doi.org/10.36721/PJPS.2021.34.6.REG.2141-2147.1

Raloxifene impedes cisplatin-induced nephrotoxicity through inhibition of Proinflammatory cytokines in female wistar rats

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Abstract: Cisplatin mediated nephrotoxicity is the main obstacle in the practice of cisplatin as a chemotherapeutic agent. Conversely, it continues to be the most commonly used anticancer agent to treat several solid tumours. This study investigated the effect of raloxifene pretreatment on nephrotoxicity mediated by cisplatin in an experimental animal model. The levels of blood urea nitrogen, creatinine, and albumin were measured to evaluate the renal damage, and the levels of proinflammatory cytokines such as interleukin 6 (IL-6), interleukin 10 (IL-10), and tumour necrosis factor-alpha (TNF-α) were measured to assess the systemic inflammation. Cisplatin in a single dose of 7.5mg/kg showed a substantial rise in serum levels of blood urea nitrogen, creatinine along with TNF-α and IL-6 and, fall in albumin and IL-10 levels. Nevertheless, there was no substantial change in a group treated with raloxifene 7.5mg/kg. We observed a substantial fall in the levels of blood urea nitrogen, creatinine along with TNF-α and IL-6 and a rise in albumin and IL-10 levels. The current study established a protective effect of raloxifene in cisplatin mediated nephrotoxicity and this is due to its potential anti-oxidant and anti-inflammatory properties. Therefore, a cisplatin induced nephrotoxicity can be prevented by the use of raloxifene as a therapeutic adjuvant.

Keywords: Cisplatin, inflammation, nephrotoxicity, raloxifene.

INTRODUCTION

Cisplatin amongst different anti-neoplastic agents' remains the most commonly used drug to treat various solid tumours such as head and neck, mammary, ovarian, and testicular tumours (Einstein and Sonpavde, 2019; Bogdanović *et al*., 2002). Cisplatin was regarded as "penicillin of cancer" by Kelland *et al* (2007) in 2007 as it is the first major chemotherapeutic agent. Furthermore, how exactly cisplatin causes its anti-neoplastic effect is unknown till date, but various reports say that it binds irreversibly to the DNA of cancerous cells and thereby interferes with the repair mechanism. However, a major limitation of cisplatin as a chemotherapeutic agent is the damage to the kidney tubules (Fang *et al*., 2021; Ali and Al Moundhri, 2006; Ramesh *et al*., 2007). This is the prominent drawback of cisplatin and causes renal damage in 27-35% of patients with just one injection (Basnakian *et al*., 2005). Hitherto, how exactly cisplatin damages renal cells have not been found? Researchers say that it distorts kidney cells and through the tubular damage it leads to inflammation, necrosis, and apoptosis (Volarevic *et al*., 2019; Faubel *et al*., 2007; Ramesh and Brian Reeves, 2006). Despite its renal toxicity, cisplatin continues to be the most widely prescribed anticancer drug for treating different sarcomas (Yao *et al*., 2007; Jamdade *et al*., 2016; Ramesh and Reeves, 2003). Thus, finding that how cisplatin causes renal damage and evolving different assisting therapies to effectively treat such glitches is of paramount significance. The combination therapies are required in order to ameliorate renal toxicity (Ramesh and Reeves, 2004; Dong and Atherton, 2007).

