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Prevention of Cadmium Toxicity by Ceftriaxone plus Sulbactam with VRP1034 in Rats

Vivek Kumar Dwivedi[1]*, Anuj Bhatanagar[2], Manu Chaudhary[1]

[1]Preclinical Division, Venus Medicine Research Centre, Baddi, H.P. - 173205, India
[2]Analytical Division, Venus Medicine Research Centre, Baddi, H.P. - 173205, India

Abstract

The aim of this study was to determine the prevention of cadmium toxicity by ceftriaxone plus sulbactam with VRP1034 drug on hematological, biochemical, lipid per-oxidation, antioxidant enzymatic activities and Cd, Zn and Fe levels in the blood and tissues of cadmium exposed rats. Twenty four male rats were divided into three groups of eight rats each. Control group received distilled water where as group II received $CdCl_2$ (1.5 mg/4 ml body weight) through gastric gavages for 21 days. Group III was received $CdCl_2$ plus treated with ceftriaxone plus sulbactam with VRP1034 for 21 days. These parameters were measured in plasma and tissues (brain, liver and kidney) of all groups. Our findings showed that a significantly decreased cadmium ($p<0.001$), malonaldialdehyde ($p<0.001$), MPO levels along with significant increased hemoglobin ($p<0.01$), red blood cell ($p<0.05$), hematocript($p<0.05$) levels and all antioxidant enzymatic activities in plasma and tissues of ceftriaxone plus sulbactam with VRP1034 treated group as compared to cadmium exposed group. δ -aminolevulinic acid dehydratase activity was significantly ($p<0.001$) increased in the blood of ceftriaxone plus sulbactam with VRP1034 treated group as compared to cadmium exposed group. The levels of hepatic and renal parameters were significantly ($p<0.001$) decreased in ceftriaxone plus sulbactam with VRP1034 treated group as compared to cadmium exposed group. So these finding concluded that ceftriaxone plus sulbactam with VRP1034 act as potent free radical scavenger and metal chelating properties that reduces free radical mediated tissue injury and prevent hepatic and renal organs dysfunction during cadmium intoxication.

Keywords: Ceftriaxone plus sulbactam with VRP1034; Cadmium; Hematological and biochemical parameters; Oxidative stress and enzymatic parameters; Hepatic and renal tissues

Abbreviations: SOD- Superoxide dismutase; CAT- Catalase; GR- Glutathione reductase; Gpx- Gluathione peroxidase; GST- Gluathione S- transferase; MDA- Malonaldialdehyde; GSH- Reduced glutahione; GSSG- Oxidised glutathione; MPO- Myloperoxidase; FDC- Fixed Dose Combination; XO- Xanthine oxidase

Introduction

Cadmium (Cd) is an industrial and environmental pollutant, arising primarily from battery, electroplating, pigment, plastic, fertilizer industries, and cigarette smoke. It is dangerous because humans consume both plants and animals that absorb Cd efficiently and concentrate it within their tissues [1]. Cd shows various mechanisms of toxicity in particular species under different experimental conditions [2-4]. It has been demonstrated that Cd stimulates free radical production, resulting in oxidative deterioration of lipids, proteins and DNA, and initiating various pathological conditions in humans and animals [4]. Cd is rapidly cleared from the blood and concentrates in various tissues. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis [4-6]. A number of cadmium-induced effects including deterioration of cell-cell adhesion, DNA-related processes; cell signaling and energy metabolism can imply that this metal acts on the different molecular targets in human organs. It is shown that cadmium can induce apoptosis in mouse liver [7].

Ceftriaxone plus sulbactam with VRP1034 is a novel fixed dose combination drug. Ceftriaxone is third generation cephalosprin class of antibiotic whereas sulbactam is β-lacatamase inhibitor. It is well reported that cephalosporins act as multidentate chelating agents [8, 9]. Beside this, cephalosporins are known as thioether containing class of antibiotics which are more effective in preventing the free radical-mediated oxidation of sulfhydryl groups in the antibiotics. Various studies have been reported that ceftriaxone and sulbactam individually showed the free radical scavenging property [10,11]. It is well known reported that sulbactam act as oxidant scavenges activity and inhibits the *invitro* neutrophil function [12]. VRP1034 is a third vector between these two drugs. VRP1034 has potential chelating and free radical scavenger properties. VRP1034 is trade secrets, which is under patent. It has already reported that a novel fixed dose combination of ceftriaxone plus sulbactam with VRP1034 drug also play significant role in various bacterial infections [13]. So the aim of this study was to investigate the prevention of cadmium toxicity by ceftriaxone plus sulbactam with VRP1034 on the hematological, biochemical and some enzymatic parameters in plasma and tissues of rats.

Material and Methods

Chemicals

All biochemicals used in the present study were procured from Sigma, St. Louis, MO, USA. δ- aminolevulinic acid (δ-ALA) was purchased from Sigma Chemical, St Louis, MO, USA. Cadmium chloride was purchased from Sigma Chemical, St Louis, MO, USA.

***Corresponding author:** Vivek Kumar Dwivedi, Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan, Baddi, H.P.– 173205, India, Tel: 91-1795-302127; Fax: 91-1795-302133; E-mail: vivekdwivedi@venusremedies.com

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Other chemicals purchased locally, were of analytical grade. Ketamine hydrochloride was purchased from Samarth Life Science Pvt Ltd. Mumbai. Other commercial kits were procured from Erba diagnostics Mannheim Gmb, Germanny.

Drugs

Ceftriaxone plus sulbactam with VRP1034 drug was obtained from Venus Remedies Ltd., Baddi, H.P. The ratio of ceftriaxone plus sulbactam with VRP1034 was 2:1 respectively.

Animal groups and treatment

The animals were obtained from the animal house facility of Venus Medicine Research Centre, Baddi, and H.P. The experiment was carried out after approval from Institutional animal ethics committee (IAEC). The IAEC number for this study was IAEC/CSV/2011-05. The study was performed on male wistar rats weighing 140 ± 10 g housed in polypropylene cages in an air-conditioned room with temperature maintained at 25 ± 2°C and 12 hrs alternating day and night cycles. Animals were allowed standard rat chow diet and sterile distilled water. Twenty four male rats were selected and divided into three groups of eight rats each which is given below.

