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Research Article

A study of cadmium acetate induced toxicity and heptoprotective activities of curcumin in albino rats

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ABSTRACT

Pollution is a worldwide environmental problem that affects our environment in a wide range. These pollutants causes a great variety of health problems. Among them, toxic metals play an important role. The one comnmon toxic metal is cadmium. Curcumin (diferuoyl methane) is a well-knowm biologically active compound in tumeric derived from the rhizome of plant *curcuma longa* . *curcuma longa* is a gold coloured spice most commonly used in food as colouring agent and also for preservation of food. The aim of our present study was to evaluate the toxicity nature of cadmium acetate and antioxidant capacity of curcumin on liver injury in albino mice. The animals were randomly divided into four groups, each with six animals. Group I (control group) received normal feed, Group II was administered with cadmium acetate (200 mg/kg) dissolved in water, Group III was administered only with curcumin and Group IV received a dose of 250 mg/kg of curcumin and 200 mg/kg of cadmium acetate. The experiment was conducted for the period of 7 days. Rats treated with cadmium acetate alone showed a increased activity of ALT, AST and a decreased activity of ALP, Protein concentrartion in serum. And also a marked decline in antioxidant enzymes like Superoxide dismutase and Catalase in cadmium acetate induced rats. However, on combined treatment of rats with curcumin and cadmium acetate provoked the above changes more significantly, when compared to each of them as alone. Hence it is suggested that curcumin has a protective effect against a cadmium acetate induced hepatic injury.

Keywords: Antioxidant enzymes; Cadmium acetate; Curcumin; Hepatotoxicity.

INTRODUCTION

In all ecosystems, the metal and metal compounds are the natural constituents present, moving between three phases like atmosphere, hydrosphere and lithosphere. Increasingly these compounds are introduced into the environment and get accumulated in abiotic systems. On exposure to heavy metals, causes harmness, especially those metal compounds which do any have any physiological role in the cell metabolism. Usually heavy metals exhibit a high atomic weight and a density greater than water. Heavy metals are the important constituents present in the waste – derived fuels. There are more than 20 heavy metals and among them lead (pd), cadmium (cd), inorganic arsenic (As) comes under toxic metals. Even at a very low concentration these heavy metals can cause damage by accumulating in food chain and enter to the body and finally gets stored in the organs like liver, kidney.

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In our environment cadmium is a toxic metal, found in air, food, water and soil. (Fridberg L *et al*., 1986). It is a heavy metal and also a non biodegradable metal (Jarup *et al*., 2000). It has a long half life in humans. Typically, cadmium is found as a mineral by combining with other elements and exists as cadmium oxide, chloride, sulfide, acetate. In this combination form, it is highly resistant to conversion, hence causes numerous health risks and eventually leads to death.

On exposure to cadmium acetate, the first organ reached after uptake from the gastro intestinal absorption is the liver. In the liver, the metal gets accumulated and induces the hepatotoxicity (Mansour , 2000). The liver injury is dominated by apoptosis and necrosis, two modes of cell death. Cadmium – causes the release of free oxygen radicals (Llobert *et al*.,1998). These free radicals cause the stimulation and destruction of sensitive macromolecules and indeed tissues (Lafuente , 2000). Cadmium acetate being a metal, it exist in a positively – charged form and binds to a negatively charged organic molecules to form complexes. These complexed forms only enter and accumulate in the body tissue, causes cadmium toxicity. On long term exposure, cadmium acetate also causes renal toxicity, and lung toxicity.

