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*Full Length Research Paper*

# Renal, Hepatic and Splenic Biotoxicity of Cadmium Sulphate in the Wistar Rats

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Heavy metals (lead, mercury and cadmium) cause problems of public health. Studies undertaken in Côte d'Ivoire within the framework of the environmental monitoring made it possible to apprehend the state of the cadmium pollution of the sediments, of the market garden grounds. Six (6) batches of rats Wistar males and females were made up. These rats were contaminated with cadmium sulfate by daily during 30 days with various proportion respectively 4; 5; 6.66; 10 and 20 mg/kg body weight (bw). The rats were weighed each week. At the end of the experiment, the serum was used for proportioning of the enzymes and of the substrates using *Cobas C311 ROCHE HITACHI*. Work completed made it possible to determine the LD<sub>50</sub> of cadmium sulfate which is of 200 mg/kg bw. The weight of the rats of the batches treated with cadmium decreased significantly compared to the batch untreated (control). Transaminases, creatinine and the urea were increased significantly ( $p < 0.05$ ) when the bilirubin and cholesterol were decreased at the two sexes. However, the effect on cholesterol is not significant. Cadmium caused liver and kidney dysfunction and it decreased the weight of rats of both sexes.

**Keywords:** Cadmium, Biochemical markers of kidneys, Biochemical markers of liver and spleen.

## INTRODUCTION

Heavy metals (lead, mercury, arsenic, cadmium...) are pollutants generated for most of the time by the human activity. They have a high toxicological impact on the plants, the products for current human consumption and on man (Amir *et al.*, 2014).

These heavy metals remain a serious problem of environment more and more worrying especially because of the affections noted on the populations which are exposed (Oumar *et al.*, 2014). With regard to cadmium, it is present in a significant way in certain food, like the seafood, meat offal, certain cereals (rice, corn...), mushrooms, vegetables and to a lesser extent,

in fish, the fruits and meat (Pascal *et al.*, 2010).

In Côte d'Ivoire, the Cadmium concentrations are 5 to 127 times more significant in the discharges than in the grounds according to Traoré *et al.* (2014). Environmental pollution with cadmium relates to especially the rubbish dump garbage of Akouedo (Abidjan, Côte d'Ivoire) where place of many studies had. Indeed, this discharge of Akouedo is known for its heavy metal threat in particular with cadmium. It is classified with the row of the wild discharges whose natural drainage is done towards the lagoon Ebrié (bay of Me Badon), to less than 2.1 km. This liquid rejection downstream from the discharge generates a significant flow (474 m<sup>3</sup> /days). Indeed, the lixiviat resulting from waste drains heavy metals (cadmium) and constitutes a potential source of underground pollution waters and surface (Adjiriet *et al.*, 2008). The populations generally

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complain about the bad smells, of diseases (respiratory, digestive, cutaneous) and the ground of the discharge contains a high heavy metal concentration (9.87 cadmium ppm) (Adjiriet *et al.*, 2008). In addition, metals and metalloids associated with the aqueous phase with the grounds can be transported through Unsaturated Zone (ZNS) either towards the plants, or towards the underground sheet of water (Adjiriet *et al.*, 2008). It is thus to fear as for the exposure of the populations to the consumption of water of well, of the market-gardening and food products cultivated on the site of the discharge. For the simple reason that this accumulation of heavy metals in the environment can be reflected on the health of the human beings and the animals (Yao and Kouassi, 2015). Several studies undertaken on the mammals showed that it causes genetic damage, the change of genes, the break of the ADN (IARC, 1993). Different experimental studies also showed that cadmium causes testicular attacks and decreases the fertility in males (Pascal *et al.*, 2010).

This is the reason why our study is focused on cadmium sulfate biotoxicity in Wistar rats.

## MATERIAL AND METHODS

### Material

#### Biological material

The biological material is the serum of rats male and female of Wistar weighing  $106 \pm 6$ g and old from 8 to 12 weeks. The rats were placed in cages furnished with litters of shaving of which revival was done each week. They were acclimatized before the beginning of handling.

#### Chemical Products

The cadmium sulfate used for the tests was dehydrated salt (MERCK), and of serial number 1.02027.0100.

