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Opposite effects of Ca^{2+} on toxicity by $CdCl_2$ on white blood cells (WBC), protein level and LD_{50} of rabbits *Oryctolagus cuniculus*

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Heavy metals and their derivatives are a special class of toxic substances. Many metals are useful for industrial, agricultural and medical applications. Indeed, they can go back through the food chain and thus achievable 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 often used in toxicological studies, and the possible neutralization using the Ca^{2+}. We are interested firstly of estimating LD_{50} of $CdCl_2$ that is in range of 70 to 150 mg/kg according DVGW and we found that the LD_{50} is 85.703 mg/kg of $CdCl_2$, this valor is augmented with the addition of 30 ppm Ca^{2+} to 108.231 mg/kg which show the protective role of Ca^{2+}. Our results also showed a significant increase in the protein level in treated rabbits with both doses, this augmentation is corrected with the addition of Ca^{2+}at all days (7^{th}, 16^{th} and 21^{st}). Impact of $CdCl_2$ on total white blood cells (WBC) number showed a decrease in treatments with 30 ppm contrarily to the rabbits treated with 60 ppm where we found an increase; these perturbations are more or less corrected with Ca^{2+}.

Key words: *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 caused toxic effects, and heavy metals are one of these harmful substances (Basketter et al., 1999).

Heavy metals known as natural metallic elements having a density exceeded 5 g/cm^3. These are most often present in the environment as traces, mercury, lead, cadmium, copper, arsenic, nickel, zinc, cobalt, manganese are example. Most of them are toxic like lead, cadmium and mercury (Veyssyre, 2000). 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 (Bliefert and Perrand, 2001).

Cadmium is one of the significant environmental pollutants and humans are exposed to it through food, water, air and heavy smoking (Friberget al., 1971; Kjellstrom, 1979; WHO, 1982).

Cadmium is highly corrosive, resistant and has been widely used in electroplating of other metals, mainly steel and iron. However, currently, only 8% of the total refined cadmium is 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 rechargeable batteries are used, for example, in mobile phones which contribute to the rapid increase in the use of cadmium in industrial countries

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were in the batteries) (Grant et al., 1995).

The mechanism of cadmium toxicity in mammals is complex, producing changes in membrane permeability (Schlaepfer, 1971; Elinder et al., 1976; Al-Haddat et al., 1981), abnormal transport of metabolites and minerals (Hadley et al., 1979; Hammer et al., 1973), antimetabolite effect (Thomas et al., 1979; Roel et al., 1981), and disturbance in cellular energy metabolism and binding to cellular respiratory components (Smith et al., 1976; Stanes et al., 1977; Di Nocola et al.,1987). Chronic feeding of cadmium at low levels to rats, rabbit, lamb and pigs causes diminished growth and feed consumption (Perry and Erlanger, 1974; Perry, 1976; Doyle et al., 1976; Kolsonis and Kluassen, 1977; Rogenfelt et al., 1984).

Rabbits have been 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 animals. As many as 76 different breeds of rabbit are known by the British Rabbit Council intended, the New Zealand White (NZW), bred in the 1920 has become the one most commonly used in research.

Historically, they have been most used for antibody development and testing as sentinels for a wide array of products (Suckow et al., 1997). The rabbit (*Oryctolagus cuniculus*) is widely used as a model for human disease because of its size, physiological attributes, and similar disease characteristics (NIAID, 2005).

In immunology, Gertz et al. (2011) worked on the regions encoding the coordinately regulated Th2 cytokines IL5, IL4 and IL13 of the rabbit *O. cuniculus* by comparing sequences of syntenic regions on chromosome 3, and they identified 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 been used for a wide-range of toxicity testing, especially on their skin (Lagomorpha, 1999; Suckow et al., 2002).