Raloxifene, also known as a selective estrogen receptor modulator (or SERM), is widely used to treat osteoporosis in postmenopausal women. Like cisplatin, it has also got side effects, but they are not as severe as that of cisplatin (Kaya *et al*., 2005; Lippuner *et al*., 2012; Shibata *et al*., 2010). Olivier *et al* in 2006 reported that raloxifene inhibits nuclear factor-κB (NF-κB) activity in cancerous cells (Olivier *et al*., 2006). It removes p65 subunits from its binding sites through the interaction of estrogen receptor α with p65 (Lippuner *et al*., 2012; Shibata *et al*., 2010; Galien and Garcia, 1997; Olivier *et al*., 2006). The NF-κB pathway is a crucial target for the number of diseases and has shown its key role in the growth and survival of cancerous cells as well (Thakur *et al*., 2015; Kumar *et al*., 2013). Other studies have reported that NF-κB has a pivotal role in inflammatory and immunological reactions and its activation causes an increase in the level of tumour necrosis factor-alpha (TNF-α) and some interleukins like IL-6, IL-1β and lower the secretion of IL-10 (Cheung *et al*., 2003; Deng *et al*., 2001; Kalaitzidis and Gilmore, 2005; Kumar *et al*., 2015). On the basis of above evidences, it is hypothesised that raloxifene pretreatment on cisplatin mediated nephrotoxicity may have ameliorating effects as raloxifene has been shown to inhibit NF-κB and this will eventually reduce the level of interleukins and TNFα which are responsible for inflammation of renal parenchyma.

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MATERIALS AND METHODS

Materials

Cisplatin and raloxifene were obtained from Sigma-Aldrich, Shanghai, China. Kits for testing Creatinine, Blood urea nitrogen (BUN), and Albumin level were acquired from Idexx VetLab®, Mumbai, India. Cisplatin injection was made in normal saline of 0.9% NaCl whereas raloxifene was dissolved in 100µl of olive oil. Always freshly prepared solutions were used for the dosing during the experiment.

Animals

The standard guidelines were observed and consent was obtained from the institutional animal ethics committee before starting the experiment. The animals (Female Wistar rats weighing 180-240g and up to 8 weeks old) were sought from the animal department. All the animals had been kept under ideal environment, surroundings and were given a normal diet (pellets) and water two times a day. The animal ethical issues had been always kept in mind and proper sanitary conditions were maintained. They were observed frequently to see if there is any discomfort, pain, or distress. In this study, 32 animals were used and were accommodated in cages made up of polypropylene. The cages were kept in a room of 12:12h light-dark cycle having a temperature of 24^0C with a relative humidity of 5%.

Study design and treatment

Four groups of animals were made arbitrarily. Group I had vehicle treated rats. Group II included rats who were treated with raloxifene 7.5mg/kg, for five days, dissolved in olive oil, and it was administered through oral gavage. Group III included cisplatin 7.5mg/kg treated rats. It was made in normal saline and was given by intra-peritoneal route. Group IV included rats pre-treated with raloxifene 7.5mg/kg dissolved in 100µl olive oil. It was given for the first five days and subsequently, a single dose of cisplatin 7.5mg/kg, dissolved in normal saline (0.9% w/v), was given by intra-peritoneal route on same day. Approval was sought from the institutional animal ethics committee for the experimental protocol. Blood was collected from the retro-orbital plexus using etherised capillaries and thiopentone sodium was used to anaesthetize the animals. Animals were sacrificed and the kidneys were collected through a midline incision. For the histological studies, 10% formalin was used to store the right kidney, whereas, for enzymatic analysis, the left kidney was kept in the deep refrigerator.

Estimation of serum albumin, blood urea nitrogen and creatinine

Centrifuge tubes were used to collect the blood samples and to centrifuge it immediately at 9000 rpm for 9 min at 4^0C. Then, the serum was separated and was stored at -90 ^{0}C till analysed. Finally, the level of blood urea nitrogen, creatinine and albumin were estimated with the help of the Idexx vet analyser.

Measurement of TNFα, IL-6 and IL-10 levels

The enzyme-linked immunosorbent assay (ELISA) kits were sought from Endogen, Woburn, MA, USA to measure the serum level of TNFα, IL-6 and IL-10. The assay was performed as per the instructions and the curve was plotted from the standards given by the manufacturer.

Histopathology of kidney

First, all rats were given light anaesthesia and were sacrificed. After surgery, through cardiac perfusion blood was collected with 0.1 MPBS (pH 7.4; 25-45 ml). In order to fix the tissue, 4% paraformaldehyde in 0.1M phosphate buffer was perfused after removing all circulating blood and adhering tissues for another 5 min. After removal of the kidney of the rats, it was sliced transversely and was embedded in paraffin for light microscopic evaluation. For each study group, at least 25 arbitrarily selected tissue sections were assessed for histopathological changes. To examine the cell structure changes, tissue sections were stained with eosin and Mayer's haematoxylin. Finally, these sections were observed under the light microscope.

