Toxic effect of combined treatment with cadmium and piroxicam on liver and kidney tissues

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A B S T R A C T

Cadmium (Cd) is a well-known hazardous environmental contaminant. Non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to have a deleterious effect on liver and kidney. Therefore, in this study, we investigated whether NSAIDS could potentiate the toxic effect of Cd on liver and kidney. Twenty male Wister rats were divided randomly into 4 groups. Group 1:the control was injected saline daily. Group 2: received cadmium (5mg/kg) daily. Group 3: served as piroxicam were injected a single dose (10 mg/kg, i.p.). Group 4: received both Cd+Px. After 14 days, all rats were euthanized and blood samples, stomach, liver, and kidney tissues were collected. Treatment with Cd or Px alone significantly increased the liver and kidney function markers. However, when Cd and Px were given in a combination, Px potentiated the Cd-induced cellular damage in liver and kidney when compared to their individual treatment in liver and kidney functions and histopathological examination. Their combination could also enhance the apoptosis.

Keywords: Cd; Non-steroidal anti-inflammatory drugs; Piroxicam; liver damage; Renal damage

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1. INTRODUCTION

Cd is one of the most hazardous heavy metals, naturally existing in the environment in air, soil, water, and in food as well. Cd is a dangerous environmental and occupational contaminant that represents a potential hazard on humans and animals health even at low levels of exposure (Singh et al., 2013). There are cumulative evidences that Cd causes long-term negative effects on health via producing a wide range of biochemical and physiological alterations in the biological system leading to various tissue injuries including hepatic, renal, and testicular injury (Abdeen et al., 2017; Claudio et al., 2016; Dai et al., 2018; Karthik Mohan and Jeyachandran Robert, 2009; Martínez-flores, 2013; Rani et al., 2014; Salama and El-Bahr, 2007; Yang and Shu, 2015). It is well known that Cd has a great affinity to various biological components such as sulfhydryl containing cellular molecules including mainly metallothionein. Cd-metallothionein complex is produced in the hepatocytes and then distributed to the kidney causing nephrotoxicity (Klaassen et al., 2009).

Cd has the ability to accumulate in the different body tissues mainly kidney and liver generating ROS which leads to induction of oxidative stress causing deleterious actions in such tissues (Nair et al., 2013). It accumulates in the cells and can directly attack the nucleus and other organelles producing DNA damage, chromosomal aberrations, mitochondrial dysfunction, ATP depletion, lipid peroxidation, and protein oxidation (Abdeen et al., 2017; Rani et al., 2014).

Px is a non-steroidal anti-inflammatory drug (NSAID) of oxicam derivatives. It is extensively prescribed over the world due to its wide pharmacological actions (Aithal, 2011;

Steinmeyer, 2000). It has gained attention as an effective anti-inflammatory drug.

Recently, the toxicological studies of combined toxicants gained a considerable attention during the last decade. There are some studies have investigated the toxicological effects of multiple toxicants on the biological system (Chouhan and Flora, 2010; El-Gamel, 2009; Fernández and Beiras, 2001; Ibraheem et al., 2016; Olszowski et al., 2016; Tsiliou et al., 2016; Zhang et al., 2013). Therefore, the current study was design to examine the modulatory effect of Cd overload Px-mediated hepato-renal toxicity.

2. MATERIALS AND METHODS:

2.1. Chemicals:

Piroxicam, Px (Feldene®, 20 mg/ ml) was obtained from Pfizer Inc, Cairo, Egypt. Cadmium, Cd was purchased from purchased from Central Drug House (p) LTD, New Delhi, India.

2.2. Experimental animals:

The present study was carried out on a total number of 20 Wister albino male rats weighing 130-150 gm. Rats were obtained from Center of Laboratory Animal, Faculty of Veterinary Medicine, Benha University, Egypt. They acclimatized for two weeks prior to the experiment (temperature ~25 °C). They received standard laboratory balanced commercial diet and water ad libitum.

2.3. Experimental design:

Rats were randomly assigned into 4 equal groups (5rats each). Group 1 (Control); rats served as vehicle control and received saline, i.p. Group 2 (Px): rats were served as Px toxic control and were injected piroxicam intraperitoneally at dose of 10 mg/kg b.wt (Villegas et al., 2002). Group 3 (Cd): rats were served as Cd toxic control and were administrated Cadmium chloride at a dose of 4 mg/kg b.wt orally daily for 14 days according to Shati (2011). Group 4 (Cd+Px); rats in this group were received both Cd (4 mg/kg b.wt., orally) and Px (10 mg/kg b.wt, i.p.). The duration of experiment was 14 consecutive days.

