IJBPAS, November, 2014, 3(11): 2362-2371

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

STUDY OF KIDNEY HISTOLOGIC CHANGES AT CISPLATIN-TREATED MICE

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INTRODUCTION

The human kidneys are primarily involved in filtering and concentrating various substances and chemical agents that may reach a high concentration and become toxic **(Loh and Cohen, 2009)**. Nephrotoxicity is an inherent adverse side effect of the anticancer drugs for solid and hematologic malignancy **(Kintzel, 2001)**. Antimetabolites, alkylating agents and anthracyclines are commonly used anticancer drugs resulting in nephrotoxicity **(Erkut *et al.*, 2008)**. Renal tubular damage is a well-known renal complication induced by anticancer drugs **(Kakihara *et al.*, 2003)**. The rate of glomerular damage may have been underestimated because tubular dysfunction can mask glomerular dysfunction **(Ikarashi *et al.*, 2004)**. The anticancer drug cisplatin (CDDP) is a very effective platinum compound in the treatment of a variety of cancer **(Kintzel, 2001)**.

Cisplatin, a widely used anti-neoplastic agent, is primarily used in the treatment of a variety of solid tumors **(Meyer and Medias, 1994)**. However, the clinical usefulness of cisplatin has been seriously restricted because of its nephrotoxic side effects **(Garnick *et al.*, 1988; Taguchi *et al.*, 2005)**. The mechanism by which cisplatin kills tumor cells is distinct from the mechanism by which it selectively kills the proximal tubule cells **(Townsend *et al.*, 2003)**. Several investigators have suggested different mechanisms by which cisplatin selectively kills the proximal tubule cells. It was hypothesized that cisplatin is activated in the kidney to toxic metabolite through a

platinum-glutathione conjugate, then to a cysteiny l-glycine-platinum-conjugate, which is further processed to a cysteine conjugate which is a metabolically reactive thiol **(Salahudeen *et al*., 1998)**. In addition, two distinct pathophysiological mechanisms have been recognized as promoters of cellular damage, i.e. inhibition of protein synthesis **(Leibbrandt *et al*., 1995; Rosenberg and Sato, 1993)** and glutathione depletion **(Bompart, 1989; Zhang and Lindup, 1993)**. Moreover, many evidences have been accumulated that this side effect is closely related to reactive oxygen species (ROS) which cause mitochondrial damage, inhibition of membraneous transport proteins and lipid peroxidation **(Kuhlmann *et al*., 1997; Baliga *et al*., 1998; Matsushima *et al*., 1998)**.

Cisplatin is one of the most efficient anti-cancer drugs that are used in the treatment of some kinds of tumors like: lung, neck and head (cancers), in particular ouario cancers. Cisplatin is used in the treatment of adrenal Carsinoma, breast, uterin, gastrointestinal, lung, prostate head and neck, germ cell tumors, neuroplastoma, and sarcoma. The effective mechanism of cisplatin is the same as other achilics: it creats links between DNA and RNA cords, then it interfere with their performance. Its side effects include: secondary anemia, kidney toxicity, blood uric acid increase or nephropathy accompanied with blood uric acid increase and otic toxicity As such, the purpose of this study is to evaluate side effect of cisplatin induced nephrotoxicity on kidney tissue in treated mice.

MATERIAL AND METHODS

In present study, 20 male mice (about 25-30gr body weight) were purchased from Animal House, Islamic Azad University. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment execution. Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals of China and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum.

Lyophilized cisplatin **(Cisplatyl 50, Laboratoire Roger Bellon, France)** was dissolved in normal saline and given i.p. in dose of 80 mg/kg bwt **(Mathe et al 2006)**.

The Mice were randomly divided into 2 groups (10 rats each) as the following:

Group 1, healthy control mice received isotonic saline solution injected i.p

Group 2, were injected 60 mg/kg dose cisplatin 3 dose at every other week injected

i.p . After 3 weeks, mice after deep anesthesia, autopsied and kidney were gathered and the samples of their kidney tissue kept in 10 %formalin for stabilization. All of samples for microscopic observation were stained with H&E and Masson's Trichorom in duration Histotechnique stages. After this stage all of samples were studied with Nikon light microscope.

RESULTS

Effects of chemical used on behavior, body weight and kidney wet weight: Animals of all groups showed no obvious symptoms or signs of toxicity throughout the experiment. Moreover; they did not exhibit any case of mortality or death. It was noticed that in cisplatin treated mice, water and pellet diet consumption was decreased, if compared to normal. Also, a significant decrease in the body weight gain after 3 weeks as compared to control was noticed.

