



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

International Journal of Recent Scientific Research
Vol. 5, Issue, 7, pp.1293-1297, July, 2014

**International Journal
of Recent Scientific
Research**

RESEARCH ARTICLE

INVESTIGATION OF RENOTOXIC EFFECTS OF CISPLATIN IN ALTERING DOSES ON EXPERIMENTAL ANIMALS

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ARTICLE INFO

Article History:

Received 11th, June, 2014

Received in revised form 21st, June, 2014

Accepted 11th, July, 2014

Published online 28th, July, 2014

Key word:

Cisplatin, Blood urea nitrogen, Urea, Lipid peroxidation, Glutathione, Catalase.

ABSTRACT

Cisplatin is the potent antineoplastic drug with significant nephrotoxic effects. The present study is to evaluate the nephrotoxic effects on varying doses of cisplatin such as 5mg/kg, 6mg/kg and 7mg/kg doses on male albino rats of Wistar strain. Various parameters determining nephrotoxicity such as blood urea nitrogen (BUN), urea, kidney weight and total body weight were assessed along with the *in vivo* antioxidant parameters like lipid peroxidation, reduced glutathione and catalase. Based on the histopathological evaluation this study has significantly shown the severity in the damage of the glomerular, tubular and epithelial cells on varying doses of cisplatin dose dependently.

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INTRODUCTION

Nephrotoxicity is one of the most common kidney problems which are mostly caused due to exposure to environmental or industrial chemicals and drugs. The chemicals like sodium oxalate, ethylene glycol, carbon tetrachloride, heavy metals such as mercury, lead, arsenic and cadmium and chemotherapeutic agents such as non steroidal anti-inflammatory drugs and aminoglycoside antibiotics induces nephrotoxicity. Kidney has high blood supply and the presence of cellular transport systems causes accumulation of these compounds within the nephron epithelial cells which lead to acute renal failure, chronic interstitial nephritis and nephritic syndrome. Kidney disease is one of the common causes for hospitalization (Arunachalam *et al.*, 2013; Ramya pydi *et al.*, 2011; Sreedevi *et al.*, 2010).

Cisplatin is a platinum compound and one of the most potent antineoplastic agents used for the treatment of solid tumours such as neck, lung, testicular, ovarian and cervical cancers. The cytotoxic mechanism involves the interaction with DNA by forming the covalent adducts between certain DNA bases and the platinum compound (Sudhavani *et al.*, 2010; Srinivasan *et al.*, 2011). Besides its clinical applications, cisplatin is associated with several dose dependent toxicities which include nephrotoxicity, hepatotoxicity, neurotoxicity (Mustafa *et al.*, 2013) and cardiotoxicity, but the prevalence of nephrotoxicity is most prominent. The general toxicity mechanism includes oxidative stress by the generation of reactive oxygen species such as hydrogen peroxide, superoxide anion and hydroxyl radicals which are generated under normal

cellular conditions and are immediately detoxified by the endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and non enzymatic compounds as reduced glutathione (GSH). But the excessive accumulation caused by cisplatin causes an imbalance in antioxidants due to loss of copper and zinc in kidneys in case of depleted SOD and leads to peroxidation of lipids and depletion of glutathione (Ammar *et al.*, 2013; Ahmed *et al.*, 2012; Srinivasan *et al.*, 2011). The synthetic and free radical scavengers such as glycine and selenomethionine show partial protection against cisplatin damage (Annie *et al.*, 2003).

Many approaches have been made since many years to alleviate these side effects. One strategy is to synthesize the novel cisplatin analogues that have lesser side effects in normal tissues which led to the synthesis of Carboplatin with less severe side effects. Another approach is made to well hydrate the patients with normal saline and mannitol. Sometimes reduction in the dose of cisplatin or discontinuation of the treatment. Despite these efforts, the side effects of cisplatin limit its use and efficacy in cancer therapy (El-sayed *et al.*, 2011; Sahar *et al.*, 2010; Xin Yao *et al.*, 2007).

According to Wasim *et al.*, 2010, the elevated levels of blood urea nitrogen and serum creatinine are the indicators of nephrotoxicity along with the acute tubular injury observed in the histopathological studies when compared with the normal histopathological studies. The main aim of the present study is to evaluate the changes in the levels of various parameters involved in nephrotoxicity on varying doses of cisplatin administration i.e 5mg, 6mg and 7mg/kg body weight of the animal.

