ORIGINAL ARTICLE

ANTIOXIDANT EFFECT OF CARNOSINE PRETREATMENT ON CISPLATIN- INDUCED RENAL OXIDATIVE STRESS IN RATS

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ABSTRACT

Cisplatin mediated nephrotoxicity is remarkably documented by reactive oxygen species. Carnosine is a naturally occurring dipeptide and has a scavenging property. The aim of present study was to assess the lipid peroxidation and antioxidant enzymes in association with oxidative stress in cisplatin –treated and 10 subsequent doses of carnosine- pretreated rats. 24 male Albino Wistar rats, were randomly divided into four groups (n=6). Group I remains untreated; Group II received Cisplatin (3 mg / kg) for 5 alternate days; Group III received Carnosine (10 mg / kg) for consecutive 10 days; Group IV received Carnosine (10 mg / kg) before administration of Cisplatin (3 mg / kg). The effects of carnosine on cisplatin-induced nephrotoxicity were evaluated by plasma creatinine, urea, malondialdehyde, nitrate; kidney tissue malondialdehyde, 4-HNE, superoxide dismutase and catalase activities. Cisplatin-induced oxidative stress was indicated by increased level of tissue MDA, 4-HNE and decreased level of tissue GSH, SOD and Catalase. Marked elevation of kidney weight and reduced body weight and pathological changes in kidney tissues were also observed in Cisplatin-treated rats. Carnosine reduced these pathological changes and counteracted the deleterious effects of cisplatin. The results divulge the beneficial effect of Carnosine pretreatment with cisplatin in experimental rat model.

KEY WORDS

Cisplatin, Carnosine, Lipid peroxidation, Antioxidant enzymes, Oxidative stress.

INTRODUCTION

In aerobic organisms, ROS (reactive oxygen species) including O_2^- (anion), H_2O_2 (hydrogen peroxide), hydroxyl radical ($\cdot OH$), and nitric oxide/ peroxynitrate ($NO/ONOO^-$) are continually generated in cells via various endogenous and exogenous sources such as mitochondrial electron transport chain, biotransformation of various xenobiotics and drugs, inflammation, UV and ionic irradiation and nitric oxide synthase. To detoxify ROS, mammalian cells have developed elaborate defense mechanisms. Oxidative stress occurs in the cells as a consequence of an imbalance between the pro-

oxidant/antioxidant systems. Oxidative stress causes damage to cellular macromolecules such as nucleic acids, proteins, and lipids are particularly more damaging because the formation of lipid peroxidation products lead to a facile propagation of free radical reactions. Abstraction of a hydrogen atom from the polyunsaturated fatty acid moiety of membrane phospholipids initiates the process of lipid peroxidation (1).

Cisplatin is a potent antitumor drug. Cisplatin-based combination chemotherapy regimens are currently used as front-line therapy in the treatment of testicular cancer, ovarian germ cell tumors, epithelial ovarian cancer, head and neck cancer, advanced cervical cancer, bladder cancer, mesothelioma, endometrial cancer, non-small cell lung cancer, malignant melanoma, carcinoids, penile cancer, adrenocortical carcinoma. The therapeutic effects of cisplatin are significantly improved by dose escalation. However, high-dose therapy with cisplatin is limited by its cumulative nephrotoxicity and neurotoxicity (2).

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It is well known that mitochondria continuously produce ROM (reactive oxygen metabolites) such as superoxide. Mitochondria also continuously scavenge ROM via the action of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase and glutathione S-transferase. Cisplatin is known to accumulate in mitochondria of renal epithelial cells (3). Several investigators have demonstrated that cisplatin induces ROS in renal epithelial cells primarily by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH (4, 5). A large number of studies have now accumulated documenting the beneficial effects of a variety of antioxidants in cisplatin-induced nephrotoxicity. Agents such as superoxide dismutase, dimethylthiourea, and GSH have been shown to reduce the degree of renal failure and tubular cell damage when administered simultaneously with cisplatin in rats (6).

