ISSN : 0974 - 7427 Volume 7 Issue 3

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 7(3), 2013 [107-113]

Impact of $CdCl_2$ on hematological and biochemical parameters of rabbits *Oryctolagus cuniculus* and opposite effect of Ca^{2+}

Rachid Rouabhi[1]*, Bouchiha Hanene[1,2], Bouchama Khaled[1], Berrebbah Houria[2], Djebar Mohamedreda[2]

[1]SNV department, SESNV Faculty, Tebessa University, 12000, (ALGERIA)

[2]Laboratory Cellular toxicology, Annaba University, 23000, (ALGERIA)

E-mail: r_rouabhi@yahoo.fr

ABSTRACT

Heavy metals and its derivatives are a special class of toxics. Many metals are useful for industrial, agricultural and medical applications. Indeed, they can go back through the food chain and thus achieve to human being.

Our aim is to study an example of heavy metals "Cadmium" $CdCl_2$at two concentrations 30 and 60 ppm on biological model*Oryctolagus cuniculus* that is very used in Toxicological studies, and the possible neutralization using the Ca^{2+}.

Our results showed a significant increase in the weight of liver, this augmentation is corrected with the addition of Ca^{2+} for the lowest concentration 30ppm and in the 07th day, but over that there is no effect of calcium on the toxicity of Cd, the biochemical and hematological parameters generally are disturbed and the effect of calcium is less or more shown in the most experiments.

KEYWORDS

Oryctolagus cuniculus;
Heavy metals;
Cadmium;
Calcium;
Hematology;
Protein;
Detoxification.

INTRODUCTION

In our environment, we are exposed to a number of natural or synthetic substances that can be caused toxic effects, and heavy metals are of these harmful substances[2] .

Heavy metals known as natural metallic elements having a density exceeded $5g/cm^3$. These are most often present in the environment astraces, mercury, lead, cadmium, copper, arsenic, nickel, zinc, cobalt, manganese are example. Most of them are toxic like lead, cadmium and mercury[32]. We are interested in our work to cadmium; it has many similarities with the physical and chemical character as zinc and is found in nature accompanying zinc.

Cadmium is highly corrosion, resistant and has been widely used in electroplating of other metals, mainly steel and iron. However, currently, only 8% of the total refined cadmium are used for veneers and coatings. Cadmium compounds (30% of its applications in developed countries) are used as pigments and stabilizers in plastics. Cadmium is also used in some alloys (3%). Small cadmium batteries, rechargeable and used, for example, in mobile phones, contribute to the rapid increase in the

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use of cadmium (in 1994, 55% of cadmium in industrial countries were in the batteries)[11].

The toxicity of cadmium varied and dependedto the chemical state and the duration and amount of exposure. It causes tubular dysfunction that result in increased secretion of low molecular weight proteins in the urine. Cadmium may also cause disturbances of calcium metabolism. High exposure to cadmium, most probably related to other factors such as dietary deficiency can lead to osteoporosis and/or osteomalacia. The inhibitory effects of cadmium on reproductive cells are proven in rodents and suspected in humans[11].

Rabbits havebeen domesticated since the sixth century, when They Were kept for food and fur. They have been also selectively bred over the years for varieties in the fur and are a popular pet.

After mice and rats, they are the most common laboratory animal. As many as 76 different breeds of rabbit are known by the British Rabbit Council intended, the New Zealand White (NZW), bred in the 1920s hasbecome the one mostcommonly used in research.

Historically, They Have beensmost used for antibody development and testing as sentinels for a wide array of products[27]. The rabbit (*Oryctolagus cuniculus*) is widely used as a model for human disease because of its size, physiological attributes, and similar disease characteristics[19].

In immunology, Gertz et *al*.[8] worked onthe regions encoding the coordinately regulated Th2 cytokines IL5, IL4 and IL13 of the rabbit *Oryctolagus cuniculus*by comparing sequences of syntenic regions on chromosome 3, and theyidentified several differences between the two donor rabbits in coding and non-coding regions of potential functional significance, confirmation awaits additional sequencing of other rabbits.

Rabbits can be restrained in stocks and easily generally docile and are cheap to maintain, they have beens used for a wide-range of toxicity testing, especially on their skin[26].

