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Hepatotoxic and Nephrotoxic Potential of an Anticancer Drug Paclitaxel after Prolong Exposure in Adult Female Rats

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Abstract: Paclitaxel (PTX) is a microtubule inhibitor commonly used as a potent chemotherapeutic agent for treatment of a variety of cancers including breast cancer. PTX is causing several serious side effects during and after chemotherapy related to metabolic and renal dysfunctions. Clinical and non-clinical studies on PTX exposure and histopathological changes in liver and kidney are limited; therefore present study was designed to evaluate hepatotoxicity and nephrotoxicity of PTX in adult female rats. In this regimen, equivalent therapeutic doses of PTX (1.6 and 3.2 mg/kg BW) were intravenously administered weekly for six weeks. After six weeks of exposure, liver and kidney of PTX exposed and control rats were extirpated, fixed in 10% neutral buffered formalin for paraffin sectioning. As per protocol, sections of liver and kidney were cut at 8 µm and finally stained with Haematoxylin and Eosin. Our results demonstrated that intermittent exposure to PTX for six weeks (once per week) induced substantive histopathological changes in liver (hepatocyte derangement, blood clotting, and CV dilatation) and kidney (glomerular atrophy, vascular congestion, dilation of distal tubules) of female rats.

Keywords: Anticancer drug, Taxol, Paclitaxel, Hepatotoxicity, Nephrotoxicity, Rats

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Introduction

The therapeutic management of various forms of cancer, systemic chemotherapy regimen is a conventional and potential process to restrain the spread of cancer in the target organ and non-target tissues. Although chemotherapy exerts specific cytotoxic effects on malignant cells, but potential inability to distinguish between normal and malignant cells is one of the biggest issues of anticancer drugs (Damia and Broggini 2004; Ménard *et al*., 2008). Unfortunately, cancer chemotherapy produces acute non-targeted organ toxicity through various mode of actions (Maor and Malnick, 2013). Among the traditional classes of anticancer agents, Paclitaxel (PTX), commonly known as Taxol, is an anti-cancer chemo-therapeutic agent that stabilizes polymerized microtubules in metaphase, preventing the progression to anaphase in rapidly dividing cells

(Schiff and Horwitz 1980; Jordon *et al*., 1993; Ikui *et al*., 2005; Risinger *et al*., 2014). It is one of the most commonly and effective chemotherapeutic drug used to treat a variety of cancers including oesophageal, ovarian, lung, cervical and breast cancer (Sparano *et al*., 2008; Suh *et al*., 2014; Miyaushiro *et al*., 2015; Langer *et al*., 2015). Approximately all classical anticancer drugs including PTX may cause mild to severe side effects associated with haematological, gastrointestinal, buccopharangeal, dermatological, peripheral neuropathic, cardiovascular, skeletal and sexual dysfunctions (Banerji *et al*., 2014; Hoke and Ray, 2014; Sullivan *et al*., 2022 ; Farrar and Jacobs, 2022) and these side effects may be expressed during and after chemotherapy and occasionally resolved if treatment regimen is changed (Reeves *et al*., 2012). However, chemotherapy of conventional antineoplastic drugs including PTX may induce lasting impact on pathophysiology of liver and kidney. These two organs are the main centres of the drug's metabolism, hence maintains the homeostasis for normal functioning. There are limited reports regarding PTX chemotherapy induced hepato-toxicity and nephrotoxicity in clinical and non-clinical investigations. Clinical exposure to anticancer drugs induced various types of hepatic ailments like hepatitis, fibrosis and cirrhosis, sinusoidal obstruction and hepatic failure, thus causing considerable hepatotoxicity (Vincenzi *et al*., 2016). Similarly, nephrotoxicity is an adverse effect of main classes of anticancer drugs namely cisplatin, methotrexate, ifosfamide, citumixab and panitumumab, mitocin C and gemcitabine, and antiangiogenesis (Lameire *et al*., 2011; Ezz-Din *et al*., 2011). In the recent past, cellular histopathological changes like sinusoidal dilatation and congestion, hepatocellular degeneration, and inflammatory infiltrations in the liver and necrosis of the terminal portion of the proximal tubule and apoptosis in the distal nephrons of the kidney were reported after chemotherapy of some classical anticancer drugs (Santos *et al*., 2020; Kerim *et al*., 2020). Karaduman *et al*. (2010) reported substantial increase of degenerative and necrotic changes in the liver of mice which indicates adverse hepatotoxic effects of PTX on a non-targeted organ (Pieniążek *et al*., 2013). In a preclinical study, Rabah *et al.* (2010) revealed that short exposure to PTX created marked degenerative changes in histopathology of kidney parenchyma as PTX induced nephrotoxicity. However, clinical and non-clinical literature indicates that PTX induced hepatotoxic and nephrotoxic studies are inadequate to draw a definite conclusion on this important issue of clinical relevance. Therefore, present study aimed to investigate the effects of clinically relevant doses of PTX on histopathological changes in liver and kidney of rats, mimicking clinical chemotherapy regimen.