STATISTICAL ANALYSIS

All values were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed to compare mean values among different groups followed by Tukey's test for multiple comparisons using GraphPad Prism 7.0 software. *P*-value <0.05 was supposed to be significant.

RESULTS

Effect of raloxifene pretreatment on cisplatin induced changes levels of creatinine, blood urea nitrogen, albumin, body weight and kidney weight

A group of animals treated with cisplatin had a substantial upsurge in serum creatinine level when matched with the normal control group. The raloxifene control group did not show a substantial change in creatinine level. However, the raloxifene pre-treatment group showed a substantial decline in the level of serum creatinine when matched with the group treated with cisplatin (fig. 1c). Similarly, a group of animals treated with cisplatin had a substantial upsurge in serum blood urea nitrogen level when matched with the normal control group. The raloxifene control group did not show a substantial change in blood urea nitrogen level. However, the raloxifene pretreatment group showed a substantial decline in the level of blood urea nitrogen when matched with group treated with cisplatin (fig. 1e). Contrary to the blood urea nitrogen and creatinine levels, the cisplatin treated group showed a substantial decline in the level of albumin. However, preatreatment of raloxifene resulted in a substantial upsurge in the level of albumin (fig. 1d).

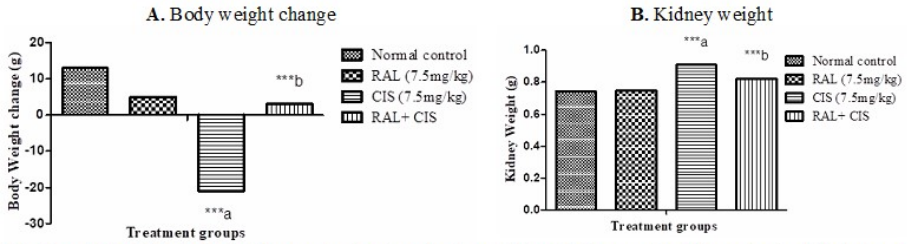


Fig. 1a and 1b: Effect of raloxifene pretreatment on body weight and kidney weight change in cisplatin induced nephrotoxicity.

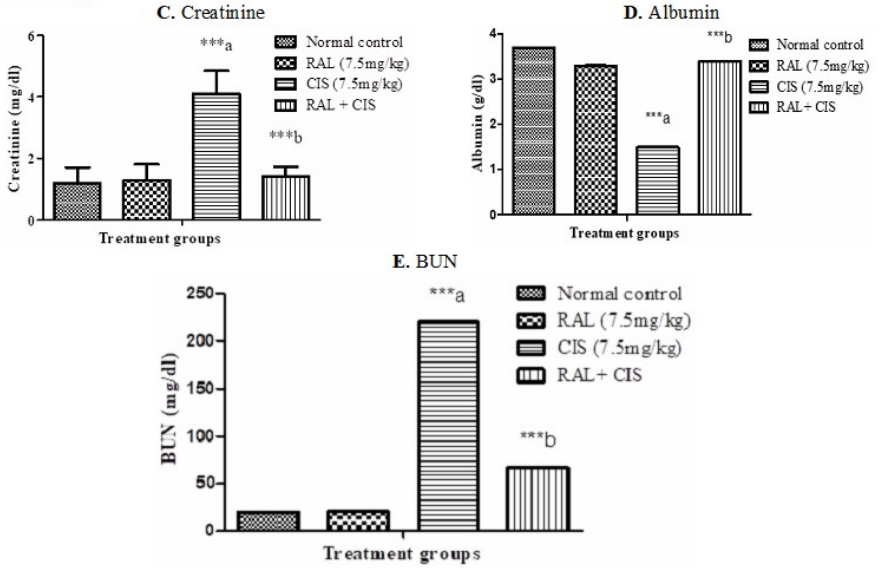
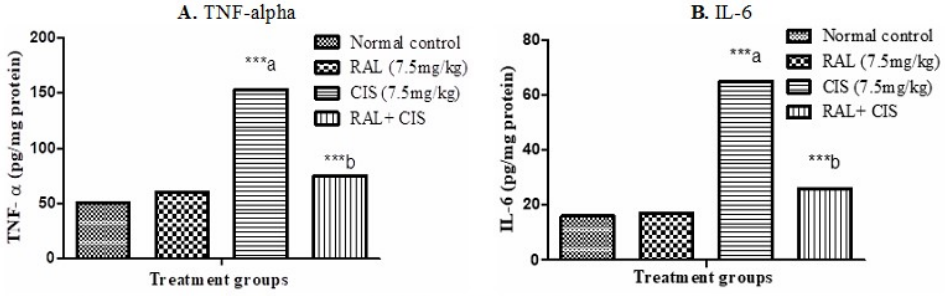