Group I: Control normal saline treated group

Group II: $CdCl_2$ induced group (1.5 mg /4 ml/kg body weight)

Group III: $CdCl_2$+Ceftriaxone plus sulbactam with VRP1034 treated group (155.0 mg/kg body weight/day)

Cadmium chloride ($CdCl_2$) was given to sixteen animals through gastric gavage daily for 21 days and eight animals received plane distilled water and served as control. Toxicity was confirmed after showing the symptoms such as loss of appetite, body weight loss, decreased hemoglobin and increased body temperature. After confirmation of toxicity, drug was given to only group III animals *via* intravenous route for 21 days treatment and further recorded the body weight, body temperature, food and water intake along with hematological parameters. All the animals were decapitated 24 hour after last treatment, 2.5 ml blood was collected in EDTA containing vials and liver, kidney and brain tissues were collected in chilled phosphate buffer saline and washed three times with chilled PBS and prepared the homogenates for the measurement of biochemical and enzymatic parameters.

Plasma preparation

1.5 ml of blood sample was centrifuged at 6000 rpm for 15 minutes and supernatant was carefully taken into other polypropylene tube and stored at 2-8°C for the measurement of antioxidant enzymatic and biochemical parameters and rest part of blood samples were used for metal estimation and Blood δ- aminolevulinic acid dehydratase activity.

Homogenate preparation

10% tissues (liver, brain and kidney) homogenate were prepared in chilled phosphate buffer-NaCl solution containing 0.15 mol/L NaCl in 0.05 mol/L, Na_2HPO_4-NaH_2PO_4 buffer (pH 7.2) and left for at least 1 hr at 2-8°C before the estimation of enzymatic and biochemical parameters.

Determination of hematological parameters

Hematological parameters were tested with automatic cell counter (Sysmex XT 2000i).

Blood δ-Aminolevulinic Acid Dehydratase (ALAD) activity

ALAD activity was assayed in the blood according to method of Berlin and Schaller [14]. For measurement of δ-ALAD activity, take 0.2 ml of blood sample and mixed with 1.3 ml double distilled water and incubate the test tubes for 10 minute at 37°C for complete hemolysis. After incubation of all test tubes, added 1.0 ml of δ-ALA standard solution and further incubate all test tubes for one hr at 37°C. The reaction was stopped after one hr by adding 1.0 ml of 10% TCA solution. All test tubes were centrifuged at 600 g for 5 to 10 min. After centrifuge, 1.5 ml of supernatant was taken in clean test tubes and added equal amount of Ehrlich reagent and absorbance was recorded at 555 nm wavelength after 5 min. The molar extension coefficient 6.1×10^4 was used for calculation.

Enzymatic parameters

All enzymatic parameters were standardized at 25°C.

Superoxide Dismutase (SOD) assay

SOD activity was determined by the Method of Misra and Fridovich [15]. The reaction mixture consisted of 1.0 ml carbonate buffer (0.2M, pH 10.2), 0.8 ml KCl (0.015M), 0.1 ml of plasma and tissue and water to make the final volume to 3.0 ml. The reaction was started by adding 0.2 ml of epinephrine (0.025M).The change in absorbance was recorded at 480 nm at 15 second interval for one minute at 25°C. Suitable control lacking enzyme preparation was run simultaneously.

One unit of enzyme activity is defined as the amount of enzyme causing 50% inhibition of auto oxidation of epinephrine.

Catalase assay

Catalase activity was measured according to procedure of Aebi [16]. 100 µl of plasma and 0.025 ml of tissues were added in clean tubes and kept on ice bath for 30 minutes at room temperature. 10 µl Triton-X was added in each plasma and tissue containing test tube. In a cuvette, 200 µl phosphate buffer (0.2M; pH 6.8), 20 µl of sample and 2.53 ml distilled water was added. The reaction was started by adding 250 µl of H_2O_2 (0.066M in phosphate buffer) and decrease in optical density was recorded at 240 nm wave length at every 15 second for one minute. The molar extinction coefficient of 43.6 M Cm^{-1} was used for determination of catalase activity.

One Unit of enzyme activity was defined as the amount of enzyme that liberates half of the peroxide oxygen from H_2O_2 in one minute at 25°C.

Glutathione Reductase activity (GR; EC.1.6.4.2)

GR activity was measured by the method of Carlberg and Mannervik [17]. The reaction mixture consisted of 1.5 ml of potassium phosphate buffer (0.2 M, pH 7.0) containing 2 mM EDTA, 0.15 ml of 2 mM NAPDH , 0.2 ml of 20 mM oxidized glutathione and added distilled water to make up the final volume of 3.0 ml. The reaction was started by adding the 0.1 ml of plasma, homogenates in the linearity range. The absorbance was measured at 340 nm for one minute at 15 sec intervals. Control lacking enzyme was run simultaneously.

One unit of GR activity is expressed as the amount of NADP formed in one minute by one ml of enzyme preparation. Calculation of the enzyme activity has been done by using the molar extinction coefficient of NADPH as $6.22\times10^{3.}$

Glutathione Peroxidase activity (GPx; EC I .ll. 1.9)

GPx activity was measured by the method described by Rotruck

et al. [18]. The reaction mixture consisted of 0.2 ml of 0.4 M Tris-HCl buffer pH 7.0, 0.1 ml of 10 mM sodium azide, 0.2 ml of homogenate, 0.2 ml glutathione, and 0.1 ml of 0.2 mM H_2O_2. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by using Elman's reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate).

Glutathione -S transferase (GST; EC 2.5.1.18) activity

Glutathione S- transferase activity in plasma and tissue spectrophotometer was measured at 480 nm and 37°C by following conjugation of the acceptor substrate1-chloro-2, 4-dinitrobenzen as described by Habig et al. [19]. The absorbance was calculated from extinction coefficient 9.6 mM/Cm.

Estimation of xanthine oxidase

XO (Xanthine oxidase) was assayed in plasma and homogenate by the method of Fried and Fried [20]. The reaction mixture consisted of 0.9 ml of 0.1 M phosphate buffer pH 7.8; 0.75 ml 10 mM EDTA; 0.15 ml 0.2 mg/ml phenazine methosulphate; 0.45 ml 4 mg/ml nitroblue tetrazolium (NBT) salt; 0.5 ml 1 mM xanthine and water to make up the volume to 3.5 ml. The reaction was started at 37°C by addition of 0.5 ml of 1 mM Xanthine for 1 min. Extinction coefficient of the reduced NBT at 540 nm is 7.2 cm/μ mole. One unit of enzyme activity has been defined as amount of enzyme that converts 1μ mole of xanthine to uric acid in one minute at specified conditions of assay.