Carnosine is a biological dipeptide predominating in long lived tissues such as skeletal muscle and brain. Carnosine was shown protective effect on diabetes (7), antioxidant effect (pre and post consecutive doses of carnosine) on a single i.p. cisplatin-induced nephrotoxicity in mice (8) and curative effect on hypercholesterolemia-induced kidney insult (9). Protective effects of carnosine supplement was also reported previously on chronic alcoholic and thioacetamide induced oxidative stress and hepatotoxicity (10). Due to multifunctional properties of carnosine, the present study was designed to manipulate the effect of carnosine as potential antioxidant agent on cisplatin-induced lipid peroxidation and renal oxidative stress.

MATERIALS AND METHODS

24 Wistar albino rats of male sex (200–260g) body weight, purchased from the animal house of ICCBS (International center for Chemical and Biological Sciences) Karachi, Pakistan for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room ($23 \pm 4^\circ\text{C}$). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines of internationally accepted principles for Laboratory use and care in animal research (Health research extension Act of 1985).

The animals were divided into four experimental groups (n=6). Each group received the following treatment- Group I: Control group remains untreated; Group II: Received Cisplatin i.p. (3mg/kg b.w.) for 5 alternate days; Group III: Received Carnosine i.p. (10mg / kg b.w.) for 10 days; Group IV: Received Carnosine (10 mg / kg b.w.) for ten days + Cisplatin i.p. (3mg/kg b.w.) for 5 alternate days.

After 48 hours of last dose of treated groups, animals were anesthetized, decapitated and blood was sampled from head wound in the lithium heparin coated tubes. A portion of blood was used to collect plasma. Kidneys were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer -70°C until analysis.

Left kidneys were quickly removed, immerse in 10 % formalin, dehydrated and embedded in paraffin, sectioned at $3\mu\text{m}$, stained with hematoxylin and eosin (H&E) and evaluated by light microscopy.

Plasma samples were assayed for Urea & Creatinine. Plasma Urea was estimated by Diacetyl Monoxime Method (11) and Creatinine by Modified Jeff's Method (12). Plasma sample was assayed for nitrate, MDA. Plasma Nitrate is estimated by ion selective electrode (ISE) method (Jenway 3345). Kidney homogenates were obtained using a tissue homogenizer Ultra Taurax T-25 Polytron at 4°C . The homogenates (1:10 w/v) were prepared by using a 100 mM KCl buffer (7:00 p H) containing EDTA 0.3 mM, All homogenates were centrifuged at 600 g for 60 minutes at 4°C and the supernatant was used for biochemical assays. 10 μl of BHT (butylated hydroxytoluene) (0.5 M in acetonitrile) was added to prevent homogenate from oxidation and the homogenate was stored at -70°C until analysis for MDA and 4-HNE. The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the method of Okhawa et al (13) and tissue 4-HNE content was measured by the method of Kinter et al (14). Catalase activity was assayed by the method of Sinha et al (15). Levels of SOD in the cell free supernatant were measured by the method of Kono et al (16) and GSH activity was determined by the procedure of Carlberg and Mannervik (17).

Results are presented as mean $\pm$ SE. Statistical significance and differences from control and test values were evaluated by Student's 't'-test. Statistical probability of $P < 0.01$, $P < 0.05$ were considered to be significant.

RESULTS

Table I and Figure I shows effects of cisplatin, Carnosine and pretreatment of Carnosine with cisplatin on kidney & body weight respectively. A marked decrease in rats' body weight was observed in cisplatin-treated rats and CDDP + Carnosine. Alone Carnosine showed increase in body weight. The kidneys of rats treated with cisplatin were enlarged. Cisplatin-treated rats showed significantly increased kidney weight ($P < 0.05$)

Table I: Effect on kidney weight & relative kidney weight in control, cisplatin treated, carnosine treated and cisplatin + carnosine- pretreated rats

Parameters	Control	Cisplatin (3mg/kg)	Carnosine (10mg/kg)	Carnosine+cisplatin
Mean kidney weight (g)	0.596±0.115	0.845 ±0.136**	0.567±0.048	0.673±0.063
Mean Relative Kidney weight10 ⁻³	2.648±0.669	3.416±0.142**	2.88±0.33	3.04±0.42

