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Analysis of cadmium chloride toxicity in rats and its amelioration with *Murraya koenigii* leaves extracts

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Abstract
Objective: The main objective of this study was to examine the protective action of aqueous extract of *Murraya koenigii* against cadmium chloride toxicity in wistar albino rats.
Methods: for *in vivo* analysis, albino rats were divided into four groups. Control, CuLE (Curry leaf extract) treated, cadmium treated and aqueous extract protected groups. The rats were treated with 6.5 mg/kg body weight cadmium chloride intraperitoneally. And oral route of administration was preferred for curry leaf extract administration.
Results: Increased levels of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase enzymes (SGPT) were recorded. The level of antioxidant enzymes, Superoxide dismutase and Catalase were also found altered with the exposure of cadmium chloride. All these changes were ameliorative when the rats were treated with curry leaf extract.
Conclusion: Based on the findings we may conclude that aqueous extract of *Murraya koenigii* can provide complete protection against cadmium chloride toxicity in rats.

Keywords: cadmium chloride, curry leaf extract, antioxidants, biomarkers

Introduction

In recent years the concentration of heavy metals in environment has increased [1]. The exposure to the toxic metals has become an increasingly recognized source of illness worldwide [2, 3]. Cadmium is a well-known heavy metal present in the environment and causes serious environmental and occupational hazard to human [4, 5]. It released into the environment by smelting and mining operations [6], fuel combustion [7], incineration of municipal waste [8], sewage sludge [9], and application of phosphate fertilizer [10]. Humans can also receive cadmium from crops and vegetables [11], tobacco [12], soil [13], and from fruits and oily seeds [14]. It may also be found in animal's milk [15]. Cadmium causes poisoning in various tissues of animals and humans [16, 17]. Uptake of Cadmium into the liver is critical for the development of overall heavy metal induced toxicity. Approximately half of the cadmium absorbed systemically is rapidly accumulated in the liver, which results in the reduced availability of cadmium to kidneys and testes, which are more sensitive to its toxic actions [18]. The cadmium toxic effects are mainly due to its inhibition of liver metabolic enzyme systems containing sulphydryl groups and uncoupling of oxidative phosphorylation in mitochondria [19], which results in increased lipid peroxidation, hepatic congestion, ischemia and hypoxia [20]. Productions of ROS (Reactive oxygen species) and oxidative tissue damage due to cadmium have been associated with hepatotoxicity [21]. Moreover, a variety of changes in the antioxidant defense enzymes are reported [22]. It has been shown that antioxidants and free radical scavengers are useful against cadmium induced toxicity [23]. Efforts have been made to find safe and potent natural antioxidants from plant sources [24].

Murraya koenigii (Family: Rutaceae) also known as Kadi patta or Curry leaf, is broadly used in Indian cookery for flavouring food items, and is a treasure of beneficial components which include glycosides, carbazole alkaloids, koenigin, phenolic compounds, flavanoids, resin and volatile oil [25-26]. Since ancient times the plant has been used in the traditional system of medicine [27]. Several pharmacological activities, antidiabetic, vasodialtory, hypocholesterolemic, antiulcer, anti-diarrheal, phagocytic, analgesic, antinociceptive and wound healing have already been reported in MK leaves. *Murraya koenigii* leaves have been reported to increase digestive secretions and relieve nausea, indigestion, and vomiting [28]. The leaf extract of *Murraya koenigii* has recently been shown to possess antioxidant potential and also has been shown to provide protection against oxidative stress [29]. The aqueous Leaf extract of *Murraya koenigii* has been shown to reduce lipid peroxidation and to decrease cellular damage, thereby protecting liver from ethanol induced toxicity [30]. Another study showed antioxidant potential of *Murraya koenigii* [31]. In vitro studies showed antioxidant and free radical scavenging activity of curry leaf extracts [32]. Scientists characterized the flavonol profile of MK by LC–MS–MS with different solvents and explained the relative antioxidant capacity[33]. According to them curry leaf has a much higher flavonol profile. Various carbazoles from curry leaf extract are known to possess antioxidative properties [34]. Keeping in view the pharmacological properties of *Murraya koenigii*, present investigation has been undertaken to assess the protective effect of *Murraya koenigii* extract on cadmium chloride induced toxicity in albino rats.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh leaves of *Murraya koenigii* were collected from different parts of Rewa district throughout the year during the course of the study. Identification of plant was done by Dr. S.N. Mishra, Retired professor, Department of Botany, Govt. Model Science College, Rewa, Madhya Pradesh.