Reduced Glutathione (GSH) measurement

Reduced glutathione was estimated by the method of Ellman [21]. 0.5 ml plasma and 0.25 ml tissue samples were mixed with equal amount of 5% (w/v) TCA reagent and kept for 10 min at room temperature, proteins were precipitated and filtrate was removed carefully after centrifuge at 3500 rpm for 15 minutes. 0.25 ml filtrate was taken and added to 2.0 ml of Na_2HPO_4 (4.25%) and 0.04 ml of DTNB (0.04%). A blank sample was prepared in similar manner using double distilled water in place of the filtrate. The pale yellow color was developed and optical density was measured at 412 nm by spectrophotometer.

Estimation of total thiol

Total thiol content was analyzed by the method of Hu [22]. 0.2 ml plasma and tissue samples were taken in test tubes and added 0.6 ml of Tris EDTA buffer (Tris 0.25 M , EDTA 20mM ; pH 8.2) followed by addition of 40 μl of 10 mM of 5,5' dithionitrobis 2-nitrobenzoic acid (DTNB in methanol) and made the total reaction volume up to 4.0 ml by adding 3.16 ml of methanol. All test tubes were sealed and the color was developed for 15-20 min, followed by centrifugation at 3000 g for 10-15 min at room temperature. The absorbance of the supernatant was measured at 412 nm wavelength.

Measurement of myleoperoxidase

Myeloperoxidase enzyme was determined by O-dianisidine method with slight modification of Kurutas et al. [23]. The assay mixture consisted of 0.3 ml of sodium phosphate buffer (0.1M; pH 6.0), 0.3 ml of H_2O_2 (0.01M), 0.2 ml of O-dianisidine (0.02M) (freshly prepared in distilled water) and make up final volume to 3.0 ml with distilled water. The reaction was started by the addition of 0.025 ml samples. The change in absorbance was recorded at 460 nm wavelength. All measurements were carried out in duplicate. One unit of enzyme activity is defined as that giving an increase in absorbance of 0.001 min^{-1}.

Measurement of lipid per-oxidation level

Free radical mediated damage was assessed by the measurement of the extent of lipid peroxidation in the term of malonaldialdehyde (MDA) formed, essentially according to method of Ohkawa et al. [24]. It was determined by thio barbituric reaction. The reaction mixture consisted of 0.2 ml samples, 0.20 ml of 8.1% sodium dodecyl sulphate (SDS), 1.5 ml of (20%, pH 3.5) acetic acid, 1.5 ml of 0.8% thio barbituric acid (TBA) and 0.6 ml distilled water and made the volume upto 4.0 ml. The tubes were kept in boiled water at 95°C for one hr and cooled immediately under running tap water. 1.0 ml of water and 5.0 ml of mixture of n-butanol and pyridine (15:1 v/v) was added and vortexed. The tubes were centrifuged at 3500 rpm for 15-20 minutes. The upper layer was aspirated out and optical density was measured at 532 nm. The molar extension coefficient 1.56×10^5 was used for calculation.

Determination of biochemical parameters

The total protein, hepatic and renal enzymes (SGOT, SGPT, Creatinine and ALP levels) were measured in the plasma sample by standard kit method.

Metal estimation

For estimation of cadmium, iron and zinc concentration in the blood and tissues, 0.5 ml of samples (blood, liver, kidney and brain) were directly mixed with 4.5 ml of acidic glycerol (1% HNO_3 and 5% glycerol mixture) and finally make up the volume 10.0 ml with distilled water. Cadmium, Iron and Zinc metal estimation were measured by using flame atomic absorption spectrophotometer (Analytikjena, model No contra A300, Germany) with hallow cathode lamp at wave length 228.8 nm (Cd), 248.0 nm (Iron) and 213.9 nm (Zinc) respectively. The direct absorption of solution was determined by atomic absorption spectrophotometer and suitable standard curves of each metal were prepared using 20 to 100 μg/ml. All chemical used for metal estimation were Merck grade.

Statistical analysis

All values were expressed as Mean ± SD. One-way analysis of variance (ANOVA) followed by Newman-Keuls comparison test was used to determine statistical difference between control vs. cadmium exposed group and cadmium exposed group vs. ceftriaxone plus sulbactam with VRP1034 treated group. P values <0.05 were considered statistically significant.

Results

Superoxide dismutase (SOD) and catalase (CAT) enzyme activities were significant decreased in ($p<0.001$) plasma, ($p<0.001$) in brain, ($p<0.001$; $p<0.01$), in liver ($p<0.001$) and ($p<0.001$; $p<0.01$) in kidney tissues of Cd exposed group as compared to control group. After treatment with ceftriaxone plus sulbactam with VRP1034 for 21 days, these enzyme activities were significantly ($p<0.01$; $p<0.05$) increased in plasma and ($p<0.001$) in brain tissues of treated group as compared to Cd exposed group. Only SOD activity was significant increased in ($p<0.001$) liver and ($p<0.001$) kidney tissue whereas the catalase enzyme activity was found insignificant ($p>0.05$) increased in liver and kidney tissue of treated group as compared to cadmium exposed group after 21 days treatment (Figures 1 and 2). Oral administration of cadmium chloride group showed a significant reduction of glutathione reductase enzyme activity in plasma ($p<0.001$), in brain ($p<0.001$) and in renal tissue ($p<0.001$) as compared to control group whereas this enzyme was insignificantly ($p>0.05$) reduced in case of liver tissue in comparison to control group. After treatment with drug for 21 days, this enzyme

activity was significantly increased only in plasma whereas in all tissue homogenate, this enzyme activity was appeared insignificant ($p>0.05$) in treated group as compared to cadmium exposed group (Figure 3). GPx activity was significantly ($p<0.001$) decreased in plasma, hepatic and renal tissues of cadmium exposed group as compared to control group whereas in brain, the enzyme activity was reduced insignificant ($p>0.05$) as compared to control group. After treatment with C+S with VRP1034 drug for 21 days, the enzyme activity was significantly elevated ($p<0.001$) in liver and ($p<0.05$) in renal tissue while the activity was found insignificantly ($p>0.05$) increased in plasma and brain tissue of treated group (Figure 4). The GSH level was significantly ($p<0.001$) reduced along with significantly increased the GSSG level and lowering their ratio in the plasma and all tissue of $CdCl_2$ induced group after 21 days as compared to control group (Figure 5). After treatment with drug, these levels were increased along with significant increased their ratio in the plasma and tissues of treated group as compared to cadmium exposed group (Tables 1 and 2).