Histopathological and Ultrastructural Findings

Examination of hematoxylin and eosin stained kidney sections of control mice after 3 weeks from the beginning of the experiment, revealed normal basic structure. Kidney sections showed a large number of renal RER corpuscles and numerous urinefrous tubules within the cortex . Renal corpuscles appeared morphologically normal with double walled Bowman's capsule surrounding the glomerulus. However, between, the two layers of Bowman's capsule is preserved a narrow urinary space.

Moreover, light microscopic preparations showed that Proximal Convoluted Tubules (PCT) of normal kidneys have narrow lumen **(Figure 1, 2)**.

In the current study, kidney sections of mice injected with cisplatin, showed more intense characteristics of chronic nephropathy when compared to controls after 3 weeks. These changes included: hypertrophied renal corpuscles, with reduced glomerular cellularity.

Moreover, it was observed that cisplatin treatment revealed focal and severe PCT tubular degenerative features including, significant reduction in the mean number of cells/tubules accompanied with a significant increase in their mean width compared to controls.

Their tubular lumen appeared wide and contained cellular debris and most of them showed swollen outlines. Light preparations showed also, mononuclear cellular infiltration among renal tubules.

According the observations, this drug Cisplatin induced necrotic effect on Proximal convoluted tubules **(Figure 3, 4)** . Epithelial cells of tubules, was seen with hyper chromatin nucleus and hyper eosinophilated cytoplasm and destructions of Brush borders observed too .Furthermore

hyaline droplets were observed in collecting ducts. Additionally Memberano-glumerolopathy and Proliferative glomerulonephritis were seen. Pathologic findings showed that renal structure is normal in the control group and there were not pathologic changes. In thecisplatin group, degenerative changes of tubular cells, acute tubular necrosis,edema, hyperemia and sever hemorrhage were more prevalent. Also, hyperemia and sever hemorrhage of glomerulus was obvious. Pathologic changes in this group included edema, moderate hyperemia and hemorrhage in the glomerulus and renal interstitial tissue with moderate degenerative changes and mild necrosis of tubular epithelium **(Figure 3, 4)**.

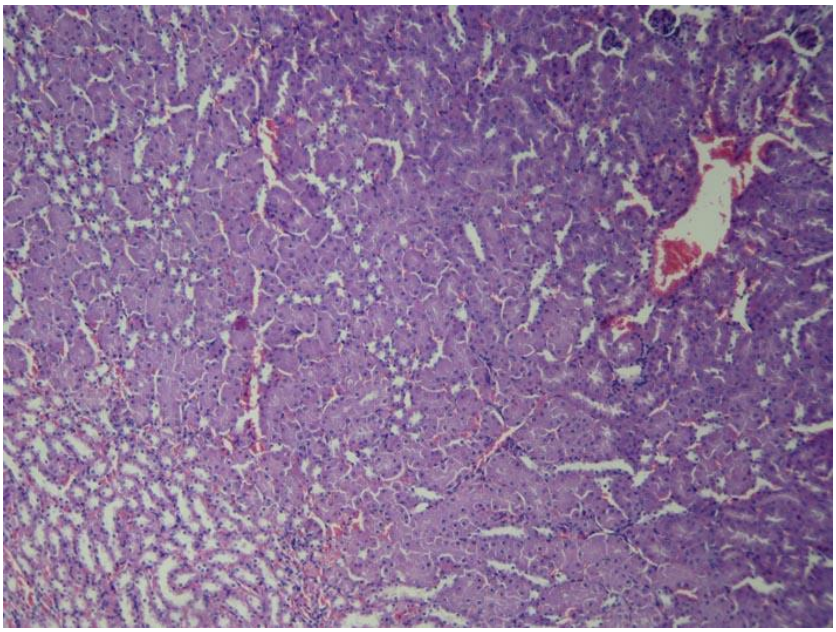

Figure 1: Microscopic view from kidney tissue of a mice belonged to control group. Renal structure is normal and there are no pathologic changes, H&E, 40×

