Role of electrolytes disturbances and Na^+-K^+-ATPase in cisplatin – induced renal toxicity and effects of ethanolic extract of *Cichorium intybus*

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Abstract: Cisplatin is known by its toxicity by disturbing electrolytes homeostasis. Thus we aimed to find out the role of herbal plant *Cichorium intybus* on Cisplatin – induced toxicity. 24 male Albino Wistar rats were randomly divided into 4 groups: Group I is termed as untreated control; Group II is Cisplatin control and received 3 mg/ kg b.w.; i.p.; Group III received *C. intybus* ethanolic extract at a dose of 500 mg/kg b.w. orally for 10 consecutive days and Group IV is Cisplatin + *C. intybus* pretreated group. *C. intybus* is given 30 minutes prior to Cisplatin. Cisplatin – induced electrolytes disturbances is indicated by increase Intra - erythrocyte sodium content, decreased plasma magnesium, calcium and Intra-erythrocyte Na^+-K^+-ATPase which implicates the renal toxicity. At a dose of 500 mg/kg b.w. of *C. Intybus* pretreatment showed partial counter action on the electrolytes imbalances and Na^+ -K^+ -ATPase activity.

Keywords: Cisplatin, electrolytes, *C. intybus* ethanolic extract.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum) therapy causes renal toxicity in a dose limiting manner (Shoaib *et al.,* 2005; Naziroglu *et al.,* 2004). A Cisplatin forms adduct with DNA which is responsible for toxic lesions in cancerous cells and non quiescent cells. However, proximal tubular cells are selectively damaged. It is suggested that programmed death cells, production of reactive oxygen species, inflammation (Lee *et al.,* 2011) and the disturbances of intracellular calcium homeostasis (Qinhong *et al.,* 2001) and vascular resistance (Pani *et al.,* 2011) are characteristic of Cisplatin toxicity (Lee *et al.,* 2011). The excretory route of Cisplatin is through Kidney therefore it is more susceptible to Cisplatin toxicity (Lee *et al.,* 2011). Cisplatin uptake in proximal tubular cells through Passive diffusion (Kroning *et al.,* 2000) and OCT2 (organic cation transporter). Ciarimboli *et al.* found that transport through these membrane protein is poly specific, electrogenic, voltage-dependent, bi-directional, pH-independent and Na-independent. Production of ROS (reactive oxygen species) production NADPH oxidase causes by increased level of intracellular calcium level which damaged mitochondria (Kawai *et al.,* 2006), cell membrane and DNA which causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations results cancer, degenerative and other diseases. S3 segment of medulla shows pathological changes (Vela *et al.,* 2011). Cisplatin causes membrane electrolytes disturbances include hypomagnesaemia, hypo-calcaemia, hypophosphataemia and hypokalaemia or hyperkalaemia (Katrin *et al.,* 2006).

Cichorium intybus Linn is a herbal medicinal plant. It has been a tonic effect upon liver and digestive tract and for ulcer as well (Varotto *et al.,* 2000). It is found to have a higher concentration of Calcium, sodium, potassium and Magnesium in plant (Sanderson *et al.,* 2002). In present study, we examined the alone and synergistic effect of Cichorium Intybus on plasma and intra-erythrocytes electrolytes and Na^+-K^+-ATPase activity, during the Cisplatin therapy in experimental rat model.

MATERIALS AND METHODS

Animals and diet

24 male Albino Wistar rats (200-260 g b.w.), purchased from the animal house of ICCBS (International center for Chemical and Biological Center Karachi, Pakistan) for the study. Animals were kept to the laboratory for one week before the start of experiment and caged in a quite temperature controlled room (23 ± 4°C). Drinking water and standard rat's diet are easily available for Rats. The experiments were followed in accordance with ethical guidelines of internationally accepted principles for Laboratory use and care in animal research (Health research extension Act of 1985)

Crude extraction of plant Cichorium intybus Linn

The herbal plant was collected from Northern area of Pakistan and identified by experts. By weight (10 kg) dried aerial parts of the plant was powdered, screened and soaked in 10 L ethanol for 1 week. The filtrate was separated and concentrated under vacuum using a rotary evaporator, gave a dark green semisolid extract (it's yield was 44.4%). Standardization of the plant extract was done by quantification of a gallic acid as a reference standard using HPLC.