Curcuma Longa, a perennial herb, grows to a height of three to five feet and a member of Zingiberaceae (ginger) family (Chainani , 2003). It is cultivated mostly in Asia, India, china and other tropical climate countries. The portion of plant most widely used in medicines in rhizome. It is boiled, cleaned and dried and used. One of the active constituents of turmeric is curcumin (diferuoyl methane). Curcumin has been found to exhibit various activities like antioxidant, (Kunchandy and Rao ,1990; Reddy and Lokesh , 1994 ; Masuda *et al*., 1993; Unnikrishnan and Rao, 1995 ; Cohly *et al* ., 1998). hepatoprotective, anti – inflammatory, anticarcinogenic (Steward *et al* ., 2010; Tennesen *et al*., 1992 ; Frank *et al*., 2003), antimicrobial (Mahady *et al* ., 2002; Han *et al*., 2005), cardiovascular, gastrointestinal effects, anti bacterial, antiamoebic, anti HIV activities(Suai *et al*., 1993) and other disorders (Amman *et al*., 1991)

The present investigation is an attempt to investigate the effect of cadmium on liver tissues of albino rats and the protective role of curcumin on cadmium acetate induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals and treatment

Adult male albino rats weighing 100-150gm were used in the present study. Ethical permission was obtained from the ethical committee before the study. The animals were housed in stainless steel cages under conventional condition (23±1^{o}c temperature, 12-h light-dark cycle, relative humidity 50 ± 10%) and also a free access to drinking water and a standard pellet diet.

All groups were treated by oral gavage once daily for 7days. At the end of the experiments, all the animals of all groups were anaesthetized using chloroform and sacrificed. The rats were divided into four experimental groups of six rats each as follows,

Group I: Normal feed

Group II: 200 mg / kg of cadmium acetate dissolved in distilled water. (Athar and Lobal , 1998, Chaung *et al* ., 2000)

Group III: 250 mg/ kg of curcumin only dissolved in glycerol.

Group IV: Pretreated with curcumin 250mg/ kg (as in group III) followed by cadmium acetate 200 mg / kg as (in group II).

The blood sample was collected by cervical decapitation and transferred to centrifuged tubes. Collected blood samples were allowed to stand in the room temperature at slanting position for half an hour, until serum was separated. Then the blood samples were centrifuged and the serum was collected. Various biochemical assays were conducted in the serum sample. The biochemical assays like AST, ALT, ALP, Protein are assayed.

Immediately after the sacrifice, one part of the liver from each animal was removed, washed and preserved in a ice cold saline solution (0.9%).

Tissue were minced and homogenised (10% w/v). Seperating in ice cold, 0.1M phosphate buffer (pH-7.4) in a potter-Elvejeum type homogenizer and the homogenate was used for the determination of antioxidant enzymes like superoxide dismutase and catalase.

Serum biochemial assays

From the collected serum, the enzymes like AST, ALT, and ALP were assayed. The enzyme aspartate transaminase (AST, E.C.2.6.1.1.) was assayed by the method of Reitman and Frankel (1957). The enzyme alanine transaminase (ALT, E.C.2.6.1.2.) was also assayed by the method of Reitman and Frankel (1957). The serum alkaline phosphatase activity (ALP E.C.3.1.3.1) was analysed by the method of king and Armstrong(1934) using disodium phenyl phosphate as a substrate, the values are expressed in IU/L. The concentration of protein was assayed by the method of Brafford, using serum bovine albumin as a standard solution and the values are expressed in gm/dl.

Tissue antioxidant assays

From the homogenate obtained by tissue centrifugation, the following antioxidant assays were made. The antioxidant enzyme superoxide dismutase (SOD E.C.1.15.1.1) was assayed by the method of Marklund and Marklund *et al*., (1974) and the activity of another antioxidant enzyme catalase (CAT E.C.1.11.16) was assayed by the mehod of sinha *et al*., (1972). The activity of both enzymes superoxide dismutase and catalase were expressed in unit/mg of protein.

Statistical analysis

The values were expressed as mean ± S.D (standard deviation). The statistical analysis of data was analyzed by one-way ANOVA followed by multiple analysis-test with $P<0.05$ and $P<0.01$ were considered as statistically significant.

RESULTS AND DISSCUSSION

Cadmium (cd) acetate is an important industrial and environmental pollutant, which affects the various organs like liver, lungs, reproductive organs, heart, brain and kidney of humans and experimental animals. Cadmium is also a toxic, non-biodegradable metal found in fossil fuel industry as well as hydrocarbon industry. It is also obtained as a byproduct of melting, smelting and other industrial process.