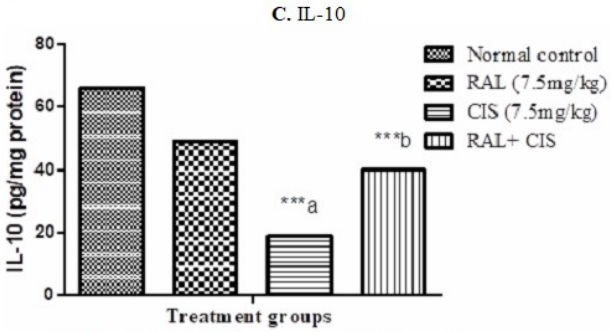
Fig. 1c, 1d and 1e: Effect of raloxifene pretreatment on creatinine, albumin and BUN in cisplatin induced nephrotoxicity.

All values are expressed as mean ± SEM (n = 8). ***P < 0.001. a vs normal control & b vs cisplatin.

Fig. 1: Effect of raloxifene pretreatment on body weight change in cisplatin induced nephrotoxicity.

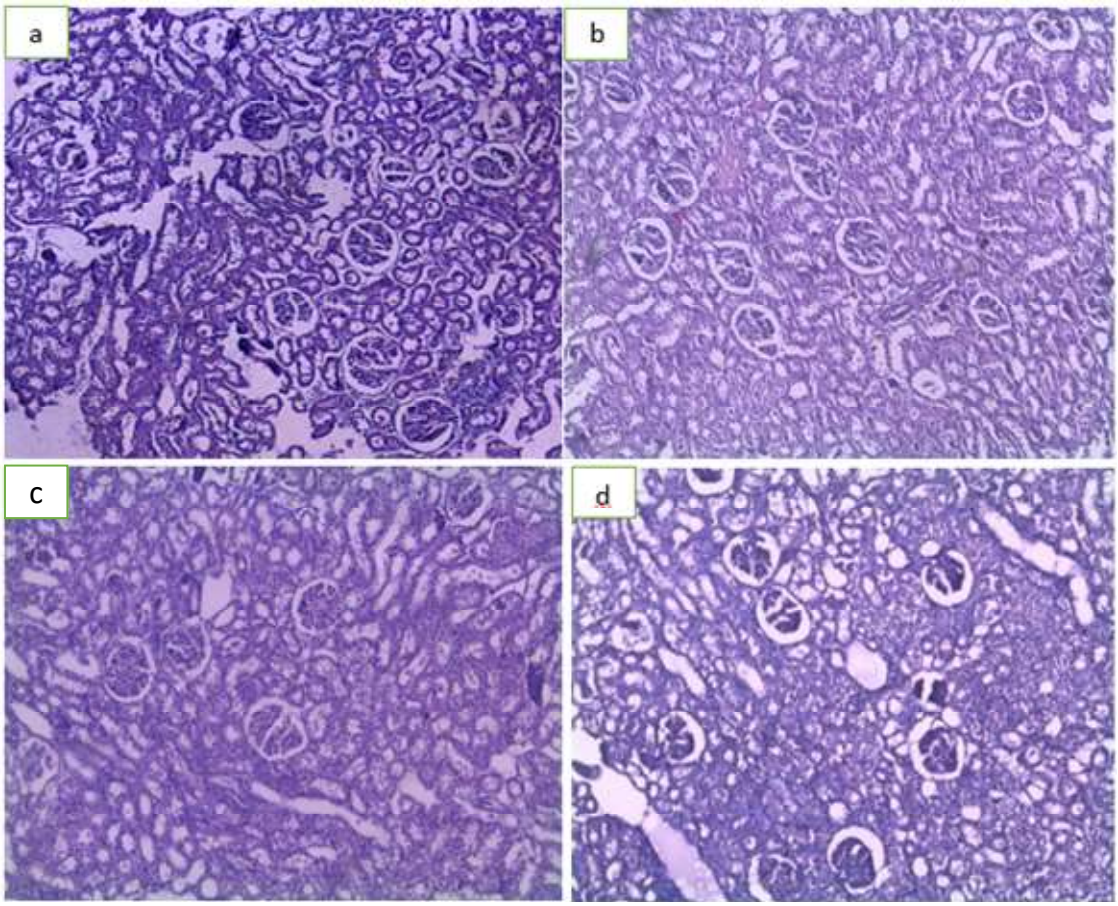


Effect of raloxifene pretreatment on TNF-alpha in cisplatin induced nephrotoxicity. All values are expressed as mean ± SEM (n = 8). ***P < 0.001. a vs normal control & b vs cisplatin.



All values are expressed as mean ± SEM (n = 8). ***p < 0.001. a vs. normal control & b vs. cisplatin.

Fig. 2: Effect of raloxifene pretreatment on TNF-alpha in cisplatin induced nephrotoxicity.



Histopathological changes in kidney after pretreatment with raloxifene. Transverse section of normal control rat kidney (a), raloxifene treated kidney (b), cisplatin treated (c), pretreatment of raloxifene (d). Sections were stained with Mayer's haematoxylin counterstained with eosin and observed under magnification of 40×

Fig. 3: Histopathological changes in kidney after pretreatment with raloxifene. Transverse section of normal control rat kidney.

The group treated with cisplatin had a substantial decrease in body weight of rats when matched with the normal control group, whereas the raloxifene pretreated group showed substantial gains in their body weights when matched to the cisplatin control group (fig. 1a). Moreover, there was a substantial increase in kidney weight of rats in the group treated with cisplatin when matched with the normal control group, whereas the raloxifene pretreatment group prevented gain in the kidney weight when matched with a cisplatin control group (fig. 1b). However, the body weight and kidney weight of rats were almost unchanged in the raloxifene control group. Based on above the observations, the pretreatment of raloxifene could be an effective way of plummeting the nephrotoxicity induced by cisplatin treatment.