### Methods

#### Preparation of the cadmium sulfate solution

The rats were weighed by batch and the average (P) for each batch was thus determined. For the preparation of the cadmium sulfate solution, the formula below was used (Michel, 2011):

$$C = \frac{D \cdot P}{V}$$

V: Volume to be managed or with gaver (ml)

D: Proportion to manage (mg/Kg body weight or 0.001 mg/g)

C: Concentration of the solution (mg/ml)

The quantity of cadmium sulfate to be weighed was thus given. This quantity was deposited in a tube falcon and was supplemented to 10 ml with water distilled to constitute the cadmium sulfate solution. For an amount of 300 mg/Kg or 0.3 mg/g of body weight and for an average weight of 78g,

$$C = \frac{0.3\text{mg/g} \times 78\text{g}}{1\text{ml}} = 23.4 \text{ mg/ml i.e. } 23.4 \text{ powder mg of cadmium for 1ml}$$

Thus, for a final cadmium sulfate solution of 10 ml, it will be necessary to weigh 234 mg.

### Study of acute toxicity by method 423 OECD

Method 423 OECD allowed to determine the lethal amount 50% (DL50) of cadmium sulfate managed by way oral examination with the rats.

### Study of the subacute toxicity of cadmium sulfate

#### Identification of the animals

Each batch consists of the same animal's sex and appreciably equal weights. The animals were marked with the tail by batch and inside each batch in order to identify them during the experimentation.

#### Methodologies of cramming

Twelve (12) Batches of five (5) rats were made up including 6 male groups and 6 groups of females. The—Groups 1 of males and females constituted the control groups where the animals received distilled water (1ml/day). Groups 2, 3, 4, 5 and 6 of each sex received respectively  $1/50^{\text{th}}$ ,  $1/40^{\text{th}}$ ,  $1/30^{\text{th}}$ ,  $1/20^{\text{th}}$  and  $1/10^{\text{th}}$  of the DL50 of the cadmium sulfate solution. They are the treated groups.

The duration of treatment of the rats with cadmium sulfate was 30 Days (Layachi, 2012) and managed volume was of 1ml per day each morning throughout treatment.

#### Weighing of the rats, taking away of blood and experimental techniques

The rats were weighed the first day (D0) of the experimentation before the force-feeding and were weighed each week (D7, D14, D21, D30). For each batch and each week of weighing, the percentage P (see formula below) is calculated where  $P_0$  represents the weight at the day 0 (D0) of the treatment and  $P_n$  weight

n days after (Galtier, 1974)

$$P = \frac{P_n}{P_o} \times 100$$

After the period of exposure (30 Days) to cadmium sulfate, the rats were sacrificed. The blood of each rat was collected immediately in the sample dry tube which was used to obtain the serum after centrifugation with 3000 rpm during 5min for the biochemical parameters with the automat *Cobas C311 ROCHE HITACHI* at the Pasteur Institute of Abidjan (Côte d' Ivoire).

### Statistical analyses of the data

Statistical analysis and layout of the curves were undertaken with *GraphPad Prism V5.01* software (Washington, USA). Groups of data were compared one-way analysis of variance (ANOVA). Due to the small population size, the non-parametric Dunnett test was performed to compare assess difference between the control group and the other groups. Differences were considered statistically significant at  $p < 0.05$ .

## RESULTS

### Determination of the DL50 by Method 423 OECD

According to the system of classification harmonized overall (SGH), cadmium is classified of category 3 whose DL50 is located between 50 and 300 mg/kg body weight (bw). Thus, the limiting DL50 is fixed at 200 mg/kg bw.

### Subacute Toxicity of Cadmium Sulfate

#### Symptomatology, Evaluation of Mortality and Determination of Maximum Tolerated Dose

The behavior of the rats presented the following characters: loss of hair as of the first week, calm, deceleration of the more visible growth for the rats exposed to 10 mg/Kg bw of cadmium and those exposed 20 mg/Kg in the males as in the females. The animals treated with cadmium ate and drank little proportionally with the amount of exposure. The animals did not make a hematuria but they urinated little. The control animals (Male and Female) ate and drank abundantly.