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MATERIALS AND METHODS

Chemicals & Drugs

Cisplatin injection available as Cytoplatin 50mg/50ml manufactured by Cipla is used. The biochemical kits used are obtained from coral and Span. All the chemicals used were of analytical grade.

Animals & Experimental design

Male albino rats of Wistar strain weighing 200-250g are purchased from Teena labs, Hyderabad. The animals are subjected to acclimatization for one week provided with food and water *ad libitum*. The animals were maintained at a controlled temperature under 12hrs dark/light cycle in the animal house at CMR College of pharmacy, Hyderabad approved by CPCSEA.

The experimental animals used in the study were divided into four groups containing six in each. The first group is the control group which is administered with normal saline. The second, third and fourth groups are treated with 5mg/kg, 6mg/kg and 7mg/kg cisplatin (*i.p* route) respectively. The drug cisplatin is administered on day one for the experimental animals. On fifth day the blood was withdrawn by retro-orbital puncture under light ether anaesthesia and the animals were sacrificed by spinal dislocation (Salma *et al.*, 2011). The body weights of all the animals were noted on first day before the administration of cisplatin and on fifth day before scarifying the animals (Kundan *et al.*, 2013).

Biochemical estimations

The serum obtained from the blood was used for the evaluation of biochemical parameters like blood urea nitrogen (BUN) and serum creatinine. Kidneys were rapidly excised, trimmed of connective tissue and the wet weights are noted. The trimmed kidneys were subjected to washing with ice-cold normal saline to make free from blood and were used for the preparation of post mitochondrial supernatant for *in vivo* antioxidant studies and histopathological studies.

In vivo antioxidant studies

Kidney homogenate is chilled in phosphate buffer pH 7.4 using a tissue homogenizer. The post mitochondrial supernatant (PMS) collected after centrifuging at 10000 rpm for 20 mins was used for *in vivo* antioxidant parameters like lipid peroxidation (Niehaus *et al.*, 1968), Catalase (Hugo, 1984) and reduced glutathione (Jollow *et al.*, 1974).

Estimation of Lipid Peroxidation (LPO)

The procedure was followed according to Niehaus *et al.*, 1968. The PMS of volume 0.5ml was allowed to react with 0.5ml tris hydrogen chloride buffer and incubated at 37°C for 2hrs followed by addition of 1ml ice cold trichloroacetic acid and centrifuged at 1000rpm for 10 mins and 1ml supernatant was added to thiobarbituric acid and boiled for 10 mins. After cooling 1ml distilled water is added and the absorbance was measured at 532 nm against the blank without tissue homogenate.

Estimation of Reduced Glutathione (GSH)

According to Jollow *et al.*, 1974, the PMS of volume 0.75ml is mixed with 0.75ml of 4% sulfosalicylic acid and centrifuged. 0.5ml of supernatant was added with 4.5ml of 0.01M DTNB

and the absorbance was measured at 412 nm against the blank without tissue homogenate.

Estimation of Catalase (CAT)

The tissue homogenate in volume 0.4 ml was diluted 20 times with phosphate buffer pH 7.0 and for 2ml of the diluted homogenate 1ml of hydrogen peroxide was added and the absorbance was measured at 240 nm against the blank without tissue homogenate (Hugo, 1984).

Statistical analysis

The statistical analysis was carried out using one way ANOVA and unpaired student's 't' test and the values were expressed as mean \pm SEM. Values are considered statistically significant at $P < 0.001$

RESULTS

Histopathological Studies

Pieces of kidney from each group were fixed immediately in 10% formalin solution for at least 24hrs then dehydrated in alcohol (50-100%), embedded in paraffin, cut into 4-5 μ m thick sections and stained with hematoxylin-eosin. The sections obtained are evaluated for the pathological signs of nephrotoxicity which includes necrosis, fatty infiltration, lymphocyte infiltration and fibrosis (Abubaker *et al.*, 2012).

Thin Sections of kidney

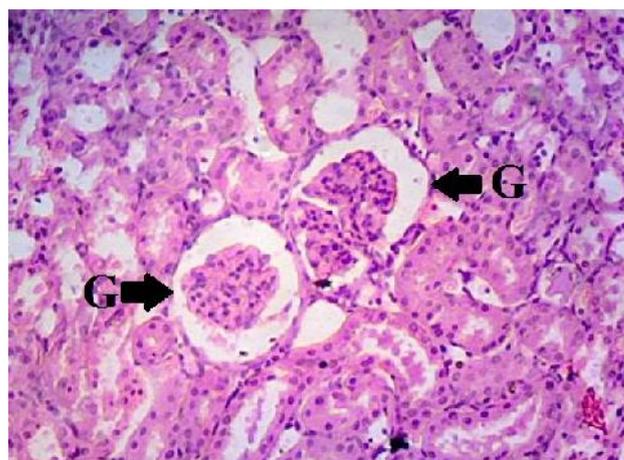


Fig 1 A photomicrograph of normal rat kidney showing normal organization of glomerular (G) and tubular epithelial cells.