Total thiol level was significantly ($p<0.001$; $p<0.001$; $p<0.01$;

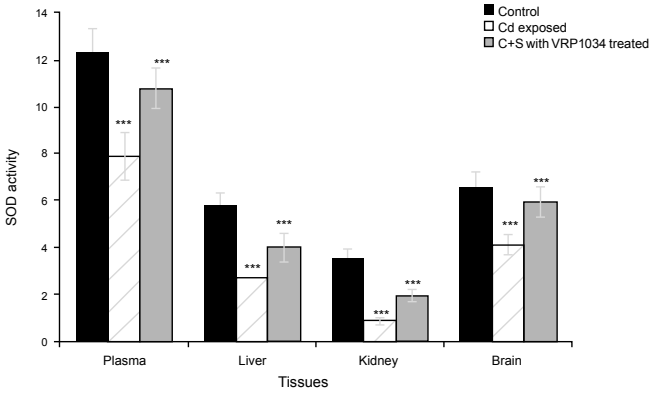

Figure 1: Effect of Ceftriaxone plus sulbactam with VRP1034 on Superoxide dimutase activity in cadmium exposed rat. All data are Mean ± SD of each group. Activity expressed in plasma (µmole/min/ml) whereas in liver, kidney and brain tissue (µmole/min/gm tissue). Data are compared between control vs Cd exposed group and Cd exposed vs C+S with VRP1034 treated group. Where $p^{***}<0.001$; (highly significant) $p^{**}<0.01$; (significant) $p^{*}<0.05$; (significant) and $p^{Ns}>0.05$.

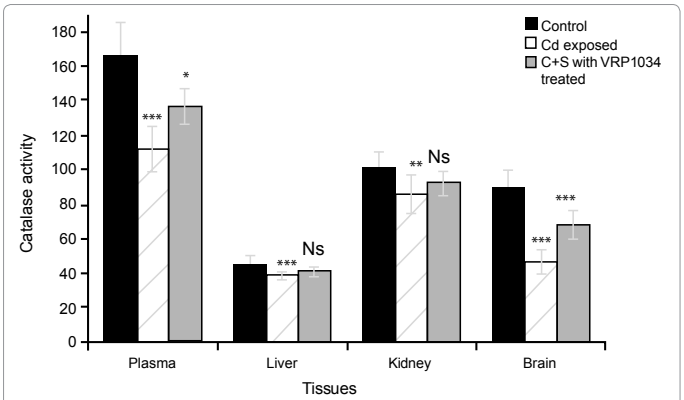

Figure 2: Effect of Ceftriaxone plus sulbactam with VRP1034 on Catalase activity in cadmium exposed rat. All data are Mean ± SD of eight animal each group. Catalase activity was expressed in plasma (µmole/min/ml) whereas in liver, kidney and brain tissue (µmole/min/gm tissue). Data are compared between control vs Cd exposed group and Cd exposed vs C+S with VRP1034 treated group. Where $p^{***}<0.001$; (highly significant) $p^{**}<0.01$; (significant) $p^{*}<0.05$; (significant) and $p^{Ns}>0.05$.

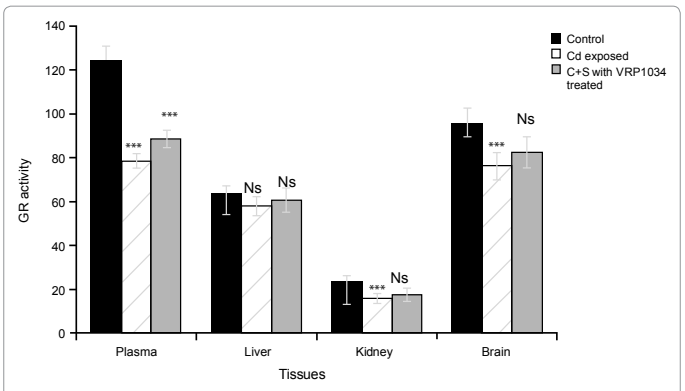

Figure 3: Effect of Ceftriaxone plus sulbactam with VRP1034 on glutathione reductase (GR) activity in cadmium exposed rat. All data are Mean ± SD of each group. Activity expressed in plasma (µmole/min/ml) whereas in liver, kidney and brain tissue (µmole/min/gm tissue). Data are compared between control vs Cd exposed group and Cd exposed vs C+S with VRP1034 treated group. Where $p^{***}<0.001$; (highly significant) $p^{**}<0.01$; (significant) $p^{*}<0.05$; (significant) and $p^{Ns}>0.05$.

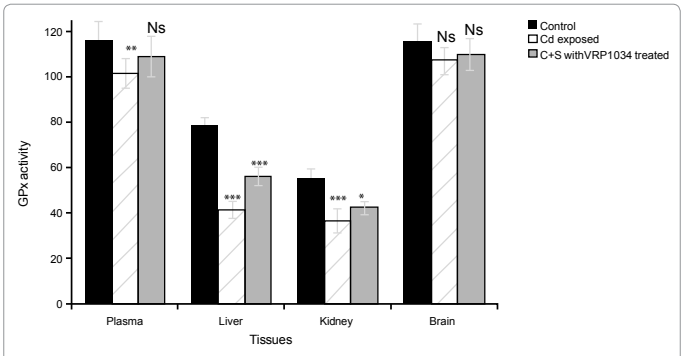

Figure 4: Effect of Sulbactomax on glutathione peroxidase (GPx) activity in cadmium exposed rat. All data are Mean ± SD of each group. GPx activity expressed in plasma (µmole/min/ml) whereas in liver, kidney and brain tissue (µmole/min/gm tissue). Data are compared between control vs Cd exposed group and Cd exposed vs C+S with VRP1034 treated group. Where $p^{***}<0.001$; (highly significant) $p^{**}<0.01$; (significant) $p^{*}<0.05$; (significant) and $p^{Ns}>0.05$.

