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# Phytoremediation of Cd induced renal toxicity in *Rattusnorvegicus* by Catharanthusroseus

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***Abstract***

*Cadmium is the second most hazardous heavy metal.It is a highly toxic metal with a very high bio-concentration factor (BCF>100). W.H.O. permissible groundwater cadmium concentration is 0.005 mg/L only, but reality is far away from this limit. It is widely distributed in the environment due to its use in industry.Cadmium is hazardous both by inhalation and ingestion and can cause acute and chronic toxicity. Present study has been designated to find out the renal changes at functional level after cadmium intoxication and protection against these changes offered by Catharanthus roseus. For the purpose albino rats were selected as the model organism. Cadmium significantly increases the level of serum proteins and nitrogenous wastes, showing reduced filtration rate of kidneys.Renal oxidative stress was determined by renal thiobarbituric acid reactive substance levels, enzymatic activity of superoxidase dismutase and glutathione peroxidase. Pretreatment with Catharanthus roseus leaf extract is effective in ameliorating the signs of nephrotoxicity. These findings conclude that Cadmium exposure affects renal functioning but Catharanthus roseus could prevent it, proving its nephro-protective potential against heavy metal toxicity.*

***Keywords:*** *Cadmium, Catharanthus roseus, Nephrotoxicity.Rattusnorvegicus*

**Introduction**

Heavy metals are the most consistent pollutants of present environment due to their non-biodegradablenature and bioaccumulation potential.Cadmium is widespread industrial and environmental pollution that may cause adverse harmful effects on human and animals.

Major sources of cadmium exposure are mining, rechargeable alkaline batteries as an electrode component, for special alloys production and also present in tobacco smoke. About the remaining part is used in coatings, pigments and electroplating and as a plastic stabilizer, vapor lamps, engraving, fertilizers and old galvanized PVC water supply pipes. Recently the rechargeable batteries, and all old electronic materials, the major component of all electronic and electrical wastes have arisen as the biggest source of cadmium exposure. These sources percolate cadmium to the groundwater, thus exposure by drinking water is major source after tobacco smoking.

Inside the body, cadmium primarily encounters with kidneys for its removal, thus are the major organs bearing the cadmium load. Kidney damage has long since been described to be the main problem for patients chronically exposed to cadmium chronic oral exposure to cadmium leads to renal failure (Anetor*.,* 2002). Impaired renal functions can be observed by measuring serum level of nitrogenous wastes, and serum proteins.

*Catharanthus roseus* (Madagascar periwinkle) is a perennial herb belonging to the family Apocynaceae. It produces over 100 different terpenoidindole alkaloids (TIAs), some of which exhibit strong pharmacological activities and are essentially used in clinical treatment of various diseases (Van Der Heijden*et al.,* 2004).Vinblastine and vincristine, which have been used clinically to treat cancers since 1950s, are the most valuable dimeric TIAs in C. roseus (Leveque, D *et al.,* 2007).

**Material and Methods:-**

Forty eight adult male albino rats (*Rattusnorvegicus*) of almost same age and weight (200 ± 10 gm), were procured from inbred colony. They were acclimatized at room temperature with 12 hr dark/light cycle and fed on standard diet and water *ad-libitum.* All experiments were performed as per animal institutional ethical committee (**360/01/CPSEA/2001**).

The experimental compound Cadmium chloride was obtained from Merck, India. The LD50 for cadmium chloride was determined by log dose / probit regression line method (Cherian *et al*., 1985) as 88 mg/kg body weight.

GC/MS analysis of leaf extract was also performed to find out its major constituents. The safe dose of *Catharanthus roseus* was calculated by performing a safety trial and it was found to be 500 mg/100 gm body weight.

Animals were divided into four groups. **Group A** (control) received distilled water only, group B treated with cadmium chloride, group C treated with *Catharanthus*

*roseus* and group D received Catharanthus roseus two hours prior to cadmium intoxication, for 1, 7, 14 and 21 days respectively (Table - 1).

To determine the renal function, the blood samples were collected from the ventricle of heart and serum was separated for the determination of

- **Serum urea** by Urea Berthelot Method (Choudhary H *et al.,* 2001)
- **Serum BUN** by Urea Berthelot Method (Choudhary H *et al.,* 2001)
- **Serum uric acid** by UricaseTrinder Method (Choudhary H *et al.,* 2001)
- **Serum creatinine** by Alkaline Picrate Method (Denham MJ *et al*., 1975).
- **Serum protein profile** by Modified Biuret and Dumas method (Dioka et al., 2004).

The results were analyzed statistically using analysis of variance (ANOVA) followed by multiple comparison of data by SNK (Student's NewmannKeul) test. Values were signified at the level of $p<0.05$ (Dumas BT *et al.,* 1975).

**Result**

Cadmium intoxication causes significant enhancement in the serum levels of nitrogenous wastes while reduction in that of serum proteins but pretreatment with *Catharanthus roseus* extract significantly retains these levels to normalcy. Whereas only *Catharanthus roseus* treated group showed no significant change as compare to control group.