Anjum (1991) 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 5 mg/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. (2005) searched the concentration of some toxic elements in *O. cuniculus* and in its intestinal cestode *Mosgovoyiactenoides*, in Dunas de Mira (Portugal), the highest quantity of Pb was found in rabbit muscle (3.81 kidney (1.02 and 0.08 ppm).

In our work, we have tried to highlight the DL_{50} of $CdCl_2$ and the effect of cadmium at two concentrations 30 and 60 ppm on rabbits (*O. 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 and 760 g and have a soft fur reddish brown, black, white and gray. Feed and water were provided *ad libitum*. Animals were kept under constant conditions of temperature environ 25 ± 3°C and humidity 35 ± 5%. The total body weight 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.

Chemicals

We used cadmium in cadmium chloride form. Aqueous solution 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 60 ppm.

Description and treatment

We have handled 63 rabbits of local breed in the region of Tebessa (*O. cuniculus*). These rabbits were divided on 6 lots of 9 rabbits and we kept nine rabbits as control. The treatments began 15th day (adaptation period of rabbits) as follows:

Lot1: controls without treatment
Lot 2: treated of 30 ppm $CdCl_2$
Lot 3: treated of 60 ppm $CdCl_2$
Lot 4: treated of 30 ppm Ca^{2+}
Lot 5: treated of 60 ppm Ca^{2+}
Lot 6: treated of $CdCl_2$ / Ca^{2+} 30 ppm
Lot 7: treated of $CdCl_2$ / Ca^{2+} 60 ppm

For DL_{50} estimation, we divided rabbits on 9 lots with 5 individuals each, chosen doses were delivered after bibliographic consultation and one lot was conserved as controls. The same work was repeated for the calcium combination with cadmium and we chose 30 ppm of calcium to test the effect.

All the animals were killed by cervical dislocation 24 h after last treatments. The blood samples were taken for estimation of WBC number.

Liver was taken out for biochemical tests, relative weight of liver was estimated by the following formula: RLW = (liver weight/body weight) × 100. Liver protein level was measured by Bradford (1976) method.

Hematological study method

Blood sampling was done at the laboratory of the University of Tebessa. The first sampling was done on the 7th day, the second to the 16th day and the last 21st day of treatment. All hematological tests were done using an automatic analyzer.

Statistical analysis

The parameters were evaluated in at least three replicates; the

means and standard error were reported. The analyses of variance were computed on statistically significant differences determined on the appropriate t-test using Minitab 16.2 software.

RESULTS

Estimation of LD_{50} and role of Ca^{2+}

Obtained results after treatments by increasing doses of cadmium chloride are shown in Table 1. Because we found the mortality in controls, so we calculate the corrected mortality according to ABBOTT's equation:

$$M_{corr}\ (\%) = [(\%M_{obs} - \%M_{cont}) / (100 - \%M_{cont})] \times 100$$

Where:
M_{corr}: corrected mortality
M_{obs}: observed mortality
M_{cont}: mortality in controls.

After addition of Ca^{2+}, we obtained the results found in Table 2.

The calculation of LD_{50} was done from Figure 1. From the two equations, we could calculate the $\log_{10}$ of 50% dose (probite = 5) as following:

From 1st equation: $\log_{10} LD_{50} = 1.932$, so DL_{50}= 85.703 mg/kg of $CdCl_2$
From 2nd equation: $\log_{10} LD_{50} = 2.034351$, so DL_{50} = 108.231 mg/kg of $CdCl_2$ after the correction from Ca^{2+}.

From these results the LD_{50} of $CdCl_2$ on rabbit is about 85.70 mg/kg and this is the first time that the exact lethal dose is published, this result is increased in the presence of calcium to about 108.23 mg/kg of $CdCl_2$ that shown a protective role of Ca^{2+}.

Effects of $CdCl_2$, on WBC number and the role of Ca^{2+}

Impact of cadmium chloride on white blood cells WBC number is shown in Table 3. In the 7th, 16th, and 21st days there was a decrease ($p<0.001$) in all treatments by 30 ppm of $CdCl_2$, contrarily to the rabbits treated with 60 ppm of $CdCl_2$.

