Histopathological and biochemical assessment of kidney damage in albino wistar rats treated with cytotoxic platinum compounds in combination with 5-FU

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Abstract: Kidney cells damage and subsequent renal adverse effects with oxaliplatin are less reported phenomena, whereas cisplatin (CDDP, first generation platinum compound) has therapeutic limitations due to renal toxicity. This experimental study reports oxaliplatin (third generation platinum compound) induced direct damage in rat kidney tissues and alterations in renal biochemical profile. Oxaliplatin was administered in albino wistar rats with 5-FU (5 Fluorouracil) to mimic as model of FOLFOX, the mainstay chemotherapeutic regimen in colorectal cancer (CRC). This study reports changes in renal biochemical profile (serum creatinine and urea) in rats treated in different treatment groups with cisplatin, oxaliplatin, 5-FU, cisplatin+5-FU and oxaliplatin+5-FU which are compared with group of rats treated with normal saline (control group). Subjective renal toxicity in tissues was compared among rats treated with oxaliplatin alone and cisplatin, with and without 5-FU by light microscopy. Cast formation, medial hypertrophy of the vessel wall, vacuolization and necrosis was seen in kidney tissues of oxaliplatin treated rat. Changes in serum creatinine well-above diagnostic risk levels were noted. Apparent tubular degenerative sequence associated with vacuolization and cast formation was observed in 5-FU treated rats. Kidney damage ensued after treatment with 5-FU and oxaliplatin are slightly comparable to massive tubular damages, hemorrhage, casts and vacuolization along with multiple foci of alterations induced by cisplatin.

Keywords: Kidney, oxaliplatin, rats, nephrotoxicity, 5 Fluorouracil.

INTRODUCTION

Nephrotoxicity is a common and dose limiting adverse effect of platinum analogues in cancer chemotherapy (Kimura *et al.,* 2015; Ju *et al.,* 2015). Oxaliplatin, a third generation platinum compound is the key chemotherapeutic agent in first line regimens for colorectal, pancreatic and gastrointestinal cancers (Marschner *et al.,* 2015; Shi *et al.,* 2015). In case of renal insufficiency in oxaliplatin treated patients, a decrease in filterable platinum removal does not significantly increase the toxicity since oxaliplatin triggers 50% renal excretion in 48 hours (Chen *et al.,* 2015). Oxaliplatin is not usually associated with significant nephrotoxicity even in sensitive populations like low age group cancer patients (Lam *et al.,* 2015). Nephrotoxicity induced by oxaliplatin is lower than cisplatin since Multidrug and toxin extrusion proteins (MATE 1 and MATE 2K), expressed in humans, transport oxaliplatin with higher affinity than cisplatin (Harrach and Ciarimboli, 2015). Oncologists often substitute oxaliplatin for nephrotoxic chemotherapeutic agents (e.g. carboplatin) to reduce renal damages (Kolomeyevskaya *et al.,* 2015). However there is an increasing concern regarding oxaliplatin induced nephrotoxicity in recent years. Kawazoe *et al* (2010) report a case of renal damage in a 77 years old Japanese male with history of chronic moderate renal impairment treated with repeated cycles of oxaliplatin shifted on dialysis. Another study shows that oxaliplatin can induce serious renal damages like acute tubular necrosis and acidosis, tubular vacuolization and acute kidney injury with hematological toxicity (Joybari *et al.,* 2014). Although toxic renal manifestations are unclear with oxaliplatin, few cases of AKI (Acute kidney injury) are suggestive of damages induced by cumulative dose of oxaliplatin given in combination with 5-FU (Márquez *et al.,* 2013). In this study we have observed oxaliplatin, cisplatin and 5-FU induced nephrotoxicity by light microscopy technique. Tissue damages are noted in platinum based treatment groups with and without 5-FU. Novelty of the research lies in the experimental design of the study based on co-administration of platinum analogues with 5FU for the first time in a rodent model.