**Table 1:-Acute & sub-acute doses (with respective units) of cadmium chloride and *Catharanthusroseus*for *Rattusnorvegicus***

| Groups Sets | Group- A (Control) | Group- B (Cadmium treated) | Group- C (Catharanthus roseus) | Group- D (Catharanthus roseus + cadmium treated) |
|---|---|---|---|---|
| Set: I Acute (1 day) | Water | 8.8 mg/kg body weight | 250 mg/kg body weight | 250 mg/kg body weight + 8.8 mg/kg body weight |
| Set: II Sub-acute (7 days) | Water | 1.26 mg/kg body weight | 250 mg/kg body weight | 250 mg/kg body weight + 1.26 mg/kg body weight |
| Set: III Sub-acute (14 days) | Water | 0.63 mg/kg body weight | 250 mg/kg body weight | 250 mg/kg body weight + 0.63 mg/kg body weight |
| Set: IV Sub-acute (21 days) | Water | 0.42 mg/kg body weight | 250 mg/kg body weight | 250 mg/kg body weight + 0.42 mg/kg body weight |

**Table 2:- Serum nitrogenous waste concentration (mg/dl) in albino rat after treatment with *Catharanthus roseus* followed by cadmium chloride** (values are expressed as Mean ±SEM)

| Serum nitrogenous waste | Treatment days | Control | Cadmium Treated | Catharanthus roseus Treated | Catharanthus roseus + Cadmium Treated |
|---|---|---|---|---|---|
| Urea | Acute (1day) | 17.42 ± 0.12 | 19.84 ± 0.62* | 17.19 ± 0.18 NS | 17.56 ± 0.07* |
| | Sub-acute (7days) | 16.56 ± 0.15 | 20.83 ± 0.34* | 17.26 ± 0.57 NS | 17.58 ± 0.12 NS |
| | Sub-acute (14days) | 17.72 ± 0.11 | 20.56 ± 0.35* | 17.61 ± 0.03 NS | 17.57 ± 0.98 NS |
| | Sub- acute (21days) | 17.37 ± 0.27 | 20.87 ± 0.41* | 17.28 ± 0.47 NS | 17.59 ± 0.29 NS |
| BUN | Acute (1day) | 6.98 ± 0.08 | 9.58 ± 0.16* | 7.64 ± 0.08 NS | 8.02 ± 0.16* |
| | Sub-acute (7days) | 7.94 ± 0.04 | 10.01 ± 0.13* | 7.98 ± 0.53 NS | 8.09 ± 0.9 |
| | Sub-acute (14days) | 8.01 ± 0.08 | 9.89 ± 0.16* | 7.37 ± 0.124 NS | 8.36 ± 0.47 |
| | Sub- acute (21days) | 7.64 ± 0.04 | 10.25 ± 0.10* | 7.98 ± 0.02 NS | 8.59 ± 0.64 |
| Uric Acid | Acute (1day) | 2.12 ± 0.09 | 2.27 ± 0.05* | 2.13 ± 0.02 NS | 2.09 ± 0.02* |
| | Sub-acute (7days) | 2.09 ± 0.13 | 2.28 ± 0.05* | 2.19 ± 0.01 NS | 2.09 ± 0.01 NS |
| | Sub-acute (14days) | 2.08 ± 0.08 | 2.29 ± 0.06* | 2.17 ± 0.03 NS | 2.07 ± 0.04 NS |
| | Sub- acute (21days) | 2.06 ± 0.08 | 2.34 ± 0.06* | 2.13 ± 0.01 NS | 2.13 ± 0.02 NS |
| Creatinin | Acute (1day) | 0.57 ± 0.17 | 1.02 ± 0.08* | 0.89 ± 0.03 NS | 0.96 ± 0.02 NS |
| | Sub-acute (7days) | 0.63 ± 0.09 | 0.99 ± 0.08* | 0.88 ± 0.04 NS | 0.98 ± 0.02 NS |
| | Sub-acute (14days) | 0.62 ± 0.08 | 0.97 ± 0.05* | 0.93 ± 0.07 NS | 0.95 ± 0.01 NS |
| | Sub- acute (21days) | 0.65 ± 0.03 | 1.01 ± 0.03* | 0.98 ± 0.06 NS | 0.98 ± 0.04 NS |