Addition of Ca^{2+} corrected this perturbation especially for the dose of 30 ppm. The analysis of variance showed a very high significant difference compared to control ($p<0.001$) and Dunnett's test showed a difference between treatments and controls.

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 observed a very high significant increase ($p<0.001$) of protein level in all samples treated by $CdCl_2$. This increase is corrected significantly by the addition of Ca^{2+}. The Dunnett's test and analysis of variance shows that there is a significant difference between Cd treatment and the control.

DISCUSSION

This study aimed to calculate the LD_{50} of $CdCl_2$ on rabbits because the data was not well persisted, and high light a possible toxicity of cadmium $CdCl_2$ on WBC and liver protein of *O. cuniculus* as a biological model, and the 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 (Vallee and Ulmer, 1972). Once in the system cadmium binds with enzymes having sulfhydryl groups (Grose et al., 1987), disturbs cell membrane permeability (Kazantzis, 1987), deposits in cellular organist (Nakano et al., 1987) and binds with nucleic acids (Scicchiltano and Pegg, 1987). 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 (Leong et al., 1959; Tseng et al., 1988). Numerous studies have shown that the primary sites of toxic action in the body are the red blood cells and hemoglobin which play more precisely the role of carrying oxygen when the iron is under ferrous form Fe^{2+} (Meyer, 1983; Perry, 1987).

Cadmium oxides ferrous iron Fe^{2+} activate molecule of hemoglobin to ferric iron Fe^{3+}, inactive form and the resulting molecule is called methemoglobin which is incapable of reversibly binding oxygen (Doland and Luban, 1987; Kawatsu et al., 1987).

Treatment with $CdCl_2$ showed a decrease in the rate of white blood cells (WBC) treated with 30 ppm and an increase with 60 ppm. These results are consistent with the results of Anibal et al. (2004) who noticed a decrease in B cells by doses of 5 and 10 ppm $CdCl_2$; and increase by 25 ppm, T cells were increased by doses of 25, 50 and 100 ppm $CdCl_2$.

Distribution of subsets of blood lymphocytes suggested that Cd prevents immune cell and hormonal response with low doses of the metal used, and opposite effects with higher doses.

The addition of Ca^{2+} associated with cadmium chloride corrected the perturbations and renormalize the rates. These results are consistent with the results of Raghpathy and Nasa (2007) who exposed rats to 25 ppm of $CdCl_2$ with drinking water during 8 weeks. Groups that were fed with a low-calcium diet (0.1 %) had increased retention of cadmium and cadmium toxicity compared to groups that were fed with a diet of high calcium (0.6%). We can explain that the calcium maintains low concentra-

Table 1. Mortality level and probite transformation.

Dose $CdCl_2$ (mg/kg)	Log_{10} dose	Rabbit number/lot	Dead Rabbits number/lot (48 h)	Mortality (%)	Corrected Mortality	Probite
00	-	5	01	20	-	-
60	1.77815	5	00	00	0	-
70	1.84510	5	01	20	0	-
80	1.90309	5	03	60	50	5.00
90	1.95424	5	03	60	50	5.00
100	2.00000	5	03	60	50	5.00
110	2.04139	5	04	80	75	5.67
120	2.07918	5	04	80	75	5.67
130	2.11394	5	04	80	75	5.67

Table 2. Mortality level after the addition of 30 ppm of Ca^{2+} and probite transformation.

Dose of $CdCl_2$ (mg/kg)	Ca^{2+} 30 ppm	Log_{10} dose	Rabbit number/lot	Dead Rabbits number/lot (48 h)	Mortality (%)	Corrected Mortality	Probite
00	00	-	5	01	20	-	-
60	30	1.77815	5	00	00	0	-
70	30	1.84510	5	00	00	0	-
80	30	1.90309	5	02	40	25	4.33
90	30	1.95424	5	02	40	25	4.33
100	30	2.00000	5	03	60	50	5.00
110	30	2.04139	5	03	60	50	5.00
120	30	2.07918	5	03	60	50	5.00
130	30	2.11394	5	04	80	75	5.67

Table 3. Effect of $CdCl_2$ on WBC and the role of Ca^{2+}.