METHODS

Study design

This animal study designed in Department of Pharmacology, University of Karachi was conducted in the animal house of DUHS (Dow University of Health Science) after institutional and ethical approval. Experimental protocols on albino wistar rats complied with the Helsinki declaration (Rickham, 1964), amended in 1996. Experiments were conducted in two stages demarcated with dosing regimens and assigned rest periods. Acute model of toxicity of each drug alone and in

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combination was developed in the first stage. In the acute model of toxicity, Group A (control), Group B, Group C and Group D were treated with normal saline (NS), 5-FU, Cisplatin and Oxaliplatin respectively. Group E was treated with the combination of 5-FU with Cisplatin. Group F was treated with the combination of 5-FU with Oxaliplatin. Changes in renal biomarkers in each treatment group were noted and compared with reference ranges of control group. Changes in renal biomarkers were assessed and compared with control groups. Histopathological assessment was made for the isolated kidney tissues of animals in each treatment group of acute toxicity models. Delayed toxicity model was developed in the second stage. A comparative profile of changes in renal biomarkers within the treatment groups was generated. The renal biomarker profile of acute model of toxicity was compared with the profile of delayed models of toxicity.

STATISTICAL ANALYSIS

Data was analyzed on SPSS version 19 with paired sample test, *p* value <0.05 was considered significant, *p* value <0.01 highly significant and *p* value <0.001 was considered very highly significant.

Animal protocols

Healthy male albino Wistar rats (inbred species) weighing between 220-250 gms were selected. The animals were housed in spacious and ventilated "Rat House" at DUHS with maintained temperature (±23°C) and relative humidity (65-75%). Diffused lighting with flickering checks was ensured for consistent Light and dark cycle (10:14 hours). Animals were kept in opaque Polypropylene cages (wire mesh tops) without in-cage shelters. To escape draughts, cage racks were aligned and positioned for proper air. Wood shavings layerings (0.5± 2 cm) provided for bedding of appropriate depth. Rat feed with 40% protein content was prepared in the labs. The animals had free access to food and tap water. The animals were adapted to the environment and accustomed to 'gentling' during rest period of seven days.

Treatments groups and dosing protocol

Thirty six animals were included in first stage of acute toxicity model development and testing. They were assigned to six treatment groups (A,B,C,D,E & F) based on treatment types as follows Group A [(0.9% normal saline (NS)) 2ml, n=6]; Group B [(5-FU) 15mg/kg, n=6]; Group C [(CDDP) 0.8mg/kg, n=6]; Group D [(Oxaliplatin) 0.8mg/kg, n=6]; Group E [(5-FU+CDDP) (15+0.8)mg/kg, n=6] and Group F [(5-FU+Oxaliplatin) (15+0.8)mg/kg, n=6].

The drugs were administered intraperitoneally (IP). The injections were carefully made at the midway of the xyphoid and the pelvic bone (lower right quadrant of the abdomen, close to the midline) and caution was taken to avoid the bladder, cecum or liver. Needle (25 needle gauge) was inserted at an angle of 30° for the shaft to reach a depth of 0.5cm. In the acute model of toxicity the doses were administered on days 1, 5, 10, 15, 20. The blood was sampled by cardiac puncture on day 25 during which the animal was kept deeply anesthetized by using chloroform. The kidneys were removed and preserved in formalin, two kidneys from each animal submitted entirely in a single jar. Representative section of kidneys cut and submitted in cassette A, B, C, D, E & F.

Dosing protocol in delayed model of toxicity comprised of similar doses of oxaliplatin, CDDP and 5-FU with alterations in dosing schedules. In the delayed model of toxicity, Group A (control), Group B, Group C and Group D were treated with 0.9% normal saline (NS), 5-FU, Cisplatin and Oxaliplatin respectively. Group E was treated with the combination of 5-FU with Cisplatin. Group F was treated with the combination of 5-FU with Oxaliplatin. The drugs were administered on day 1 and day 5 every week for four weeks. Rest period of ten days was assigned after every two weeks. Blood sampling, scheduled 30 days after the last dose was conducted by cardiac puncture.

Biochemical assessment

Sampled blood was collected in anticoagulant tubes, 3 ml in Green top Heparinized tube, 3ml in Lavender top EDTA tube and 3ml in Light blue top Citrate tube. Plasma and blood cells were separated by centrifugation for 10 minutes (1000-2000×g) in a refrigerated centrifuge machine.Ten minutes centrifuge time helps to attain designated plasma for serological testing. Plasma was immediately transferred into polypropylene tubes with Pasteur pipettes at a temperature of 2-8°C.

