Prevention of Cadmium Toxicity by Ceftriaxone plus Sulbactam with VRP1034 in Rats
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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 CdCl₂ (1.5 mg/4 ml body weight) through gastric gavages for 21 days. Group III was received CdCl₂ 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), malonaldehyde (p<0.001), MPO levels along with significant increased hemoglobin (p<0.01), red blood cell (p<0.05), hematocrit(p<0.05) levels and antidote to the different molecular targets in human organs. It is well known that ceftriaxone plus sulbactam with VRP1034 treated group as compared to cadmium exposed group. δ-aminovaleric acid dehydroxalase 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<0.01) 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 activity; Hepatic and renal tissues

Abbreviations: SOD- Superoxide dismutase; CAT- Catalase; GR- Glutathione reductase; Gpx- Glutathione peroxidase; GST- Glutathione S- transferase; MDA- Malonaldehyde; GSH- Reduced glutathione; GSSG- Oxidised glutathione; MPO- Myeloperoxidase; 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 cephalosporin class of antibiotic whereas sulbactam is β-lactamase inhibitor. It is well reported that cephalosporins act as multideterminate chelating agents [8, 9]. Beside this, cephalosporins are known to 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 invivo 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-aminovaleric acid (δ-ALA) was purchased from Sigma Chemical, St Louis, MO, USA. Cadmium chloride was purchased from Sigma Chemical, St Louis, MO, USA.

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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 as given below.

Group I: Control normal saline treated group
Group II: CdCl₂ induced group (1.5 mg /4 ml/kg body weight)
Group III: CdCl₂+Ceftriaxone plus sulbactam with VRP1034 treated group (155.0 mg/kg body weight/day)

Cadmium chloride (CdCl₂) was given to sixteen animals through gastric gavage daily for 21 days and eight animals received placebo 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 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 1.5 ml of supernatant was taken in clean test tubes and centrifuge, 1.5 ml of supernatant was carefully taken into other polypropylene tube and stored at 2-8°C for the measurement of antioxidant enzymatic parameters.

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 extinction coefficient 6.1×10³ 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 H₂O₂ (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 H₂O₂ 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 NADPH, 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 NADPH 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 6.22×10³.

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

GPx activity was measured by the method described by Rotruck et al.
Measurement of myeloperoxidase

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 H₂O₂ (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 malondialdehyde (MDA) formed, essentially according to method of Ohkawa et al. [24]. It was determined by thiobarbituric 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 acetatic acid, 1.5 ml of 0.8% thiobarbituric 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 extinction coefficient 1.56×10⁵ 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% HNO₃ 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<0.01) in brain, (p<0.01; p<0.05), in liver (p<0.001) and (p<0.01; p<0.05) 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<0.01) 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 (p<0.001) increased in plasma and all tissue of CdCl10 exposed group (Tables 1 and 2). The GSH level was significantly (p<0.001) increased along with significant increased their ratio in the plasma and tissues of treated group as compared to control group. Where p<0.001; (highly significant) p<0.01; (significant) p<0.05; (ns) p>0.05.

Figure 1: Effect of Ceftriaxone plus sulbactam with VRP1034 on Superoxide dismutase 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>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>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; (significantly) and p>0.05.

Figure 4: Effect of Sulbactam 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>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>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 malonaldehyde (MDA) formed. There was highly significant (p<0.001) increased malonaldehyde (MDA) and myeloperoxidase (MPO) levels in plasma and tissues of CdCl₂ 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, 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 CdCl₂ 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 CdCl₂ 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 CdCl₂ 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 CdCl₂ 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 cysteolic protein to which cadmium binds. It is well known that heavy metals impairs hem 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 hemoglobin (Hb) levels along with increased cadmium concentration in blood and all tissues of CdCl₂, 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 hem synthesis pathway that leads to anemia. The heme metabolic pathway 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 CdCl₂ for 21 days via gastric gavage a significant higher the malonaldehyde (a marker of free radical), myeloperoxidase 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

<table>
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<tr>
<th>Groups</th>
<th>Plasma</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
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<td></td>
<td>GSH</td>
<td>GSSG</td>
<td>GSH</td>
<td>GSSG</td>
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<tr>
<td>Control group</td>
<td>3.96 ± 0.96</td>
<td>0.81 ± 0.17</td>
<td>4.89 ± 0.88</td>
<td>0.55 ± 0.11</td>
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<tr>
<td>Cadmium exposed group</td>
<td>1.55 ± 0.48</td>
<td>1.03 ± 0.64</td>
<td>2.25 ± 1.01</td>
<td>0.89 ± 0.091</td>
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<td>Ceftriaxone plus sulbactam</td>
<td>2.09 ± 0.85</td>
<td>0.78 ± 11</td>
<td>3.96 ± 0.66</td>
<td>0.64 ± 0.10</td>
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<tr>
<td>with VRP1034 treated group</td>
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</tbody>
</table>