In the females, no mortality was noted. On the other hand at the males, in batch 6 (20 mg/Kg bw), two mortalities were noted. Therefore, the maximum tolerated dose to the males was 10 mg/Kg bw and for the females, it was 20 mg/Kg bw, according to the method of Galtier, (1974) modified.

### Effect of Cadmium on the Body Weight of the Rats

Generally, the cadmium sulfate reduced the body weight of the males rats tested compared to the control males rats that weighed D7 = 115.244 ± 4.96g; D14 = 130.598 ± 8.27g; D21 = 142.088 ± 12.11g; D30 = 148.03 ± 11.93g. Thus, according to figure 1, this reduction was significant for the rats of batch 2 in D21(128.106 ± 5.84g;  $p < 0.05$ ) and D30 (129.922 ± 7.37g;  $p < 0.05$ ), of batch 3 in D21 (127.116 ± 7.13g;  $p < 0.05$ ), of batch 4 in D14 (115.034 ± 2.95g;  $p < 0.05$ ), D21 (122.084 ± 3.56g;  $p < 0.05$ ) and J30 (129.922 ± 4.05g;  $p < 0.05$ ), of batch 5 since D14 (110.470 ± 11.17g;  $p < 0.001$ ) until D30 (116.306 ± 7.68g;  $p < 0.0001$ ) and of batch 6 since D7 (102.634 ± 8.61g;  $p < 0.001$ ) until D30 (110.577 ± 6.16g;  $p < 0.0001$ ). The rate in body weight reduction of the male animals is dose-dependent and this reduction is high to the animals of batch 6 in D30 (25.30%;  $p < 0.0001$ ) (Figure 1).

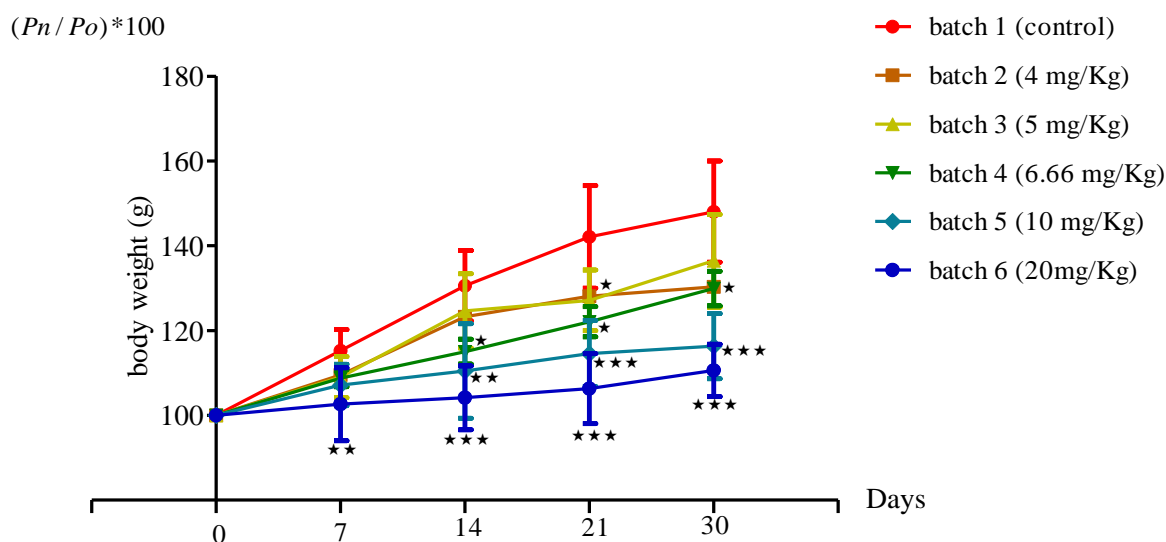
The females rats also knew a reduction in their body weight compared to the control batch that weighted D7 = 117.446 ± 2.32g; D14 = 130.986 ± 5.10g; D21=135.108 ± 4.74g; D30=140.822 ± 5.49g. According to figure 2, this reduction was significant for batch 2 in D7 (104.104 ± 2.58g;  $p < 0.0001$ ) and D14 (113.016 ± 5.71g;  $p < 0.001$ ), batch 3 in D7 (106.848 ± 3.02g;  $p < 0.001$ ), batch 5 in D7 (103.312 ± 3.95g;  $p < 0.0001$ ) and D14 (108.846 ± 9.78g;  $p < 0.001$ ) and batch 6 since D7 (95.472 ± 6.64g;  $p < 0.0001$ ) until D30 (103.924 ± 5.43g;  $p < 0.0001$ ). In the females, this reduction in the body weight is not dose-dependent, but remains very high in female animals from D14 to D30 at about 25% (figure 2).