Values are mean±SE; Significant difference between control, cisplatin, c.intybus extract and c.intybus extract +cisplatin treated rats by t-test **P<0.05

and relative kidney weight but result was not significant as compared to control. Alone Carnosine showed significantly increased relative kidney weight ($P < 0.05$). After 5 alternate days cisplatin-treated rats showed glomeruli congestion, marked ATN in proximal and distal tubule, decrease in the height of epithelial cells, shedding of atypical cytoplasm and loss of brush boarder (Fig II B). Lumen wide opens and marked congestion in vessels (++++) were also seen. Pretreatment with carnosine markedly prevented congestion in glomeruli and vessels (+) and other alterations (Fig. II D). Alone carnosine had showed no alterations (Fig II C).

Cisplatin treated rats showed a markedly increased level of plasma urea (76.45 ± 7.41 , $P < 0.01$) as compared to control (Table II). Alone Carnosine showed slightly increased urea level but the results were not significant. Results were not different in CDDP+Carnosine group as compared to control showing correction of urea level. Table II shows a significant effect on plasma creatinine level in cisplatin and pretreatment with Carnosine. Creatinine level was significantly increased (11.85 ± 1.56 , $P < 0.01$) in cisplatin treated rats as compared to control. Pretreatment with Carnosine partially prevented increase in plasma creatinine level in CDDP+Carnosine group.

Body weight was measured in 5 alternate days

Fig I: Effect on body weight in control, cisplatin treated, carnosine treated and cisplatin + Carnosine- pretreated rats

Carnosine Alone group showed slightly increased creatinine level (5.45 ± 1.19 , $P < 0.01$) as compared to control.

Increased level of plasma MDA was observed in cisplatin-treated rats (19.26 ± 0.71 , $P < 0.05$). A significant decreased in plasma MDA was noted after Carnosine alone (7.28 ± 1.53 , $P < 0.01$). The MDA level was also decreased in CDDP+Carnosine pretreated rats but results were not significant (Table II). Table II shows oxidative status in rat kidney after cisplatin, Carnosine and cisplatin with Carnosine treatment. Tissue MDA level increased in cisplatin- treated rats but results were not significant. The increase was prevented by Carnosine in CDDP + Carnosine group (0.779 ± 0.30 , $P < 0.01$). Carnosine Alone treatment showed slight decreased MDA level (1.25 ± 0.17 , $P < 0.05$) as compared to control.

Fig II: Histology of rat kidney in Control, Cisplatin-treated, Carnosine-treated and Cisplatin + Carnosine-pretreated rats

Fig II A: Normal kidney histology from control rats; Fig II B: Represent histological abnormalities after 5 alternate days of cisplatin administration; Fig II C: Carnosine did not produce any histological alterations; Fig II D: Represent histological abnormalities and their prevention by carnosine

A significant increase in tissue 4-HNE was observed in cisplatin-treated rats (412.13 ± 69.09 , $P < 0.05$) as compared to control, showing oxidative stress (Table II). In

Table II: Effect on Renal function, Oxidative Status & Antioxidant Enzymes in Control, Cisplatin-treated, Carnosine-treated and Cisplatin + Carnosine pre-treated rats (n=6)

Parameters	Control	Cisplatin (3mg/kg)	Carnosine(10mg/kg)	Carnosine+Cisplatin
Plasma Urea (mg/dl)	39.52±2.53	76.45±7.41*	42.93±5.53	40.38±10.94
Plasma Creatinine (mg/dl)	1.87±0.52	11.85±1.50*	5.45±1.19*	5.81±5.02
Plasma MDA(nm/ml)	17.66±1.32	19.26±0.71**	7.28±1.53*	14.23±4.09
Tissue MDA (nm/g of tissue)	1.81±0.55	2.13±0.55	1.25±0.17**	0.77±0.30*
Plasma Nitrate (mg/L)	19.51±0.93	23.49±1.97*	17.24±3.21	20.31±5.77
Tissue 4-HNE (nm/ g of tissue)	298.85±19.6	412.13±69.09**	306.81±48.08	124.45±36.18*
Tissue Catalase (mmol/ g of tissue)	0.537±0.05	0.258±0.02*	0.467±0.17	0.379±0.14
Tissue SOD (U/ g of tissue)	28.76±3.05	19.83±2.67*	29.36±5.76	24.04±4.51
Tissue GSH (U/ g of tissue)	4.25±0.90	1.508±0.66*	2.63±0.38*	2.73±0.92