Materials and Methods

Animals and Ethical clearance:

Adult female Wistar rats of 8-10 weeks of age, weighing 180±10 g were used. All experimental rats were housed in plastic cages with rice bran as the bedding material and maintained under standard laboratory conditions (22±2^{0}C, 24±2 % RH and 12h L/D cycle). Tap water was provided *ad libitum* throughout the experiment. Animals were used in accordance with the animal welfare act and protocol for use of experimental rats was approved by the Institutional Animal Ethics Committee (IAEC), UoA.

Rationale and calibration of drug doses:

Paclitaxel (PTX) manufactured by Beta Drugs Pvt. Ltd. and marketed by Cadila Pharmaceuticals Ltd. Ahmedabad, India, was purchased from the local pharmaceutical market. PTX solution 30 mg/5 ml was diluted in 0.9% normal saline prior to infusion. The equivalent therapeutic doses of PTX (1.6 mg/kg BW and 3.2 mg/kg BW) were calibrated on the basis of per kg body weight (Polomano *et al*., 2001). Further, selected drug doses were again adjusted according to body weight gain/reduced after one week of exposure.

Experimental design:

All female rats were randomized into three

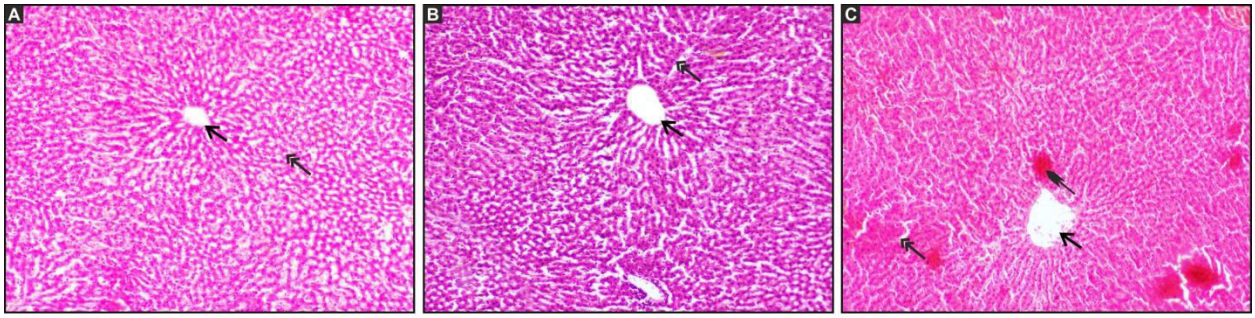

Fig. 1: Histopathological changes in liver. (A) Control; (B) PTX (1.6 mg/kg) exposed; (C) PTX (3.2 mg/kg) exposed. PTX induced dilation of central vein (CV), congestion of blood and dilation of sinusoidal space marked with arrow. 10X magnification; ↖central vein, ↖ sinusoidal space, ↖ congestion of blood.

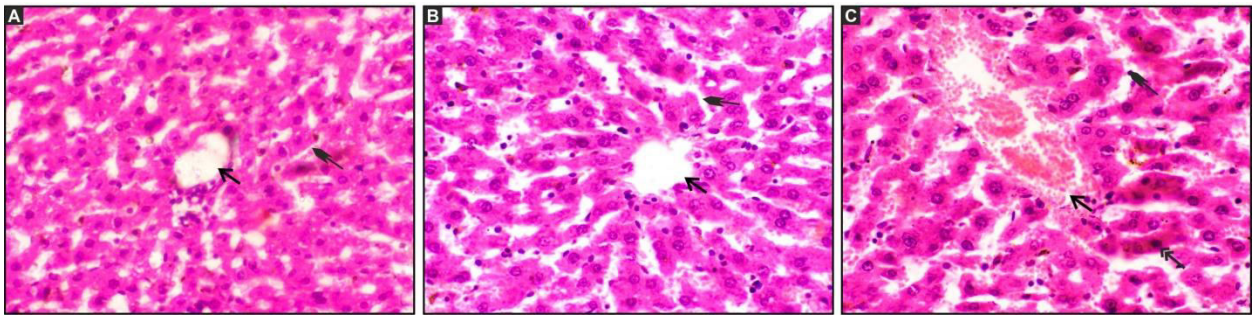

Fig. 2: Histopathological changes in liver at higher magnification. (A) Control; (B) PTX (1.6 mg/kg) exposed; (C) PTX (3.2 mg/kg) exposed. PTX induced dilation of central vein (CV), degeneration of hepatocytes, and congestion of blood in central vein and dilation of sinusoidal space marked with arrow. 40 X magnification; ↖ central vein, ↖ sinusoidal space, ↖degeneration of hepatocytes.