Sample (mm^3)	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
7^{th} day	6416 ± 52	4533 ± 50***	8400 ± 52***	6530 ± 51	6690 ± 56	6566 ± 76	6600 ± 52
16^{th} day	5966 ± 76	4166 ± 52**	7500 ± 54**	5901 ± 52	6603 ± 52	6166 ± 73	6483 ± 54
21^{st} day	6433 ± 51	4233 ± 53***	7648 ± 40**	6033±58	6566 ± 10	6245 ± 44	7000 ± 52

** $p<0.01$ high difference according to the control. *** $p<0.001$ very high difference according to the control.

tions of metal in the cytosol. These results are consistent with the work of Zoghlami et al. (2006) where they have shown that cadmium causes an inhibition of weight gain, which depends on the concentration of the metal and the organ in question. In the presence of 20 µM of $CdCl_2$, the addition of calcium from 0.1 to 10 mM $CaCl_2$ in the middle improved the production of biomass in conjunction with an improvement in the mineral composition of the plant and increased the content of photosynthetic pigments. Taken together, these results suggest that the negative impact of cadmium on some process of growth and development can be mitigated by adequate intake of calcium in the culture medium.

In this present work, when the body of animals was dissected, in most cases, their abdominal cavities were filled with fluids, liver were shrunk, having numerous white spots probably fats infiltrations. At sub-lethal doses there were no changes in body weights suggesting that the doses were not strong enough to produce the known cadmium symptoms as skeleton deformation and renal disorders (Princi, 1947). However, metal after ingestion induces the increase of relative liver weight. These results are in concordance with the results of Grose et al. (1987). Goering and Curtin (1984) reported moderate to severe hepatic injury, evident by cells swelling after cadmium administration to immature rats. Cadmium was