2.4. Sampling

After the end of the experiment, a blood sample was collected directly from the caudal vena cava, apart was taken in heparinized tubes for hematological studies and the other part was kept at room temperature without anticoagulant for serum separation intended for the biochemical studies. Liver and kidney tissues were also collected for oxidative cascade determination and histopathological and immunohistochemical examinations.

2.5. Serum biochemical studies:

Serum AST, ALT, ALP (Reitman and Frankel, 1957), total protein (Lowry et al., 1951), albumin (Doumas et al., 1971), creatinine (Larsen, 1972), urea (Coulombe and Favreau, 1963), were determined using diagnostic kits obtained from Laboratory Biodiagnostics Co, Cairo, Egypt.

2.7. Histopathological and immunochemical examinations:

Tissue samples were collected from liver and kidney and fixed in 10% formalin for 24 h and processed using conventional paraffin-embedding techniques. For light microscopic examination, paraffin blocks were cut into 5 µm thick sections and stained with H&E (Bancroft et al., 1996). For immunohistochemical examination; Liver and kidney sections were deparaffinized and dehydrated graded alcoholic solutions. Antigen was retrieved by heating citric acid solution for 5 min. A 3% H_2O_2solution was used to inactivate the endogenous peroxidases. Then, the slides were blocked in 5% BSA blocking solution for 20 min. Next, the slide was incubated with anti-Bax or anti-Bcl2 primary monoclonal antibody with dilution of 1:100 at 4 °C overnight followed by an incubation with avidin-biotin complex (ABC kit, Vector Laboratories) at 37°C for 1 h. The reaction product was visualized by 3,3-diaminobenzidine tetrahydrochloride (DAB), and the slide was counterstained with Mayer's hematoxylin.

2.8. Statistical analysis:

Statistical analysis was performed using SPSS (Version 20.0; SPSS Inc., Chicago, IL, USA). The

significant differences between groups were evaluated by one way ANOVA using Duncan test as a post hoc. Results are expressed as mean ± SE. All values at $P \leq 0.05$ were considered significant.

3. RESULTS:

3.1. Serum biochemical analysis:

The concentrations of the AST, ALT, ALP, creatinine and urea in serum were increased in both Px and Cd treated groups and this increase was significantly compared to control group. While, concentrations of the total protein and albumin in serum were decreased in both Px and Cd treated groups and this decrease was significantly compared to control group. In Px plus Cd treated group, AST, ALT, ALP, creatinine and urea in serum were significantly increased compared to control, Px and Cd treated groups The effect on biochemical parameters were shown in Table 1.

3.2. Histopathological findings:

To confirm the data mentioned above, we examined the histopathological changes occurred in the liver and kidney tissues after treatment with Px and/or Cd. In liver tissue, the control group showed uniform polyhedral hepatocytes, normal sinusoids, and portal veins as shown in Fig. 1A. On the other hand, Cd or Px treatment alone induced severe lymphocytic infiltrations, fatty degenerations, pyknotic nuclei, and dilatation of portal vein with occluded sinusoids suggesting a severe damage occurred (Fig. 1B and C). Interestingly, combined toxicity of Cd+Px showed marked damage in the hepatic cells when compared to their individual treatment (Fig. 1D). In kidney tissue, the control group exhibited normal glomerular, tubular, and interstitium structure (Fig. 2A). While, in Cd or Px group, we observed damage in renal tubular epithelia, hydropic degenerations and cytoplasmic vacuolation, tubular dilatation, and severe interstitial lymphocytic infiltration (Fig. 2B and C, respectively), importantly, Cd showed more damage than Px. However, a combined toxicity of Cd and Px potentiate their damage indicated by severe destruction of the renal architecture (Fig. 2D). The histopathological findings support the afore-mentioned data (Table 1).

3.3. Immunohistochemical study:

Changes in Bax expression after treatment with Cd and/or Px in liver and kidney tissues (Figures 3-4), respectively.

Table 1: Effect of piroxicam (Px) and/or cadmium (Cd) treatment on serum biochemical parameters in rats (n=7).