Raloxifene pretreatment, cisplatin and level of TNF-α

Based on the scientific literature, it is quite evident that tumour necrosis factor-alpha has an important role in damaging the kidney with cisplatin administration. Therefore, curbing the TNF-α secretion can prevent this renal damage. The group treated with cisplatin showed a substantial upsurge in TNF-α level when matched with the normal control group. However, pretreatment of raloxifene substantially reduced the level of TNF-α in the group treated with cisplatin and this established that renal damage can be prevented by curbing TNF-α secretion (fig. 2a).

Raloxifene pretreatment, cisplatin and levels of IL-6 and IL-10

There was a noteworthy upsurge in the level of IL-6 in the cisplatin control group when matched with the normal control group, whereas the raloxifene control group did not show any substantial change in the level of IL-6 (fig. 2b). Raloxifene pretreatment considerably decreased the level of IL-6 in the cisplatin treated group when matched with a group of cisplatin control. Moreover, there was a substantial decline in the level of IL-10 in the cisplatin control group when matched with the normal control group. However, the raloxifene control group did not show any substantial change in the level of IL-10 (fig. 2c). Raloxifene pretreatment showed a substantial upsurge in the level of IL-10 in the cisplatin treated group

when matched to the group of cisplatin control, suggesting that IL-6 and IL-10 also play a vital role in nephroprotection.