2.2 Preparation of aqueous Curry leaf extract

For the preparation of aqueous extract, the fresh leaves of *Murraya koenigii* were collected and washed with normal water and kept at room temperature for 1 hour with its bottom covered with blotting paper to soak the excess water. The leaves were then dried in a hot air over at 50ºC for two hours till they were dry and crispy. The dried leaves were crushed into coarse dust with the help of mortar and pestle and then grinned in mechanical grinder to make the fine dust. The leaf powder was stored in airtight Tarson bottles at refrigerator until further use.

For the preparation of aqueous extract, the leaf powder was soaked in double distilled water overnight (7.5gm /100 ml of Double D/W), filtered through muslin cotton cloth and the filtrate was centrifuged at 5000 RPM for 10 min using a REMI cold centrifuge. The pellet was discarded and the supernatant, thus obtained was again filtered, collected in polypropylene tubes and frozen at refrigerator. The content of the tube was then dried and the dried material was stored until further use. A definite amount of the aqueous extract was always freshly dissolved in double distilled water to give a particular concentration. Any leftover of this solution was always discarded.

2.3 Chemicals used

All the chemicals used in the present study were of analytical grade.

2.4 Experimental Animals

All the experiments were carried out using wistar albino rats of either sex with body weight 160-180 gm. The animals were handled as per the guidelines of animal ethical committee, Government of India. The efforts were made to minimize the number of animals used.

2.5 Experimental design for in-vivo studies

The rats were randomly divided into four groups. (n=6). The treatment of rats was carried out as per the schedule mentioned below.

- Group I: Control Group
- Group II: Aqueous CuLE treated rats, dose 100 mg / kg body weight, administered orally every day for a period of 15 days.
- Group III: Cadmium chloride ($CdCl_2$) treated rats, route of administration intraperitoneal, dose 6.5 mg /kg body weight for a period of 15 days.
- Group IV: Aqueous CuLE and Cadmium treated rats, $CdCl_2$ was administered intraperitoneally at a dose of 6.5 mg/kg body weight for 15 days and CuLE was administered orally at a dose of 100 mg/kg body weight prior to the administration of cadmium chloride.

At the end of treatment period, animals of each group were kept fasted overnight. The body weight of animals of each group were measured and recorded individually. Blood was collected in two different set of tubes for biochemical estimations.

2.6 Measurement of the activities of the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT)

The values of SGOT and SGPT were measured using enzymes estimation kits. Enzyme activity was expressed as IU/L.

2.7 Measurement of Total Protein

The protein contents of the different samples were determined by the method of Lowry *et al.* (1951) using Bovine Serum albumin (BSA) as standard [35].

2.8 Determination of Superoxide dismutase (SOD) and Catalase (CAT) activities

The values of SOD and Catalase were estimated using lab kits. The enzyme activity was expressed as units/min/mg of protein and µmoles of H_2O_2 consumed/min/mg tissue protein respectively.

2.9 Statistical analysis

Each experiment was repeated at least three times with different rats. Data are represented as mean ± S.E.M of 6 animals.

3. Results

3.1 Survival and Mortality

No mortality occurred in any of the treatment groups during the whole experimental period.

3.2 Biomarkers of organ damage

Table No-01 illustrates the level of serum specific markers, namely SGOT and SGPT which were found to be significantly higher in cadmium treated groups when compared to control. The activities of these enzymes were found to be significantly decreased in the animals pre-treated with aqueous extract of *Murraya koenigii*. However, the extract itself did not alter the activities of these enzymes. The results indicate that the aqueous CuLE do possess the capability to provide protection against Cd induced hepatic damage.

