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Ameliorative effect of riboflavin on the cisplatin induced nephrotoxicity and hepatotoxicity under photoillumination

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ABSTRACT

Cisplatin is a widely used anticancer drug. It is documented that it elicits major side effects like nephrotoxicity and hepatotoxicity due to oxidative stress forcing the patients to limit its clinical use in long term treatment. Riboflavin (vitamin B₂) is a strong photosensitizer because it generates reactive oxygen species (ROS) upon photoillumination. We have tried to trap its photosensitizing property to ameliorate the cisplatin induced nephrotoxicity and hepatotoxicity in mice. They were treated with riboflavin and cisplatin separately as well as with their combination under photoilluminated condition. The status of major antioxidant enzymes, antioxidant proteins, functional markers, lipid peroxidation and protein oxidation was studied in liver, kidneys and serum samples of all the groups. Cisplatin treated group showed significantly compromised level of antioxidant enzymes and the proteins with higher extent of lipid and protein oxidation. Similar but less pronounced pattern was observed in the riboflavin treated group. The groups treated with the combination of cisplatin and riboflavin showed all the parameters tended towards normal levels in a dose dependent manner. Hence, it can be hypothesized that riboflavin shows ameliorative effect on the cisplatin induced nephrotoxicity and hepatotoxicity under the mentioned treatment conditions.

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1. Introduction

Cisplatin (cis-diamminedichloroplatinum II, CIS) is platinum coordinated complex based anticancer drug used against many human cancers including oral, lung, head and neck cancer, metastatic tumors of testis and ovaries, advanced bladder cancer and many other solid tumors (Gottfried et al., 2008; Turk et al., 2008). In spite of its effective anticancer behavior, it exerts many unwanted side effects including nephrotoxicity, hepatotoxicity, ototoxicity, emetogenesis myelosuppression and spermiotoxicity (Tarlacali-sir et al., 2008; Liao et al., 2008; Atessahin et al., 2006; Mc Keage, 1995). Hence, these major side effects limit the clinical use of the drug.

The anticancer property of CIS comes from its ability to bind to N-7 of purine bases of cellular DNA leading to formation of mono-adducts which are later transformed into inter- and intra-strand cross links by reaction of second reactive site of the drug with the second nucleobase (Hah et al., 2006; Fichtinger-Schepman et al., 1985). This is inhibitory to fundamental cellular processes

including replication, transcription, translation and DNA-repair in many cell types (Suo et al., 1999). Beside these, CIS generates oxidative and nitrosative stresses (Srivastava et al., 1996; Xiao et al., 2003) because of depletion or inhibition of antioxidant enzymes and proteins which results into nephrotoxicity and hepatotoxicity as major side effects of the drug (Iseri et al., 2007; Naziroglu et al., 2004).

Riboflavin (RF) or vitamin B₂ is chemically 6,7-dimethyl-9-D-1-ribityl isoalloxazine. It is a natural constituent of all forms of life, exists in the two co-enzymatic forms – flavinadenine dinucleotide (FAD) and flavinadenine mononucleotide (FMN) that are used in metabolic redox reaction by different enzyme-systems. It also acts as photosensitizer via radical species or via the generation of singlet oxygen. It reacts via its singlet and triplet excited states with molecular oxygen leading to generation of superoxide anion, riboflavin radical (Kumari et al., 1996) and generates hydroxyl radical in presence of transition metal ions (Yoshida et al., 2003). These species exert a collective damaging effect on several biologically important molecules (Jazzer and Naseem, 1994, 1996) and can also affect the drugs as well as the medicines (Cosa, 2004). Apart from being an essential vitamin, its photosensitizing property makes it an effective agent for photodynamic therapy in killing tumors (Edwards et al., 1994), inactivation of neurotoxin A (Eubanks et al., 2005), treatment of neonatal hyperbilirubinemia, blue nevi, pigmented skin lesions (Sato et al., 2000) and sterilization of blood

Abbreviations: BUN, blood urea nitrogen; CIS, cis-diamminedichloroplatinum II; ROS, reactive oxygen species; RF, riboflavin; –SH, sulfhydryl groups.