Values are mean ± SE; Significant difference between control, cisplatin, carnosine and carnosine +cisplatin treated rats by t-test **P<0.05, *P<0.01

CDDP+Carnosine group, 4-HNE level was decreased (124.45 ± 36.18 , $P < 0.01$) as compared to control group. Carnosine Alone treatment had showed similar result as that of control. Nitrate level in plasma was increased (23.49 ± 1.97 , $P < 0.01$) (Table II) in cisplatin treated rats, indicating nitrosative stress. CDDP+Carnosine group showed no change as compared to control. Carnosine Alone treatment showed no significant result. Tissue catalase level decreased significantly (0.25 ± 0.025 , $P < 0.01$) in cisplatin treated group as compared to control (Table II). Carnosine prevented decreased catalase level in CDDP+Carnosine group. While Carnosine alone showed no significant effect.

Table II shows a significant effect of cisplatin treated (19.83 ± 2.67 , $P < 0.01$) rats. Cisplatin treatment decreased the SOD level significantly. In CDDP+Carnosine group the SOD level was restored. Carnosine alone showed no significant effects. Table II shows a significant decreased in GSH level in cisplatin-treated rats (1.50 ± 0.66 , $P < 0.01$) as compared to control. A decrease was partially prevented by carnosine in CDDP+Carnosine group. Carnosine alone showed a slight decreased GSH level (2.63 ± 0.38 , $P < 0.01$) as compared to control group.

DISCUSSION

Using an experimental model of CDDP-induced nephrotoxicity in rats, we evaluated the renal activity of the key antioxidant enzymes (GSH, SOD, Catalase), the lipid peroxides (LPOs) MDA, 4-HNE content of tissue as an index of lipid peroxidation. In the present study, the rats treated with cisplatin showed a decrease in body weight (Fig I). Mora et al suggested that CDDP-induced weights loss might be due to gastrointestinal toxicity and by reduced ingestion of food. Our results

demonstrated that repeated treatment with CDDP (3mg/ kg) for 5 alternate days induced biochemical and histological signs of renal injury (Fig II) with significant reduction in the activity of Catalase, SOD and GSH (Table II).

The impairment of kidney function by cisplatin is recognised as the main side effect and the most important dose-limiting factor associated with its clinical use. Several investigators (18) reported that the alterations induced by cisplatin in the kidney functions were characterized by signs of injury such as increase of products of LPO (lipid peroxide) and changes in GSH levels in kidney tissue, creatinine and urea levels in plasma. The present study showed that administration of cisplatin to rats caused a reduction in glomerular filtration rate, which correlated with increased creatinine and urea in plasma (Table II). The alterations in glomerular function in cisplatin-treated rats may also be secondary to ROS (reactive oxygen species) (19) which induce mesangial cells contraction, altering the filtration surface area and modifying the ultrafiltration coefficient factors that decrease the glomerular filtration rate (10).

The impairment in kidney function was accompanied by an increased MDA contents in kidney tissue (3,19). Our results are also in accordance with previous studies as shown in (Table II). These biochemical parameters were well correlated with the renal histological results. Although the exact mechanism of CDDP-induced nephrotoxicity is not well understood, several investigators have shown that CDDP nephrotoxicity is associated with LPO in renal tissue. LPO is ascribed to a free radical-mediated chain reaction that damages cell membranes (20) and inhibition of this process by carnosine is mainly attributed to the ability of scavenger of free radicals. It uniquely present double antioxidant action,

scavenging ROS, preventing lipid peroxidation of cell membranes, and regaining those already oxidized (10). Carnosine Alone showed significantly decreased MDA level in plasma and tissue as compared to control. It is suggested that exogenously injected carnosine may be responsible for decreased MDA levels in carnosine alone –treated rats (Table II). It is known that carnosine is an endogenously synthesized dipeptide which is present in brain, cardiac muscle, kidney, stomach, olfactory bulbs and in large amounts in skeletal muscle (21). It is reported previously that 4-HNE, the major α,β unsaturated aldehyde formed by the degradation of both $\omega 3$ and $\omega 6$ poly unsaturated fatty acid during lipidperoxidation is fairly stable and present in relatively higher amounts in biological membranes under the conditions of oxidative stress (1).