Parameters	Control	Px	Cd	Px+Cd
AST (U/L)	69.65 ± 6.66^{c}	141.68 ± 3.64^{b}	147.68 ± 11.1^{b}	189.37 ± 7.83^{a}
ALT (U/L)	24.72 ± 1.72^{d}	49.92 ± 3.53^{c}	67.31 ± 4.58^{b}	91.24 ± 1.84^{a}
ALP (U/L)	184.26 ± 15.8^{c}	441.81 ± 28.7^{b}	354.43 ± 17.6^{b}	582.79 ± 23.5^{a}
T. protein (g/dl)	8.51 ± 0.17^{a}	6.68 ± 0.27^{b}	5.72 ± 0.16^{c}	4.53 ± 0.16^{d}
Albumin (g/dl)	3.50 ± 0.07^{a}	2.72 ± 0.08^{b}	2.24 ± 0.16^{c}	1.53 ± 0.12^{d}
Creatinine (mg/dl)	0.73 ± 0.02^{d}	0.88 ± 0.07^{c}	1.09 ± 0.02^{b}	1.33 ± 0.03^{a}
Urea (mg/dl)	32.22 ± 2.05^{c}	68.58 ± 5.08^{b}	72.90 ± 2.89^{b}	95.69 ± 3.88^{a}

Data are expressed as the mean±SE. Different superscript letters in the same row indicate statistical significance at $P \leq 0.05$.

Figure 2:

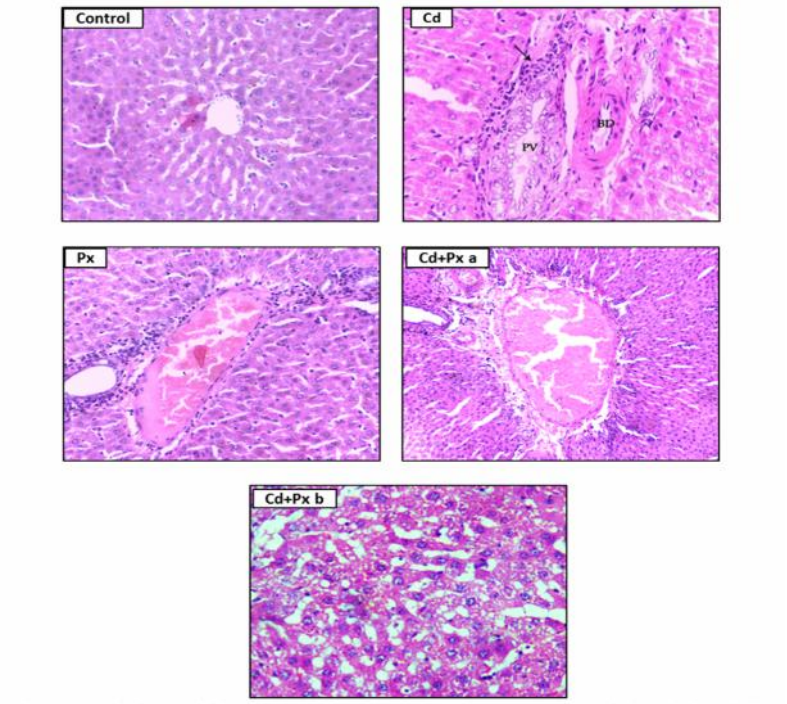

Figure 2. Histology of liver tissue after treatment after treatment with cadmium (Cd), piroxicam (Px), or their combination (Cd+Px). Control: Liver section from saline-treated rats,

Figure 3:

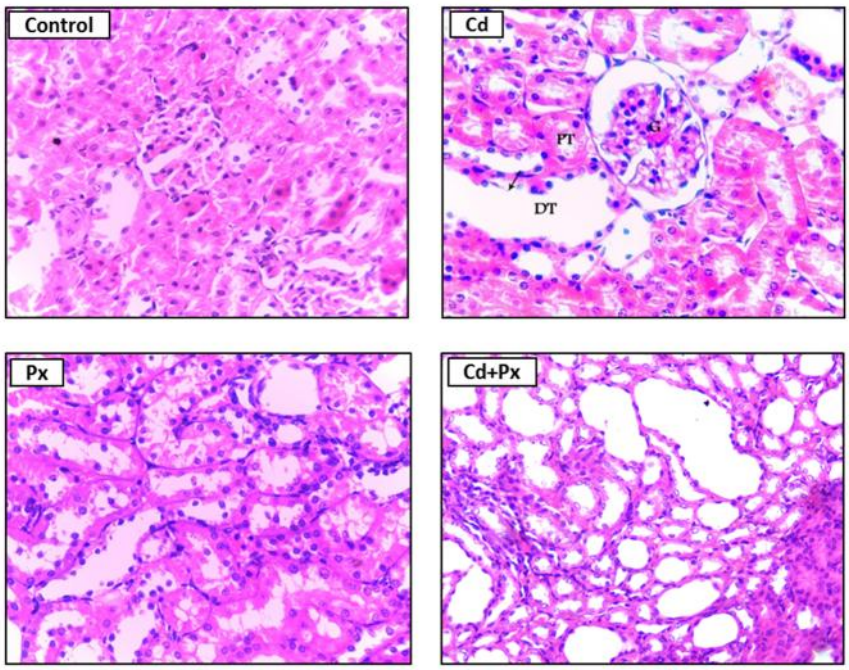

Figure 3. Histology of kidney tissue after treatment after treatment with cadmium (Cd), piroxicam (Px), or their combination (Cd+Px). Control: Kidney section from

Figure 4:

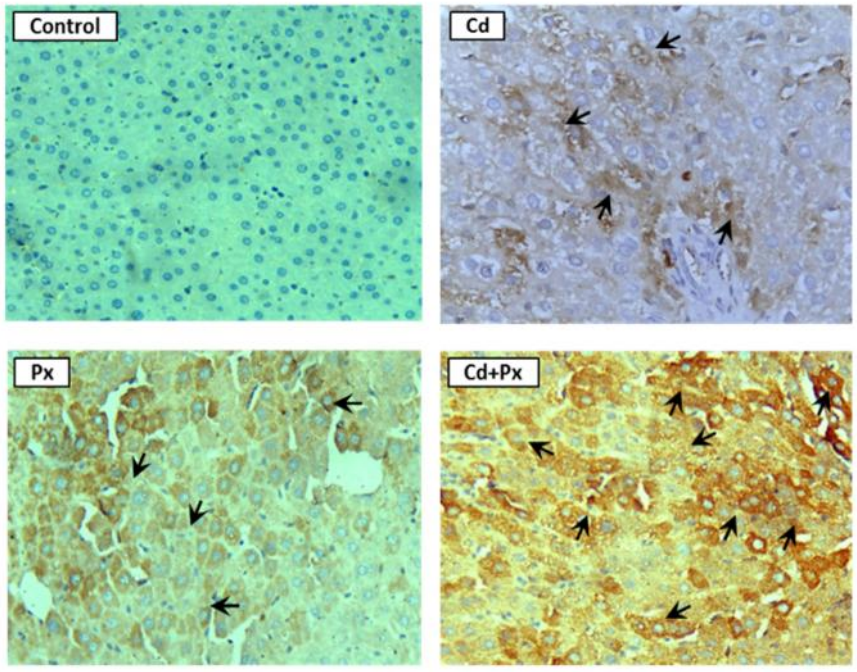

Figure 4: Changes in liver Bax expression after treatment with cadmium (Cd), piroxicam (Px), or their combination (Cd+Px). Immunostaining was performed

Figure 5:

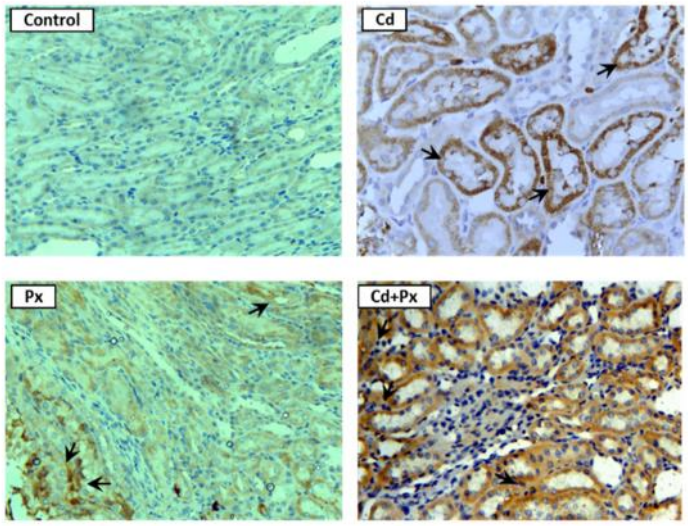

Figure 5: Changes in kidney Bax expression after treatment with cadmium (Cd), piroxicam (Px), or their combination (Cd+Px). Immunostaining was performed using anti-Bax antibody and developed with DAB. The positive staining of Bax is pointed by arrows and indicated by a brown color. (magnification: X200)