Raloxifene, cisplatin and changes in kidney histology of rat

The intact renal tubule along with glomeruli was observed in group I treated with normal saline. The renal cortex of rats showed a single layer of the epithelium of intact tubule. (fig. 3a). Moreover, there was increased tubular space and epithelial cells showed desquamation in renal tubules along with vacuolation (fig. 3a). However, there was evidence of improved renal toxicity with the least damage to the kidney tubules in the raloxifene pre-treated group (fig. 3d). Group II of raloxifene control did not show any effect on the histology of kidney (fig. 3b). Hence, the findings of biochemical parameters were also supported by the histopathological changes in the cisplatin induced renal damage.

DISCUSSION

This study was carried out to find out the effect of pretreatment of raloxifene on cisplatin mediated renal damage. Many preclinical studies have established this damage as a dose limiting side effect. We found out that raloxifene pretreatment reduced cisplatin induced renal damage to a certain amount. Researchers found that the cisplatin induces the renal damage via activation of nuclear factor κB (NF-κB) which in turn causes synthesis of different proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) and decrease the synthesis of interleukin 10 (IL-10) (Tsuruya *et al*., 2003; Liu *et al*., 2012; Miller *et al*., 2010; Schrier, 2002). The group treated with cisplatin showed an upsurge in TNF-α and IL-6 levels. Along with this, it showed an upsurge in the biomarkers of kidney damage such as blood urea nitrogen (BUN), creatinine and albumin levels. However, raloxifene pretreatment substantially reduced TNF-α and IL-6 levels and also showed an upsurge in IL-10 levels. Furthermore, the histopathological studies also proved the improvement in kidney tubules in the raloxifene pretreated group when compared with the cisplatin control group with decreased levels of blood urea nitrogen and creatinine.

Irrespective of this distinguishing renal damage, this anti-cancer drug has been widely used to treat several tumours, including breast cancer, ovarian and testicular cancers along with head and neck tumours. Till now, no one has discovered the precise mechanism of cisplatin mediated renal toxicity(Liu *et al*., 2012). However, it has been found that cisplatin acts on tumour necrosis factor receptor (TNFR) 1 and TNFR 2 through mitogen-activated protein kinase (p38) ensues inflammation and apoptosis. Inflammation is caused by means of TNFR 2 by the synthesis of chemokines and cytokines, which results in damage to tissues and ultimately accelerate the acute renal toxicity (Ramesh and Reeves, 2003).

A single dose of cisplatin accelerates the renal damage frequently in proximal convoluted tubules and certain portions of distal convoluted tubules and this is evidenced by reducing blood urea nitrogen and creatinine clearance. Here, we corroborate that pretreatment of raloxifene has substantially enhanced urea and creatinine clearance with an upsurge in albumin level. Moreover, this study provides a piece of evidence that pretreatment of raloxifene has substantially reduced TNF-alpha level, causing a noteworthy drop in renal damage accompanied by a drop in IL-6 level and an upsurge in IL-10 level. The cisplatin induced renal damage and its amelioration by pretreatment of raloxifene was warranted by histopathological outcomes. Research suggests that raloxifene removes the p65 (RelA) subunit of NF-κB via interaction of estrogen receptor α, thus prevents stimulation of proinflammatory cytokines and reduce their levels (Frasor *et al*., 2015).

CONCLUSION

The quality of life of cancer patients is miserable and that too with a very short life expectancy. Breast cancer patients have a limited number of interventions to treat with cisplatin being the most widely used one. And this becomes very inconsolable for breast cancer patients if they are suffering from kidney disorders because cisplatin deteriorates the condition of such patients causing nephrotoxicity. Hence, this study would be very promising for the patients who are suffering from breast cancer along with kidney disorders. Also, raloxifene has its own anticancer effects and that will lead to additive anti-cancer effects. From this research, we suggest further studies using different animal models, so that this combination can be introduced in a clinical trial for human use.

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