

CADMIUM ACETATE INDUCED NEPHROTOXICITY AND PROTECTIVE ROLE OF CURCUMIN IN RATS

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ABSTRACT

Cadmium, found to be a toxic metal causes damage to organs such as kidney, lungs, bones, liver, brain and reproductive organ testes. But the most affected organ is kidney on cadmium exposure. In this study, cadmium toxicity and curcumin antioxidant activity against nephrotoxicity were analysed. Here twenty four rats were taken and divided into four groups,each group comprised of six animals each. The first group served as control group with a normal feed and water. The second group received cadmium acetate in distilled water(200 mg/kg of body weight) for 7 days. The third group received only curcumin, dissolved in glycerol (250 mg/kg of body weight) for the same experimental days. Finally, the fourth group is the pretreated group. Rats were treated with curcumin and cadmium acetate continuously for 7 days. At the end of experiment, animals were sacrificed and the blood sample, tissue samples analysed. The level of urea and creatinine in serum were found to be increased, the antioxidant enzymes like superoxide dismutase, catalase were decreased in tissue sample. All these, confirmed the protective activity of curcumin on induced nephrotoxicity.

Keywords: nephrotoxicity, superoxide dismutase, catalase, urea, creatinine.

INTRODUCTION

Heavy metals include both non-toxic and toxic elements iron (Fe), cobalt (co), copper (cu), manganese (mn), molybdenum (mo), and zinc (zn) are the trace elements and they are required in a very minute amount,whereas other metals are non-essential, toxic to animals and even fatal when accumulated. For example, mercury (hg), arsenic (as)/ lead (pb), plutonium (pu), vanadium (v), tungsten (w) and cadmium (cd). Cadmium, a heavy metal obtained as a by product of zinc, is one of the most toxic environmental and industrial pollutants, obtained through consumption of foods and drinking water, inhaled from air or cigarette smoking or from ingestion of contaminated soil and dust¹. It is a non biodegradable metal, volatile element, non essential, non beneficial to plants, animals and humans, toxic with a very long biological half life². Once cadmium entered into the body, gets accumulated in kidney, skeleton and vital organs like lungs and liver. In the kidney, proximal tubular cells are most primarily affected due to the accumulation.it belongs to group II of biochemical processes leading to abnormalities and in some cases causes³ fetal consequences even in a very low concentration.^{4,5,6}

Kidneys are the primary target organ for cadmium poisoning. Kidney are the two bean shaped organs located at the rear of the abdominal cavity. Each kidney has a convex and concave surface, surrounded by a tough fibrous tissue called renal capsule. Kidney mainly regulates electrolytes, acid - base balance and blood pressure, excretion of waste (urine), secretion of hormones including calcitriol, erythropoietin and enzyme renin. They are the important organ involved in metabolism, detoxification, storage and excretion of metabolites which are harmful to the body.

Cadmium is a toxic metal ions found in our environment causes a serious hazards even allergy at low concentration. It is a non-biodegradable, volatile element. Non beneficial to plants, animals and humans.

MATERIALS AND METHODS

Test animals

Totally 24 adult male - rats weighing about grams were used in the present work. In this work, four groups were taken, each group comprised of about six male healthy rats. The rats were selected and kept in a controlled conditions of about 12 hours dark and 12 hours light (alternative dark light cycle 12:12). All the animals were fed with a standard laboratory feed and water *libitum*. Care and treatment of the animals were performed according to ethical committee approval.

Treatment

Out of the four groups, three groups were used for treatment while one group serves as control.The control group received normal water and feed (group I). The induced group (group II) received cadmium acetate continuously for 7 days (200 mg/kg of body weight dissolved in water). The third group received only curcumin for 7 days (250 mg/kg of body weight dissolved in glycerol). Finally, the last group (group IV) received curcumin in the same concentration as third group, followed by cadmium acetate (200mg/kg of body weight dissolved in glycerol) about 7 days.