In our current study, cadmium acetate was administered to the rats of about four sets, each containing 6 rats were marked for identification and used for the analysis. The administered cadmium acetate gets accumulated in the tissue of liver (Frank, *et al*., 2003) when compared to the control animals and also our

Table 1: Experimental groups of six rats each

Parameters	Group I	Group II	Group III	Group IV
AST	83.10 ± 7.24	102.92 ± 3.99^{+a}	87.29 ± 7.05	93.35 ± 0.69^{+++a}
ALT	22.01± 4.67	37.53 ± 4.69^{+a}	31.57 ± 5.61	31.57 ± 5.61^{+++a}
ALP	110.0 ± 12.70	76.95 ± 1.28^{+a}	116.75 ± 11.35	95.23 ± 0.77

Table 2: The concentration of protein in serum of four experimental rats

Parameters	Group I	Group II	Group III	Group IV
PROTEIN	7.33 ± 0.26	3.47 ± 0.10	7.55 ± 0.66	6.3 ± 0.10^{++a}

investigation were made to study the protective effect of curcumin on these hapatotoxic rats.

Here, we evaluate the functions of liver by measuring the activity of serum enzymes like Aspartate transaminase, Alanine transaminase, alkaline phosphatase and antioxidant enzymes like superoxide dismutase, catalase in tissue sample.

Cadmium acetate intoxication produced a significant elevation of serum liver enzymes, aspartate transaminase, alanine transaminase compared to normal (group I) control rats(Table 1). Generally, aspartate transaminase, alanine transaminase enzymes gets increased under liver damage.(Hungl *et al*., 2006) Aspartate transaminase, alanine transaminase are the two specific marker enzymes elevated under cadmium acetate induced toxicity (Kowakzyk et al ., 2003; Guilhermino *et al*., 1998). This is because, the accumulated cadmium acetate destructs the hepatocytes(Annio *et al*., 1979) by disrupting their tight junction resulting in a damaged cell membrane. It causes an increased permeability to the hepatocyte membrane. Thus, consequently both the enzymes aspartate transaminase and alanine transaminase are released into the blood stream and thus the elevated level (kataoka *et al*., 2002; Philippe *et al* ., 1997). All these mentioned changes were significantly reduced to normal upon curcumin pretreatment (Pulla Reddy *et al*., 1996; Arafa HM., 2005) before cadmium acetate toxication in rats. (Group IV). (Table 1, fig 1).

On administration of cadmium acetate, the alkaline phosphate enzyme gets significantly decreased when compared to the normal group I animals. Alkaline phosphatase is a metalloenzyme, containing Mg, Zn ions as metals. On cadmium acetate, administration these two metal ions are replaced by cadmium. Hence, without the two metal ions, the activity of alkaline phosphatase enzyme gets decreased in cadmium acetate toxicated rats(group II). But all these conditions were reversed back near to normal in cucumin pretreated rats (group IV) when compared control rats (Table 1, fig1)

All values are mean ± S.D (n=6 animals / group), analysed by one way analysis of variance test (ANOVA) followed by Bonforrni multiple comparison test.

AST – Asparatate transaminase,

ALT – Alanine transaminase,

ALP – Alkaline phosphatise.

AST, ALT, ALP are the enzymes expressed in IU/L.

a – comparison are made with Group I,

+ - 0.001 significant value,

+++ - 0.05 statistically significant value.