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Study design

Twenty four rats were divied into four groups, six rats in each group and received the following treatment:

Group I: Control group remains untreated

Group II (Nephrotoxic): (+ve Cisplatin control) received Cisplatin intraperitoneally at a dose of (3 mg/kg body weight) for 5 alternate days

Group III: Antioxidant treated group and received C.Intybus orally (500 mg/kg body weight) for 10 consecutive days

Group IV: Synergistically treated group and received Cisplatin i.p. (3 mg/kg body weight) for 5 alternate days + *C. intybus* orally (50 mg/kg body weight) for 10 consecutive days. Extract was given thirty minutes prior to Cisplatin.

The rats were sacrificed after 48 hrs of last dose. The blood was collected in a lithium heparinized coated tubes, mixed gently, plasma separated by using centrifuge at a speed of 2000 rpm for 20 minutes. Plasma was stored at -70°C in disposable Eppendorff tubes.

The RBC's was washed and processed for analysis of electrolytes and ATPase.

Analytical method

Assessment of electrolytes homeostasis plasma separation for electrolytes estimation

Plasma Sodium and Potassium was estimated by flame photometry (Corning 410), Estimation of Plasma Calcium ion by Ion Selective Electrode Method Jenway (Ion Meter 3345).

Estimation of intra-erythrocyte sodium and potassium (Fortes and Starkey, 1977)

RBC's were washed three times at room temperature with $MgCl_2$ solution (112 M), centrifugation at 12000 rpm at 4°C for 5 minutes. Packed cell volume was estimated by microhaematocrit capillary tubes.

Lyses the cell suspension with 0.01 mL of saponin solution (20 % in $MgCl_2$ 112 mM) then determine the intra- erythrocytes sodium and potassium by taking 0.3 mL of lysate to 10 mL of lithium nitrate diluent (15 mM). Intensities of erythrocyte sodium and potassium was calculated as mM by using flamephotometery.

Erythrocyte membrane preparation

The washed RBC's then treated with 25 volumes of 0.011 M Tris-HCl buffer at pH = 7.4 at 4°C, 12000 rpm for 30 minutes 3 X. The membrane yield was ~4 mg protein/mL of Tris buffer. Biuret method was used to detect the concentration of protein (Savory *et al.,* 1968) and stored at -80°C until the analysis.

Erythrocyte Na^+-K^+-ATPase activity measurement (Denis et al., 1996)

Briefly a mixture consists of 92 mM Tris-HCl (pH = 7.4), 100 mM NaCl, 20 mM KCl, 5 mM $MgSO_4.H_2O$ and 1 mM EDTA were preincubated with membrane pellete (400 µg) for 10 minutes at 37°C. Na-K-ATPase activity was calculated by measuring the differences of the release of inorganic phosphate with and with out incubated for 10 minutes with 1Mm Ouabain (Na-K-ATPase inhibitor). Activity was corrected to a nanomolar concentration of inorganic phosphate released and calculated as nm/mg protein/hour.

Estimation of plasma magnesium (Hallry and Sky Peck, 1964)

Briefly, 0.2 mL of plasma was taken and mixed with 1.8 mL of TCA (5% w/v) to make protein free filtrate. Then take out 1 mL of filtrate and add 1.5 mL of titan yellow (0.05%) and 0.5 mL of NaOH (4 N). A blank was prepared by taking 1 mL of deionized water & treated similarly. The color intensity was measured against blank at 540 nm after 15 minutes incubation at room temperature on Schimadzu-spectrophotometer UV-120-01.

STATISTICAL ANALYSIS

Results are presented as mean ± SD. Statistical significance and difference from control and test values were evaluated by Student's t-test. P-values of $P<0.001$, $P<0.01$ and $P<0.05$ were considered significant. Significant difference between Control with Cisplatin, CIE and Cisplatin + CIE-pretreated rats by t-test represented by $^{**}P < 0.05$, $^{*}P < 0.01$, $^{***}P<0.001$. Significant difference between +ve Control Cisplatin with CIE and Cisplatin + CIE-pretreated rats by t-test represented by $^{+++}P<0.001$, $^{++}P < 0.05$, $^{+}P < 0.01$

RESULTS

Intra-erythrocytes sodium level in control, Cisplatin, Cichorium intybus extract (CIE) and CDDP +CIE

Intra-erythrocytes sodium levels were increased in Cisplatin treated rats ($P<0.05$). CIE treated rats showed decreased level when compared with cisplatin control ($P<0.01$) while no change were observed in CDDP+ CIE pretreated rats when compared with cisplatin control (fig. 1).