**Table 3:- Effect of *Catharanthus roseus*against cadmium intoxication on serum protein - Profile (g/dl) of albino rats (**Values are expressed as mean ± SEM**)**

| Serum Proteins | Treatment Day | Control | Cadmium Treated | Catharanthus Treated | Cadmium + Catharanthus Treated |
|---|---|---|---|---|---|
| **Total Proteins (g/dl)** | Acute (1day) | 7.01 ± 0.02 | 6.43 ± 0.02* | 7.83 ± 0.01 NS | 6.78 ± 0.17* |
| | Sub-Acute (7days) | 7.04 ± 0.01 | 5.89 ± 0.07* | 8.29 ± 0.07 NS | 6.98 ± 0.48* |
| | Sub- Acute (14days) | 6.98 ± 0.17 | 5.16 ± 0.09* | 7.89 ± 0.23 NS | 7.67 ± 0.24 |
| | Sub- Acute (14days) | 6.94 ± 0.12 | 4.51 ± 0.08* | 7.87 ± 0.09 NS | 7.51 ± 0.31 |
| **Albumins (g/dl)** | Acute (1day) | 4.27 ± 0.19 | 4.17 ± 0.09* | 4.68 ± 0.23 NS | 4.17 ± 0.09* |
| | Sub-Acute (7days) | 4.68 ± 0.17 | 3.89 ± 0.17* | 4.47 ± 0.19 NS | 4.29 ± 0.06* |
| | Sub- Acute (14days) | 4.89 ± 0.41 | 3.78 ± 0.08* | 4.87 ± 0.17 NS | 4.67 ± 0.07 |
| | Sub- Acute (14days) | 4.87 ± 0.39 | 3.69 ± 0.07* | 4.89 ± 0.21 NS | 4.69 ± 0.08 |
| **Globulin (g/dl)** | Acute (1day) | 4.09 ± 0.09 | 3.98 ± 0.01* | 4.17 ± 0.09 NS | 4.49 ± 0.09 NS |
| | Sub-Acute (7days) | 3.89 ± 0.09 | 3.19 ± 0.03* | 4.08 ± 0.008 NS | 4.27 ± 0.009 NS |
| | Sub- Acute (14days) | 4.17 ± 0.08 | 2.81 ± 0.03* | 4.27 ± 0.07 NS | 4.28 ± 0.09 NS |
| | Sub- Acute (14days) | 4.08 ± 0.05 | 2.87 ± 0.02* | 4.29 ± 0.05 NS | 4.63 ± 0.03 NS |
| **A/G ratio** | Acute (1day) | 2.34 ± 0.02 | 2.09 ± 0.01* | 2.21 ± 0.04 NS | 2.13 ± 0.02 |
| | Sub-Acute (7days) | 2.41 ± 0.04 | 2.64 ± 0.03* | 2.38 ± 0.03NS | 2.42 ± 0.09 |
| | Sub- Acute (14days) | 2.09 ± 0.03 | 2.24 ± 0.18* | 2.09 ± 0.03 NS | 2.12 ± 0.05 |
| | Sub- Acute (14days) | 2.11 ± 0.04 | 2.63 ± 0.04* | 2.18 ± 0.01 NS | 2.09 ± 0.03 |

## Discussion

Most studies on cadmium toxicity, therefore center on the detection of early signs of kidney dysfunction which results from oxidative damage by producing ROS, and decreasing the biological.Its absorbed from the alimentary tract, metal forms durable combination with the protein thionein forming metallothionein, which play an important role in further metabolism of this metal. Cadmium-Metallothionein complex reaches kidneys for its elimination, so considered to be the most susceptible organs for metals (Hollis L *et al.,*2001).

The present study demonstrated that Cd is renal toxicant and *Catharanthus roseus* by its antagonistic properties can reduce cadmium toxicity on serum parameter level related to renal function. Serum creatinine, urea and uric acid is used for assessing renal glomerular function. For this purpose the level of Cd toxicity futher suggest that during the sub-acute and acute doses the accumulated metal in the renal cortex also results in disturbance of normal function of kidney, serum nitrogenous wastes and Protein profiles are generally considered (Marya RK., 2003). According to the Anetor (2002) and Dioka *et al*. (2004), the serum nitrogenous waste concentration is determined largely by the efficiency of renal clearance. Accumulation of cadmium in the kidney results in sodium and potassium serum levels significantly increase and loss of tubular function, leading to tubular proteinuria. Cadmium interferes with glomerular filtration rate (GFR) and retention of sodium and decreased water excretion. Actually cadmium increase sodium reabsorption. Cadmium also leads to cell membrane depolarization and decrease cell membrane resistance and stimulates potassium channels and increase extracellular potassium concentration (Jungwirth A, 1990; Newman DJ., 1999).Serum protein concentration in Cd treated group was significantly lower than control group. These observed decrease serum protein following Cd in serum proteins following Cd exposure may be due to many reasons. One of the most toxic effects of Cd on kidney is proteinuria.

GC-MS analysis of essential oil of *Catharanthusroseus*reveals that terpenoids and phenylpropanoids are its major ingredients. Due to these components *Catharanthus roseus* extract good antioxidant activity, which compensate with oxidative stress induced by cadmium. This compensation may overcome the glomerular endothelial and tubular injury of kidney leading to the retention of renal functions thus normalized level of serum nitrogenous wastes and protein profiles.

The findings of study conclude that *Catharanthus roseus* is an effective nephroprotective agent. Further separation and characterization of its active constituent may establish it as a future novel drug against toxic heavy metals.

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