Serum creatinine levels were quantitatively determined with Biosience Kit Jaffe Colorimetric Kinetics by using standard kid method. Urea was quantitatively determined with Bioscience kit Urease-GLDH Kinetic employing *in vitro* diagnostic (IVD) procedure and using standard kit method.

Light microscopy

After removal of the kidneys, each kidney was marked in the median through the tip of the papilla and renal pelvis. To allow optimal examination of the renal pelvis, papilla and junction with the ureter the transverse section of the kidney were made. The histological assessment of a large tissue area including both renal poles is permitted by the longitudinal section. The capsules were not removed. The concretions and urothelial changes in the regions of the renal pelvis close to the poles was hence possible. To attain a full length of the renal papilla in section a slightly paramedian cut at trimming was made. Tissue samples were fixed in 10% formalin in saline, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in molten paraplast wax blocks at 57-61°C, 4-5

micron thick section cut were stained by H&E (Hematoxylin & Eosin) and Periodic Acid Schiff (PAS). Prepared slides were assessed for structural evaluation under a bright field light microscope by trained pathologist unaware of the treatments (blinded assessment).

RESULTS

Comparative changes in the renal status of the rats in each treatment group of both the toxicity models assessed by changes in the levels of Serum biomarkers, Creatinine and Urea is shown in fig. 1 and fig. 2 respectively.

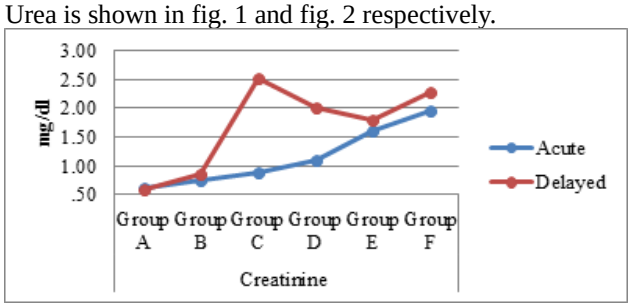

Fig. 1: Comparative difference in serum creatinine levels in all treatment groups of acute and delayed model of toxicity

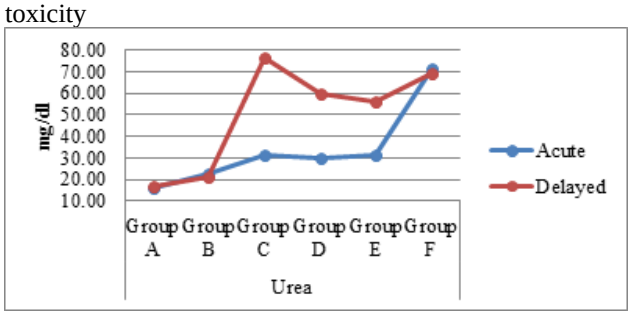

Fig. 2: Comparative difference in urea levels in all treatment groups of acute and delayed model of toxicity

Acute renal toxicity in experimental rat models-biochemical parameters

Table 1 shows that the difference in the levels of serum creatinine measured in the rats of each treatment group in the acute toxicity model in comparison with the control group is significant in groups B and C ($p<0.05$), highly significant in group D ($p<0.01$) and very highly significant in both group E and F ($p<0.001$). The blood urea levels in the treatment group after acute toxicity induction was also significantly higher than the blood Urea levels of the control group in group B, whereas the difference was very highly significant in groups C, D, E and F ($P<0.001$). fig. 1 shows that the rise in the serum creatinine level is markedly higher in group F in comparison to the rest of the treatment groups.

Delayed renal toxicity in experimental rat models-biochemical parameters

Table 2 shows that the difference in the levels of serum creatinine measures in the rats of each treatment group in the delayed toxicity model in comparison with the control group is significant ($p<0.05$) in group D, very highly significant in groups C,E and F ($p<0.01$) and non-significant in group B. The blood urea levels in the treatment group after delayed toxicity induction was also significantly higher than the blood urea levels of the control group. fig. 1 shows that the rise in the serum creatinine level is markedly higher in group C followed by group F in comparison to the rest of the treatment groups.