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products (Cui et al., 2008). The present study was designed to enlarge the therapeutic window of RF to regulate the nephrotoxic and hepatotoxic effects of CIS in mice as animal model system. It is a preliminary attempt to suggest alternative regime for the cancer patients undergoing CIS based chemotherapy based on our earlier in vitro observation.

2. Materials and methods

2.1. Chemicals

Riboflavin, cisplatin, reduced and oxidized glutathione, nicotinamide adenine dinucleotide phosphate reduced (NADPH), nicotinamide adenine dinucleotide reduced (NADH) were bought from Sigma–Aldrich Chemical Company, USA. Succinic acid, potassium dihydrogen and monohydrogen phosphate, glycine, pyrogallol, hydrogen peroxide, trichloroacetic acid (TCA) and ethylenediaminetetra-acetic acid (EDTA) were purchased from Qualigens Fine Chemicals, Mumbai, India. Folin's phenol reagent, bovine serum albumin (BSA), β -mercaptoethanol, sulphosalicylic acid were bought from Sisco Research Laboratory, Mumbai, India. *p*-Nitrophenyl phosphate (pNPP), 1-chloro-2,4-dinitrobenzene (CDNB) and thiobarbituric acid (TBA) were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Rest all other chemicals used were of analytical grade.

2.2. Animal treatment strategy

Thirty-six adult Swiss albino male and adult mice (48–50 g, 6 months old) were bought from the central animal house, Jamia Hamdard University, New Delhi, India. They were kept and treated under humane and hygienic conditions ($25 \pm 5^\circ\text{C}$ with 12 h day:night cycle) in accordance with the institutional guidelines. They were allowed to acclimatize for 10 days prior to the treatment on standard pellet mice diet (Ashirwad Industries, Chandigarh, India) and fresh drinking water ad libitum. All the animals were randomly divided into six groups – control normal was named as group I, control as group II, riboflavin (RF, 2 mg/kg body weight) treated as group III, cisplatin (CIS, 2 mg/kg body weight) treated as group IV and combination of cisplatin and riboflavin in ratio of 1:0.5 and 1:1 as group V (CIS + RF1) and group VI (CIS + RF2). Each group had five to six mice. Among them, groups II, III, IV, V and VI were exposed to visible light (Philips tube light, 40 W, 5 cm distance) during day-time for 12 h except group I. All the doses were injected intraperitoneally with 40 units (1 ml) insulin syringe and, saline was used as vehicle of RF and CIS. In the combinational groups V and VI, RF was injected prior to CIS at the interval of 30 min. The control, group II was given only saline of equal volume to that in the treated groups, whereas control normal, group I was kept as such.

The treatment was continued for 1 month at the rate of 2 doses/week. The dorsal surface of all the mice (except group I) was mildly shaved for maximum possible penetration of light through the skin. The strategy, dose and the duration of treatment were chosen to study the chronic effect of the treatment at moderately toxic dose of the drug. The treatment was scheduled in such a way that all the animals were sacrificed on the same day under light chloroform anesthesia. Their kidneys, livers and blood (with anticoagulant) were collected. The organs were washed with saline (with EDTA) and then kept in cold saline buffer in deep freeze.

2.3. Preparation of samples

The blood was centrifuged at 2500 rpm (1500g) for 10 min to collect their serum which was later stored in cold. Their kidneys and livers were also homogenized separately at 5000 rpm (3000g) in potassium-phosphate buffer (pH 7.36, 0.1 M) and their supernatants were taken-out for biochemical assays and estimations. Prior to it, 1 ml of homogenate of each mouse is saved for reduced glutathione (GSH) estimation. All the samples were labeled properly and kept in -20°C for further analysis.

2.4. Effect of the treatment on body weight of the mice

The body weight of all mice was noted before and after the treatment to see effect of the treatment on the animals.

2.5. Assay of antioxidant enzymes

The activity of different antioxidant enzymes were assayed with standard protocols. Cu Zn superoxide dismutase (CuZnSOD) was assayed by autoxidation of pyrogallol (Marklund and Marklund, 1974) while that of catalase (CAT) was done by decomposition of hydrogen peroxide (Aebi, 1984). The activity of glutathione-S-transferase (GST) was measured by Habig et al. method (1974). Glutathione reductase (GR) activity was carried-out by the method involving oxidation of NADPH into NADP⁺ in presence of oxidized glutathione (Carlberg and Mannervik, 1985). Assay of alkaline phosphatase (ALP) was done by Shah et al. method (1979).