groups, Group I: control/vehicle-treated (n=6); Group II: 1.6 mg/kg BW PTX treated (n=6); Group III: 3.2 mg/kg BW PTX treated (n=6). After mild anaesthesia (ketamine 2.0 mg/kg BW, i.p.), Group-II and Group-III rats were individually placed in a constrainer chamber and selected doses of PTX, 1.6 mg/kg BW and 3.2 mg/kg BW were administered intravenously (i.v.) in the rat's lateral tail vein through a catheter, once a week for six weeks. Similarly, Group-I rats were exposed with equal amount of 0.9% saline.

Procedure for histopathological study:

After 6 weeks of intermittent exposure to PTX, all control and PTX exposed rats were perfused transcardially with saline followed by 10% neutral formalin under mild anaesthesia, and their liver and kidney were extirpated and fixed in the same fixative for histopathological examinations. Further, all dissected rats were euthanized with phenobarbital anaesthesia (200 mg/kg BW), and disposed off as per guidelines of IAEC. As per protocol, prefixed tissues of liver and kidney were processed for paraffin block preparation, and sections were cut at 8 µm by rotatory microtome; and finally stained with Hematoxylin and Eosin and examined under light microscope.

Results

Effect of PTX on histology of the liver:

In PTX exposed (1.6 mg/kg) rats, there was a moderate dilation of central vein (CV), congestion of blood, dilation of sinusoidal space and mild hepatic cord disarray, whereas these changes were found severe with loss of hepatic lobules in 3.2 mg/kg PTX exposed rats in comparison with control rats (Figs. 1, 2).

Effect of PTX on histology of the kidney:

The control group rat kidneys had normal glomeruli, tubules, interstitium, and blood arteries. The 1.6 mg/kg PTX-treated group showed proteinaceous casts in the tubular lumen after treatment. Additionally, it was discovered

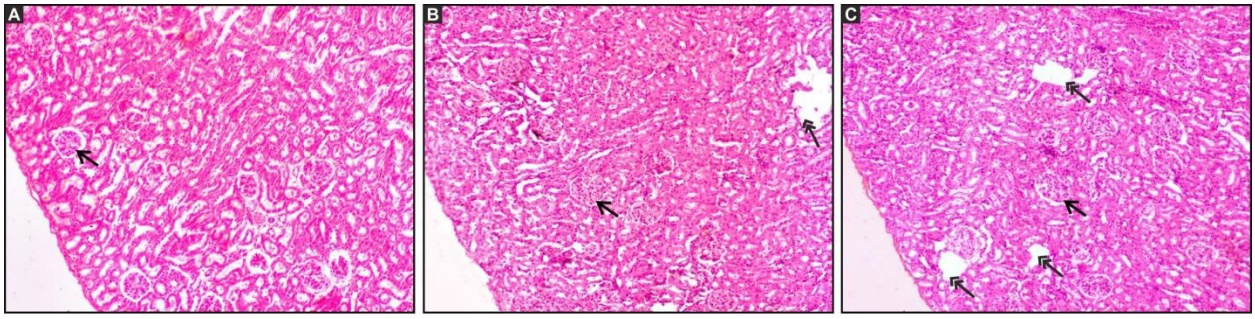

Fig. 3: Histopathological changes in rat's kidney. (A) Control; (B) PTX (1.6 mg/kg) exposed; (C) PTX (3.2 mg/kg) exposed. PTX induced marked disorganization of Bowmen's capsule and distal renal tubules marked with arrow. 10X magnification; ↖Bowmen's capsule, ↖ degeneration of renal corpuscle.