Cadmium sulfate induced a slowdown in the growth of animals

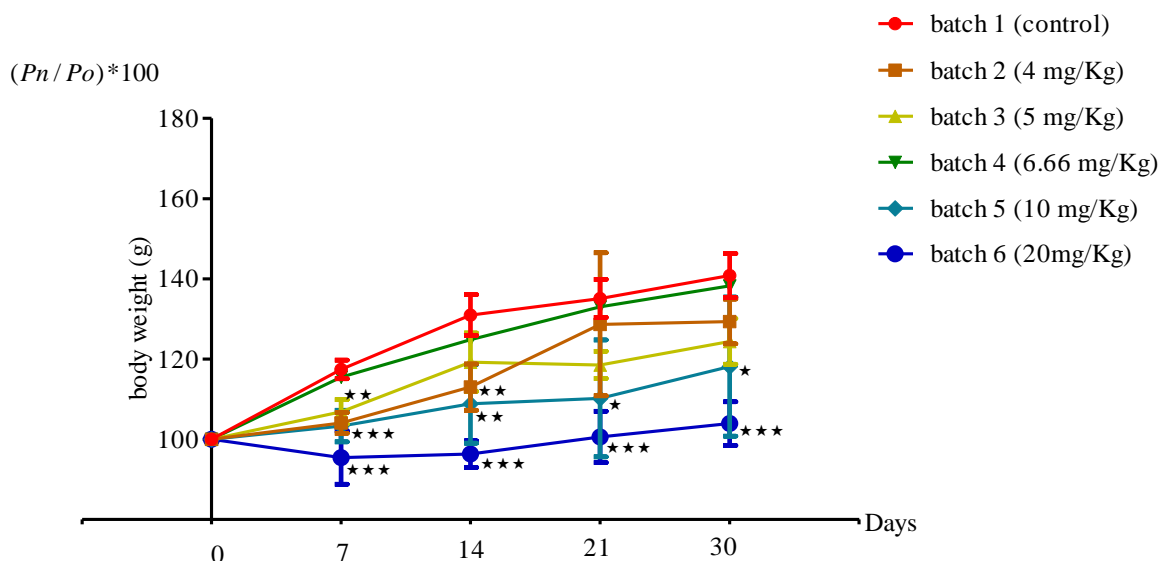
### Action of Cadmium on the Hepatic Function

Table I as well shows an increase in the activity of the ALT in the males as in the females compared to the control batches (male: 42.66 ± 9.796 UI/L and female: 40.17 ± 1.88 UI/L). This increase is significant in the males rats for batches 2 (70.62 ± 20.09 UI/L;  $p < 0.001$ ), batch 5 (74.47 ± 9.05 UI/L;  $p < 0.0001$ ) and batch 6 (161.4 ± 1.13 UI/L;  $p < 0.0001$ ). In the females, the increase in the activity of the ALT is significant for the batches 5 (60.18 ± 12.34 UI/L;  $p < 0.0001$ ) and batch 6 (77.64 ± 17.80 UI;  $p < 0.0001$ ).