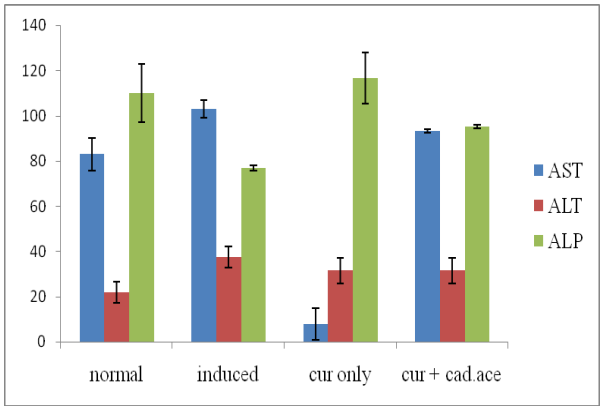

Figure 1: level of serum enzymes in Group I, Group II, Group III and Group IV rats

(Table 2, fig 2) cadmium acetate administration produced a significant fall in serum protein concentration (group II) as compared to normal rats (group I). The decreased serum concentration of protein due to disturbance in protein metabolism by either in free form or bound form or in the form of metallothionin formation. This fall in level was brought back to normal level in pretreated groups (group IV). This may be due to binding of curcumin with metallothionin.

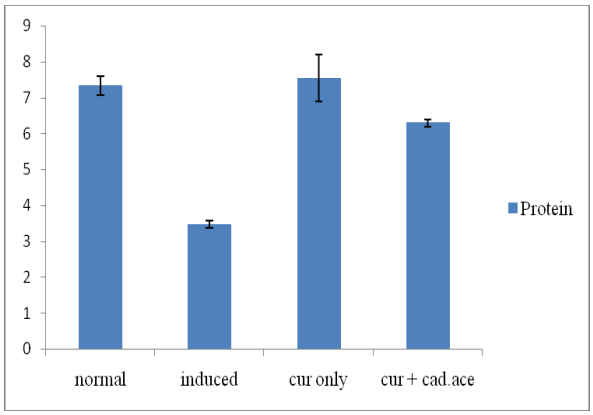

Figure 2: The concentration of protein in Normal, induced, only curcumin and pretreated groups

Table 3: The activity of antioxidant enzymes like SOD and CAT in liver tissue

Parameters	Group I	Group II	Group III	Group IV
SOD (Liver)	5.54 ± 0.31	2.99 ± 0.04	4.97 ± 0.09^{++a}	4.71 ± 0.19^{+a}
CAT (Liver)	163.78 ± 2.45	148.05 ± 1.44	163.88 ± 3.01	156.10 ± 1.23^{+a}

The level of antioxidant enzymes like superoxide dismutase, catalase in normal, cadmium acetate treated, curcumin alone treated, pretreated rats were depicted in Table 3, figure 3. These antioxidant activities were assayed in tissue sample (liver) of four groups.

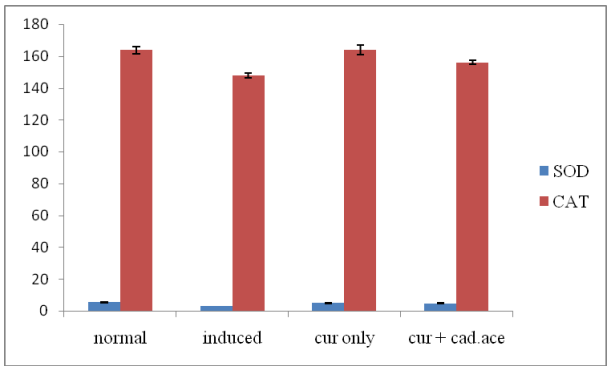

Figure 3: The activity of antioxidant enzymes in four experimental groups

The activity of superoxide dismutase and catalase were found to be decreased in cadmium acetate induced animals and these were reverted in treated rats. Superoxide dismutase is a metalloenzyme containing copper and zinc as cofactor, which are the important metals required for the activity. The cadmium acts as an antagonist to these cofactors hence the level gets decreased. The reduced activity of catalase is due to decreased absorption of iron, and essential trace elements required for activity of catalase.

The decreased level of antioxidant enzymes comes near to normal in curcumin treated rats because, curcumin replaces all the cadmium ions with specific cofactors. (Srinivasan *et al.*, 2006; Wohaeib and Godin, 1987) As a conclusion it was found that the pretreatment with curcumin reduced the cadmium acetate induced hepatotoxicity in rats.

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