2.6. Estimation of lipid peroxidation and protein oxidation

The extent of lipid peroxidation was estimated by the method of Beuge and Aust (1978) involving the measurement of total melondialdehyde (MDA) which is the major product of lipid peroxidation. Protein oxidation was measured by estimation of total carbonyl content (Levine et al., 1994) that is the final product of the oxidation.

2.7. Estimation of GSH level and –SH groups

The level of reduced glutathione (GSH) was estimated by method of Jollow et al. (1974) while that of total sulfhydryl groups (–SH) was measured by Sedalk and Lindsay method (1968).

2.8. Estimation of urea as kidney function marker

The level of urea was estimated in the serum by the commercially available diagnostic kits (Span Diagnostics Limited, India).

2.9. Estimation of GOT and GPT as liver function markers

The activity of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) in the serum was assayed by commercially available estimation kits (Span Diagnostics Limited, India).

2.10. Statistical analysis

All the data have been expressed as in mean \pm standard error of mean (SEM) for 5–6 different preparations in duplicate. Their statistical significance was evaluated by one-way ANOVA software. The probability of occurrence was selected at $p \leq 0.05$. The treatment and the experiments were repeated twice to check reproducibility of the results.

3. Results

The animals were given 8 intraperitoneal doses of the treatment. They were sacrificed all together on the same day. Major target organs of the treatment – kidney and liver and the blood of all mice were collected. Their organs were homogenized and blood was centrifuged to get the supernatants and the serums, respectively, at -4°C and was stored at -20°C after that. Their biochemical study and analysis were carried-out to see whether the groups fifth and sixth showed any recovery or healing from the CIS-induced nephrotoxicity and hepatotoxicity. Minor fluctuations in readings were observed on repetition of the assays and the estimations. All parameters have been expressed in mean \pm SEM with % change (in parentheses marked by ^a) from the control (group II) in the all the tables for groups III and IV and % recovery (in parentheses marked by ^b) for groups V and VI as compared to the fourth group.

3.1. Effect of the treatment on body weight

Before starting of the treatment, weight of all the mice was in the range of 49–50 g/mouse but after the treatment, there was noticeable decline in all the groups except group I. The average body weight of groups III and IV decreased by 4.05% and 14.65%,

Table 1
Effect of the treatment on body weight (in g) of the mice (6 mice per group).

Groups	Before treatment	After treatment	% Change
I	49.8 \pm 0.50	50.25 \pm 0.32	+0.90
II	50.2 \pm 0.50	49.50 \pm 0.50	–1.39
III	49.4 \pm 0.30	47.40 \pm 0.25	–4.05 ^a
IV	49.5 \pm 0.80	42.25 \pm 0.32	–14.65 ^{a,c}
V	49.6 \pm 0.70	44.12 \pm 0.23	–11.05 ^{b,c}
VI	50.0 \pm 0.30	46.25 \pm 0.32	–7.50 ^{b,c}

^a % Change from control.

^b % Change from CIS.

^c Significantly different at $p < 0.05$.

respectively, compared to the weight before the treatment. The hair quality of the fourth group was also not as smooth and lustrous as that in the groups II and III of mice. These could be the obvious side effects of chemotherapy (Table 1).

3.2. Effect of the treatment onto the antioxidant status

Four major antioxidant enzymes – CuZnSOD, CAT, ALP and GR, were assayed in all organ samples. It was observed that group I