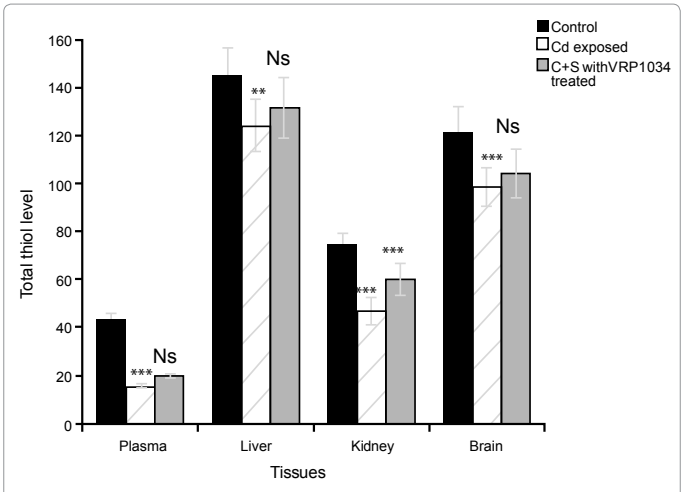

Figure 5: Effect of Ceftriaxone plus sulbactam with VRP1034 on Total thiol level in cadmium exposed rat. All data are Mean ± SD of each group. The level expressed in plasma (µmole/min/ml) whereas in liver, kidney and brain tissue (µmole/min/gm tissue). Data are compared between control vs Cd exposed group and Cd exposed vs C+S with VRP1034 treated group. Where $p^{***}<0.001$; (highly significant) $p^{**}<0.01$; (significant) $p^{*}<0.05$; (significant) and $p^{Ns}>0.05$.

p<0.001) lowered in plasma, brain, liver and renal tissue of cadmium exposed group. Total thiol level was significantly elevated in plasma and tissues of treated group after treatment with ceftriaxone plus sulbactam with VRP1034 drug for 21 days (Table 2). Lipid peroxidation was assayed in the term of malonaldialdehyde (MDA) formed. There was highly significant (p<0.001) increased malonaldialdehyde (MDA) and myloperoxidase (MPO) levels in plasma and tissues of $CdCl_2$ exposed group as compared to control group after 21 days. After treatment with ceftriaxone plus sulbactam with VRP1034 drug, the MDA and MPO parameters were significantly lowered (p<0.001; p<0.05) in plasma, (p<0.01; p>0.05) in brain, (p<0.001) in liver and (p<0.001; p>0.05) in renal tissue of treated group as compared to cadmium chloride group (Table 3). The xanthine oxidase (XO) and glutathione-S transferase (GST) enzyme activities were significantly (p<0.001) altered in the plasma and tissues of $CdCl_2$ induced group as compared with control group. The GST activity was significant increased (p<0.001) along with significant (p<0.001) reduction of XO activity in plasma and tissues of treated group as compared to $CdCl_2$ induced group after 21 days treatment. The GST and XO activities were found insignificant in the renal and brain tissues as compared to induced group (Table 4).

Hepatic parameters (SGOT, SGPT) were significantly increased in the plasma and liver tissue while renal parameters (creatinine) were increased in renal tissue of cadmium exposed group as compared to control group. These parameters were significantly lowered in plasma and tissues after treatment with ceftriaxone plus sulbactam with VRP1034 drug for 21 days (Table 5). Hematological parameters (Hb, RBC, WBC and HCT) were significant altered in the cadmium exposed group as compared to control group (Table 6). These parameters were improved after treatment with ceftriaxone plus sulbactam with VRP1034 for 21 days. The δ-ALAD enzyme activity was significantly (p<0.001)inhibited along with significant changes in the hemoglobin, RBC, WBC and HCT levels in the blood of cadmium exposed group as compared to control group. This activity and hematological parameters were significantly improved after treatment with ceftriaxone plus sulbactam with VRP1034 in the treated group as compared to cadmium exposed group (Table 6). Cadmium concentration was found significant (p<0.001) increase in blood and all tissues of $CdCl_2$ induced group in comparison to control group while in brain tissue of control group, the concentration was not detected. The concentration of Cd was significantly (p<0.001) minimized by intravenous route of ceftriaxone plus sulbactam with VRP1034 in treated group after 21 days. After treatment with drug, the concentration was reduced in brain tissue as compared to $CdCl_2$ induced group (Table 7). The depletion of Zinc and Fe levels were observed in liver, brain and renal tissues whereas the zinc level was increased in blood of cadmium exposed group as compared to control group. These levels were significant increased in plasma and all tissue of treated group after treatment with ceftriaxone plus sulbactam with VRP1034 drug (Table 8).

Discussion

Cadmium from the environment enters into body through lungs and intestines. It is then transported into the blood and accumulates in the liver where it induces the synthesis of metallothionein (MT), a cytosolic protein to which cadmium binds. It is well known that heavy metals impairs heam synthesis in the bone marrow and causes an increase of protoporphyrin-IX in erythrocytes. So our findings showed that, there was significantly decreased δ- ALAD enzyme activity and heamoglobin (Hb) levels along with increased cadmium concentration in blood and all tissues of $CdCl_2$ induced group as compared to control group. Due to inhibition of δ- ALAD enzyme, Hb level was reduced in induced group which confirmed that Cd metal interference with heam synthesis pathway that leads to anemia. The heme metabolic path way is highly susceptible to alterations induced by metal ions. Kannan and Flora [25] have suggested that the hemoglobin level and δ-aminolevulinic acid dehydratase enzyme activity were inhibited during heavy metal exposure in rats. Cd induced oxidative stress by increased lipid peroxidation level and inhibition of antioxidant enzymes required to prevent the oxidative stress cadmium toxicity [26]. In our experiment, the administration of $CdCl_2$ for 21 days *via* gastric gavage a significant higher the malonaldialdehyde (a marker of free radical), myloperoxidase levels and XO (free radical generating enzyme) along with significant reduction of endogenous antioxidant enzymes activities (SOD, CAT, GR, GPx, total thiol, GSH) in the plasma and tissues of cadmium chloride induced group as compared to control group. The level of GSSG and their ratio (GSH/GSSG) was also significantly reduced in the induced group as compared to control group after

Groups	Plasma		Brain		Liver		Kidney	
	GSH	GSSG	GSH	GSSG	GSH	GSSG	GSH	GSSG
Control group	3.96 ± 0.96	0.81 ± 0.17	4.89 ± 0.88	0.55 ± 0.11	5.78 ± 0.55	0.64 ± 0.06	2.60 ± 0.04	0.54 ± 0.13
Cadmium exposed group	1.55 ± 0.48***	1.03 ± 0.64^{ns}	2.25 ± 1.01***	0.89 ± 0.091***	2.69 ± 0.33***	0.91 ± 0.05***	1.87 ± 0.08***	0.71 ± 0.15*
Ceftriaxone plus sulbactam with VRP1034 treated group	2.09 ± 0.85^{ns}	0.78 ± 11^{ns}	3.96 ± 0.66***	0.64 ± 0.10***	4.00 ± 0.62***	0.77 ± 0.08***	2.39 ± 0.17***	0.60 ± 0.09^{ns}

All data are Mean ± SD of each group. The GSH and GSSG levels were expressed in the plasma (mg/dL) whereas in the tissues (µmole/gm tissue). Newman keuls test was performed for statistical significance between control group **vs** Cd exposed group and Cd exposed group **vs** ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001 (highly significant) **p<0.01(significant); p<0.05 (significant) ^{Ns}p>0.05 (non significant).