Renal toxicity in experimental rat models-histopathological parameters

Light microscopy images fig. 3(A) [0.9%Normal Saline treatment group (Acute model)] and fig. 3(B) [0.9%Normal Saline treatment group (Acute model)] show normal morphology of the glomeruli and renal tubules and thin basement membrane of both the structure. Associated with normal glomeruli and tubular lining, normal Bowman's space and unremarkable parenchyma is seen. Thickened vessel wall is not seen in both the photomicrographs. The bright field light microscopy photomicrographs figs. 3(C-E) [5-FU treatment group (Acute model in fine resolutions)] show multiple foci of changes showcasing renal damage in 5-FU treated rats. The glomeruli appear to be normal but altered parenchyma and altered epithelial cells are seen. Vacuolization and cast formation is there with apparent tubular degenerative sequence. The membrane of the glomeruli is not thick but thickened blood vessel walls can be marked. Parenchyma and epithelial cell loss is visible. fig. 4(A) [Cisplatin treatment group (Acute model)] shows marked dilatations and widening of Bowman's space. fig. 4(B) [Cisplatin treatment group (Acute model)] shows damaged tubules and cast. Tubular parenchyma is severely altered. Loss of epithelial cells is seen. Cast formation and vacuolization is apparent. In fig. 4(C) [Cisplatin treatment group (Acute model)] massive tubular damage, multiple foci of alteration, vacuolization, hemorrhage and cast are seen. The bright field light microscopy photomicrographs fig. 4(D) [Oxaliplatin treatment group (Acute model)] shows marked dilatation of Bowman's capsule. The normal and damaged Bowman's capsule with normal and widened space is comparatively shown in fig. 4(D). fig. 4(E) [Oxaliplatin treatment group (Acute model)] show casts in the renal tubules, altered structure and multiple foci of patchy necrosis, whereas, prominent cast formation is also visible. In fig. 4 (F) [Oxaliplatin treatment group (Acute model)], tubular epithelial cells vacuolization, epithelial cell loss and necrosis, casts and architectural loss is prominent.

The bright field light microscopy images figs. 5(A) and 5(B) [5-FU+Cisplatin treatment group (Acute model)] show thickened vessel walls, dilatation of the Bowman's space. Medial hypertrophy of the vessel wall, cast formation, vacuolization and necrosis are prominent in the

Table 1: Comparative change in renal biomarkers in all treatment groups of acute model of toxicity

Paired Samples Test							
ACUTE TOXICITY			Paired Differences		t	df	p-value
			Mean	Std. Deviation			
Renal	Urea	Group A - Group B	-7.000	2.530	-6.778	5	0.001
		Group A - Group C	-15.833	3.656	-10.608	5	0.000
		Group A - Group D	-13.833	2.994	-11.316	5	0.000
		Group A - Group E	-15.500	3.507	-10.826	5	0.000
		Group A - Group F	-55.500	3.507	-38.763	5	0.000
	Creatinine	Group A - Group B	-0.133	0.121	-2.697	5	0.043
		Group A - Group C	-0.283	0.214	-3.248	5	0.023
		Group A - Group D	-0.483	0.204	-5.800	5	0.002
		Group A - Group E	-1.000	0.245	-10.000	5	0.000
		Group A - Group F	-1.333	0.151	-21.693	5	0.000

p value < 0.05 (significant), p value < 0.01 (highly significant), p value < 0.001 (very highly significant)

Table 2: Comparative changes in renal biomarkers in all treatment groups of delayed model of toxicity

Paired Samples Test							
DELAYED TOXICITY			Paired Differences		t	df	p-value
			Mean	Std. Deviation			
Renal	Urea	Group A - Group B	-4.500	1.378	-7.997	5	0.000
		Group A - Group C	-59.833	2.858	-51.286	5	0.000
		Group A - Group D	-43.500	1.975	-53.955	5	0.000
		Group A - Group E	-39.333	3.445	-27.969	5	0.000
		Group A - Group F	-52.667	8.430	-15.303	5	0.000
	Creatinine	Group A - Group B	-0.267	0.528	-1.237	5	0.271
		Group A - Group C	-1.950	0.327	-14.602	5	0.000
		Group A - Group D	-1.417	0.471	-7.370	5	0.001
		Group A - Group E	-1.217	0.117	-25.493	5	0.000
		Group A - Group F	-1.700	0.303	-13.729	5	0.000