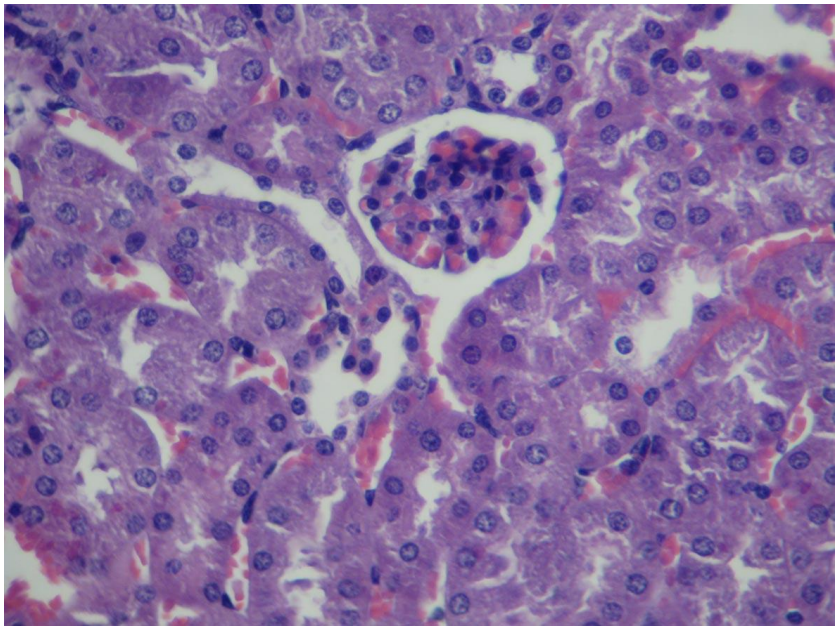

Figure 2: Microscopic view from renal cortex of a mice belonged to control group. Renal cortex is normal and there are no pathologic changes. H&E, 40×

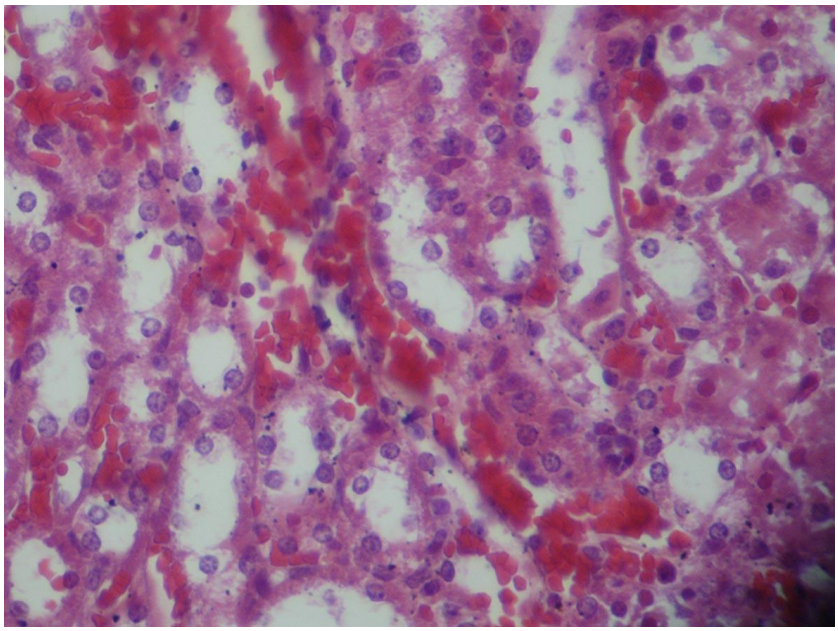

Figure 3: Microscopic view from renal medulla of a mice belonged to cisplatin group. Hyperemia and sever hemorrhage in the renal interstitial tissue with sever degenerative changes with necrosis of tubular epithelium is obvious. H&E, 40×