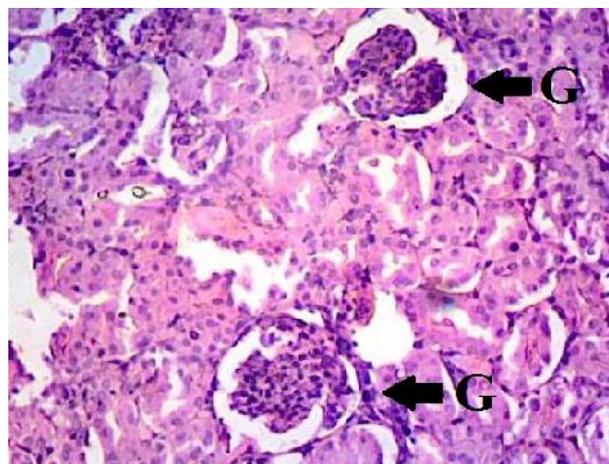


Fig 2 A photomicrograph of rat kidney treated with Cisplatin 5mg/kg showing glomerular congestion(G) but not significant

Table 1 Effect of Cisplatin treatment in varying doses on body weight, BUN and serum creatinine

Group	Treatment group	Changes in kidney weight (g)	Changes in body weight (%)	BUN(mg%)	Serum Creatinine (mg%)
1	Normal saline	0.43 ± 0.05	10.9 ± 3.55	18.5 ± 2.72*	0.7 ± 0.27*
2	Cisplatin 5mg/kg	0.69 ± 0.06*	-11.7 ± 2.52*	31.8 ± 1.72*	2.5 ± 0.10*
3	Cisplatin 6mg/kg	0.70 ± 0.07*	-11.9 ± 2.10*	31.5 ± 2.45*	2.1 ± 0.16*
4	Cisplatin 7mg/kg	0.75 ± 0.07*	-13.1 ± 2.78*	33.5 ± 2.67*	2.9 ± 0.14*

Values were expressed as mean ± SEM (n=6 animals per group). *P<0.001 Vs Group 1

Table 2 Effect of Cisplatin treatment in varying doses of *In vivo* antioxidant studies

Group	Treatment group	LPO nmol/mg of tissue	GSH nmol/mg of tissue	CAT nmol/mg of tissue
1	Normal saline	0.26 ± 0.03	0.70 ± 0.02	0.83 ± 0.008
2	Cisplatin 5mg/kg	0.82 ± 0.02*	0.60 ± 0.03*	0.66 ± 0.01*
3	Cisplatin 6mg/kg	0.83 ± 0.01*	0.52 ± 0.01*	0.60 ± 0.01*
4	Cisplatin 7mg/kg	0.94 ± 0.01*	0.34 ± 0.01*	0.47 ± 0.01*

Values were expressed as mean ± SEM. *P<0.001 Vs Group 1

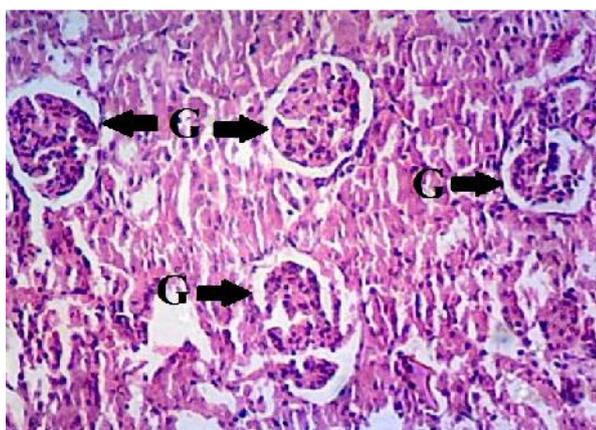


Fig 3 A photomicrograph of rat kidney treated with 6mg/kg cisplatin showing glomerular congestion, glomerular atrophy, disappearance of nuclei in the tubular cells and epithelial damage