Intra-erythrocytes potassium level in control, Cisplatin, Cichorium intybus extract (CIE) and CDDP +CIE

Intra – erythrocytes potassium was decreased ($P<0.05$) when compared with control. CDDP + CIE pretreated rats showed slightly decreased potassium level ($P<0.05$) when compared with cisplatin control while no changes were observed in alone CIE treated rats.

Intra-erythrocytes Na^+-K^+-ATPase level in Control, Cisplatin, Cichorium Intybus extract (CIE) and CDDP +CIE

Cisplatin treated rats showed marked decrease level of Na^+-K^+-ATPase level when compared with control ($P<0.01$), while alone CIE treated rats slightly decreased Na^+-K^+-ATPase ($P<0.01$). No significant changes were observed when compared with cisplatin control.

Plasma Mg^{++} level in control, Cisplatin, Cichorium intybus extract (CIE) and CDDP +CIE

Plasma Mg^{++} level was decreased when compared with control ($P<0.01$). CIE treated rats showed decrease Mg^{++} level when compared with cisplatin control ($P<0.05$) while no changes were observed in CDDP + CIE when compared with cisplatin control (fig. 4).

Plasma Ca^{++} level in control, Cisplatin, Cichorium intybus extract (CIE) and CDDP +CIE

Fig. 5 showed decreased Ca^{++} level in cisplatin treated rats when compared with control ($P<0.05$), while no changes were observed in CDDP + CIE when compared with cisplatin control.

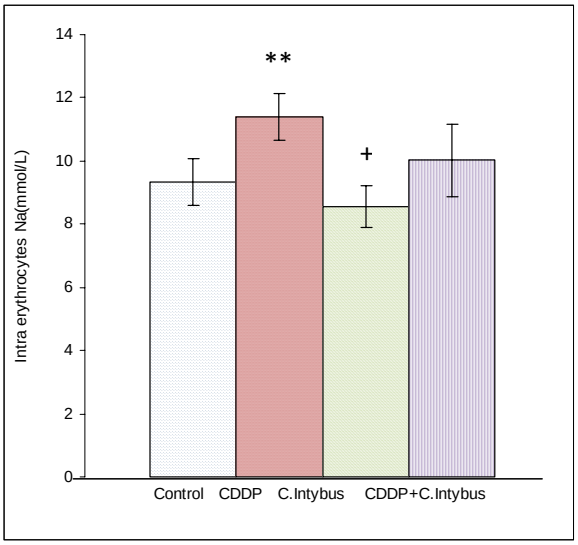

Fig. 1: Intra-erythrocytes Na^+ level in Control, Cisplatin, *C. intybus* and CDDP + *C. intybus* - pretreated rats.
**P<0.05 when compared with control, + P<0.01 when compared with cisplatin control

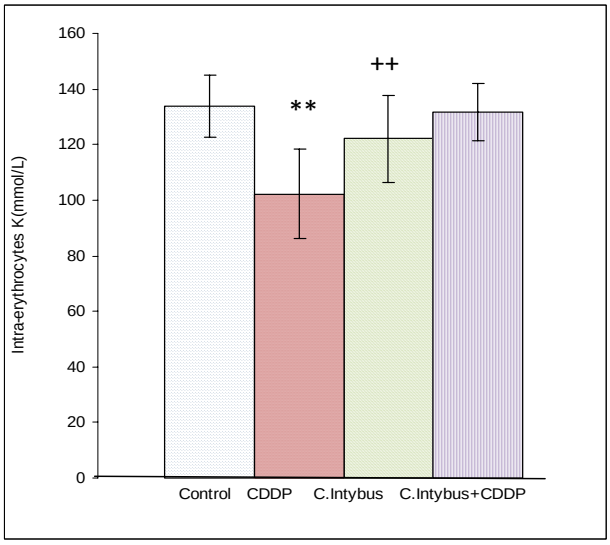

Fig. 2: Intra-erythrocytes K^+ level in Control, Cisplatin, *C. intybus* and CDDP + *C. intybus* - pretreated rats
**P<0.05 when compared with control, ++ P<0.05 when compared with cisplatin control