Several studies demonstrated that CDDP-induced acute nephrotoxicity is mediated by depletion of renal GSH and by impaired activity of SOD and glutathione as well as an increase in renal lipid peroxidation (19, 22, 23, 24). The role of NO and RNS in cisplatin-induced nephrotoxicity has not been completely established. It has been shown that the renal content of nitrate/nitrite is increased in cisplatin-treated rats suggesting that NO is increased in these animals (25, 26). The increase in renal ONOO⁻ induced by cisplatin may be secondary to the increase in NO and O₂ production (Table II). In fact, there are evidences of the renal increase in NO production in cisplatin nephrotoxicity (27). The O₂^{•-} increase in cisplatin-nephrotoxicity may be simply consequence of the mitochondrial dysfunction (4) and the decrease in superoxide dismutase activity (28). One of the most important intracellular antioxidant systems is the glutathione redox cycle. GSH is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism. The depletion in the renal GSH level has been observed in rats in response to oxidative stress caused by CDDP treatment (29). It has been suggested that cisplatin is able to generate reactive oxygen species, and that is also inhibits the activity of antioxidant enzymes in renal tissue such as CAT, SOD, GSH-Px (30, 18). The antioxidant property of carnosine is important manifestation as it directly interacts with lipid peroxidation products (30). Taking into account the biochemical parameters and histological findings it is suggested that increased level of 4-HNE and MDA in cisplatin-treated rats are responsible for renal oxidative stress. Pretreatment of carnosine significantly reduced the lipid peroxides and restored endogenous antioxidant enzymes. Carnosine is naturally occurring dipeptide, subsequent doses may interfere with the normal biological functions. Dose adjustment should be monitored carefully to manipulate the

oxidant/antioxidant status. The present results and previous findings show that increased lipid peroxides implicate the renal oxidative stress. Moreover, combined therapy of carnosine with cisplatin successively attenuates the antioxidant system.

REFERENCES

1. Yusong Y, Rajendra S, Abha S, Sanjay A, Yogesh CA. Lipid peroxidation and cell cycle signaling : 4-hydroxynonenal, a key molecule in stress mediated signaling. *Acta Biochimica Polonica* 2003; 50: 319-36.
2. Langerak AD, Dreisbach LP. *Chemotherapy Regimens and Cancer Care*. Landes Bioscience; Georgetown, Texas, 2001.
3. Santos NA, Bezerra CS, Martins NM, Curti C, Bianchi ML, Santos AC. Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. *Cancer Chemother Pharmacol* 2008; 61(1): 145-55.
5. Huang Q, Dunn RT, Jayadev S, DiSorbo O, Pack FD, Farr SB, Stoll RE, Blanchard KT. Assessment of cisplatin-induced nephrotoxicity by microarray technology. *Toxicol Sci* 2001; 63: 196-207.
6. Tsuji T, Kato A, Yasuda H, Miyaji T, Luo J, Sakao Y, Ito H, Fujigaki Y, Hishida A. The dimethylthiourea-induced attenuation of cisplatin nephrotoxicity is associated with the augmented induction of heat shock proteins. *Toxicol Appl Pharmacol* 2009; 234(2): 202-8.
7. Janssen B, Hohenadel D, Brinkkoetter P, Peters V, Rind N, Fischer C, et al. Carnosine as a protective factor in diabetic nephropathy. *Diabetes* 2005; 54: 2320-27.
8. Soliman K, Al Ansary A, Aly HF, Mahmoud S. Effects of carnosine on liver enzymes of infected hamsters. *Egypt J Schistosomiasis Infect Endem Dis* 2004; 26: 1-18.
9. Liu WH, Liu TC, Yin MC. Beneficial effects of histidine and carnosine on ethanol-induced chronic liver injury. *Food Chem Toxicol* 2008; 46(5): 1503-9.
10. Aydogan S, Yapislar H, Artis S, Aydogan B. Impaired erythrocytes deformability in H(2)O(2)-induced oxidative stress: protective effect of L-carnosine. *Clin Hemorheol Microcirc* 2008; 39(1-4): 93-8.
11. Butler AR, Hussain I, Leitch E. The chemistry of the diacetyl monoxime assay of urea in biological fluids. *Clin Chim Acta* 1981; 112 : 357-60.
12. Spierto FW, Macneil ML, Burtis CA. The effect of temperature and wavelength on the measurement of creatinine with the Jaffe's procedure. *Clin Biochem* 1979; 12: 18-21.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-8.
14. Kinter M In, Punchard NA, Kelly GJ Eds. *Free Radicals-A Practical Approach*, Oxford University Press, Oxford 1996; 136.
15. Sinha KA . Colorimetric assay of catalase. *Anal Biochem* 1972; 47: 389-94.
16. Kono Y. Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys* 1978; 186: 189-95.
17. Carlberg I, Mannervik B. Glutathione reductase. *Methods. Enzymol* 1985; 113: 484-90.
18. Naziroglu M, Karaoglu A, Aksoy AO. Selenium and high dose Vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats. *Toxicol* 2004; 195: 221-30.