All these procedure were carried out orally. At the end of the experimental period, the animals were anaesthetized and sacrificed. Blood were collected and allowed to stand in standing position without any disturbances for about half-an-hour until the serum gets separates. Immediately, after sacrifice, kidney were taken, blotted with two filter papers to remove the excess blood, stored in 10% formalin solution for further analysis.

Serum collection

After half an hour, time interval, the blood samples were centrifuged at 1000rpm for 5 minutes. The serum gets collected at the supernatant. From the serum, following biochemical tests were performed. The concentration of urea, creatinine were measured.

Homogenate preparation

One kidney from each animal was immediately removed and washed using saline solution. Tissues were crushed and homogenized in a potter - elvehjem type homogenizer in ice - cold 0.1M phosphate buffer (pH 7.4). the homogenate was used for the determination of superoxide dismutase and catalase.

Serum sample analysis.

Function of kidney were assessed by measuring the concentration of urea and creatinine in serum. The concentration of urea was determined by the method of diacetyl monoxime using urea as a standard solution. The concentration of urea are expressed in mg/dl.⁷ The level of creatinine was assayed by the method of Jaffe's alkaline picrate method⁸ and the concentration of creatinine are expressed in mg/dl.

Antioxidant Assay

By using the homogenate, the activity of antioxidants, superoxide dismutase and catalase were assayed. Superoxide dismutase (SOD

EC.1.15.1.1) was determined by the method of Misra and Fridovich⁹. The activity of catalase was done by the method of Aebi 1983¹⁰. The units are expressed in unit/mg of protein.

Statistical analysis

Results of all the analysis were expressed as mean \pm S.D. The data were statistically analysed by using one way ANOVA variable followed by multiple analysis test. The significance was found to be $p < 0.005$ and $p < 0.001$.

RESULTS AND DISCUSSION

Heavy metal ions even at a very low level can cause serious health problems including humans and animals. It affects the growth and development, cancer formation, damage to organs, nervous system damage and in severe cases it leads to death. The sources of cadmium are mining, industry, burning coal, house hold wastes, cigarette smoking, fish and plants grown in cadmium-laden soil etc. From all these sources, cadmium gets entry into air, water, soil, and food.¹¹ Long term exposure to lower level of cadmium in contaminated air, water, soil and food leads to accumulation of cadmium in the kidneys and possibly causes kidney diseases.¹²

The present study was undertaken to evaluate the function and structure of kidney in cadmium acetate induced and treated rats.

Table 1 and figure 1 represents the levels of urea and creatinine in serum of four groups. On cadmium acetate exposure, the level of urea and creatinine were found to be increased in the serum (group II). These increased level were brought back to near normal on pretreatment with curcumin (group IV).

In liver, the protein molecules undergoes metabolism to form a final product of ammonia and carbon dioxide. The formed ammonia gets converted into urea in the liver. From the liver, urea freely diffuses, transported in blood to kidneys, where excreted in the urine. A small amount of urea enters into the intestine during its transported and gets broken down into carbon dioxide and ammonia by the activity of bacterial enzyme. Finally, ammonia is either lost in the feces or absorbed into the blood. creatinine is also another nitrogenous waste product that gets eliminated by the kidney. The excretion gets suppressed due to the insufficiency.

Table 1: The level of urea and creatinine in serum of four experimental groups.

Groups	Urea (mg/dl)	Creatinine(mg/dl)
Normal	15.46 \pm 0.29	0.66 \pm 0.045
Induced	17.63 \pm 0.24	1.10 \pm 0.030
Curcumin only	15.36 \pm 0.37	0.70 \pm 0.047
Curcumin+Cadmium acetate	16.34 \pm 0.36*	0.81 \pm 0.11**

All the values are analysed by ANOVA followed by multiple comparison test.

n = 6 animals per each group.

Comparison are made with normal group.