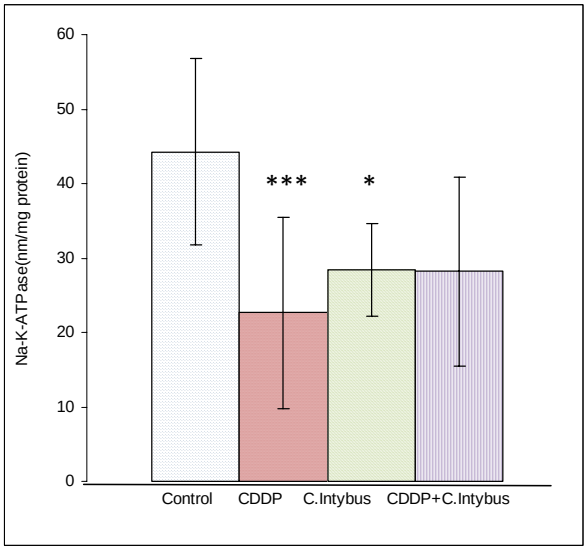

Fig. 3: Intra-erythrocytes Na^+-K^+-ATPase level in Control, Cisplatin, *C. intybus* and CDDP + *C. Intybus* - pretreated rats.
***P<0.001 when compared

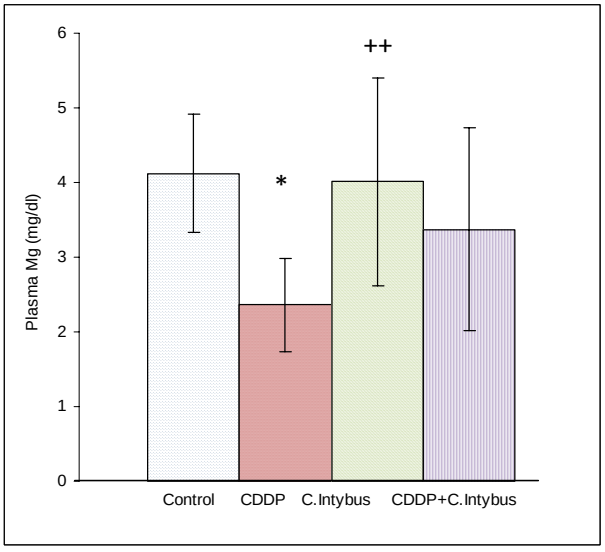

Fig. 4: Plasma Mg^{++} level in Control, Cisplatin, *C. intybus* and CDDP + *C. intybus* - pretreated rats.
*P<0.01 when compared with control, ++ P<0.05 when compared with cisplatin control

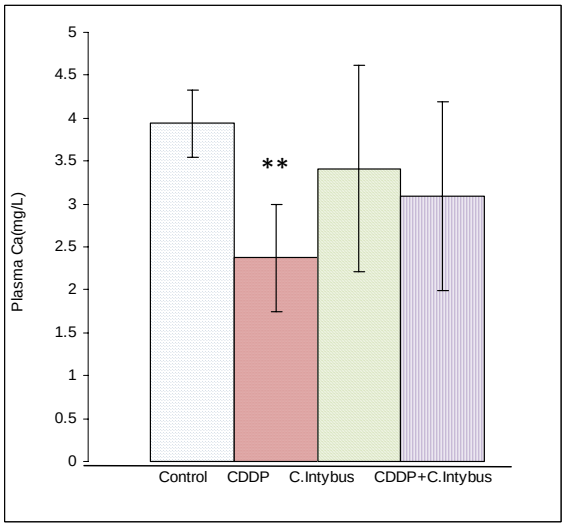

Fig. 5: Plasma Ca^{++} level in Control, Cisplatin, *C. intybus* and CDDP + *C. intybus* - pretreated rats.
**P<0.05 when compared with control.

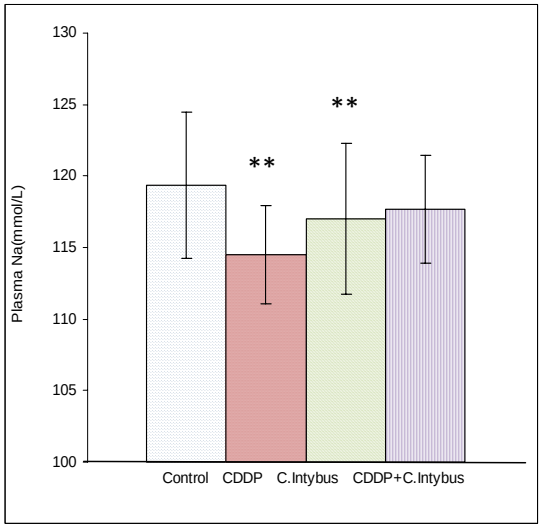

Fig. 6: Plasma Na^+ level in Control, Cisplatin, *C. intybus* and CDDP + *C. intybus* - pretreated rats.
**P<0.05 when compared with control.