Table 1: Effect of ceftriaxone plus sulbactam with VRP1034 on GSH, GSSG levels in cadmium exposed group after 21 days treatment.

Groups	**Plasma**		**Brain**		**Liver**		**Kidney**	
	GSH/GSSG	Total thiol	GSH/GSSG	Total thiol	GSH/GSSG	Total thiol	GSH/GSSG	Total thiol
Control group	4.88	4.33 ± 0.98	8.89	121.4 ± 10.85	9.03	145.36 ± 10.88	4.81	75.12 ± 4.52
Cadmium exposed group	1.5	1.57 ± 0.34***	2.52	98.7 ± 8.17***	2.97	124.21 ± 11.20**	2.63	46.89 ± 5.87***
Ceftriaxone plus sulbactam with VRP1034 treated group	2.68	1.99 ± 0.73^{ns}	6.19	104.3 ± 10.21	5.19^{ns}	131.66 ± 12.55^{ns}	3.98	60.24 ± 6.54^{***e}

All data are Mean ± SD of eight animals of each group. The thiol level was expressed in the plasma (µmole/min/ml) whereas in the tissues (µmole/gm tissue). Newman keuls test was performed for statistical significance between control group **vs Cd** exposed group and Cd exposed group **vs** ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001 (highly significant) **p<0.01(significant); *p<0.05 (significant) ^{Ns}p>0.05 (non significant).

Table 2: Effect of ceftriaxone plus sulbactam with VRP1034 on redox state and total thiol level in cadmium exposed group after 21 days treatment.

Groups	Plasma		Brain		Liver		Kidney	
	MDA	MPO	MDA	MPO	MDA	MPO	MDA	MPO
Control group	153.11 ± 7.38	5.63 ± 1.28	83.41 ± 5.86	23.10 ± 3.54	342.1 ± 10.59	15.46 ± 0.75	3.56 ± 0.42	10.21 ± 2.45
Cadmium exposed group	274.30 ± 10.29***	8.44 ± 0.98**	115.20 ± 6.01***	32.45 ± 2.09***	398.5 ± 15.44***	40.22 ± 1.39***	0.884 ± 0.13***	17.50 ± 1.12***
Ceftriaxone plus sulbactam with VRP1034 treated group	210.64 ± 11.52***	6.25 ± 1.99*	103.36 ± 8.28**	29.42 ± 2.63ns	361.87 ± 8.56***	33.98 ± 1.76***	1.96 ± 0.22***	15.26 ± 2.37ns

All data are Mean ± SD of each group. The MDA and MPO levels were expressed in the plasma (µmole/min/ml) whereas in the tissues (µmole/gm tissue). Newman keuls test was performed for statistical significance between control group **vs** Cd exposed group and Cd exposed group **vs** ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001 (highly significant) **p<0.01(significant); *p<0.05 (significant) Nsp>0.05 (non significant).

Table 3: Effect of ceftriaxone plus sulbactam with VRP1034 on oxidative stress parameters in cadmium exposed group after 21 days treatment.

	Plasma (nmole/min/ml)		Liver (nmole/min/gm tissue)		Kidney (nmole/min/gm tissue)		Brain (nmole/min/gm tissue)	
	XO	GST	XO	GST	XO	GST	XO	GST
Control group	135.4 ± 8.56	14.33 ± 1.52	44.10 ± 2.65	25.12 ± 1.87	58.84 ± 4.23	36.41 ± 3.78	111.02 ± 7.49	9.11 ± 1.02
Cd exposed group	296.3 ± 10.22***	18.96 ± 1.10***	87.33 ± 6.02***	51.05 ± 2.64***	64.20 ± 3.63*	42.16 ± 2.81***	144.89 ± 9.48***	13.40 ± 1.47***
Ceftriaxone plus sulbactam with VRP1034 group	168.7 ± 14.31***	13.74 ± 0.55***	71.82 ± 4.49***	37.0 ± 2.09***	56.22 ± 1.77***	40.33 ± 1.59ns	139.45 ± 8.80ns	10.00 ± 1.12***

All data are Mean ± SD of each group. The XO and GST levels were expressed in the plasma (µmole/min/ml) whereas in the tissues (µmole/gm tissue). Newman keuls test was performed for statistical significance between control group **vs** Cd exposed group and Cd exposed group **vs** ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001 (highly significant) **p<0.01(significant); *p<0.05 (significant) **Ns**p>0.05 (non significant).

Table 4: Effect of ceftriaxone plus sulbactam with VRP1034 on xanthine oxidase (XO) and gluathione s transferase (GST) in cadmium exposed group after 21 days treatment.

Groups	Plasma				Liver		Kidney
	SGOT	SGPT	Creatinine	ALP	SGOT	SGPT	Creatinine
Control group	10.10 ± 2.45	12.97 ± 2.63	1.02 ± 0.12	19.56 ± 3.54	20.48 ± 0.85	8.63 ± 1.21	0.86 ± 0.053
Cadmium exposed group	88.56 ± 4.56***	115.12 ± 1.07***	7.54 ± 1.75***	5.98 ± 0.63***	73.63 ± 1.55***	35.28 ± 2.01***	5.84 ± 0.13***
Ceftriaxone plus sulbactam with VRP1034 treated group	34.12 ± 5.47***	66.39 ± 3.25***	3.23 ± 0.22***	14.25 ± 1.14***	41.21 ± 0.99***	18.56 ± 2.84***	1.96 ± 0.22***

All data are Mean ± SD of each group. These parameters were expressed in the plasma (IU/L) whereas in the tissues (mMole/gm tissue). Newman keuls test was performed for statistical significance between control group **vs** Cd exposed group and Cd exposed group **vs** ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001 (highly significant) **p<0.01(significant); *p<0.05 (significant) **Ns**p>0.05 (non significant).

Table 5: Effect of ceftriaxone plus sulbactam with VRP1034 on hepatic and renal parameters in cadmium exposed group after 21 days treatment.

Groups	Parameters				
	Hb (g/dL)	RBC (10^6/µL)	HCT %	WBC (10^3/µL)	δ- ALAD in blood (nmole/min/ml erythocyte)
Control	13.93 ± 0.19	7.36 ± 0.10	39.38 ± 0.66	5.12 ± 0.81	9.14 ± 0.71
Cd-exposed	9.27 ± 0.55***	7.01 ± 0.08*	36.89 ± 0.43**	11.25 ± 1.31***	3.55 ± 0.61***
Cd+Ceftriaxone plus sulbactam with VRP1034	11.22 ± 0.99**	7.20 ± 0.06 ns	37.78 ± 0.6*	7.84 ± 0.58**	6.74 ± 0.50***

All data are Mean ± SD of each group. Newman keuls test was performed for statistical significance between control group **vs** Cd exposed group and Cd exposed group **vs** ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001 (highly significant) **p<0.01(significant); *p<0.05 (significant) **Ns**p>0.05 (non significant).