Table 1: Effect of aqueous extract of *Murraya koenigii* on the values of SGOT, SGPT and Total protein in hepatic tissues of rats

Parameters	Groups			
	Control	CuLE	Cadmium	CuLE + Cadmium
SGOT (IU/L)	6.89 ± 0.161	6.26 ± 0.214	16.06 ± 0.189*	9.04 ± 0.118**
SGPT (IU/L)	7.34 ± 0.242	7.06 ± 0.132	13.04 ± 0.144*	9.13 ± 0.121**
TP (Mg/dl)	10.2 ± 0.39	9.1 ± 0.36	6.6 ± 0.44*	8.4 ± 0.29**

Values are expressed as Mean ± S.E.M of 6 animals in each group.
*P<0.001 compared to control; **P<0.001 compared to Cadmium treated group.

3.3 Status of antioxidant enzymes

Table No-02 indicates that treatment of rats with 6.5 mg/kg body weight cadmium chloride intraperitoneally caused alteration in the values of SOD and Catalase in the hepatic tissues. Pretreatment of CuLE at the dose of 100 mg/kg body weight protected the value of antioxidant enzymes from being altered.

Table 2: Effect of aqueous extract of *Murraya koenigii* on the values of CAT and SOD in hepatic tissues of rats

Parameters	Groups			
	Control	CuLE	Cadmium	CuLE + Cadmium
CATALASE (µmoles H_2O_2 consumed/min/mg protein)	164.02±4.14	172.27±5.06	122.51±6.08*	151.49±5.03**
SOD (Units/min/mg protein)	3.64 ± 0.069	3.03 ±0.085	5.46±0.136*	4.03 ± 0.054**

Values are expressed as Mean ± S.E.M of 6 animals in each group.
*P<0.001 compared to control; **P<0.001 compared to Cadmium treated group.

Figures (1-5): Effect of aqueous extract of *Murraya koenigii* (100 mg/kg) on the values of different parameters in cadmium chloride treated rats.