Anjum[7] found that rabbits are excellent models for investigation of heavy metals effects on liver functions and drug metabolisation enzyme system, where he found that the addition of $CdCl_z$ to the rabbits pretreated with phenobarbitone and promethazine with dose of 5mg/kg of weight increases the activities of serum GOT, LDH and ICDH 49%, 73% and 32%, respectively. Activity of AP was decreased 69% in the phenobarbitone. In pretreated promethazine rabbits, cadmium chloride administration decreased the activities of serum GOT, GPT, LDH and AP, 56%, 35%, 27% and 25%.

Eira et *al*.[6] searched the concentration of some toxic elements in *Oryctolagus cuniculus* and in its intestinal cestodeMosgovoyiactenoides, in Dunas de Mira (Portugal), The highest quantity of Pb was found in rabbit muscle (3.81 ppm) while highest Cd and Hg values were found in kidney (1.02 and 0.08 ppm).

In our work we have tried to highlight the effect of cadmium at two concentrations 30 and 60 ppm on rabbits (*Oryctolagus cuniculus*) and the possible role of calcium in the phenomenon of detoxification.

MATERIALS AND METHODS

Biological material

For our experiments, we chose to work on rabbits of local strain in the region of Tebessa east-north Algeria. All rabbits were males weighing between 260 g and 760 g and have a soft fur reddish brown, black, white and gray. They had access to water and their food for all times. Animals were kept under constant conditions of temperature environ 25±3°C and humidity 35±5 %. The total body weight of body was daily recorded before and during the experiments. There was a gain in body weight and increase of food consumption indicating the good conditions of laboratory.

Chemical

We used a cadmium under Cadmium Chloride form. Aqueous solutions of cadmium chloride salt was administered by oral system. Control rabbits were kept untreated and their body weight was recorded daily, we selected two doses 30 and 60ppm.

METHOD

Description and treatment

To begin our experience, we have handled 63 rabbits of local breed in the region of Tebessa (*Oryctolagus cuniculus*). These rabbits were divided on 6lots of 9 rabbits and we kept nine rabbits as control. The treatments began 15 days (adaptation period of rabbits) as

follows.

Lot1 : as controls without treatment
Lot2 : treated of 30 ppm $CdCl_2$
Lot 3 : treated of 60 ppm $CdCl_2$
Lot4 : treated of 30 ppm Ca^{2+}
Lot 5 : treated of 60 ppm Ca^{2+}
Lot6 : treated of $CdCl_2$ / Ca^{2+} 30 ppm
Lot7 : treated of $CdCl_2$ / Ca^{2+} 60 ppm

All the animals were killed by cervical dislocation 24 hours after last treatments. The blood samples were for estimation of hematological parameters.

Liver was taken out for biochemical tests, relative weight of liver was estimated by the following formula: RLW=(liver weight/body weight) X100. Liver protein level was measured by Bradford (1976) method.

Hematological study method

The hematological parameters hemoglobin, MetHb and a parameter considered as an early marker of inflammation (erythrocyte sedimentation level). Blood sampling was done at the laboratory of the University of Tébessa. The first sampling on 7thday, the second to the 16thday and the last 21stday of treatment.

Determination of methemoglobin (MetHb)

The procedure consists of two steps

First step

0.2 ml of heparinized blood and 5 ml of isotonic chloride (9%), Centrifugation at 5000 rpm for 5 minutes, Base with 5 ml of ice water for 15 minutes, Complete with 10 ml of Sorensen's buffer and agitation followed by centrifugation 5000 rpm for 5 minutes then read the optical density of the supernatant at 632 nm (ODA).

Add a drop of the mixture (KCN [0.1 M] + CH3COOH [12%]) to the supernatant and read the optical density (ODB)

Second step

Add to 2ml of supernatant 1.5 ml of Fe (CN) 6 K3 (brown color), and read the optical density (ODC).

Add a drop of mixture (KCN [0.1 M] + Fe (CN) 6 K3) (red color), and read the optical density (ODD).

The level of methemoglobin is obtained by the following formula:

MetHb = [(OD A-ODB) / (OD C-OD D)] X 100

RESULTS

Effects of $CdCl_2$, on hemoglobin (Hb) and the role of Ca^{2+}

TABLE 1 shows the effects of $CdCl_2$, and the role of Ca^{2+} in the 7th, 16thand21stdays of treatment. There is a decrease of hemoglobin levels in all treated animals with $CdCl_2$ (P=0.001). The addition of Ca^{2+}increases this level as controls level. The Dunnett's test confirms that there is a difference compared to controls of treated by $CdCl_2$.