Plasma Na^+ level in control, Cisplatin, Cichorium intybus extract (CIE) and CDDP +CIE

Slightly decreased plasma Na^+ level was observed in cisplatin treated rats and CIE treated rats when compared with control (P<0.05) while no changes were observed in CDDP+CIE pretreated rats when compared with cisplatin control (fig. 6).

Plasma K^+ level in control, Cisplatin, Cichorium intybus extract (CIE) and CDDP +CIE

Fig. 7 showed increased plasma K^+ level when compared with control in cisplatin treated rats (P<0.01). CIE treated rats showed decreased K^+ level when compared with cisplatin control (P<0.05) while no changes were observed in CDDP+CIE pretreated rats when compared with cisplatin control.

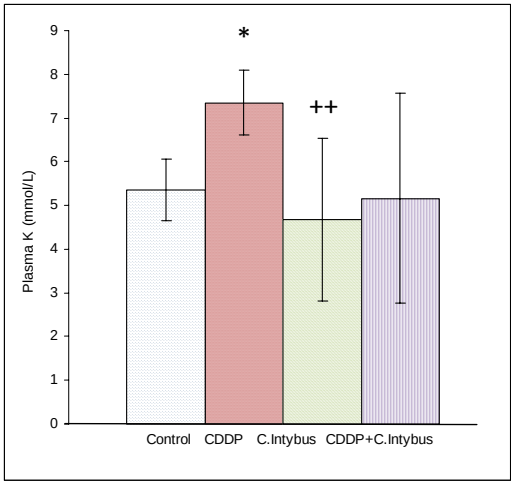

Fig. 7: Plasma K^+ level in Control, Cisplatin, *C. intybus* and CDDP + *C. intybus* - pretreated rats.
*P<0.01 when compared with control, ++ P<0.05 when compared with cisplatin control

DISCUSSION

The nephrotoxic cisplatin treated group showed increased intra-erythrocytes sodium (P<0.05; fig. 1), decreased plasma calcium (P<0.05), magnesium (P<0.01) (figs. 4 and 5) and intra-erythrocytes potassium (P<0.05) and Na^+-K^+-ATPase (P<0.001) (figs. 2 and 3) may involve in renal cell toxicity. Similarly decreased plasma potassium (P<0.01) (fig. 7) and increased sodium (P<0.05) (fig. 6) was also observed.

Hypocalcaemia is caused by excessive urinary loss and decreased renal up-take during high dose Cisplatin treatment. Proximal tubular damage leads to decreased reabsorption of cations. Acute nephrotoxicity presents with increased creatinine and persistent protein and electrolyte losses. Chronic nephrotoxicity is characterized by a decrease of glomerular filtration rate and a slightly elevated but persistent magnesium, potassium and calcium excretion (Cornelison and Reed, 1993). Presently we found the decreased calcium and magnesium level in nephrotoxic Cisplatin group which is responsible for decreased Na^+-K^+-ATPase level. Guarino *et al.*, 1979 have implicated this inactivation of ATPase as responsible for the kidney toxicity so prominent after Cisplatin treatment.

It is found that Mg act as a substrate for ATPase and it's deficiency is responsible for ATPase decreased level and shows reduced activity (Lajer *et al.,* 2005). The present study shows an association of decreased Magnesium and Calcium level with Na^+-K^+-ATPase, which may be the cause of renal toxicity as shown in fig. 8.