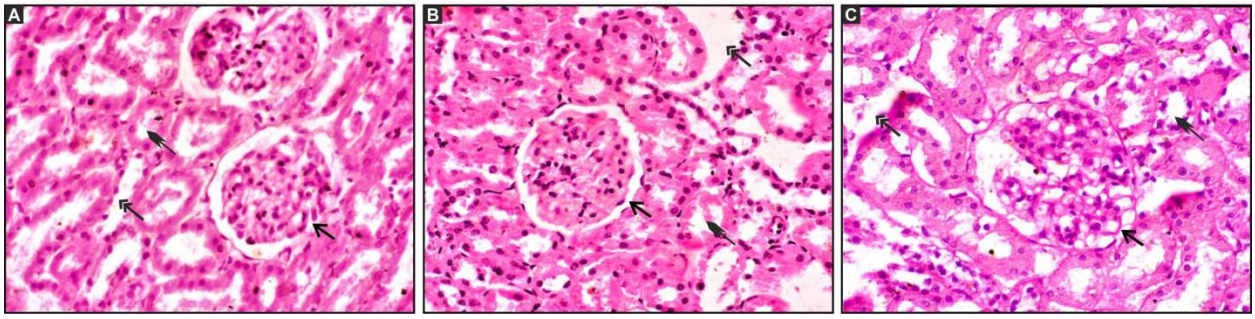

Fig. 4: Histopathological changes in rat's kidney. (A) Control; (B) PTX (1.6 mg/kg) exposed; (C) PTX (3.2 mg/kg) exposed. PTX induced marked disorganization of Bowmen's capsule, distal renal tubules and degenerative changes in kidney parenchyma marked with arrow. 40X magnification; ↖ Bowmen's capsule, ↖ widening of proximal renal tubule, ↖ widening and degeneration of distal renal tubule.

that some tubules had tubular cells that had detached from the basement membrane. The 3.2 mg/kg PTX-treated animals showed marked disorganization and mild to moderate tubular necrosis in their kidneys, particularly in the proximal tubule (Figs. 3, 4).

Discussion

The present study revealed that prolonged exposure to PTX at selected equivalent therapeutic doses induced hepatotoxicity and nephrotoxicity in experimental rats. The present findings corroborate well with those investigators who have reported considerable hepatotoxicity and nepherotoxicity in PTX exposed adult subjects (Vincenzi *et al*., 2016). Cosan *et al*. (2008) reported that exposure to PTX damaged the liver and kidney structures (histopathological changes) and functions (liver and kidney function tests) in rats. Rabah *et al.* (2010) also demonstrated that short exposure (48 h) of PTX (1.7 mg/kg dose) induced nephrotoxicity with noticeable degenerative changes (loss or injury of renal tubule epithelial lining and brush border; necrosis and apoptosis in renal tubules, atrophy in glomerular tufts, vascular congestion and degeneration of renal blood vessels) in the histopathology of kidney of adult male mice. In clinical trials, mild impaired liver functions with elevated enzymes were noticed after chemotherapy with PTX (Navarro and Senior, 2006; Mandaliya *et al*., 2015). In preclinical studies on rodent, some researchers reported histological abnormalities in liver of rats induced by some other classical chemotherapeutic drugs, cisplatin, doxorubicin and 5-flurouracil (El-Sayyad *et al*., 2009; Aikemu *et al*., 2016). Moreover, cisplatin-induced nephrotoxicity and hepato-toxicity endowed with alterations in histology of kidney and liver was also found in mice

(Nakashima-Kamimura *et al*., 2009; Sloop *et al*., 2019). It is established that individual chemotherapy with PTX or in combination with other conventional anticancer drugs of different inducing mechanisms may induce structural and functional impairments in some vital organs like liver and kidney resulting into disturbances of homeostasis and physiology of the subjects irrespective of age, drug doses, exposure time and sexual category (Dai *et al*., 2015). The results of the present study corroborate well with the findings of other clinical and preclinical investigations especially on histopathological changes in liver and kidney which ultimately disturbed the behavioural functions related to locomotory responses, exploration, anxiety, depression and cognition (data not included in this study).

In the current investigation, it was found that prolonged exposure (six weeks, once per week) to PTX even at lower dose, i.e. 1.6 mg/kg BW which strictly mimics with equivalent therapeutic dose, caused mild to moderate hepatotoxicity (congestion of CV, loss of hepatocytes, sinusoidal space enlargement, and minor hepatic cord disruption) and nephrotoxicity (altered glomerular and tubular components of the kidney parenchyma, focal necrosis in the form of vacuolization in renal tubules) with clinical characteristics, whereas higher dose (3.2 mg/kg BW) of PTX induced dose-dependent severe dystrophy in cellular organization of liver and kidney under light microscopic observations.

Since the effectiveness of PTX for treating a range of tumour malignancies has been established (Wang *et al*., 2000; Zang *et al*., 2010), but its impact on renal structure and function seems to be of clinical significance. Hence, kidney and liver functions must be closely monitored at the beginning and during chemotherapeutic regimen of the conventional antineoplatic drugs including PTX.

Conclusion

The present study concluded that prolonged PTX exposure induced dose-dependent mild degenerative changes in liver and kidney at minimum tolerated dose (1.6 mg/kg BW), whereas 3.2 mg/kg dose of PTX induced remarkable degenerative changes in liver and kidney when compared with control or vehicle treated group. Therefore, cautions must be taken before exposure or initiation of chemotherapy with PTX in adult female subjects.

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