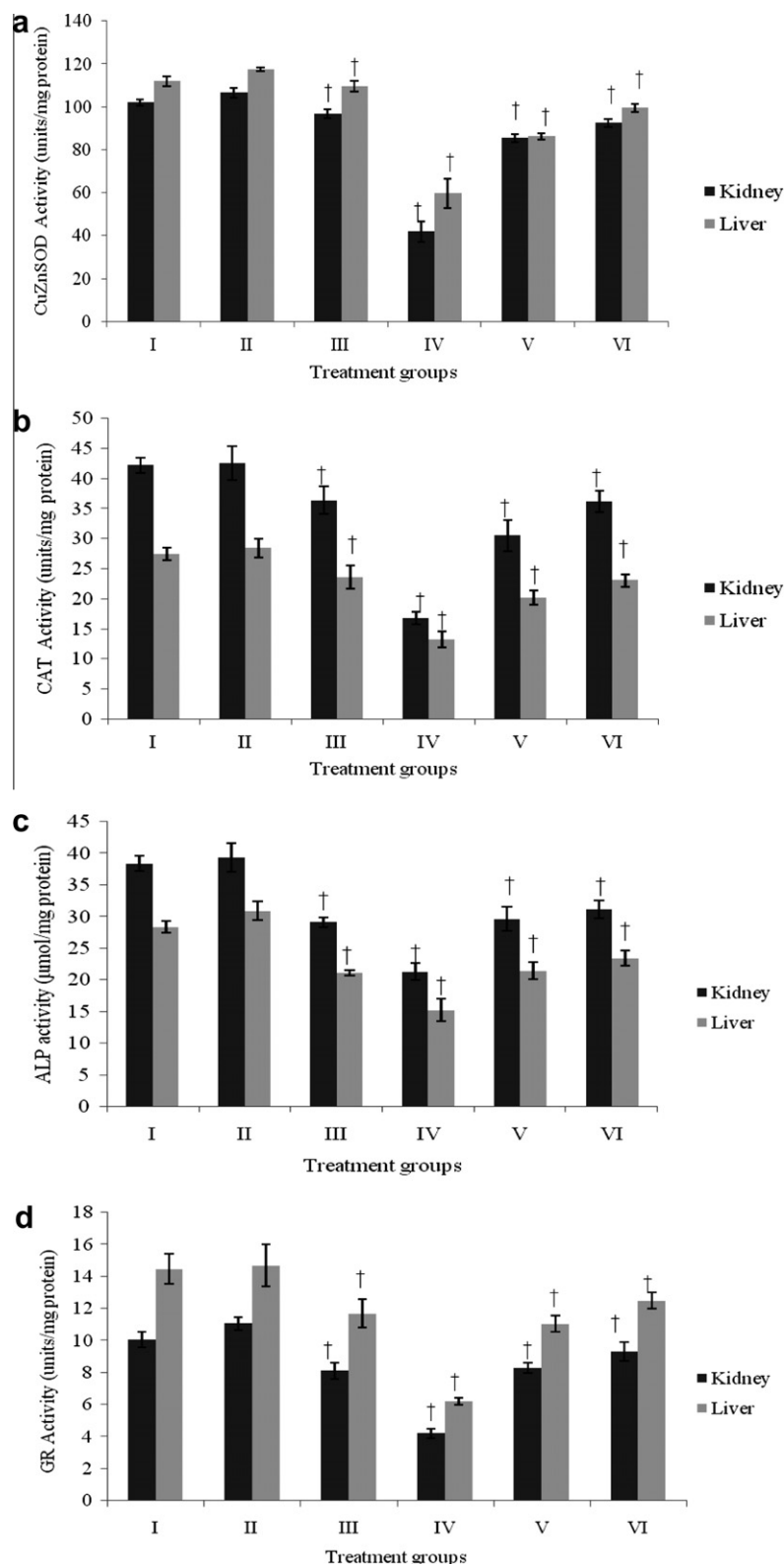


Fig. 1. Effect of the treatment on specific activity of antioxidant enzymes in target organs. (a) CuZn SOD; (b) CAT; (c) ALP; (d) GR. CuZn SOD, copper zinc superoxide dismutase; CAT, catalase; ALP, alkaline phosphatase; GR, glutathione reductase; mg, milligram of protein in the sample. Results have been expressed in mean \pm SEM of different samples. †Significantly different at $p < 0.05$.

which was neither under any treatment nor under photoillumination, showed maximum activity of all the enzymes in both kidney and liver samples, followed by the second group which was kept under photoillumination but was given no treatment. The specific activity of all the enzymes decreased most significantly in the CIS-treated group, whereas the RF treated group also showed a mild dip in all the enzymes with respect to the control group II. The combinational groups V and VI, showed a recovery in all the enzymatic activities in a dose dependent manner. All the enzymes showed better recovery of activity in kidneys in case of the combinational groups except ALP which showed better recovery in their liver samples. The most effected enzyme among all after CIS treatment was GR which showed inhibition by 62% and 55% in kidney and liver, respectively, followed by 61% decline of SOD and CAT both in kidney. Group III also showed the decline in the enzymes but it was not as pronounced as in group IV. As far as recovery is concerned, SOD, GR and CAT showed highest % recovery in kidney of group VI by 121%, 121% and 116%, respectively (Fig. 1a–d).