The decreased activity of ATPases consequently effect the membrane permeability and gradients specially

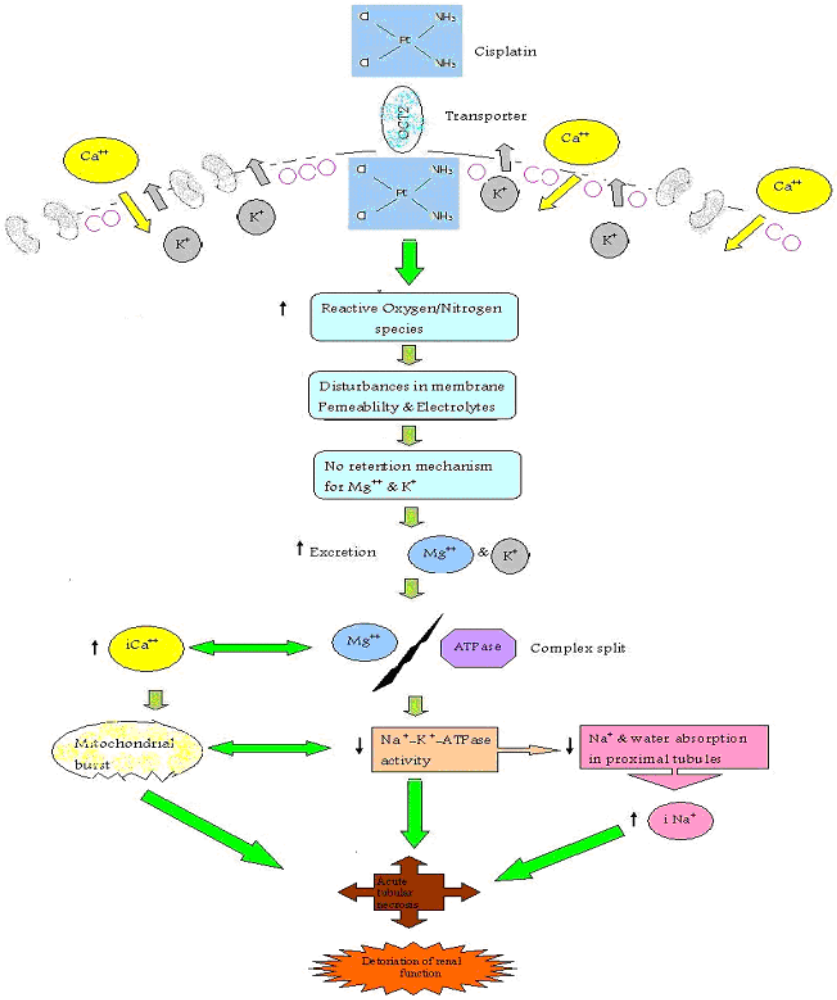

Fig. 8: Proposed mechanism of cisplatin induced nephrotoxicity and electrolytes disturbances.

potassium gradient thus decreased cellular K^+ content (Della *et al.,* 2011). Toxicity induced by Cisplatin treatment is due to the disturbances in the electrolytes homeostasis which finally reduced the activity of Na^+-K^+-ATPase activity and leading to cell death (Stakisaitis *et al.,* 2010; Ciarimboli, 2011). The study suggests that electrolytes imbalance particularly increase Intra-erythrocyte sodium content, decreased plasma magnesium, calcium and Intra-erythrocyte Na^+-K^+-ATPase may implicates the nephrotoxicity.

Our results previously reported the protective role of Cichorium Intybus in Cisplatin induced nephrotoxicity. It is found that increased ROS (reactive oxygen species) formation implicates the membrane electrolytes disturbance (Shafaq and Tabassum, 2009).

Treatment with polyphenolic extract of *C. Intybus* at a dose of 500 mg/kg b.w. partially protected the ion homeostasis altered by Cisplatin administration. Increased doses and time duration of herbal polyphenolic extract could function to reverse the toxic effect.

REFERENCES

Ciarimboli G (2011). Role of organic cation transporters in drug-induced toxicity. *Drug Metab Toxicol.,* **7**(2): 159-174.

Ciarimboli G, Ludwig T, Lang D, Pavenstädt H, Koepsell H, Piechota HJ, Haier J, Jaehde U, Zisowsky J and Schlatter E (2005). Cisplatin nephrotoxicityis critically mediated via the human organic cation transporter 2. *Am. J. Pathol.,* **167**: 1477-1484.

Cornelison TL and Reed E. (1993). Nephrotoxicity and hydration management for Cisplatin, carboplatin, and ormaplatin. *Gynecol. Oncol.,* **50**: 147-158.

Della Coletta Francescato H, Cunha FQ, Costa RS, Barbosa Júnior F, Boim MA, Arnoni CP, da Silva CG and Coimbra TM (2011). Inhibition of hydrogen sulphide formation reduces cisplatin-induced renal damage. *Nephrol. Dial. Transplant.,* **26**(2): 479-488.