As for the AST, the concentration of 5mg/kg bw induced a significant reduction in the activity as well in the males (223.2 ± 35.18 UI/L;  $p < 0.05$ ) that in the females (228.75 ± 15.20 UI/L;  $p < 0.001$ ) compared to the activity at the control rats (male: 316.76 ± 60.03 UI/L; females: 307.82 ± 34.77 UI/L). The high concentrations did not induce a significant variation of the AST activity in female rats, whereas in the males, this high concentration (batch 6: 20mg/kg bw) induced a



**Figure 1.** Effect of cadmium on the body weight of male rats  
Significance level: \* = p<0.05; \*\* = p<0.001; \*\*\* = p<0.0001 with n = 5



**Figure 2.** Effect of cadmium on the body weight of female rats  
Significance level: \* = p<0.05; \*\* = p<0.001; \*\*\* = p<0.0001 with n = 5

Table 1. Effect of cadmium sulfate on the liver enzymes in the rats

Sex	Male		Female	
Parameter	ASTL (UI/L)	ALTL (UI/L)	ASTL (UI/L)	ALTL (UI/L)
LOT 1 (Témoïn)	316.76 ± 60.03	42.66 ± 9.796	307.82± 34.77	40.17± 1.88
LOT 2 (4mg /Kg)	352 ± 48.74	70.62 ± 20.09**	334.40± 3.81	54.00 ± 12.44
LOT3 (5mg /Kg)	223.2± 35.18*	37.60± 10.37	228.75± 15.20**	39.75 ± 2.05
LOT 4 (6.66mg /Kg)	339.7±54.76	58.6 ±0.61	315.53± 7.87	54.72±9.05
LOT 5 (10mg /Kg)	374.4 ± 57.23	74.47±9.05***	324.02± 33.30	60.18 ± 12.34*
LOT 6 (20mg /Kg)	1025.5± 66.04***	161.4 ± 1.13***	333.16± 51.83	77.64± 17.80***

Significance level: ¶\* = p<0.05; ¶\*\* = p<0.001; ¶\*\*\* = p<0.0001 with n = 5

significant increase ( $1025.5 \pm 66.04$  UI/L;  $p < 0.0001$ ) (Table I) The AST and ALT activities were dose-dependent in male rats, but on the other hand, the ALT activity was not significantly varied for the female rats.

### Action of Cadmium on the Renal Function

The tested concentrations induced a non-significant increase in creatinin in the rats regardless of sex from the values in the control groups (Table 2). However, according to table II, the high concentrations (6.66; 10 and 20 mg/kg bw) induced respectively a significant increase in urea in the males rats ( $0.50 \pm 0.06$  g/L,  $p < 0.001$ ;  $0.51 \pm 0.06$  g/L,  $p < 0.001$  and  $0.498 \pm 0.025$  g/L,  $p < 0.0001$ ). However, only the high concentration (20mg/kg bw) induced a significant increase in the females ( $0.524 \pm 0.08$  g/L;  $p < 0.05$ ) compared to the control groups values (male:  $0.38 \pm 0.06$  g/L and female:  $0.3914 \pm 0.01$  g/L). The change of renal function both in male and female rats, is correlated to the variation of the value of the urea

### Action of Cadmium on the Splenic Function ¶

The rate of total bilirubin was decreased significantly for all the test concentrations (4; 5; 6.66; 10 and 20 lg/kg bw) both in the male rats ( $1.35 \pm 0.7$ g/L;  $p < 0.0001$ ;  $1.60 \pm 0.31$  g/L;  $p < 0.001$ ;  $1.76 \pm 0.27$  g/L;  $p < 0.0001$  and  $0.21 \pm 0.09$  g/L;  $p < 0.0001$ ) than in females compared to the control rats groups (male:  $2.43 \pm 0.68$  g/L and female:  $1.62 \pm 0.26$  g/L) (table 3). This reduction is dose-dependent in the male rats.

For total cholesterol, table 3 shows non-significant variation both in the two sex. The disturbance of the splenic function in the rat would be due to the variation of the rate of the total bilirubin.

