks intraperitoneally for cancer induction [11], in addition to carcinogen, group C were also injected intraperitoneally with doxorubicin drug (25 mg/kg body weight) once a week for two months, while group D was only recived doxorubicin drug (25 mg/kg body weight) once in a week interperitoneally for two months [12].

2- Sample collection and haematological , biochemical tests: The body weight of the animals were recorded before and after the injection. (6 ml) of rat blood were drawn, (1 ml) of it was placed in plastic tubes with anti-coagulant EDTA for some blood analyses such as calculating the haemoglobin concentration (Hb) [13] and packed cell volume (PCV) [14]. The rest of the blood was placed in tubes without anticoagulant and centrifuged to obtain blood serum for biochemical tests of kidney function such as urea [15], uric acid [16], albumin [17], globulin [18], total protein [19] and creatinine [16].

3- The histological study: two weeks after, the carcinogen-injected rats were investigated to detect the occurrence of cancer, while the other groups were exaimend a week after the last dose, the rats kidney were then sectioned and fixed in 10% formalin for the purpose of the histological study [20] .

4- Statistical analysis: Using the 2008 statistical package (Social Science, version 20(SPSS)) program, the statistical analysis of the current study results was carried out to study the effect of the materials used in the experiment and to determine the significant differences between the average probability level ($p \leq 0.05$) [21].

Results and Discussion

The current study showed a significant reduction in the weight of the rats group treated with the carcinogen compared with the other groups and the control group ($p \leq 0.05$) as shown in figure (1-A). The reason is that during the treatment period, the negative effect of the carcinogen on the animals causes a decrease in their nutrition, an increase in the production of free radicals, and therefore an increase in lipid peroxidation, as the decrease is due to the loss of fatty tissue in the muscle [22, 23]. Doxorubicin drug also has an effect on the DNA of the cancer cell, which leads to apoptosis of the cancer cell and its non-proliferation [24). It was also observed that there was a significant decrease below the level $p \leq 0.05$ in some haematological parameters such as Hb concentration and PCV in cancer-induced group (B group) compared with control group and as shown in figure (1-B), this is due to the effect of tumour cells on intestinal cells and microvilli enzymes in the intestine impact their effect on absorption and metabolism of iron [22]. Tumor cells also cause side effects on the kidneys, such as cases of bleeding and a decrease or lack of erythropoietin hormone secretion that is important for blood cell production. In addition , the tumor cells collecting in the organs may be cause ulcers and scarring , especially in the gastrointestinal tract , leading to malabsorption of iron and other minerals , as well as a decrease in body proteins as a result of lack appetite and difficulty in feeding and the occurrence of cachexia condition in carcinogenic rats , an example of which is the transferrin protein, which has the ability to bind to iron and transporting it to the inside of the cells and since iron is important in the formation of blood cells, so the lack of this protein causes anaemia. In addition to a decrease erythropoietin hormone, which is secreted by the adrenal gland and responsible for the process of formation of blood cells (erythropoiesis) due to the effect of the carcinogen on the kidney tissue [25]. A significant decrease was also observed in group C and D compared with the control group due to the effect of this drug on the kidneys and induced alterations in kidney functioning, as well as their effects on erythropoietin hormone secretion [26]. Doxorubicin disrupts the production of blood cells and cause blood clotting disorders and anaemia [27].

Consistent with our results, [28] observed that rats treated with this drug exhibited a sharp decrease in red blood cell number and haemoglobin level.

Current results also observed a significant decrease ($p \leq 0.05$) in the concentration of Urea, uric acid , albumin, globulin , total protein and creatinine as shown in figure (1-C,D,E) for the group treated with the carcinogen compared to control group, and this may due to the effect of the carcinogen on the kidney tissue [22], while the group that subjected to the carcinogen and drug, it was proven that there was a significant increase in the previous concentration of above tests compared to the carcinogen group only. As for the drug treated group only, due to the increased production of free radicals in the body, there was an insignificant decrease in the concentration of above parameters compared to the control group, causing tissue damage, especially the kidney tissue, causing a nephrotoxicity condition [29]. Proteinuria causes mesangial cell injury, which in turn causes mesangial proliferation and increased mesangial matrix production by doxorubicinin causing glomerular expansion [30] .