Denis R, Cloudie A, Azulay JP and Vague P (1996). Erythrocyte Na^+-K^+-ATPase activity, metabolic control and neuropathy in IDDM patients. *Diabetes Care,* **19**(6): 564-568.

Fortes KD and Starkey BJ (1977). Simpler flame photometric determination of erythrocyte sodium and potassium. The reference range for apparently healthy adults. *Clin. Chem.,* **23**: 257-258.

Guarino AM, Miller DS, Arnold ST, Pritchard JB, Davis RD, Urbanek MA, Miller TJ and Litterst CL (1979). Platinate toxicity: Past, present, and prospects. *Cancer Treat. Rep.,* **63**: 1475.

Hallry H and Sky Peck HH (1967). Method for the estimation of serum magnesium. *Clin. Chem*., **10**: 391.

Katrin Hoffmann, Angela Marten, Katja Lindel, Fritz S, Jager D, Buchler MW and Schmidt J (2006). Major combined electrolyte deficiency during therapy with low-dose Cisplatin, 5-Fluorouracil and interferon alpha: report on several cases and review of the literature. *BMC Cancer,* **6**: 128.

Kawai Y, Nakao T, Kunimura N, Kohda Y and Gemba M (2006). Relationship of intracellular calcium and oxygen radicals to Cisplatin-related renal cell injury. *J. Pharmacol. Sci.,* **100**: 65-72.

Kroning R, Lichtenstein AK and Nagami GT (2000). Sulfur-containing amino acids decrease Cisplatin cytotoxicity and uptake in renal tubule epithelial cell lines. *Can. Chemother. Pharmacol.,* **45**: 43-49.

Lajer H, Kristensen M, Hansen HH, Nielsen S, Frokiaer J, Ostergaard LF, Christensen S, Daugaard G and Jonassen TE (2005). Magnesium depletion enhances Cisplatin-induced nephrotoxicity. *Cancer Chemother. Pharmacol.,* **56**: 535-542.

Lee JW, Nam WJ, Han MJ, Shin JH, Kim JG, Kim SH, Kim HR and Oh DJ (2011). Role of IL-1α in cisplatin-induced acute renal failure in mice. *Korean J. Intern. Med.,* **26**(2): 187-194.

Naziroglu M, Karaoglu A and Aksoy AO (2004). Selenium and high dose Vitamin E administration protects Cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicology*, **195**: 221-230.

Pani SR, Mishra S, Sahoo S and Panda PK (2011). Nephroprotective effect of Bauhinia variegata (Linn.) whole stem extract against cisplatin-induced nephropathy in rats. *Indian J. Pharmacol.,* **43**(2): 200-202.

Qihong Huang, Robert T. Dunn, II, Supriya Jayadev, DiSorbo O, Pack FD, Farr SB, Stoll RE and Blanchard KT (2001). Assessment of Cisplatin-induced nephrotoxi-city by Microarry Technology. *Toxicol. Sci.,* **63**: 196-207.

Sanderson MA, Labreveux M and Hall MH (2002). Forage yield and persistence of chicory and plantain. *Crop. Sci.,* **43**: 995-1000.

Savory J, Pu P H and Sunderman FW (1968). A Biuret method for determination of protein. *Clin. Chem.,* **14**: 1168-1171.

Shafaq Noori and Tabassum Mahboob (2009). Ameliorative effect of ethanolic extract of *Cichorium Intybus* on Cisplatin-induced nephrotoxicity in rats. *Pak. J. Sci. Ind. Res.,* **52**(4): 208-216.

Shoaib M, Zehra T, Saeed S and Khan AN (2005). Assessing tubular damage due to Cisplatin Nephrotoxicity, using Tc-99m MAG3. *World J. Nuc. Med.,* **4**: 99-103.

Stakisaitis D, Dudeniene G, Jankunas RJ, Grazeliene G, Didziapetriene J and Pundziene B (2010). Cisplatin increases urinary sodium excretion in rats: Gender-related differences. *Medicina* (Kaunas), **46**(1): 45-50.

Varotto S, Lucchin M and Parrin P (2000). Immature embryos culture in Italian red Chicory (Cichorium intybusc). *Plant Cell Tiss. Org. Cult.,* **62**: 75-77.

Vela L, Contel M, Palomera L, Azaceta G and Marzo I (2011). Iminophosphorane-organogold(III) complexes induce cell death through mitochondrial ROS production. *J. Inorg. Biochem.,* **105**(10): 1306-1313.