TABLE 1 : Effect of $CdCl_2$ on hemoglobin Hb and the role of Ca^{2+} (g/dl).

Sample (g/dl)	Control	$CdCl_2$ 30ppm	$CdCl_2$ 60ppm	Ca^{2+} 30ppm	Ca^{2+} 60ppm	$CdCl_2$/Ca^{2+} 30ppm	$CdCl_2$/Ca^{2+} 60ppm
7th day	11.600±0.530	8.000±0.500*	7.600±0.530*	11.00±0.500*	11.66±0.290	11.530±0.500	11.400±0.530
16th day	11.530±0.500	9.200±0.260**	7.260±0.250**	12.530±0.550*	11.850±0.300*	10.460±0.0.500*	12.570±0.560*
21st day	11.430±0.513	8.800±0.755*	8.400±0.535*	11.27±0.374	12.50±0.500	11.610±0.548*	11.140±0.17

*** (p<0.05) There is a significant difference between control and treated sample; ** (p<0.01) there is very significant difference between control and treated samples.**

Effects of $CdCl_2$, on Sedimentation speed (SV) level and the role of Ca^{2+}

First hour

The effects of chloride cadmium on 1st hour sedimentation speed level of red blood cells and the role of calcium in the detoxification is showed in the TABLE 2. In the 7thday there was an increase of this parameter in organisms treated by(30 and 60 ppm)$CdCl_2$, Ca^{2+} (30 ppm) and combined treatment (30, 60 ppm),in the rest of parameters there is a decrease (P = 0.301). at the 16th day there was an increase in the two $CdCl_2$ concentrations, Anova test shows that there is no difference in the 7thand 16thday compared to the controls, except the 21st where there a difference.

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TABLE 2 : Effect of $CdCl_2$ on the sedimentation speed levelof the RBC at 1st hour (mm/h).

Samples (mm/h)	Control	$CdCl_2$ 30ppm	$CdCl_2$ 60ppm	Ca^{2+}30ppm	Ca^{2+}60ppm	$CdCl_2/Ca^{2+}$ 30 ppm	$CdCl_2/Ca^{2+}$ 60 ppm
7th day	2.000±1.000	3.330±1.582	4.330±2.517	4.330±2.528	1.660±1.155	3.330±1.528	3.000±1.000
16th day	2.000±1.000	3.000±1.000±	2.330±1.155	1.330±1.528	1.330±1.528	1.330±0.577±	3.660±1.155
21st day	2.000±1.000	2.000±1.000	3.000±2.000	5.000±1.000**	1.660±1.155	1.330±0.577*	1.000±0.000*

**(p<0.01) there is a high significant between control and treated samples.

Second hour

The effects of $CdCl_2$, on the level of sedimentation at the second hour are shown in (TABLE 3). In general manner, there is a big increase of the sedimentation speed level in all cadmium treatments, but the role of calcium is very clear where there is a neutralization effect. Dunnett's test confirms that there is a difference compared to the control, except to all treated with Ca^{2+}($p>0.05$).

Effects of $CdCl_2$ on methemoglobin level and the possible role of Calcium

The results shown in (figure 1) illustrated the effects of chloride calcium on the level of methemoglobin and the role of calcium in the correction of effects. There was an increase in all $CdCl_2$ treated versus control ($p<0.001$). The role of Ca^{2+} was very clear in the correction of this intoxication. Dunnett's test confirms that

TABLE 3 : Effect of $CdCl_2$ on the sedimentation speed rate of RBC in the second hour (mm/h).

Samples (mm/h)	Control	$CdCl_2$ 30 ppm	$CdCl_2$ 60 ppm	Ca^{2+} 30 ppm	Ca^{2+} 60 ppm	$CdCl_2/Ca^{2+}$ 30 ppm	$CdCl_2/Ca^{2+}$ 60 ppm
7th day	2.330±0.577	5.330±2.082*	7.000±1.000**	2.360±1.155	2.000±0.000	3.660±1.528	3.640±1.528
16th day	2.340±2.517	4.990±1.155*	6.000±1.000*	1.660±2.082	1.660±1.528	2.250±1.000	3.660±1.528
21st day	2.330±0.577	3.240±1.000	4.660±1.528*	2.690±1.155	2.660±1.155	2.330±0.577	1.660±0.577

organisms treated by cadmium are differences to the control.