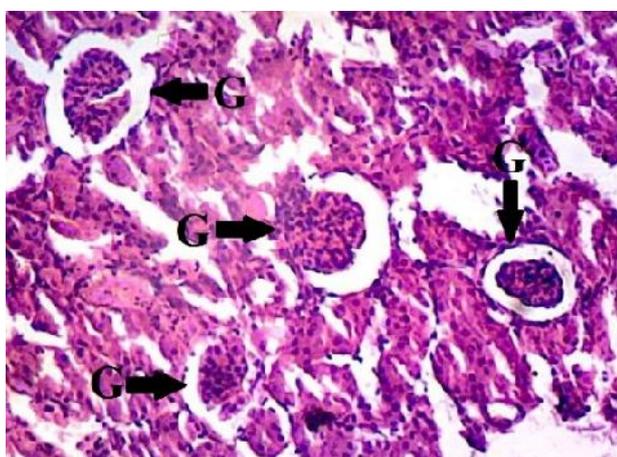


Fig 4 A photomicrograph of rat kidney treated with cisplatin 7mg/kg showing significant glomerular congestion (G), glomerular atrophy, disappearance of nuclei in the tubular cells and epithelial damage.

DISCUSSION

Kidney is an essential organ which has a prominent role in maintaining body homeostasis by excreting the metabolic waste products and conserving necessary products depending on the needs of the body. Renal failure is a common clinical syndrome of nephrotoxicity which causes a rapid decline in renal functioning due to decreased glomerular filtration rate (GFR) and the inability of the kidney to excrete the toxic metabolic substances produced in the body resulting in

abnormal retention of renal biomarkers i.e. serum creatinine and blood urea nitrogen (Arunachalam *et al.*, 2013).

Nephrotoxicity is a complex process which has been reported to involve DNA damage, caspase activation, mitochondrial dysfunction, formation of reactive oxygen and nitrogen species, polymerase overactivation, and inflammation leading to necrosis and apoptosis. (Dong *et al.*, 2007). Glomerular, tubular and interstitial cells frequently encounter with significant concentrations of medications and their metabolites, which can induce changes in kidney function and structure. The renal tubular cells such as proximal tubular cells of inner cortex and outer medulla which are the major sites for cisplatin induced renal damage absorb high amounts approximately five times of drug compared with extracellular concentration after its administration which promotes the generation of ROS (Hao pan *et al.*, 2009).

Cisplatin can induce nephrotoxicity after its single Intraperitoneal administration, which is a rapid process involves reaction with the proteins in the renal tubules which occurs mostly in the first hour of administration (Sreedevi *et al.*, 2010). Approximately, 25-35% of the patients treated with single dose (50-100mg/m²) of cisplatin show renal dysfunctioning associated with high mortality (Gholamreza *et al.*, 2010; Sahar *et al.*, 2010).

Cisplatin is stable in the blood stream but it is hydrolyzed in the chloride-poor cellular environment and the hydrolyzed metabolite binds DNA, RNA, proteins, and phospholipids, causing cytotoxicity (Miller *et al.*, 2010). Recent studies suggest that inflammatory mediators such as macrophages and leukocytes are released due to cisplatin induced damage. Tumour necrosis factor (TNF)- mediated apoptic pathway is reported to be one of the major parts in cisplatin induced damage (Sudhavani *et al.*, 2010; Srinivasan *et al.*, 2011).

The oxidative stress is associated with lipid peroxidation on cellular lipids, which is determined by measurement of thiobarbituric acid reacting substance. The concentration of LPO products reflects the degree of oxidative stress and the malondialdehyde(MDA) concentration is a measure for lipid peroxidation. The levels of the products of LPO was significantly increased in varying doses of cisplatin (Niehaus *et al.*, 1968). GSH is a major non-protein thiol which is the endogenous antioxidants that counter balances free radical mediated damage. It is involved in the protection of normal cell structure and function by maintaining homeostasis. In this

study the increasing dose of cisplatin increases the amount of free radicals produced which significantly decreases the levels of this endogenous antioxidant as it is involved in scavenging the reactive oxygen species (Jollow *et al.*, 1974). While CAT is a heme protein which is localized to microperoxisomes reduces hydrogen peroxide by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisome. The enzyme catalyses the decomposition of hydrogen peroxide to water and oxygen and thus protecting the cell from oxidative damage. The increase in the levels of the hydroxyl radicals counteract with CAT and cause its significant decrease according with the dose (Hugo, 1984).