4. **DISCUSSION**

The concentrations of the AST, ALT, ALP, creatinine and urea in serum were increased in Px and/or Cd treated groups and this increase was significantly compared to control group. While, concentrations of the total protein and albumin in serum were decreased in Px and/or Cd treated group and this decrease was significantly compared to control group. The aminotransferases (ALT, AST, ALP), are among serum biomarkers of hepatic function, with their increase in the serum indicating hepatic damage (Kleiner et al., 2014). Significant changes were

observed in ALT, AST and ALP level in rats treated with Px, which indicated that Px at given dose produced adverse effect on liver. Similarly, significant increased levels of AST and ALT have been observed in Px at dose 15 mg/kg for 14 days in rats (Abatan et al., 2006) and Px (6.6 mg/kg) for 15 day in mice (Sahu, 2016).

Normal levels of protein restoring are an important factor for liver recovery (Navarro and Senior, 2006). Levels of total protein and albumin were related to hepatic cells function (Huo et al., 2011). The total protein concentration was significantly decreased in Px treated group. A low protein level is observed in hepatopathy. Loss of protein during inflammation or ulceration of the gastrointestinal tract could lead to impaired absorption as well as loss of serum protein due to injured mucosal cells (Braamskamp et al., 2010). Damage to the kidney was also responsible for the loss of plasma protein and causes their low concentration (Jain, 1986). The reduction in proteins might be resulted from hepatocytes impairment and reduced in amino acid availability and/or in hepatic protein capability (Gomes et al., 1999). Significant increase was observed in serum creatinine and urea concentrations in Px treated group as compared to control which considered as an index of nephrotoxicity. An increase in serum urea and creatinine concentrations were recorded in diclofenac treated rats (El-Shafei and Saleh, 2016).

Cd is one of the most toxic pollutants among other environmental pollutants. Cd is known to induce oxidative damage by enhancing production of ROS inside the cell. Liver and kidney are the main target organs for Cd (Abernethy et al. 2010). Cd has a great affinity for SH-containing molecules inside the cell such as glutathione (GSH) and metllothionein (MT). MT is a cysteine-SH rich protein which is important in detoxification of Cd through formation of Cd-MT complex. Because liver and kidney are rich in MT, they are known to be the major target organs for Cd accumulation (Hollis et al. 2001; Massanyi et al. 2003). Hence, SH group is involved in the function of many enzymes, the Cd-SH complex possibly disturb many functions of cell mainly mitochondrial dysfunction. Interacting of Cd with the redox system in the mitochondria enhances over production of ROS and their release into the cytoplasm inducing apoptotic cascade (Radosavljevici et al. 2012). In the current study, firstly, the hepatotoxic effect of Cd was demonstrated by elevated ALT, AST, and ALP levels. Cd-induced lipid peroxidation in liver resulted in increasing the permeability of the cell membrane of hepatocytes and release of transaminases (ALT and AST) into the blood. The increase in synthesis of ALP indicates hepatic toxicity and biliary obstruction (Toppo et al. 2015). In addition, our results revealed reduction in the serum total protein and albumin, which possibly due to the reduced ability of liver to synthesize proteins because of Cd insult. These data have been also confirmed by histopathological examination of liver.

Cd increased the levels of serum creatinine and urea. Since Cd impaired the glomerular filtration, the creatinine and urea accumulated in the blood. Proximal tubular epithelial cells are rich in mitochondria comparing to other renal cells. These mitochondria are needed for high-energy production required in reabsorption of albumin by endocytosis and amino acids with other molecules by active transportation (Birn and Christensen 2006; Tojo and Kinugasa 2012). Because, mitochondria are the main target for Cd inducing oxidative damage (Radosavljevici et al. 2012), the proximal convoluted tubule is the most affected part in the kidney as confirmed by our histopathological examination. In consistence with the previous studies, the decreased serum total protein and albumin indicates loss of these components in the urine as a result of Cd induced glomerular and tubular injury (Takeda et al. 2003; Birn and Christensen 2006).

5. CONCLUSION

When Cd and Px are given in combination, they exert much toxicities than their individual administration.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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