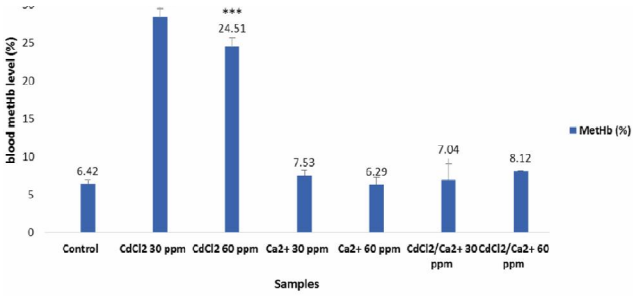

Figure 1 : Effect of $CdCl_2$ on methemoglobin level and the role of Ca^{2+}.

Effects of $CdCl_2$ on relative liver weight and the role of Ca^{2+}

Impact of chloride cadmium on relative liver weight is shown in the TABLE 4. All treatments by $CdCl_2$ showed an augmentation in RLW, the Ca^{2+} decrease and correct this amount according to the treatments ($p>0.05$).

Effect of $CdCl_2$ on hepatic protein level and the role of Ca^{2+}

The protein content of rabbit liver was determined using Bradford method, and the effects of $CdCl_2$, and the possible opposite effect of $Ca^{2+}$are given in the figure 2. We observe a very high significant increase ($p<0.001$)of protein level in all treaties by $CdCl_2$. This increase is correct significantly by the addition of Ca^{2+}. The Dunnett's test and analysis of variance shows that there is a significant difference between Cd treaties and control.

TABLE 4 : Effects of $CdCl_2$ on relative liver weight (g).

Samples x100 (g)	Control	$CdCl_2$ 30ppm	$CdCl_2$ 60 ppm	Ca^{2+} 30 ppm	Ca^{2+} 60 ppm	$CdCl_2/Ca^{2+}$ 30 ppm	$CdCl_2/Ca^{2+}$ 60 ppm
7th day	0.020±0.003	0.035±0.002**	0.040±0.002***	0.022±0.003	0.020±0.002	0.020±0.004	0.024±0.003
16th day	0.020±0.002	0.032±0.001**	0.044±0.001***	0.026±0.001	0.028±0.001	0.023±0.001	0.025±0.002
21st day	0.025±0.005	0.039±0.004**	0.049±0.005***	0.029±0.004	0.030±0.003	0.026±0.002	0.027±0.004

***(P<0.001) very high significant difference

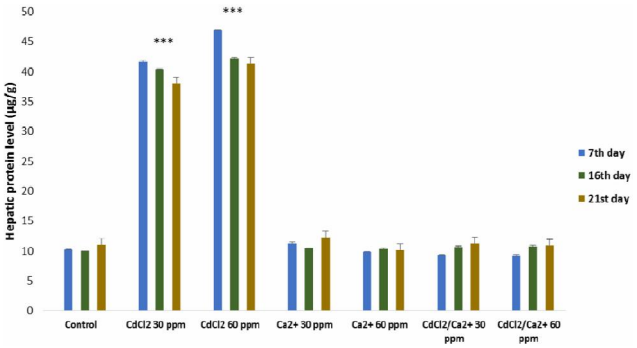

Figure 2 : Effects of 30 and 60 ppm of $CdCl_2$ on hepatic protein level (µg/g) and the role of Ca^{2+}.

DISCUSSION

This study aimed to highlight a possible toxicity of cadmium $CdCl_2$ onsome hematological and biochemical parameters of *Oryctolagus cuniculus* as a biological model, andthe possible role of Calcium Ca^{2+} in the detoxification and neutralization of these effects.

Cadmium is reported having no known beneficial functions in animal life[31]. Once in the system cadmium binds with enzymes having sulfhydryl groups[13], disturbs cell membrane permeability[15], deposits in cellular organist and binds with nucleic acids[25]. All the biological functions like excretion, digestion, respiration and reproduction are affected by the intoxication by cadmium causing the death of organism.