3.3. Effect on activity of GST

GST is considered as the main detoxifying enzyme for drugs and xenobiotic compounds along with other supporting enzymes in liver and kidney. Hence its activity level was taken as toxicity mar-

ker in the target organs. The RF treated group III showed drop in activity by 13% in kidney and 8% in liver, whereas CIS-treated group IV exhibited 38% decrement in kidney and 51% in liver. The % recovery was 28 and 47 in group V and; 40 and 71 in group VI in kidney and liver, respectively (Table 2).

3.4. Effect on the lipid peroxidation and the protein oxidation

MDA levels and carbonyl contents are considered as final products for estimation of lipid and protein oxidation, respectively. Group III showed the lipid peroxidation enhancement by 24% in kidney and 19% in liver while group IV showed tremendous increment of the same by 131% and 191% in kidney and liver, respectively. In groups V and VI, its level decreased significantly showing the recovery of 44–48% in kidneys and 54–59% in liver with increasing dose of RF (Table 3).

CIS and RF treatment also caused protein oxidation in the mice. The carbonyl content was enhanced in group III by 50% in kidney and 27% in liver while it shot up to 154% and 191% in kidney and liver, respectively, in the fourth group. But, there was also appreciable recovery in the groups V and VI. The % recovery in the fifth group was 44 in kidney and 56 in liver while it was 53 and 62 in kidney and liver, respectively, in the sixth group (Table 3).

Table 2

Effect of the treatment on the major detoxifying enzyme, GST.

GST (units/mg)	I	II	III	IV	V	VI
Kidney	85.00 ± 6.29	87.76 ± 7.39	76.46 ± 5.23 (–13) ^{a,c}	54.75 ± 4.03 (–38) ^{a,c}	70.13 ± 2.86 (+28) ^{b,c}	76.60 ± 3.89 (+40) ^{b,c}
Liver	119.41 ± 5.03	121.45 ± 5.65	111.60 ± 4.87 (–8) ^{a,c}	59.65 ± 6.25 (–51) ^{a,c}	87.50 ± 6.22 (+47) ^{b,c}	102.09 ± 7.04 (+71) ^{b,c}

GST, glutathione-S-transferase; mg, milligram of protein in the sample.

Results have been expressed in mean ± SEM of different samples.

^a Values in parenthesis indicate % change from control.

^b Values in parenthesis indicate % change from CIS.

^c Significantly different at $p < 0.05$.

Table 3

Effect of the treatment on extent of oxidation of the macromolecules.

End-products	I	II	III	IV	V	VI
<i>Lipid peroxidation/total MDA level (nmol/mg)</i>						
Kidney	0.50 ± 0.02	0.51 ± 0.07	0.63 ± 0.03 (+24) ^{a,c}	1.18 ± 0.06 (+131) ^{a,c}	0.66 ± 0.06 (–44) ^{b,c}	0.61 ± 0.09 (–48) ^{b,c}
Liver	0.64 ± 0.03	0.67 ± 0.05	0.80 ± 0.07 (+19) ^{a,c}	1.95 ± 0.15 (+191) ^{a,c}	0.90 ± 0.02 (–54) ^{b,c}	0.81 ± 0.04 (–59) ^{b,c}
<i>Protein oxidation/total carbonyl content (nmol/mg)</i>						
Kidney	5.88 ± 0.04	5.94 ± 0.09	8.90 ± 0.13 (+50) ^{a,c}	15.11 ± 0.2 (+154) ^{a,c}	8.50 ± 0.24 (–44) ^{b,c}	7.11 ± 0.30 (–53) ^{b,c}
Liver	9.87 ± 0.24	9.99 ± 0.38	12.69 ± 0.3 (+27) ^{a,c}	29.05 ± 0.3 (+191) ^{a,c}	12.83 ± 0.6 (–56) ^{b,c}	11.05 ± 0.41 (–62) ^{b,c}

MDA, melondialdehyde; nmol/mg, nanomoles per milligram of protein in the samples.