Table 6: Effect of ceftriaxone plus sulbactam with VRP1034 on hematological and δ ALAD parameters in cadmium exposed group after 21 days treatment.

Groups	Blood (µg/ml)	Liver (µg/gm tissue)	Kidney (µg/gm tissue)	Brain (µg/gm tissue)
Control group	0.15 ± 0.011	0.68 ± 0.13	0.12 ± 0.01	ND
Cd exposed group	11.79 ± 2.4***	16.87 ± 1.17***	13.49 ± 2.21***	3.89 ± 0.27
Ceftriaxone plus sulbactam with VRP1034 treated group	6.75 ± 0.89***	10.11 ± 0.83***	9.55 ± 1.07***	0.62 ± 0.12***

All data are Mean ± SD of each group. Newman keuls test was performed for statistical significance between control group *vs* Cd exposed group and Cd exposed group vs ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001(highly significant) **p<0.01(significant); *p<0.05(significant) Nsp>0.05 (non significant).

Table 7: Effect of ceftriaxone plus sulbactam with VRP1034 on cadmium level in blood and tissues after 21 days treatment.

administration of $CdCl_2$ for 21 days *via* gastric gavages. The depletion of GSH, increase in GSSG and lowering of GSH/GSSG ratio in blood, liver, brain and renal tissue were consistent with the accumulation of cadmium in these tissues. These alterations were seemed to be due to the generation of free radicals. Bray and Taylor [27] reported that the depletion of GSH and increase GSSG and their ratio in the liver, kidney and brain tissues due to generation of oxidative stress. These results were accordance with other researcher. Various studies have suggested that cadmium metal can cause oxidative stress by interaction with -SH groups of major intracellular defense glutathione [28-30]. Other study has also reported that the increased lipid peroxidation in tissues of rats by a lower the tissue GSH level during cadmiun intoxication [31]. Cd exposed group decreases the endogenous antioxidant enzymes (SOD, Catalase, GR and GPx) activities along with increased lipid

Blood (µg/ml)			Liver (µg/gm tissue)		Kidney (µg/gm tissue)		Brain (µg/gm tissue)	
	Zinc	Fe	Zinc	Fe	Zinc	Fe	Zinc	Fe
Control group	4.11 ± 0.28	2.89 ± 0.21	38.96 ± 2.45	15.65 ± 1.02	20.11 ± 1.25	9.61 ± 1.58	10.25 ± 0.98	20.12
Cd exposed group	6.37 ± 0.55***	0.33 ± 0.014***	21.04 ± 3.22***	8.87 ± 1.56***	18.96 ± 1.93^{ns}	3.67 ± 0.59***	6.69 ± 0.71***	6.78 ± 1.15***
Ceftriaxone plus sulbactam with VRP1034 group	5.02 ± 0.19***	0.72 ± 0.011***	24.56 ± 1.5*	10.23 ± 2.10^{ns}	19.44 ± 2.78^{ns}	5.03 ± 0.97*	9.85 ± 1.28***	13.87 ± 2.31***

All data are Mean ± SD of each group. Newman keuls test was performed for statistical significance between control group *vs* Cd exposed group and Cd exposed group *vs* ceftriaxone plus sulbactam with VRP1034 treated group. Where ***p<0.001(highly significant) **p<0.01(significant); *p<0.05(significant); ^{Ns}p>0.05(non significant).

Table 8: Effect of ceftriaxone plus sulbactam with VRP1034 on Zinc and Fe levels in blood and tissues after 20 days treatment.

peroxidation and MPO levels in plasma, liver, brain and renal tissue as compared to control group. Similarly, there was significant reduction of total thiol level in plasma and all tissues of cadmium exposed group as compared to control group. The thiol level decreases in cadmium exposed group due to binding of thiol group with cadmium metal.

Various studies have reported that alteration in endogenous antioxidant enzyme activity in different organisms following cadmium exposure as a consequence of different period of time, the amount of cadmium and the organs [32,33]. Similar results have reported in cadmium exposed rats [6,34,35]. Asagba [36] and Guilhermino [37] have reported that cadmium alter the hematological parameters in cadmium exposed rat. So in the study, there was significant reduction in the hematological parameters (Hb, RBC and HCT) in the cadmium exposed group. The hepatic and renal parameters were also significant increased in cadmium toxicity induced group as compared with control group. It suggested that renal impairment affect due to cadmium toxicity that causes alteration in proximal tubules of kidney tissue. These findings also suggesting that, cadmium metal causes increased lipid peroxidation that imbalance the antioxidant defense system which leads to organs damage and dysfunction. δ- ALAD enzyme activity decreased in blood along with significant decreased the Zn and Fe levels in blood and tissue of cadmium exposed group as compared to control group. The inhibition of δ- ALAD enzyme in cadmium exposed group due to interference of heme synthesis pathway. The inhibition of micronutrient (Zinc, Fe) levels in cadmium exposed group due to cellular inhibitory action of these metals themselves.

After treatment with ceftriaxone plus sulbactam with VRP1034 drug for 21 days treatment, these antioxidant enzyme activities along with free radical generating enzyme xanthine oxidase, lipid peroxidation, MPO levels ,hepatic and renal parameters were significantly improved in plasma and tissues of treated group as compared to Cd exposed group. The GSH/GSSG ratio, δ- ALAD, Cadmium concentration and micronutrients level were also improved in plasma and tissues of treated group as compared to Cd exposed group. Due to increased δ-ALAD, SOD, Catalase antioxidant enzymes activities and decreased lipid peroxidation, MPO and cadmium level in the blood and tissues, it is confirmed that ceftriaxone plus sulbactam with VRP1034 having antioxidant and chelating properties. Ceftriaxone and sulbactam individually interact with heavy and trans metals and form complex which chelate out from sulfydryl group of antibiotics. But due to presence of VRP1034 in ceftriaxone plus sulbactam, this combination showed the synergistic effect that enhanced the free radical scavenging and chelating ability properties. Various studies have been reported that ceftriaxone and sulbactam individually showed the free radical scavenging property [10,11]. Our previous finding also reported that ceftriaxone plus sulbactam with VRP1034 is effective drug for removal of arsenic intoxication in rats [38]. In our best knowledge, there is no as such articles are available on combination of two antibiotic (beta lactam and betalactamase inhibitor) act as metal chelating properties. So on basis of above results and findings it is concluded that ceftriaxone plus sulbactam with VRP1034 is protective and effective drug for removal of heavy metal from body that plays a significant role in the improvement of endogenous antioxidant defense system along with reduces oxidative stress and protect from hepatic and renal injury during cadmium toxicity.