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 (non significant).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
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<td>GSH/GSSG</td>
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<td>Control group</td>
<td>4.88</td>
<td>4.33 ± 0.98</td>
<td>8.89</td>
<td>121.4 ± 10.85</td>
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<tr>
<td>Cadmium exposed group</td>
<td>1.5</td>
<td>1.57 ± 0.34</td>
<td>2.52</td>
<td>98.7 ± 8.17</td>
</tr>
<tr>
<td>Ceftriaxone plus sulbactam</td>
<td>2.68</td>
<td>1.99 ± 0.73</td>
<td>6.19</td>
<td>104.3 ± 10.21</td>
</tr>
<tr>
<td>with VRP1034 treated group</td>
<td></td>
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</tr>
</tbody>
</table>

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 (non significant).
administration of CdCl₂, 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 seen 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 cadmium intoxication study has also reported that the increased lipid peroxidation in tissues (SOD, Catalase, GR and GPx) activities along with increased lipid peroxidation in tissues (MDA) and MPO levels 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) “p>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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma (nmole/min/ml)</th>
<th>Liver (nmole/min/gm tissue)</th>
<th>Kidney (nmole/min/gm tissue)</th>
<th>Brain (nmole/min/gm tissue)</th>
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<tr>
<td></td>
<td>MDA</td>
<td>MPO</td>
<td>MDA</td>
<td>MPO</td>
</tr>
<tr>
<td>Control group</td>
<td>153.1 ± 7.38</td>
<td>83.41 ± 5.86</td>
<td>23.10 ± 3.54</td>
<td>15.46 ± 0.75</td>
</tr>
<tr>
<td>Cadmium exposed group</td>
<td>274.3 ± 10.29</td>
<td>115.20 ± 6.01</td>
<td>342.1 ± 10.59</td>
<td>35.6 ± 0.42</td>
</tr>
<tr>
<td>Ceftriaxone plus sulbactam</td>
<td>210.64 ± 11.52</td>
<td>103.36 ± 8.28</td>
<td>361.87 ± 8.56</td>
<td>3.96 ± 0.22</td>
</tr>
<tr>
<td>with VRP1034 treated group</td>
<td>62.5 ± 1.99</td>
<td>29.42 ± 2.63</td>
<td>33.98 ± 1.76</td>
<td>15.26 ± 2.37</td>
</tr>
</tbody>
</table>

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) “p>0.05 (non significant).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>XO</th>
<th>GST</th>
<th>XO</th>
<th>GST</th>
<th>XO</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>135.4 ± 8.56</td>
<td>14.33 ± 1.52</td>
<td>144.3 ± 2.65</td>
<td>25.12 ± 1.87</td>
<td>36.64 ± 3.78</td>
<td>9.11 ± 1.02</td>
</tr>
<tr>
<td>Cd exposed group</td>
<td>296.3 ± 10.22</td>
<td>18.96 ± 1.10**</td>
<td>87.33 ± 6.02**</td>
<td>51.05 ± 2.64**</td>
<td>42.16 ± 2.81**</td>
<td>13.40 ± 1.47**</td>
</tr>
<tr>
<td>Ceftriaxone plus sulbactam</td>
<td>168.7 ± 14.31**</td>
<td>13.74 ± 0.55**</td>
<td>71.82 ± 4.49**</td>
<td>37.0 ± 2.09**</td>
<td>56.22 ± 1.77**</td>
<td>10.00 ± 1.12**</td>
</tr>
<tr>
<td>with VRP1034 group</td>
<td>88.56 ± 4.56</td>
<td>115.12 ± 1.07**</td>
<td>7.54 ± 1.75**</td>
<td>5.98 ± 0.63**</td>
<td>73.63 ± 1.55**</td>
<td>5.84 ± 0.13**</td>
</tr>
</tbody>
</table>