Results have been expressed in mean ± SEM of different samples.

^a Values in parenthesis indicate % change from control.

^b Values in parenthesis indicate % change from CIS.

^c Significantly different at $p < 0.05$.

Table 4

Effect of the treatment on antioxidant proteins.

Antioxidants	I	II	III	IV	V	VI
<i>Level of GSH (nmol/mg)</i>						
Kidney	37.61 ± 1.04	37.55 ± 1.49	30.24 ± 0.63 (–20) ^{a,c}	17.65 ± 1.37 (–53) ^{a,c}	28.61 ± 1.36 (+62) ^{b,c}	31.31 ± 1.49 (+77) ^{b,c}
Liver	59.71 ± 0.93	57.34 ± 0.58	52.19 ± 1.37 (–9) ^{a,c}	29.65 ± 1.92 (–48) ^{a,c}	48.57 ± 0.35 (+64) ^{b,c}	51.50 ± 0.37 (+74) ^{b,c}
<i>Total –SH groups content (nmol/mg)</i>						
Kidney	0.50 ± 0.02	0.47 ± 0.01	0.39 ± 0.01 (–17) ^{a,c}	0.23 ± 0.02 (–55) ^{a,c}	0.36 ± 0.01 (+57) ^{b,c}	0.42 ± 0.01 (+83) ^{b,c}
Liver	0.72 ± 0.02	0.68 ± 0.02	0.60 ± 0.01 (–12) ^{a,c}	0.42 ± 0.02 (–43) ^{a,c}	0.56 ± 0.01 (+33) ^{b,c}	0.63 ± 0.02 (+50) ^{b,c}

GSH, reduced glutathione; –SH, sulfhydryl groups; nmol/mg, nanomoles per milligram of protein in the samples.

Results have been expressed in mean ± SEM of different samples.

^a Values in parenthesis indicate % change from control.

^b Values in parenthesis indicate % change from CIS.

^c Significantly different at $p < 0.05$.

3.5. Effect of the treatment on –SH groups and GSH level

Both, –SH and GSH are indicative parameters of redox status of any living system that help to counter oxidative stress along with the antioxidant enzymes. GSH level was affected by the treatment as group III showed decrement by 20% and 9% in kidney and liver, respectively. Hitherto, group IV showed much lower level of the same by 53% in kidney and 48% in liver. The recovery shown by the group fifth and sixth was 62% and 77% in kidney; 64% and 74% in liver, respectively (Table 4).

The –SH level, in group III was also observed to decrease by 17% in kidney and 12% in liver, whereas group IV showed decline by 55% and 43% in kidney and liver, respectively, of the same As % recovery is concerned, it was 57% in kidney and 33% in liver of group fifth although the sixth group showed better recovery, i.e., by 83% in kidney and 50% in liver (Table 4).

3.6. Effect of the treatment on kidney function markers in serum

Urea was chosen as kidney function marker in serum samples. Its level was found to be 34% higher in the third group's serum while group IV showed 98% increment in the serum as compared to the control (Fig. 2). The graph also shows the recovery of the marker towards the normal levels after the combinational treatment as shown by groups V and VI. The group fifth exhibited the level of urea recovered up to 28% from CIS induced damage in serum while it showed better improvement in sixth group by 37% in the serum (Fig. 2).

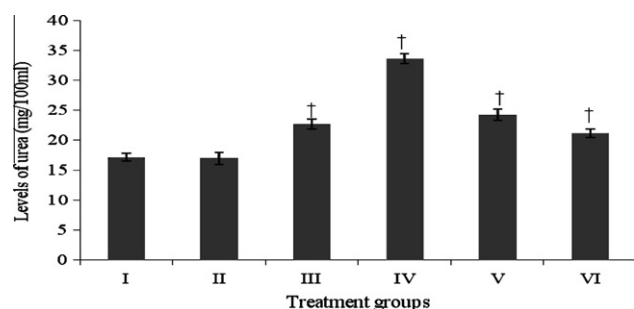


Fig. 2. Effect of treatment on urea, a serum kidney function marker. mg/100 ml, milligram per 100 milliliters of the samples. Results have been expressed in mean \pm SEM of different samples. [†]Significantly different at $p < 0.05$.