In the present study, it is determined that the blood parameters related to nephrotoxicity include blood urea nitrogen and serum creatinine have shown the elevated levels in the cisplatin treated groups when compared with control group. According to the results illustrated the weight of the animal and the percentage change in body weight has significantly decreased on cisplatin treatment dose dependently on comparison with control rats. These findings have shown the nephrotoxic effect of cisplatin which has produced marked reduction of rat's body weight with a significant increase in relative kidney weight.

Generally, the animals increase in the body weight along the duration of time as in case of the group one. The loss of weight is closely related to the gastrointestinal toxicities and concomitant loss of animal appetite with subsequent reduction of food ingestion or due to excessive loss of water, salts and proteins possibly due to the tubular damage with subsequent dehydration and weight loss. The rise in weight of kidney might be due to the inflammatory mechanisms. The glomerular damage caused in cisplatin treated group might be due to ROS generation and that possibly elevate the serum creatinine and blood urea levels. The oxidative stress inhibits the antioxidant enzymes and generates the ROS that destroys the lipid, protein and DNA components of the cell with subsequent enzymatic inactivation and mitochondrial dysfunction which would be the major pathway of nephrotoxicity (Dilek *et al.*, 2013; Nasr, 2013).

Cisplatin is one of the commonly used drugs for inducing nephrotoxicity. It can be potentially used to evaluate the nephroprotective effect of various natural and synthetic compounds. The microscopical evaluation of the sections revealed the marked tubular degeneration, necrosis and desquamation of the tubular epithelial cells with cystic formation, interstitial cellular infiltration, wide capsular space and congested glomerular capillary tufts which was dose dependent. Cisplatin 7mg/kg dose could be the optimum dose for inducing nephrotoxicity in rats with less mortality. The higher doses can significantly show high mortality (Marcos *et al.*, 2009) and several experimental deviations.

CONCLUSION

From the study it can be concluded that increasing doses of cisplatin significantly decreases the levels of various *in vivo* antioxidants such as CAT & GSH and increase the levels of nephrotoxicity evaluating parameters like BUN and serum creatinine. Cisplatin can be extensively used for inducing nephrotoxicity and studying various nephroprotective compounds.

References

- Abubaker Siddiq, Shanmukha I, Rajendra S.V and Ramachandra Setty S, (2010). Protective effect of *Spathodea campanulata* bark against paracetamol-induced nephrotoxicity in rats. *Int J of PharmTech Research*, 4: 398-493,
- Ahmed MA and Sajida HI, (2012). Protective Effect of Ginger Extract Against Cisplatin-Induced Hepatotoxicity and Cardiotoxicity in Rats. *Iraqi J Pharm Sci*. 21(1): 27-33.
- Ammar AH and Sabah NA, (2013). Evaluation of the protective properties of amlodipine, on cisplatin induced cardiotoxicity in male rats. *Glo J of Med res Interdisciplinary*. 13(2): 23-28,
- Annie Shirwaikar, Malini S and Chandrika Kumara S, (2003). Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats, *Int J of Exp Bio*. 41: 58-62,
- Arunachalam Kumar, Suchetha Kumari N, Prima D'Souza and Divya Bhargavan (2013). Evaluation of renal protective activity of *adhatoda Zeylanica* (medic) leaves extract in wistar rats. *Nitte Univ J of Hea Sci*. 3(4): 45-56,
- Dilek Pandir and Ozlem Kara, (2013). Cisplatin-induced kidney damage and the protective effect of bilberry (*Vaccinium myrtillus* L.): an experimental study. *Turk J Med Sci*, 43: 951-956,
- Dong Z and Atherton SS, (2007). Tumor necrosis factor in cisplatin nephrotoxicity: A homebred foe. *Kid Int*, 72: 5-7,
- El-Sayed E. El-Awady, Yasser M. Moustafa, Dina M. Abo-Elmatty and Asmaa Radwan, (2011). Cisplatin-induced cardiotoxicity: Mechanisms and cardioprotective strategies. *Eur J of Pharmacol*, 650: 335-341,
- Gholamreza Karimi, Alireza Khoei, Abbas Omidi, Mahmudreza Kalantari, Javad Babaei, Elahe Taghiabadi and Bibi Marjan Razavi, (2012). Protective effect of aqueous and ethanolic extracts of *Portulaca Oleracea* against Cisplatin induced nephrotoxicity. *Ira J of Basic Med Sci*, 13(2): 31-35,
- Hao Pan, Partha mukhopadhyay, Mohanraj Rajesh, Vivek Patel, Bani Mukhopadhyay, Bin Gao, Gyrogy Hasko and Pal Pacher, (2009). Cannabidiol attenuates cisplatin induced nephrotoxicity by decreasing Oxidative/Nitrosative stress, Inflammation and cell death. *J of Pharmacol Exp Ther*, 328(3): 708-714,
- Hugo EB, (1984). Oxidoreductases acting on groups other than CHOH: catalase. In: Colowick SP, Kaplan NO, Packer L, editors. *Methods in Enzymology*, 105(7): 121-5,
- Jollow DJ, Mitchell JR, Zampaglione N and Gillette JR, (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11(3):151-169,
- Kundan G. Ingale, Prasad A. Thakurdesai, and Neeraj S. Vyawahare, (2013). Protective effect of *Hygrophila spinosa* against cisplatin induced nephrotoxicity in rats. *Ind J Pharmacol*. 45(3): 232-236,
- Marcos Rabelo de Freitas, Viviane Carvalho da Silva, Gerly Anne de CB, José Valdir de CJ, Raimundo Martins GJ, Ronaldo de AR, (2009). Distortion-product otoacoustic emissions and auditory brainstem responses sensitivity assessment in cisplatin-induced ototoxicity in rats. *Braz J Otorhinolaryngol*, 75(4):476-84,