*indicates 0.01 significance,

**indicates 0.005significance.

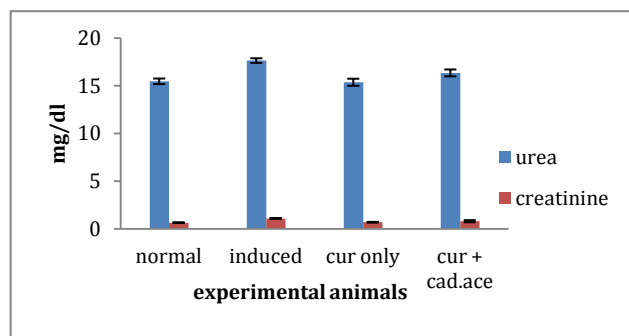


Figure 1: shows the concentration of urea and creatinine in serum of experimental animals.

In cadmium administered rats, the cadmium gets accumulated in the kidney, hence there is a defect in glomerular filtration. According to Gilrolami,¹³ the cadmium administration leads to a elevated level of creatinine and urea in urine. Due to the defect in filtration, the level of creatinine and urea gets increased in serum (group II) when compared to the normal (group I). These marked increase were reduced by treatment with curcumin (group IV). According to Lall et al (1997)¹⁴ rise in creatinine value is an indication of renal – tubular damage due to cadmium induced nephrotoxicity.¹⁵

Table 2 and figure 2 depicts the level of antioxidant enzymes, superoxide dismutase and catalase in tissue (kidney) sample. Cadmium enhances the peroxidation of membrane lipids and causes injury to the cellular components, due to interaction of cadmium ions with the cell organelles. Cadmium also depletes glutathione and protein bound sulphhydryl groups and results in a enhanced production of reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide. These reactive oxygen species causes an increased lipid peroxidation.^{16,17} The free radicals are scavenged by two enzymes superoxide dismutase and catalase in a normal condition. But, here, the cadmium binds to the imidazole group of the His-74 in SOD, which is a vital cofactor for the hydrogen peroxide metabolism¹⁸. Thus, the activity of SOD gets decreased under cadmium toxicity. Likewise, the enzyme catalase also carries a important cofactor, iron a essential trace elements required for metabolism of free radicals. Cadmium disturbs the absorption of iron from intestine, lead to a decreased level of iron in circulation. This leads to a decreased level of catalase in tissue. When rats were treated with curcumin, the observed antioxidant levels were normalized since curcumin has the property of antioxidant activity.^{19,20,21}

Table 2 :Depicts the activity of antioxidant enzymes SOD and CAT in tissue sample.

Groups	SOD	CAT
Normal	5.72 \pm 0.11	62.23 \pm 2.46
Induced	2.62 \pm 0.42	50.60 \pm 0.86
Curcumin only	6.30 \pm 0.62	62.08 \pm 1.29
Curcumin+Cadmium acetate	4.56 \pm 0.32*	56.07 \pm 1.46*

All the values are analysed by ANOVA followed by multiple comparison test.

n = 6 animals per each group.

Comparison are made with normal group.

*indicates 0.01 significance,

+indicates 0.01 significance.

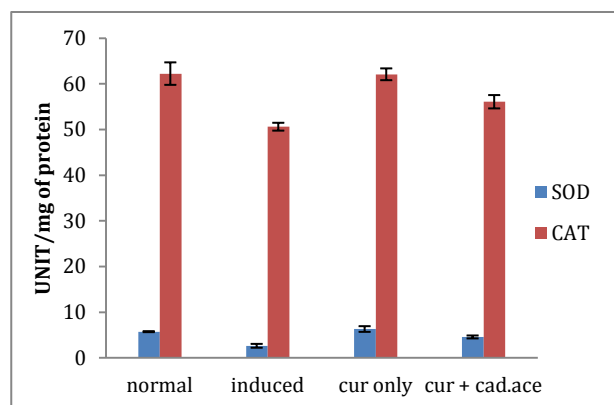


Figure 2 :Shows the activity of antioxidant enzymes in kidney tissue.

From all these , it was propounded that curcumin has the capacity to cure the cadmium acetate induced nephrotoxicity in rats.

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