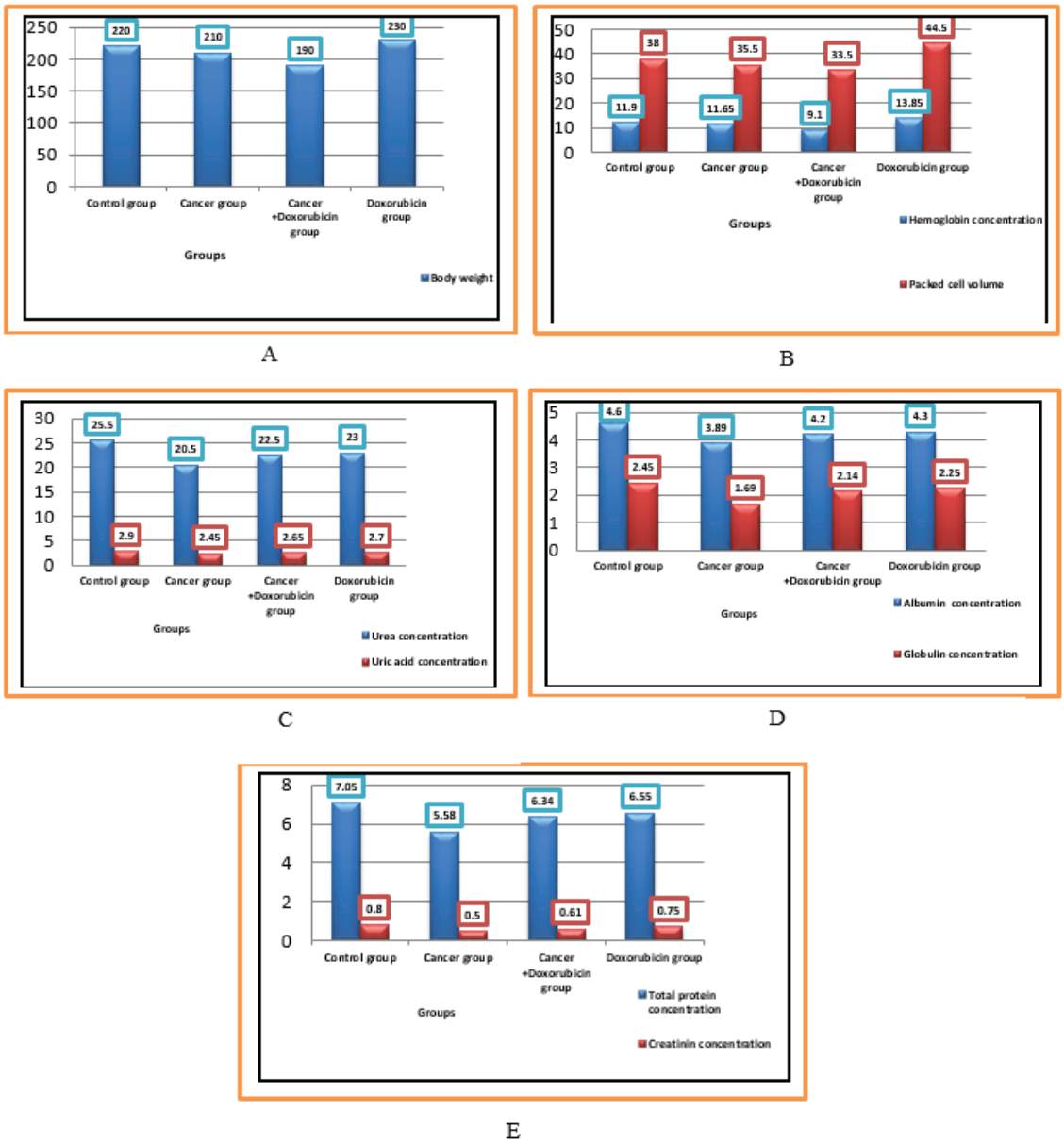

Figure (1): A): Body weight for experimental groups.

B) Hemoglobin concentration (Hb): Packed cell volume (PCV)

C) Urea , Uric acid concentration for experimental groups.

D) Albumin, Globulin concentration for experimental groups.

E) Total protein , Creatinine concentration for experimental groups

Histological study :

The microscopic examination of the kidney tissue of the control group reveal normal shape and structure , as shown in figure (2-A), while the histological examination of the group treated with carcinogen (figure2-B,C,D), showed the presence of bleeding between the renal tubules , lymphocytes infiltration, glomeruli condense, destroyed of urinary tubules, degeneration of cells lining the renal tubules, dilation of the tubules, presence of non- living materials in tubules lumen, expansion of bowman space, destroyed basement membrane, destroyed urinary tubules, and epithelial cells degeneration due to the effect of the carcinogen on the renal cells causing tissue damage [22]. Regarding to the group treated with the carcinogen and doxorubicin drug, the following effect were observed, presence case degeneration in renal tubules, cell necrosis, lysis of cortical tubules, lymphocyte infiltration, glomerulus destroy ,tubules destroy, glomerular thickening. Basement membrane breakdown, epithelial cell degeneration tubules expansion and bleeding between the cells (figure2-E,F,G), these disorders may due to the effect the carcinogen and drug on the kidney tissue [22, 26,31, 32, 33].