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) “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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma (IU/L)</th>
<th>Liver (µMole/gm tissue)</th>
<th>Kidney (µMole/gm tissue)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGOT</td>
<td>SGPT</td>
<td>Creatinine</td>
<td>ALP</td>
</tr>
<tr>
<td>Control group</td>
<td>10.10 ± 2.45</td>
<td>12.97 ± 2.63</td>
<td>1.02 ± 0.12</td>
<td>19.56 ± 3.54</td>
</tr>
<tr>
<td>Cadmium exposed group</td>
<td>88.56 ± 4.56</td>
<td>115.12 ± 1.07**</td>
<td>7.54 ± 1.75**</td>
<td>5.98 ± 0.63**</td>
</tr>
<tr>
<td>Ceftriaxone plus sulbactam</td>
<td>34.12 ± 5.47**</td>
<td>66.39 ± 3.25**</td>
<td>3.23 ± 0.22**</td>
<td>14.25 ± 1.14**</td>
</tr>
<tr>
<td>with VRP1034 group</td>
<td>11.22 ± 0.99**</td>
<td>7.20 ± 0.65**</td>
<td>37.78 ± 0.6**</td>
<td>7.84 ± 0.58**</td>
</tr>
</tbody>
</table>

All data are Mean ± SD of each group. These parameters were expressed in the plasma (IU/L) 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) “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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb (g/dL)</th>
<th>RBC (10⁶/µL)</th>
<th>HCT %</th>
<th>WBC (10⁶/µL)</th>
<th>5- ALAD in blood (nmole/min/ml erythocyte)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>13.93 ± 0.19</td>
<td>7.36 ± 0.10</td>
<td>39.38 ± 0.86</td>
<td>5.12 ± 0.81</td>
<td>9.14 ± 0.71</td>
</tr>
<tr>
<td>Cd-exposed</td>
<td>9.27 ± 0.55</td>
<td>7.01 ± 0.08</td>
<td>36.89 ± 0.43</td>
<td>11.25 ± 1.31</td>
<td>3.55 ± 0.61</td>
</tr>
<tr>
<td>Cd+Ceftriaxone plus sulbactam</td>
<td>11.22 ± 0.99</td>
<td>7.20 ± 0.65</td>
<td>37.78 ± 0.6</td>
<td>7.84 ± 0.58</td>
<td>6.74 ± 0.50</td>
</tr>
</tbody>
</table>

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) “p>0.05 (non significant).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood (µg/ml)</th>
<th>Liver (µg/gm tissue)</th>
<th>Kidney (µg/gm tissue)</th>
<th>Brain (µg/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA</td>
<td>MPO</td>
<td>MDA</td>
<td>MPO</td>
</tr>
<tr>
<td>Control group</td>
<td>0.15 ± 0.01</td>
<td>0.68 ± 0.13</td>
<td>0.12 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Cd exposed group</td>
<td>11.79 ± 2.47</td>
<td>16.87 ± 1.17**</td>
<td>13.49 ± 2.21**</td>
<td>3.89 ± 0.27</td>
</tr>
<tr>
<td>Ceftriaxone plus sulbactam</td>
<td>6.75 ± 0.89**</td>
<td>10.11 ± 0.83**</td>
<td>9.55 ± 1.07**</td>
<td>0.62 ± 0.12**</td>
</tr>
</tbody>
</table>

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) “p>0.05 (non significant).

Blood (µg/ml)                     Liver (µg/gm tissue)                   Kidney (µg/gm tissue)                   Brain (µg/gm tissue)  
<table>
<thead>
<tr>
<th></th>
<th>Zinc</th>
<th>Fe</th>
<th>Zinc</th>
<th>Fe</th>
<th>Zinc</th>
<th>Fe</th>
<th>Zinc</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>4.11 ± 0.28</td>
<td>2.89 ± 0.21</td>
<td>38.96 ± 2.45</td>
<td>15.65 ± 1.02</td>
<td>20.11 ± 1.25</td>
<td>9.61 ± 1.58</td>
<td>10.25 ± 0.98</td>
<td>20.12</td>
</tr>
<tr>
<td>Cd exposed group</td>
<td>6.37 ± 0.55</td>
<td>0.33 ± 0.014</td>
<td>21.04 ± 3.22</td>
<td>8.87 ± 1.56</td>
<td>18.96 ± 1.93</td>
<td>3.67 ± 0.59</td>
<td>6.69 ± 0.71</td>
<td>6.78 ± 1.15</td>
</tr>
<tr>
<td>Ceftriaxone plus sulbactam with VRP1034 group</td>
<td>5.02 ± 0.19</td>
<td>0.72 ± 0.011</td>
<td>24.56 ± 1.5</td>
<td>10.23 ± 2.10</td>
<td>19.44 ± 2.76</td>
<td>5.03 ± 0.97</td>
<td>9.85 ± 1.28</td>
<td>13.87 ± 2.31</td>
</tr>
</tbody>
</table>

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 (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 (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 effect 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 sulfhydryl 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

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