## DISCUSSION

For the population, the principal ways of contamination to cadmium are food, water and cigarette smoke. According to conditions of exposure to metal, 5% of introduced cadmium is absorbed by the gastro-intestine tract in the form of salt, while 90% of inhaled cadmium is absorbed by pulmonary way (Jarup, 2002). The experimental study showed that the cadmium sulfate cause losses of weight and a deceleration of the growth. That can be explained by the fact why the rats treated with cadmium ate little compared to the control animals noted at the time of the study of Wang *et al.* (2014) according to the dose of exposure. Consequently, for Sajjadet *et al.* (2014), cadmium would have caused a loss of appetite in the rats (Sajjadet *et al.*, 2014). Indeed, there is a correlation between the loss of body weight

and the cadmium concentration in the hypothalamus and the pituitary gland in the animals treated with cadmium (Sajjadet *et al.*, 2014). For several authors, other factors can explain this loss of weight. In the guinea-pigs, the loss noted during the exposure to cadmium 5 mg/Kg is explained by damage of the system glucocorticoid, essential hormone in the regulation of glucose and the metabolism of the lipids and proteins (Mouhamed and Azab, 2014) necessary to the loss and the profit of weight. These results of loss of weights noted at the time of the study are in agreements with those of Sandrita *et al.*, (2003) where the mice were watered with water enriched with cadmium chloride (10 mg/L) during 8 weeks. Losses of weight, weaknesses and mortalities were noted. Indeed, Already with 0.15mg/kg cadmium sulfate considered as proportions pathological at the time of the study of Berroukche *et al.* (2014) showed a similar loss of weight where the weakest concentration was 4 mg/kg body weight.

The biochemical parameter makes it possible to evaluate possible toxic effects of an agent on the physiological functions of the organism responsible for many diseases (Smaoui *et al.*, 2000). The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are used in animal toxicology like markers of the dysfunction of liver (Hannah *et al.*, 2002). The quantification of the activity of these enzymes in the animals is a marker of the exposure to pollutants (Hannah *et al.*, 2002) observed in fish.

The results of the study showed a significant increase in the enzymatic activity of AST and ALT in the serum of the contaminated rats with cadmium sulfate compared to the control group in the males as in the females. Increased in these activities of enzymes in the blood, represented their leakage of tissue into plasma following a hepatic lesion responsible for the deterioration of the membrane permeability (Layachi and Kechrid, 2013). This damage and the relaxation of the enzymes in blood is due to an accumulation of cadmium sulfate in the liver. In his study, Nashwa (2013) put in obviousness two ways characterizing the hepathotoxicity of cadmium. On a side it causes an inflammation leading to damage of the liver and kidneys (Fahim *et al.*, 2012) characterized by the high rate of white globules (Mouhamed and Azab, 2014), and other, it destroys the cells of the liver directly.

In their studies (Egwurugwu *et al.*, 2007), the Wistar rats exposed to 200 mg/L showed a similar rise in transaminases compared to the control group rats like in fish (Jisha *et al.*, 2013) and in chickens with a concentration of 100 ppm cadmium (Bharavi *et al.*, 2011). The rate of accumulation of heavy metals in the animals varies according to the species and on the individuals in the population, but, also depends on the sex, the age, the size and the food (Bedii and Kenan, 2005; Haki *et al.*, 2005).

Cadmium is biotoxic environmental pollution; it

**Table 2.** Effect of cadmium on kidney markers in rat

Sexs	Male		Female	
	Urea (g/L)	Creatinin (mg/L)	Urea (g/L)	Creatinin (mg/L) ¶
LOT 1 (Témoin)	0.38± 0.06	5.4± 0.54	0.391± 0.01	4.75± 0.96
LOT 2 (4mg /Kg)	0.42± 0.06	6 ± 1	0.395± 0.06	6.5 ± 2.38
LOT3 (5mg /Kg)	0.47±0.04	5.1± 0.57	0.393± 0.05	5± 1.41
LOT 4 (6,66mg /Kg)	0.50± 0.06**	6 ± 1	0.4768±0.06	6.2 ± 0.44
LOT 5 (10mg /Kg)	0.51 ± 0.06**	5.5 ± 0.58	0.482± 0.09	5.4± 0.55
LOT 6 (20mg /Kg)	0.498 ± 0.025*	6.33± 1.15	0.524± 0.08*	5.2± 0.84