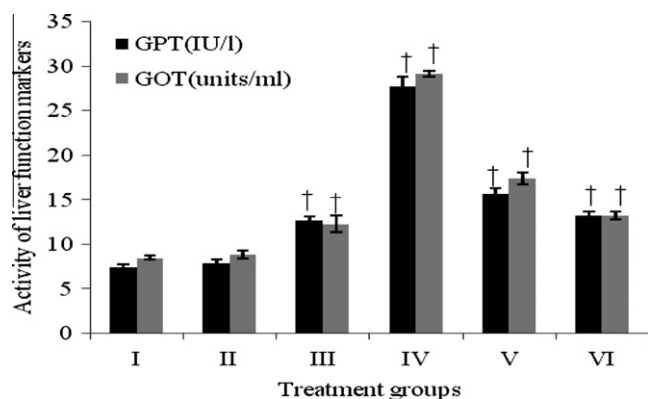


Fig. 3. Effect of treatment on liver function markers in serum. IU/L, international units per liter of the sample; GPT, glutamate pyruvate transaminase; GOT, glutamate oxaloacetate transaminase. Results have been expressed in mean \pm SEM of different samples. [†]Significantly different at $p < 0.05$.

The levels of creatinine and BUN were also affected following similar pattern as urea (data not shown).

3.7. Effect of the treatment on liver function markers in serum

The level of glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT) was estimated in serum samples as the liver function markers.

The treatment had markedly affected the liver specific enzymes. There was increase in the level of GPT by 252% in serum of group IV. Group III also showed the increased level of GPT by 36% in their serum samples. In case of GOT, the third group serum samples showed minor raised level by 39% while the fourth group exhibited significant increment up to 228% in serum (Fig. 3).

This graph also suggests that liver function markers are elevated in the serum due to release of the enzymes from damaged liver. This was not so in the CIS–RF combinational treatment as shown by groups V and VI. Group VI showed better recovery dependent on the dose of RF (Fig. 3).

4. Discussion

Cisplatin (CIS) is one of the most widely used anticancer drug for the treatment of various cancers and solid tumors (Sweetman, 2002). However, its major side effects – nephrotoxicity and hepatotoxicity are the main limiting factors of its clinical use for long term treatment (Antunes et al., 2000; Zicca et al., 2002). Various treatment-strategies and curing agents have been tried and used to monitor or control its side effects since its discovery. Earlier studies from our lab have shown that photoilluminated riboflavin (RF) can ameliorate the toxic effects of CIS via interaction at molecular level in situ in keratinocytes (Husain and Naseem, 2008). The present part of our work is an attempt to see whether our in situ observation holds true in vivo or not.

Administration of CIS to mice resulted in decrease in the body weight of the animals. This weight loss observed in the CIS-treated group may be because of reduced appetite and enhanced catabolic rate which are considered as the obvious side effects of chemotherapy. It could be also due to CIS-induced dysfunction of the gastrointestinal system. The treatment affects the target organs – kidneys and liver as evidenced by their elevated functional markers in the serum of the CIS-treated group. This is a clear indication of nephrotoxicity and hepatotoxicity caused by CIS as the markers are released by the damaged organs in the circulatory system. A marked recovery was observed in the markers post-combination treatment by CIS with RF. Several possible mechanisms have been proposed to explain the pathological status of kidney and liver post-CIS treatment (Martin et al., 2008; Tikoo et al., 2007).

In earlier studies, it is documented that CIS is accumulated in its target organs by covalently binding with their proteins (Perra et al., 1992). This can affect their antioxidant enzymes which are the first line of defense against any oxidative insult to the cells. This may be the primary factor behind the alteration in the parameters observed in group IV. It is obvious from our results that CIS caused nephrotoxicity and hepatotoxicity evidenced by marked decline in activity of the antioxidant enzymes (SOD, CAT, ALP and GR). RF being photosensitive gets activated under photoillumination and generates free radicals. Hence, the effect of RF on all the parameters observed in the RF treated group III was similar to group IV but not as pronounced as seen in the later indicating that RF is toxic under light but not as much as CIS.