p value < 0.05 (significant), p value < 0.01 (highly significant), p value < 0.001 (very highly significant)

slide. Loss of parenchyma and architectural distortion are marked. The bright field light microscopy photomicrographs fig. 5 (C) and 5 (D) [5-FU+Oxaliplatin treatment group (Acute model)] show Bowman's capsule dilatation, focal tubular epithelial loss in some areas and also cast formation. Focal areas of vacuolization are noticeable. Renal damage and structural alterations are present but not severe.

DISCUSSION

Oxaliplatin, a third generation platinum analogue has similar mechanism of action as cisplatin (Yu *et al,* 2015). Cisplatin induces tubular cell injury and death by accumulation in renal tubular cells hence imparting DNA damage and associated response (Zhu *et al,* 2015). Animal studies show that cisplatin is metabolically activated and converted into a more potent toxin (cysteinyl-glycine-conjugates) inside the kidney cells which are further metabolized to highly reactive thiols by cysteine-S-conjugate beta-lyase (Miller *et al,* 2010). Nephrotoxicity induced by oxaliplatin is less than cisplatin since Multidrug and toxin extrusion proteins (MATE 1 and MATE 2K), expressed in humans, which transport oxaliplatin with higher affinity than cisplatin (Harrach and Ciarimboli, 2015). In the absence of protein transporters in rodent model of toxicity in this study, degree of oxaliplatin induced kidney damages is comparable to cisplatin induced nephrotoxicity. Theiner *et al* (2015) investigated drug accumulation in medulla and cortex correlated with nephrotoxicity, reporting that there is a 10 fold increase of oxaliplatin in cortex over medulla, a similar distribution as cisplatin but no evident sign of oxaliplatin induced nephrotoxicity.

Histopathological assessment of kidney tissues in oxaliplatin treated rats (Acute treatment schedule) is indicative of multiple foci of patchy necrosis, cast formation and alterations in normal parenchyma. Risk of oxaliplatin induced kidney cell damages can result in significant nephrotoxicity in susceptible patients subjected to aggressive schedules of definitive