- Miller RP, Tadagavadi RK, Ramesh G and Reeves WB, (2010). Mechanisms of Cisplatin nephrotoxicity. *Toxins (Basel)*, 2(11): 2490-2518,
- Mustafa Gulec, Elif Oral, Onur B. Dursun, Atakan Yucel, Ahmet Hacimuftuoglu, Fatih Akcay and Halis Suleyman, (2013). Mirtazapine protects against cisplatin-induced oxidative stress and DNA damage in the rat brain. *Psych and Clin Neurosci*, 67: 50–58,
- Nasr AY (2013). Effect of misoprostol on ultrastructural changes of renal tissues in cisplatin-treated adult rats. *J Cytol Histol* 4: 175
- Niehaus WG and Samuelson B, (1968). Formation of malondialdehyde from phospholipids arachidonate during microsomal lipid peroxidation. *Eur J Biochem* 6:126-130,
- Ramya Pydi, I. Rajalakshmi, S Indumathy and S Kavimani. (2011). Nephroprotective Medicinal Plants - A Review. *Int J Univ Pharm and Lif Sci*. 1(2): 266-281,
- Sahar Khalil, Abdel-Gawad and Abeer AK Mohamed. (2010) Silymarin administration protects against cisplatin-induced nephrotoxicity in adult male albino rats. *Egypt. J. Histol*. 33(4): 683 – 691,
- Salma Khanam, Nitha. P. Mohan, Kshama Devi and Rokeya Sultana, (2010). Protective role of *tinospira cordifolia* against cisplatin- induced nephrotoxicity. *Int J Pharm Pharm Sci*,3(4): 268-270,
- Sreedevi A, Bharathi K and Prasad K.V.S.R.G, (2011). Effect of decoction of root bark of *Berberis aristata* against Cisplatin-induced nephrotoxicity in rats. *Int J Pharm and Pharm Sci*, 2(3): 51-56,
- Srinivasan KK, Jessy Elizabeth M, Joseph K, Dinakaran Vachala S and Shivanna M, (2011). Effect of ethanol extract of *Graptophyllum pictum (L.) Griff.* on cisplatin induced nephrotoxicity in rats. *Herba polonica*, 57(2): 51-65,
- Sudhavani V, Chinnikrishnaiah V, Raghu moorthy V, Raghavendra HG and Ranganayakulu D, (2010). Nephroprotective activity of *Merremia emarginata* burm against cisplatin induced nephrotoxic rats. *Journal of advances in drug Research*, 1(1): 27-34,
- Wasim ahmad, N.A. Khan, Gufran ahmad and Shamshad ahmad, (2010). Effect of kaknaj (*Physalis alkekengi* Linn fruit) on gentamicin induced renal impairment in rats. *Hipp J of una med*, 5(3): 10,
- Xin Yao, Kessarini panichpisal, Neil kurtzman and Kenneth hugent, (2007). Cisplatin nephrotoxicity: A review, *Ame J of Med Sci*, 334(2):115-124,