GST is considered as the main detoxifying enzyme for drugs and xenobiotic compounds along with other supporting enzymes in

liver and kidney. Hence its activity level was taken as toxicity marker in the target organs. Its level in group IV also showed significant decrement in activity (Hanigan et al., 2001). The multiple doses of CIS given may have exerted abundant toxic burden on the enzyme so much that it was either insufficient to counter the toxicity or it may have been inhibited or its expression may have been affected.

Reduced glutathione (GSH) is considered as a sensitive oxidative stress marker because it helps to maintain the integrity of mitochondria and cell membrane. Its compromised level in the cells may deteriorate the membrane permeability and risks the cellular defense against ROS resulting in oxidative injuries (Younes and Siegers, 1981). Many glutathione based antioxidant enzymes and proteins (GST, GR, GSH and –SH) are important to maintain redox status of the cells. All these enzymes utilize glutathione (GSH) in the reactions they catalyze which may lead to depletion of GSH and –SH groups in the living system in the condition of oxidative stress. This may be the possible reason for decreased level of GSH and –SH level in target organs in CIS treated mice (Cayir et al., 2009). On the other hand, GSH may also cause metal reduction as thiol-part of it is highly reactive to the alkylating agents like CIS. There are also evidences that CIS can bind to GSH and gets metabolized via γ -glutamyl transpeptidase pathway which may also contribute to nephrotoxicity and hepatotoxicity in mice due to the depletion of GSH in the target organs (Hanigan et al., 2001). From literature, it is clear that CIS induces lipid peroxidation by excessive free radical generation (Younes and Siegers, 1981) that can lead to oxidation of cell membrane lipids and the lipid of membrane surrounding organelles in the target organs deteriorating the membrane functions. Hence, it is possible that CIS gets access to the nuclear and the organelle DNA (Corde et al., 2003) and proteins directly or indirectly (Perra et al., 1992) which may either inactivate or degrade or hamper their activity and hence it can effect their biological functions as well. This could be the ultimate possible reason for decline in the activity of antioxidant enzymes and depletion in the levels of protective groups (GSH and –SH) in group IV.

Groups V and VI showed significant recovery in all the parameters taken in the experiments in a dose dependent manner. The antioxidant enzymes and proteins tended to rise towards normal levels along with recovery in the extent of lipid peroxidation, protein oxidation and the level of target organ function markers (including BUN and creatinine in serum samples) to appreciable levels in a dose dependent way. This observation in both the groups V and VI could be explained on the basis of previous studies demonstrating the ameliorative effect of RF on cisplatin induced damage to the system in vitro as well as in situ (de Souza et al., 2006; Husain and Naseem, 2008). We have previously shown recovery from oxidative damage in the presence of CIS–RF combination compared to RF alone or CIS alone through UV–vis and fluorescence spectra (Husain and Naseem, 2008). We have also demonstrated the recovery to protein damage when exposed to this combination supporting our in vivo results. Previously, it has been demonstrated that the photoproducts of RF can act as adjuvants with chemotherapy leading to the activation of Fas ligand – Fas mediated apoptosis in human prostate cancer cell line (PC3) as well in leukemia (de Souza et al., 2006; de Souza Queiroz et al., 2007).

In our case, when CIS is used in combination with RF, the toxicity of the drug is possibly minimized via interaction of CIS with the excitable electrons of the alloxazine ring of RF when exposed to light, there by inhibiting the ROS generation. At the same time, RF may be helpful in inducing apoptosis in cancer cells via induction of Fas–Fas ligand mediated caspase pathway, possibly making CIS–RF together, a more effective chemotherapeutic combination against solid tumors.

5. Conclusion

It may be hypothesized that the excitable electrons in alloxazine ring of RF may interact with the active groups of CIS at the molecular level which may possibly lead to decreased production of ROS by CIS thereby reducing the oxidative stress as well as suppressing the riboflavin prooxidant potential leading to decreased ROS generation in the target organs and correspondingly improved status of the organs effected by CIS toxicity. Hence, our work opens a novel window to manage CIS-induced nephrotoxicity and hepatotoxicity in the cancer patients undergoing chemotherapy with CIS that can lessen their suffering and may prolong their survival. Our findings can also help to design better and safer platinum based anticancer drugs but more in vivo studies and clinical trials in the cancerous mice strains or patients are required to be done in future to establish this hypothesis.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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