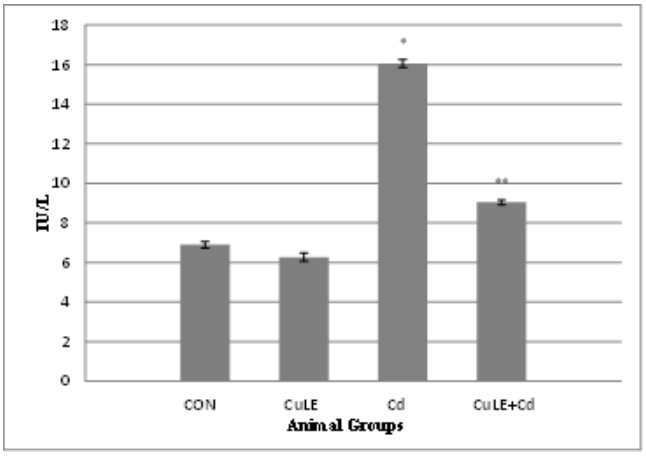

Fig 1: Effect of CuLE on the value of SGOT

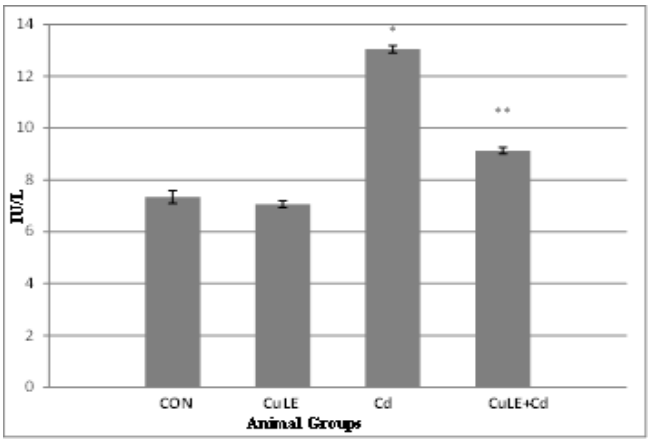

Fig 2: Effect of CuLE on the value of SGPT

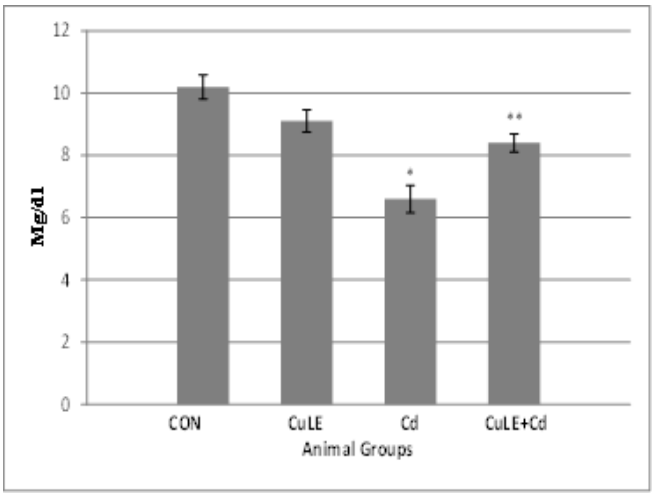

Fig 3: Effect of Cu LE on the value of TP

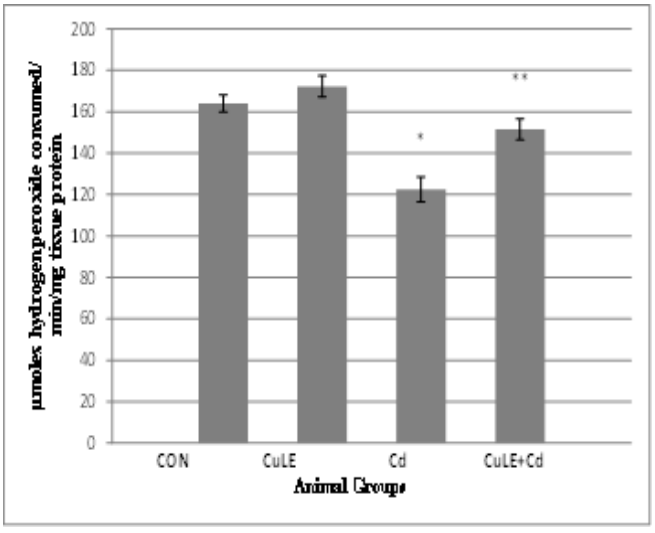

Fig 4: Effect of Cu LE on the value of CAT

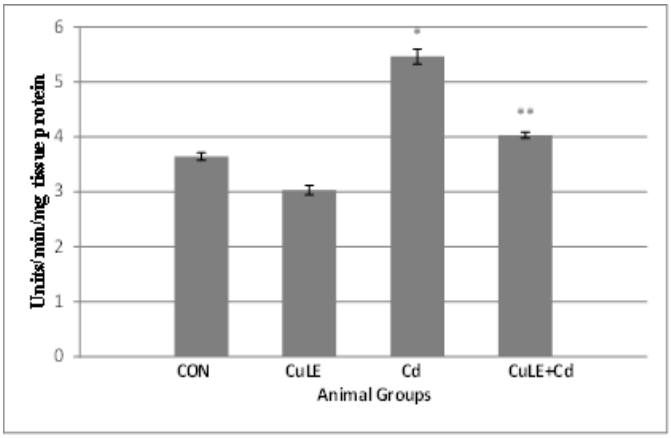

Fig 5: Effect of CuLE on the value of Superoxide dismutase

CON= Control, CuLE= Curry leaf extract; Cd= Cadmium; CuLE+ Cd= Curry leaf extract + Cadmium

Values are expressed as Mean ± SEM. *P<0.001 compared to control; **P <0.001 compared to Cadmium treated group.

4. Discussion

The present study depicts the protective activity of aqueous extract of *Murraya koenigii* against cadmium induced hepatic tissue damage in experimental rats.