Blood is one of the most sensitive indicators of many metabolic disorders[17,29]. Numerous studies have shown that the primary site of toxic action in the body is the red blood cells and hemoglobin which more precisely the role of carrying oxygen when the iron is under ferrous form Fe^{2+} [18,20].

Cadmium oxides ferrous iron Fe^{2+} active molecule of hemoglobin to ferric iron Fe^{3+} inactive and the resulting molecule is called methemoglobin which is incapable of reversibly binding oxygen[5,14]. Our results showed a toxic effect of cadmium translated by the increase in methemoglobin (MetHb); these effects are corrected by the adding of the Calcium Ca^{2+}. In adults, there are enzyme systems such as NADH-MetHb reductase;formed methemoglobin eliminating the absence of this enzymatic equipment in the fetus resulting in cyanosis and high neonatal mortality. Ampy and Williams[1] showed that nitrates generate a cascade of physiological phenomena affecting in most cases the blood tissue but also other organs (kidney, spleen and liver).

The addition of Ca^{2+} reduced the effect of $CaCl_2$, corrected the level of MetHb, probably by conserving the enzymatic compartment, and enhanced the eliminating of $CdCl_2$ by exocytosis phenomenon.

Our results showed adecrease of hemoglobin level in treated animals by cadmium, Sutton[28] found a decrease in hemoglobin and hematocrit in female mice that are exposed to a dose of 6 mg/kg of Cd. Prigge[21] exposed female rats at concentrations Cd from 25 to 50 mg/m3 (as Cd oxide) for 100 days also cause a reduction in growth and an increase in hemoglobin and hematocrit.

The addition of Ca^{2+} to the cadmium's treated animals returns the amount of Hb to normal level and corrected the effect of this metal. Several hypotheses, sometimes contradictory, have been proposed to explain the inhibitory action of calcium on the collection and accumulation of cadmium. Knowing that Cd^{2+} ions can be transported through the protein calcium channels in the membranes of root cells[30] a possible competition between the two ions can occur for the same absorption sites[34,16]. Calcium is a competitive inhibitor of cadmium uptake in *Rhytidiadelphus squarrosus*[33].

In present work when the body of animals were dissected, in most cases, their abdominal cavities were filled with fluids, liver were shrunk, having numerous white spots probably fats infiltrations. At sublethal doses there was no changes in body weights suggesting that the doses was not strong enough to produce the known cadmium symptoms as skeleton deformation and renal disorders[22]. However, metal after ingestion induces the increase of relative liver weight this results are in concordance with the results of Grose et *al*.[13]. Goering and Curtin[10] reported moderate to severe hepatic injury, evident by cells swelling after cadmium administration to immature rats. Cadmium was reported to inhibit protein synthesis at cellular level[35] which probably returns on hepatic weight.

The liver shows a change in weight after ingestion of cadmium. Borzelleca et *al*.[4] found effects on growth and organ weights of spargue-Dawley rats who ingested doses of Cd about 1.1 to 14 mg/kg for 10 days in their drinking water, and from 15 to 65 mg/kg, organ weights

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were also modify. The presence of Ca^{2+} combined with $CdCl_2$ corrected the decrease of liver weight to normal level, this due probably by the competition and the role of calcium in the cadmium extracellularmovement, and the sedimentation speed confirm that there was a hepatic injury; this is corrected by the calcium combination.

The rate of hepatic protein was increased in the animals treated with CdCl2, this augmentation is the results of the resistance enzymes secretion[24]. The cadmium perturbs all metabolic ways in the organism, and the Ca2+ correct these perturbation.

The presence of calcium associated with doses of cadmium in our experiments induced a correction of cadmium effects at 30 and 60ppm. These results are consistent with the results of Raghpathy and Nasa[23] were exposed rats to 25 ppm of $CdCl_2$ with drinking water hanging 8 weeks, groups fed with low calcium diet (0.1 %) had increased retention of cadmium and cadmium toxicity compared to groups that were fed with diet of high calcium (0.6%). Wecan say that the calcium maintains low concentrations of metal in the cytosol. These results are consistent with the work of Zoghlami et al.[36] have shown that cadmium causes an inhibition of weight gain that depends on the concentration of the metal in the organ.

As conclusion, many negative effects of cadmium can be corrected and neutralized with the best amount of calcium.

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