Different pathological changes have been observed for the group treated with doxorubicin drug, including thickening of the basement membrane, metaplasia of the epithelial lining of the tubules, bowman space expansion, complete dissolution in the lining of tubules and necrosis of medulla tubules, expansion of their lumen (figure2-H,I,J). High dose of doxorubicin causes acute toxic side effects in kidney tissue which in turn induced nephrotoxicity [29,33,34, 35, 36]. In addition , doxorubicin drug causes cell damage due to oxidative stress and this is consistent with [32]. The presence of protein in the kidney causes mesangial cell injury, which in turn causes mesangial proliferation and increased mesangial matrix production, causing doxorubicin glomular expansion. [35].

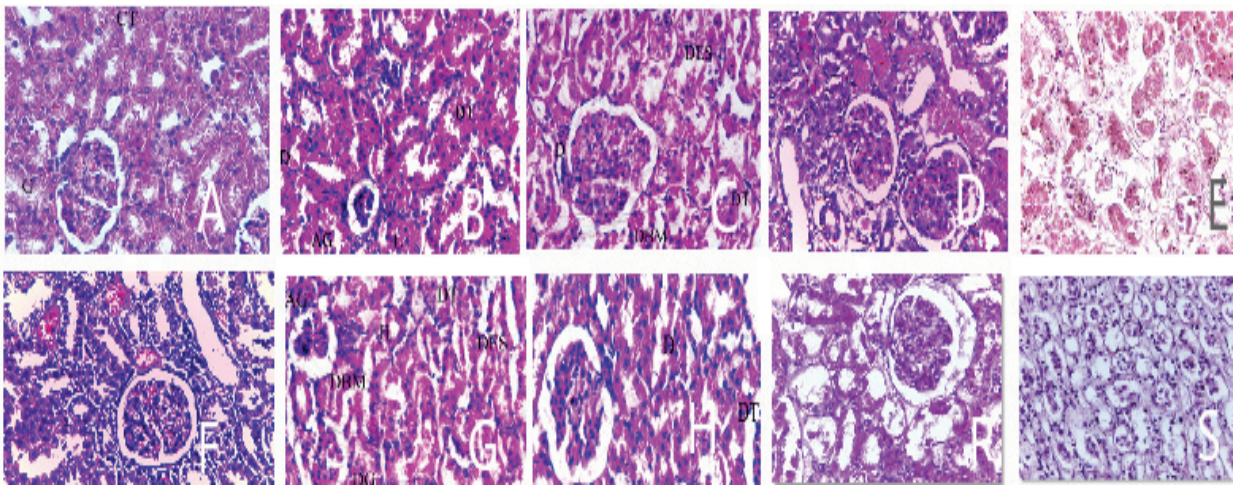

Figure (2): A):kidney rat control group illustrates the normal shape of the kidney, glomular(G),urinary tubules(CT) (H&E) (400X).

B):kidney rat carcinogen group illustrate lymphocyte infiltration(IL), glomeruli condense and destroy(AG,DG) , destroyed urinary tubules(DT) , endothelial cell degeneration(D) (H&E) (400X).

C):kidney rat carcinogen group illustrate destroyed basement membrane(DBM) , destroyed urinary tubules(DT) , epithelial cells degeneration (D) (H&E) (400X).

D):kidney rat carcinogen group illustrate dilation of the tubules(TD) , presence of non- living materials in tubules lumen(N), expansion of bowmans space(EB), hemorrhage(H),Congestion(CON) (H&E) (400X).

E):kidney rat carcinogen and doxorubicin drug group illustrate cell necrosis(N), lysis of corticol tubules (L) (H&E) (400X).

F):kidney rat carcinogen and doxorubicin drug group illustrate epithelial cells degeneration ,tubules degeneration and expansion ,hemorrhage (H&E) ,(400X) . G) : kidney rat carcinogen and doxorubicin drug group illustrate lymphocyte infiltration(IL), glomerulus destroy(DG) ,tubules destroy(DT) , glomerular thickening(AG), Basment membrane breakdown(DBM), epithelial degeneration(DES) (H&E) (400X).

H):kidney rat doxorubicin drug group illustrate tubules destroy(DT), epithelial cells degeneration(D) (H&E) (400X).

I):kidney rat doxorubicin drug group illustrate bowman space expands(BE) , completely dissolves in tubules lining(DT) (H&E) (400X).

J):kidney rat doxorubicin drug group illustrate necrosis of medulla tubules(N) , expansion of their lumen€ (H&E) (400X).

Conclusion

The study showed the effect of carcinogen (azoxymethane (AOM)) on the hematological parameters and biochemical tests specially kidney function. Effects carcinogen upon the kidney tissues. Side effects for doxorubicin on kidney tissue .

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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