Significance level:¶\* = p<0.05;¶\*\* = p<0.001;¶ with n = 5

**Table 3.** Effect of cadmium on spleen markers in rat

Sexe	Male		Female	
	Bilirubin (g/L)	Cholesterol (g/L)	Bilirubin (g/L)	Cholesterol (g/L)
LOT 1 (Témoin)	2.43±0.68	0.946± 0.06	1.62± 0.26	0.66± 0.10
LOT 2 (4mg /Kg)	1.35± 0.7***	0.96± 0.22	0.99±0.13***	0.72± 0.13
LOT3 (5mg /Kg)	1.60± 0.31**	0.72± 0.09	1.08±0.08**	0.85± 0.33
LOT 4 (6.66mg /Kg)	1.76±0.27*	0.88±0.06	1.09± 0.09**	0.63±0.09
LOT 5 (10mg /Kg)	1.46 ± 0.29***	0.91±0.22	1.14± 0.01**	0.73± 0.17
LOT 6 (20mg /Kg)	0.21± 0.09***	0.97± 0.38	1.16± 0.41*	0.54± 0.11

Significance level:¶\* = p<0.05;¶\*\* = p<0.001;¶\*\*\* = p<0.0001 with n = 5

accumulates in tissues mainly in the liver, the kidneys, osseous marrow, the reproductive bodies and in the immune system (Egwurugwu *et al.*, 2007). In addition, the major body implied in the toxicity of cadmium is liver (Singh *et al.* 2013). What is confirmed by Sandrita *et al.* (2003) where the mice contaminated with 10 mg/L cadmium recorded 0.032µg/g and 0,12 cadmium µg/g respectively in the liver and the kidneys.

The results of the study showed in animals of both sexes, a significant increase in levels of creatinine and urea in the blood compared to control group. This increase in these two parameters testifies to a renal insufficiency (Boujelbeneet *et al.*, 2002). Indeed, a significant rate of the blood volume passes by the kidneys, them making thus extremely vulnerable to the cytotoxic effect of many pollutants such as cadmium, lead and mercury (Boujelbeneet *et al.*, 2002). During their experiment on the evaluation of the toxicity of cadmium sulfate, the control group of Wistar rats recorded the presence of cadmium in their kidneys (Asagba and Obi, 2004), reflecting food and water pollution. Kidneys are the main targets after ingestion of cadmium (Wang *et al.*, 2014) where 50 % of metal accumulate with a half-life from 10 to 35 years and create kidney damage (Poontaweet *et al.*, 2016). Cadmium causes a tubular kidney dysfunction and a decrease in glomerular filtration rate thus leading to kidney failure reported by Fahim *et al.* (2012). This renal failure is evaluated by creatinine and urea determination (Fahim *et al.*, 2012). The results showed that the

increase of creatinin is not significant ( $p > 0.05$ ) in the females rats. Our results corroborate those of Fahim *et al.* (2012) and Wang *et al.* (2014) who have not observed the changes of creatinine level in the blood after 28 days of cadmium treatment.

The increase of uremia translated some kidney damage and is an acute exposure marker. As for creatinine, its increase is a sign of the kidney function failure (Abdel-Moneim and Ghafee, 2007). Therefore, the exposure of the rats to cadmium in this study causes a disturbance of the renal function in the males.

The bilirubin is the breakdown product of red blood corpuscles out-of-date or damage. These red blood corpuscles will release heme and the globine (Basso *et al.*, 1991). In the liver, bilirubin binds to other molecules before being excreted in the bile. The results obtained showed a significant reduction in the bilirubin compared to the control group in both rats sexes. These results confirmed the total correlation between bilirubin and cadmium where Zeneli *et al.*, (2009) noted that the high cadmium concentration in blood is due to cigarette smoke. In fact, this cadmium inhibits the synthesis of bilirubin. The bilirubin rate reduction in blood may be caused by anemia (Zeneli *et al.*, 2009) due to cadmium.

## CONCLUSION

Cadmium cause a weight decrease noted by a deceleration of the growth Cadmium causes slower

growth through decreased weight, anemia, renal failure and liver damage. These observed pathologies are the risks to people living in a polluted environment to cadmium. This is the place to draw the attention of clinicians so that incorporation metal settings to blood checkups including cadmium for better management of heavy metal poisoning.

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