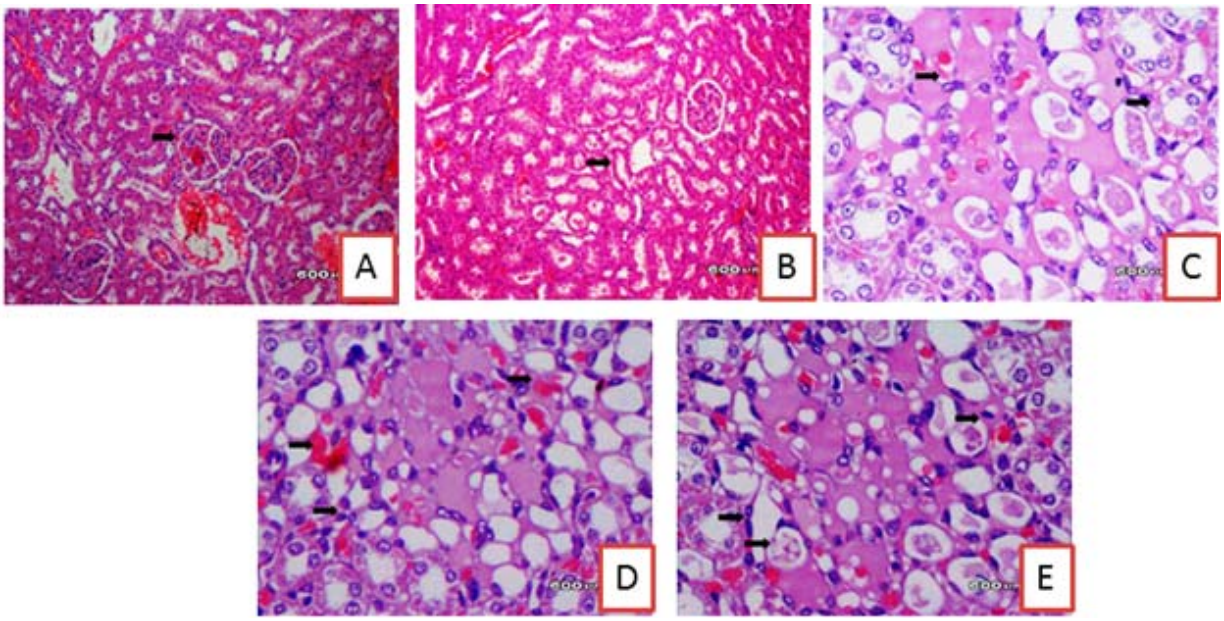

Fig. 3: Histopathological effects of 0.9%Normal Saline (A, B) and 5-FU (C-E) on rat kidney

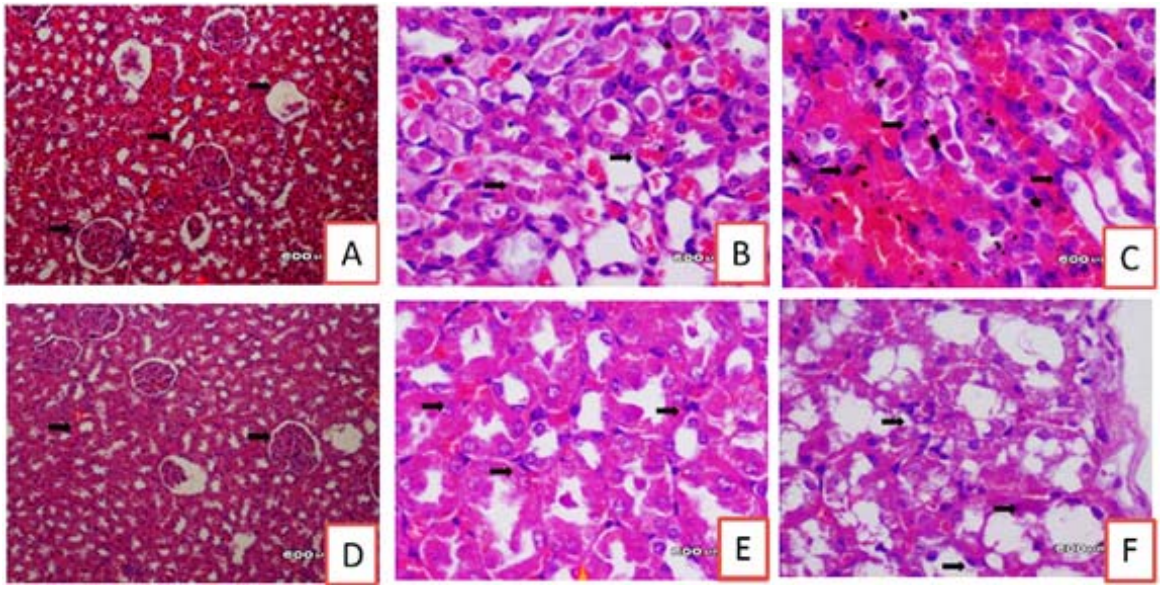

Fig. 4: Histopathological effects of Cisplatin (A-C) and Oxaliplatin (D-F) on rat kidney

chemotherapy. There are few and far between report of oxaliplatin induced renal damages consistent to the pattern of kidney cell toxicity reported in our study. Severe acute tubular vacuolization and necrosis after oxaliplatin infusion is reported in colorectal cancer patients (Filewod and Lipman, 2013; Joybari *et al*, 2014). Labaye *et al* (2005) reported a case of anuric acute renal failure associated with tubular renal necrosis. Ito *et al* (2012) reported a case of acute kidney injury associated with thrombocytopenia, acute renal failure and hemolytic anemia during chemotherapy with oxaliplatin, leucovorin and 5 Fluorouracil. The patient recovered with corticosteroid treatment, hemodialysis and plasma exchange.