19. Somani SM, Husain K, Whitworth C, Trammel GL, Malafa M, Rybak LP. Dose-dependent protection by lipoic acid against cisplatin-induced nephrotoxicity in rats: antioxidant defense system. *Pharmacol Toxicol* 2000; 86(5): 234-41.
20. Velmurugan B, Santhiya ST, Nagini S. Protective effect of S-allylcysteine and lycopene in combination against N-methyl-N-nitro-N-nitrosoguanidine-induced genotoxicity. *Pol J Pharmacol* 2004; 56: 241-5.
21. Gulshanara B, Adam C, Michael L. Physiological role of carnosine in Contracting Muscle. *Int J Sports Mus Exer Metabol* 2005; 15: 493-514.
22. Saad SY, Al-Rikabi AC. Protection effects of taurine supplementation against cisplatin-induced nephrotoxicity in rats. *Chemotherapy* 2002; 48(1): 42-8.
23. Borrego A, Zamora ZB, Gonzalez R, Romay C, Menendez S, Hernandez F, et al. Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats. *Mediators Inflamm* 2004; 13(1): 13-9.
24. Gonzalez R, Borrego A, Zamora Z, Romay C, Hernandez F, Menendez S, et al. Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats. *Mediators Inflamm* 2004; 13(5-6): 307-12.
25. Yildirim Z, Sogut S, Odaci E, Iraz M, Ozyurt H, Kotuk M, Akyol O. Oral erdosteine administration attenuates cisplatin-induced renal tubular damage in rats. *Pharmacol Res* 2003; 47: 149-56.
26. Ozen S, Akyol O, Iraz M, Sogut S, Ozugurlu F, Ozyurt H, et al. Role of caffeic acid phenethyl ester, an active component of propolis, against cisplatin-induced nephrotoxicity in rats. *J Appl Toxicol* 2004; 24: 27-35.
27. Mora Lde O, Antunes LM, Francescato HD, Bianchi Mde L. The effects of oral glutamine on cisplatin-induced nephrotoxicity in rats. *Pharmacol Res* 2003; 47: 517-22.
28. Kadikoylu G, Bolaman Z, Demir S, Balkaya M, Akalin N, Enli Y. The effects of desferrioxamine on cisplatin-induced lipid peroxidation and the activities of antioxidant enzymes in rat kidneys. *Hum Exp Toxicol* 2004; 23: 29-34.
29. Silva CR, Gregg Antunes LM, M deP Bianchi. Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. *Pharmacol Res* 2001; 43: 561-6.
30. Martinis BS, M deP, Bianchi. Effect of Vitamin C supplementation against cisplatin-induced toxicity and oxidative DNA damage in rats. *Pharmacol Res* 2001; 44 (4): 317-20.