Cadmium accumulates in tissues predominantly by binding to sulfhydryl group of proteins [36] and produces toxicity by inducing reactive oxygen species production through fenton reaction [37], elevating cytoplasmic Ca^{2+} levels by inhibiting Ca^{2+} export from cytoplasm [38] and reducing the antioxidant defense systems [39], there by produces oxidative stress.

In our present study, the serum levels of organ specific marker enzymes (SGOT, SGPT) were found increased indicating hepatic damage with the exposure of cadmium chloride. The present dose of cadmium (i.e., 6.5 mg/kg b.w for a period of fifteen days) not only produced significant changes in the parameters studied in comparison to control animals, but also there was no animal mortality during the entire treatment period. Increased levels of SGOT and SGPT point toward hepatic damage. Leakage of intracellular or membrane enzymes into the blood stream in large quantities indicates a loss of functional integrity of membrane architecture [40]. Administration of aqueous curry leaf extract, orally (i.e., 100 mg/kg b.w, fed orally) attenuated the cadmium-induced elevation of the serum levels of these marker enzymes which indicates the hepatoprotective activity of CuLE. This hepato-protection might have been due to the presence of phytochemical(s)/ phytonutrient(s) in the curry leaf extract.

The level of tissue total protein was also found altered with the exposure of cadmium chloride in rats. However, pretreatment of rats with aqueous curry leaf extract (100 mg/kg body weight) orally, attenuated the toxic effect of cadmium chloride and a protective value of total protein was recorded.

Catalase reduces the tissue injury by removing the H_2O_2. It catalyzes the conversion of hydrogen peroxide into water. SOD catalyzes the destruction of superoxide anion free radical by dismutation and H_2O_2 formation [41]. We demonstrated an increase in SOD activity with a concomitant decrease in the activity of catalase in the cadmium-treated rats. This indicates that H_2O_2 accumulated in the hepatic tissue, contributes partly to the damage caused by cadmium to this organ. Ikediobi *et al*. recorded increased activity of SOD in rat liver cells treated with cadmium and explained it as a response to accumulation of ROS (particularly H_2O_2 and O_2.-) in the cytosol induced by Cd. According to Ikediobi *et al*. cells can sense ROS and can induce specific responses [42]. Time-dependent leakage of Mn SOD from the mitochondria into the cytosol and a possible non-specific interaction with Cd provide rational explanations for a dramatic increase in SOD activity following prolonged exposure of cells to Cd. Catalase contains iron in its active site. The decreased activity of catalase may be due to the direct binding of the metal to the active site of the enzymes, or cadmium may decrease iron availability to the enzyme, or due to the increased usage of the enzyme in scavenging free radicals induced by the metal. Administration of aqueous CuLE protected the activities of SOD and CAT from getting altered in the hepatic tissue of cadmium-treated rats. The protection afforded by the aqueous CuLE may be due to the ability of the extract to reduce the accumulation of free radicals generated following cadmium treatment. The phyto-constituents in the extract may scavenge the ROS or may inhibit their formation. They may also reduce the levels of the pro-oxidants by up-regulation of the expression of the antioxidant enzymes and needs further investigation.

5. Conclusion

Administration of cadmium chloride at a dose of 6.5 mg/kg body weight, intraperitoneally, to the rats caused oxidative stress induced damage in the hepatic tissues. Pre-treatment of rats with the aqueous Curry leaf extract at a dose of 100 mg/kg body weight, fed orally ameliorated all these changes brought by cadmium. Aqueous CuLE seems to provide complete protection to the hepatic tissues of the rats against cadmium-induced oxidative stress through its direct as well as indirect antioxidant activity and also through its possible cadmium chelating properties. The results obtained here may be of future therapeutic significance particularly in the areas where man is chronically exposed to cadmium either occupationally or environmentally. As Curry leaves are part of a regular diet in India and many parts of the world, it may be used as a nutritional supplement to combat oxidative stress-induced tissue damage in the people exposed to cadmium.

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