In our study,light microscopy assessment of samples show that kidney damages in rats treated with the combination of 5-FU and Oxaliplatin (acute treatment schedule) are more intense than kidney damages in rats treated only with oxaliplatin. Aside from patchy necrosis, Bowman's capsule dilatations focal tubular epithelial loss and prominent cast formation is revealed in the photomicrographs. The creatinine levels are markedly high in rats treated with the combination regimen (fig. 2). induced nephrotoxicity in cancer patients can adversely implicate conditions like tumor lysis syndrome (Farooqi *et. al*, 2015; Davidson *et. al*, 2004), especially in case of large tumors. Therefore, nephrotoxicity should be carefully monitored not only in patients treated with

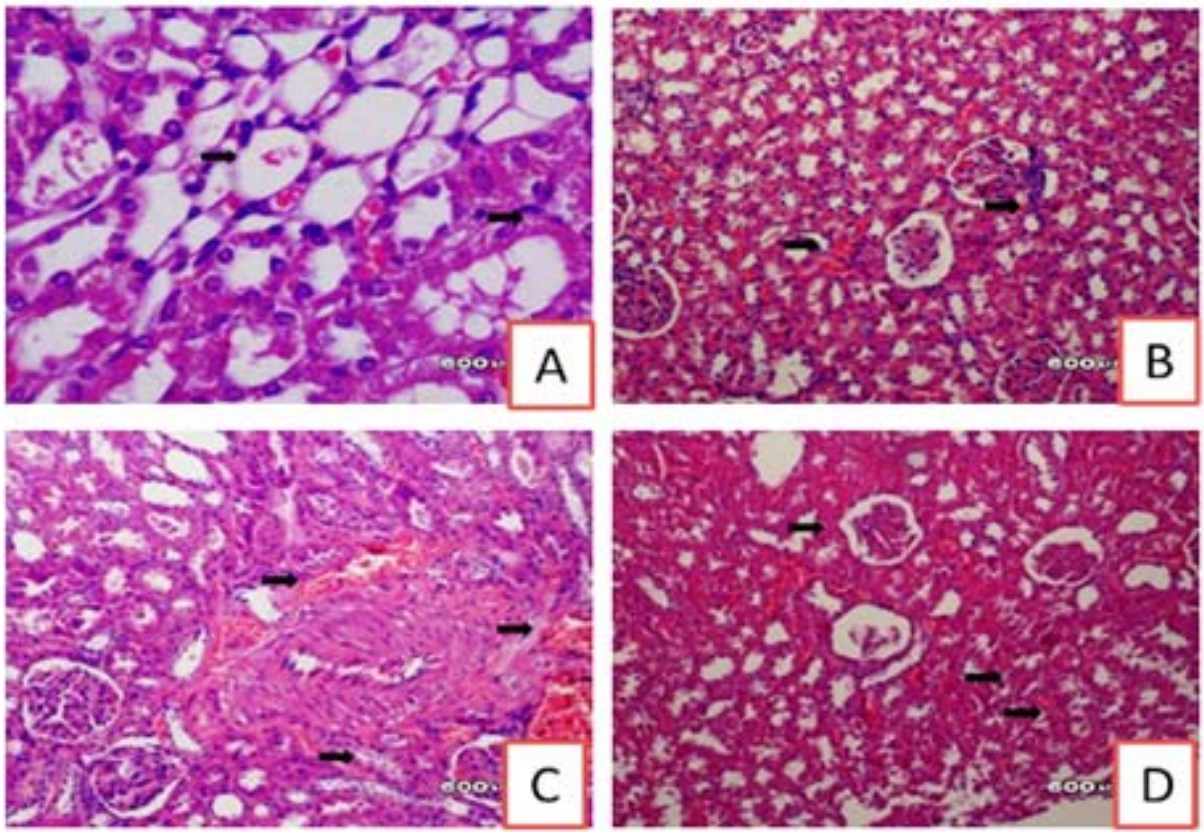

Fig. 5: Histopathological effects of 5FU+Cisplatin (A, B) and 5FU+Oxaliplatin (C, D) on rat kidney

cisplatin based regimens but also in patients treated with oxaliplatin based regimen particularly those which also include 5-FU as a combination drug.

CONCLUSION

Substantial risk of acute tubular necrosis associated with oxaliplatin treatment can lead to kidney injuries in patients with renal insufficiency after multiple cycles of chemotherapy. Although oxaliplatin induced renal toxicity is markedly lower than cisplatin, it is rendered significant in combinations of oxaliplatin and 5 Fluorouracil.

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