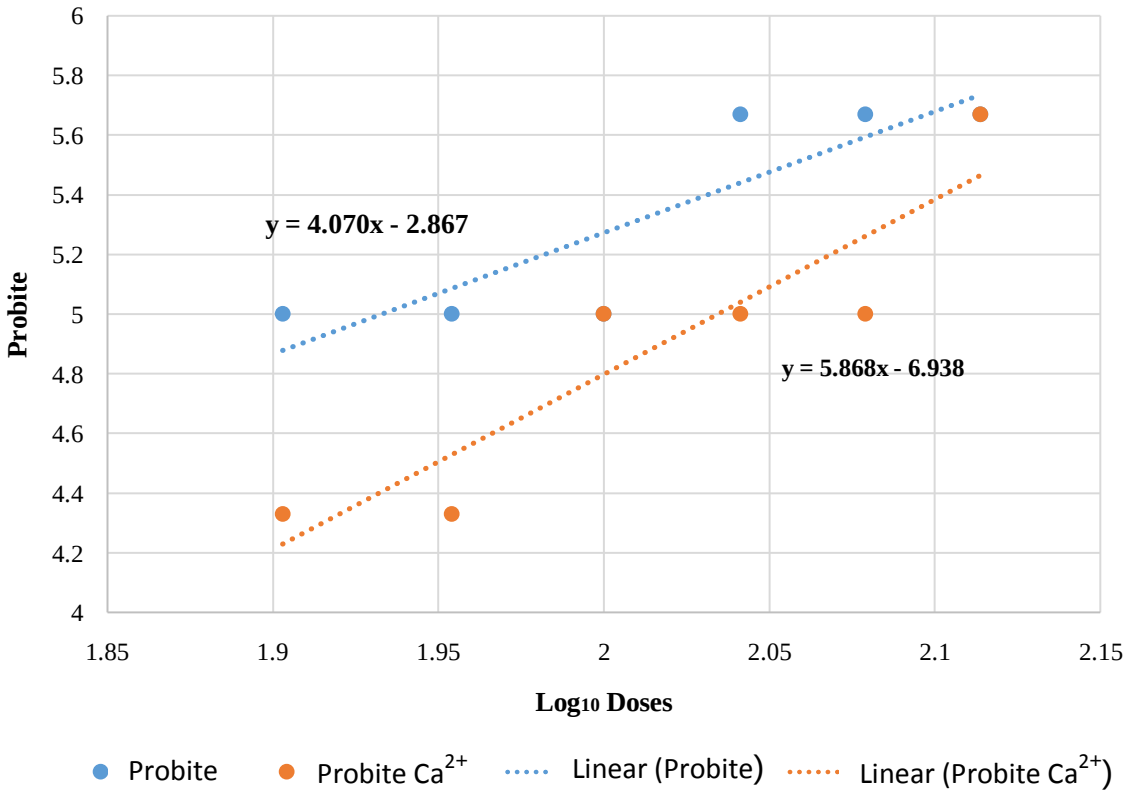

Figure 1. Probites before and after Ca^{2+} addition according to the log_{10} of doses.

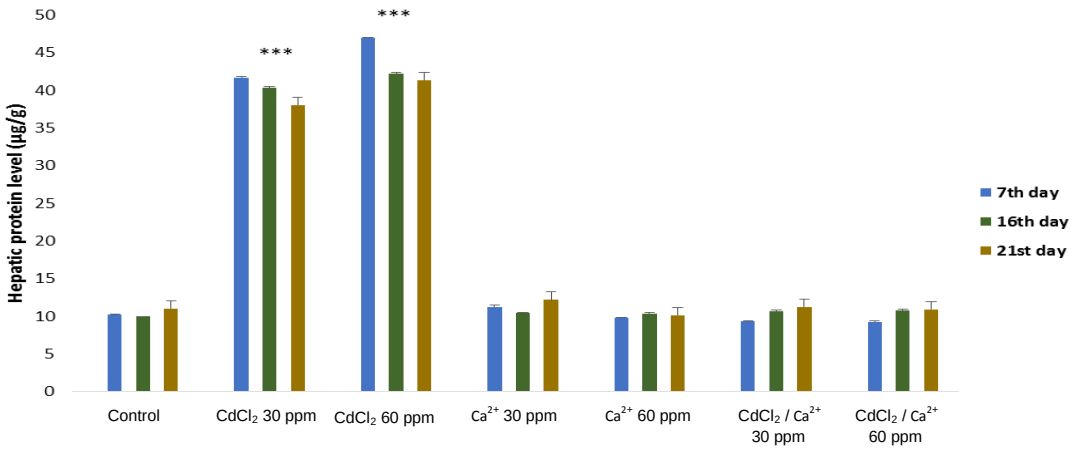

Figure 2. Effects of 30 and 60 ppm of $CdCl_2$ on hepatic protein level (µg/g) and the role of Ca^{2+}.

reported to inhibit protein synthesis at cellular level (Xiao and Lui, 1987) which probably returns on hepatic weight. The rate of hepatic protein was increased in the animals treated with $CdCl_2$; this augmentation is the results of the resistance enzymes secretion (Rouabhi et al., 2008). The cadmium perturbs all metabolic ways in the organism, and the Ca^{2+} corrected these perturbations. The presence of calcium associated with doses of cadmium in our experiments induced a correction of cadmium effects at 30 and 60 ppm. These results are consistent with the results of Raghpathy and Nasa (2007) who 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%). We can say that the calcium maintains low concentrations of metal in the cytosol. These results are consistent with the work of Zoghlami et al. (2006) who have shown that

cadmium caused 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 suitable amount of calcium.

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