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References

1. Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 18: 321-336.
2. Iscan M, Coban T, Eke BC (1994) Differential combined effect of cadmium and nickel on hepatic and renal glutathione S-transferases of the guinea pig. Environ Health Perspect 9: 69-72.
3. Zikic RV, Stajn A, Saicic ZS, Spasic MB, Ziemnicki K, et al. (1996) The activities of superoxide dismutase, catalase and ascorbic acid content in the liver of goldfish (*Carassius auratus* gibelio Bloch.) exposed to cadmium. Physiol Res 45: 479-481.
4. Waisberg M, Joseph P, Hale B, Beyersmann D (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology 192: 95-117.
5. Shaikh ZA, Vu TT, Zaman K (1999) Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol Appl Pharmacol 154: 256-263.
6. Casalino E, Calzaretti G, Sblano C, Landriscina C (2002) Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. Toxicol 179: 37-50.
7. Waalkes MP, Diwan BA (1999) Cadmium-induced inhibition of the growth and metastasis of human lung carcinoma xenografts: role of apoptosis. Carcinogenesis 20: 65-70.
8. Anacona JR, Osorio I (2008) Synthesis and antibacterial activity of copper (II) complexes with sulphathiazole and cephalosporin ligands. Transition Met Chem 33: 517-521.
9. Anacona JR, Rodriguez A (2005) Synthesis and Antibacterial Activity of Ceftriaxone Metal Complexes. Transition Met Chem 30: 897-901.
10. Lapenna D, Cellini L, De Gioia S, Mezzetti A, Ciofani G, et al. (1995) Cephalosporins are scavengers of hypochlorous acid. Biochem Pharmacol 49: 1249-1254.
11. Gunther MR, Mao J, Cohen MS (1993) Oxidant-scavenging activities of ampicillin and sulbactam and their effects on neutrophil functions. Antimicrob Agents Chemother 37: 950-956.
12. Santos JI, Arbo A (1989) The *in vitro* effect of sulbactam on polymorphonuclear leukocyte function. Diagn Microbiol Infect Dis 12: 147S-152S.
13. Dwivedi VK, Chaudhary M, Soni A, Yadav J, Tariq A (2009) Diffusion of Sulbactomax and ceftriaxone into cerebrospinal fluid of meningitis induced rat model. Int J Pharmacol 5: 307-312.
14. Berlin A, Schaller KH (1974) European standardized method for the determination of delta aminolevulinic acid dehydratase activity in blood. Z Klin Chem Klin Biochem 12: 389-390.
15. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation

of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247: 3170-3175.

16. Aebi H (1984) Catalase. In: Methods in Enzymol, Packer L (ed.). Academic Press: Orlando, FL 125– 126.
17. Carlberg I, Mannervik B (1985) Glutathione reductase. Meth Enzymol 113: 485-90.
18. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, et al. (1973) Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 588-590.
19. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130-7139.
20. Fried R, Fried LW (1945) Xanthine Oxidase (Xanthine Dehydrogenase) Methods. Enzymat Anal 2: 644-649.
21. ELLMAN GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82: 70-77.
22. Hu ML (1994) Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 233: 380-385.
23. Kurutas EB, Arican O, Sasmaz S (2005) Superoxide dismutase and myeloperoxidase activities in polymorphonuclear leukocytes in acne vulgaris. Acta Dermatovenerol Alp Panonica Adriat 14: 39-42.
24. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-358.
25. Kannan GM, Flora SJ (2004) Chronic arsenic poisoning in the rat: treatment with combined administration of succimers and an antioxidant. Ecotoxicol Environ Saf 58: 37-43.
26. Kelley C (1999) Cadmium therapeutic agents. Curr Pharm Des 5: 229-240.
27. Bray TM, Taylor CG (1993) Tissue glutathione, nutrition, and oxidative stress. Can J Physiol Pharmacol 71: 746-751.
28. Valko M, Morris H, Cronin MT (2005) Metals, toxicity and oxidative stress. Curr Med Chem 12: 1161-1208.
29. Tzure-Meng WU, Yi-Ting HSU, Tse-Min LEE (2009) Effects of cadmium on the regulation of antioxidant enzyme activity, gene expression, and antioxidant defenses in the marine macroalga Ulva fasciata. Botanical Studies 50: 25-34.
30. Flora SJ, Mittal M, Mehta A (2008) Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J Med Res 128: 501-523.
31. Ramos O, Carrizales L, Yanez L, Mejia J, Batres L, et al. (1995) Arsenic increased lipid peroxidation in rat tissues by a mechanism independent of glutathione levels. Environ Health Perspect 1: 85-88.
32. Tandon SK, Singh S, Prasad S, Khandekar K, Dwivedi VK, et al. (2003) Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. Toxicol Lett 145: 211-217.
33. Jurczuk M, Jakoniuk MJ, Rogalska J (2006) Glutathione related enzyme activity in the liver and kidney of rats exposed to cadmium and ethanol. Polish J Environ Stud 15: 861-868.
34. Casalino E, Calzaretti G, Sblano C, Landriscina V, Felice Tecce M, et al. (2002) Antioxidant effect of hydroxytyrosol (DPE) and Mn2+ in liver of cadmium-intoxicated rats. Comp Biochem Physiol C Toxicol Pharmacol 133: 625-632.
35. Sen Gupta R, Sen Gupta E, Dhakal BK, Thakur AR, Ahnn J (2004) Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Mol Cells 17: 132-139.
36. Ogheneovo Asagba S (2010) Biochemical changes in urine and plasma of rats in food chain-mediated cadmium toxicity. Toxicol Ind Health 26: 459-467.
37. Guilhermino L, Soares AM, Carvalho AP, Lopes MC (1998) Effects of cadmium and parathion exposure on hematology and blood biochemistry of adult male rats. Bull Environ Contam Toxicol 60: 52-59.
38. Dwivedi VK, Arya A, Gupta H, Bhatnagar A, Kumar P, et al. (2011) Chelating ability of Sulbactomax drug in arsenic intoxication. Afri J Biochem Res 5: 307-3014.