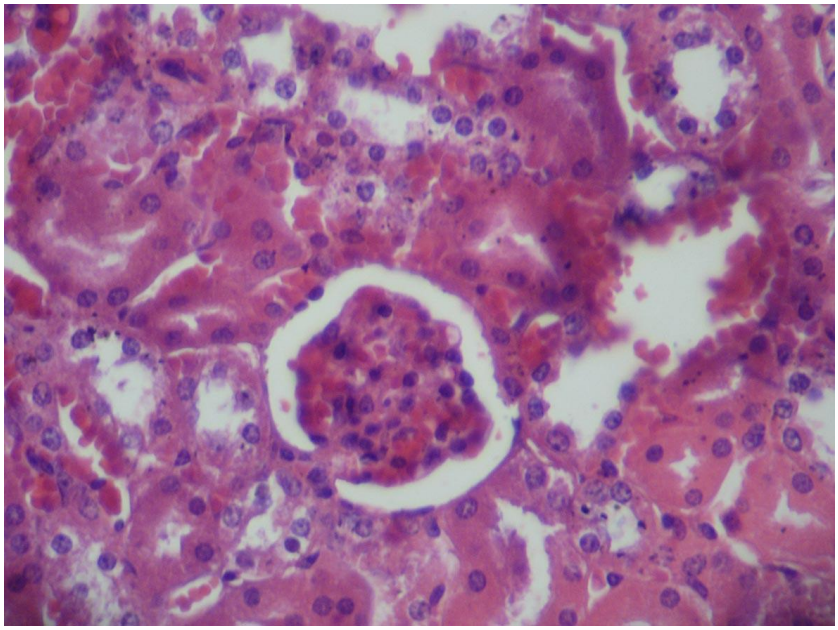

Figure 4: Microscopic view from renal cortex of a mice belonged to cisplatin group. Hyperemia and sever hemorrhages of glomerulus and renal interstitial tissue with sever degenerative changes and necrosis of tubular epithelium is obvious. H&E, 40×

CONCLUSION

The exact mechanisms of nephrotoxicity induced by CDDP are still not fully elucidated. **Stewart *et al*. (1982)** reported that cisplatin is preferentially taken up and accumulated in the kidney cells. Nevertheless the major site of renal injury is the proximal convoluted tubule as reported by **Kuhlmann *et al*. (1998)**. In kidneys have been implicated in the pathogenesis cisplatin induced renal injury **(Yilmaz *et al*., 2004)**. Many toxic effects of cisplatin are now well known including neurological and those on the gastrointestinal tract. The dangers of prescribing these anti cancer agents to patients with impaired liver function are always emphasized but hepato-toxicity of these drugs is often overlooked. It was revealed that nephrotoxic effect of the cisplatin was obvious has in renal tissue particularly, The existing of proteinuria after cisplatin administration is the major consequences of drug induced Glomerolopathy . Cisplatin-induced nephrotoxicity at proved to be dose related. These and other reports with studies performed on various animal models showed a strong relationship between the administered dose and uremia, as well as dose-related structural alterations detected on microscopic kidney examination. These alterations consisted predominantly in tubular necrosis. As could be expected from animal studies, early clinical reports showed similar toxicity in humans **(Higby et al. 1974)**. In fact, the reduced number of nephrons will produce a higher flow of ultrafiltrate per tubule with lower tubular cisplatin concentration and probably less

tubular "contact" between the toxin and tubular epithelium.

Cisplatin is an effective chemotherapeutic agent for a wide variety of tumors **(Park *et al.*, 2009)**. Nevertheless, it has several side effects including hepatotoxicity **(Mansour *et al.*, 2006; Pratibha *et al.*, 2006)**. The alterations in renal structures detected in mice modelscorrelate well with the nephrotoxic effects of cisplatin in patients treated with antitumor agent **(Daugaard *et al.*, 1988a, 1988b)**. In the present investigation, a 3 dose of cisplatin (60 mg/kg, i.p.), in mice resulted in the deterioration of renal corpuscle structure and increased tubular necrosis after 3 weeks. Moreover, cisplatin administration revealed that most renal corpuscles appeared hypertrophied with diminished glomeruli.

thus, cisplatin toxicity in PCT is morphologically characterized by tubular necrosis. In the present study, cisplatin caused structural alterations characteristics of acutetubular necrosis in both PCT and DCT after 3 weeks. Who stated that male Wistar rats receiving single dose of cisplatin (3 mg/kg) for 5 days showed severe tubular necrosis among kidney sections. Moreover, in the present work, PCT showed cytoplasmic - debris, denudation of PCT basement membrane, swollen PCT cell with open face and pyknotic nuclei, vacuolated cytoplasm; intercellular edema and mononuclear infiltration. DCT appeared with few tubular changes. Thus, our description were in general agreement with those reported by **Chirino *et al.* (2004)**, who observed PCT tubular necrosis, cytoplasmic vacuolization and intercellular edema in cisplatin treated to body male Mice (at dose level 7.5 mg/kg, i.p., for 3 days). Similarly, **Morigi *et al.* (2004) and Behling *et al* (2006)**, reported similar description in acute cisplatin nephrotoxicity. After 3 weeks from the beginning of the present experiment, histological features of chronic nephropathy as indicated by degenerated and highly congested glomeruli were detected among kidney sections of this group confirming our results. In the end we recommended the massive water taking and co-administration of and urocozoric agent for decreasing of nephrotoxic side effects of cisplatin.

ACKNOWLEDGEMENTS

We thank the Lebanese University for financial support.

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