

**Effects of cadmium on some haematological and biochemical parameters of
*Clarias gariepinus***

Naglaa F. Elarabany^{1*}, Mohammed H. Bahnsawy¹ and Mona Alfakheri^{1, 2}

1- Zoology Department, Faculty of Science, Damietta Univ., Damietta, 34514, Egypt

2- Zoology Department, Faculty of Science, Sabha Univ., Sabha, Libya

*elarabany@du.edu.eg

ABSTRACT

This study aimed to determine the effects of sub-lethal concentrations of cadmium (CdCl₂) on some haematological and biochemical parameters in 172 specimens of *Clarias gariepinus*. Fishes were captured from Manzala Lake, Egypt. They were acclimatized and fed with commercial fish diet for one month before starting the experiment. After determination of LC₅₀, Two experimental groups were exposed to two sub-lethal concentrations (10% and 30% of LC₅₀) of cadmium chloride along with control group. Fishes from different experimental groups were sacrificed after 60 days of exposure. Haematological parameters including erythrocytes, Hb, PCV, thrombocyte, leucocytes, and differential count of WBCs were estimated. Also, some biochemical parameters such as sodium, potassium, calcium, glucose, total protein, albumin, AST, ALT, ammonia, urea, uric acid, total bilirubin, creatinine, total lipids, cholesterol, triglycerides and cortisol and oxidative stress enzymes such as GSH, CAT, SOD, and MDA were measured. Group treated with 10 % of LC₅₀ of Cd showed significantly lower concentrations of Hb, thrombocytes and SOD activity and significantly higher concentrations of eosinophil, MDA, and triglyceride. On the other hand, group of 30 LC₅₀ of Cd showed a significantly lower concentration of sodium and significantly higher activities of CAT, ALT and ammonia concentrations. The investigated two sub-lethal concentration (10 % and 30% of LC₅₀ of Cd) groups showed a significantly lower activity of GSH and lower concentrations of potassium and cholesterol, besides these showed a significantly higher concentrations of uric acid and creatinine. Serum calcium, glucose, albumin, AST, urea and total lipids did not show any significant differences in the estimated treatment concentrations.

Key words: Cadmium, oxidative stress, haematotoxicity, African catfish, *Clarias gariepinus*.

INTRODUCTION

Heavy metals generally have different effects on fishes depending on the type of heavy metal, its concentration in water and the period of exposure (Boscher *et al.*, 2010). Many studies have shown that bioaccumulation of heavy metals affects fish growth rate and the quality of their meat, beside to its significant effect on fish physiological and chemical status (Soegianto *et al.*, 2010). Some heavy metals are essential in the environment system such as copper, chromium, and iron, while others such as cadmium and lead are not necessary and are toxic even in low concentrations (Rubio *et al.*, 2016).

Cadmium is a highly toxic heavy metal, produced from liquid waste, paint, and mining extracts (Perez and Wallace, 2004). Studies showed that fish exposed to different concentrations of cadmium can be found at different rates in some organs such as liver, kidneys, and gills (Klinck *et al.*, 2007), however, exposure to this metal causes changes in metabolism as well as many biochemical and physiological effects (Szebedinszky *et al.*, 2001). Fishes convert the foreign compounds enzymatically into less harmful compounds, reactions are often the most important processes xenobiotic biotransformation (Persch *et al.*, 2017).

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Clarias gariiepinus, the African catfish, has a great nutritional importance as an economical source of protein in Egypt, being one of the most common freshwater fish, therefore it's a good animal model for investigating the impact of various environmental pollutants (Darwin *et al.*, 2017). This species is resistant to environmental stress and can adapt to different types of food in the environment and it can be available at night or day (Qu *et al.*, 2014).

Exposure to cadmium leads to accumulation in fish tissue, later, many serious disorders appear in the metabolism, behavior change, abnormalities in movement, and obvious effects on blood (Gabriel *et al.*, 2004). Joshp *et al.* (2002) studied the effect of cadmium on catfish *Clarias batrachus* and their results showed that Cd caused a reduction in hemoglobin and erythrocytes count and glucose, cholesterol, and protein levels. Also, Cd decreased MCHC and PCV in *Heterobranchus bidorsalis* (Kori-Siakpere *et al.*, 2006). This metal had been proved to lower GSH activity (Amaeze *et al.*, 2015).

Due to the lack of studies on African catfish and its economic importance as food source in Egypt, this study aimed to measure the changes of haematological, biochemical parameters and oxidative stress enzymes in this fish after its exposure for 60 days to two different doses of of LC₅₀ of cadmium chloride.

MATERIALS AND METHODS

1 Determination of LC₅₀

One hundred and seventy-two African catfish, *Clarias gariiepinus* (mean body weight is 150± 25 g) were obtained from Manzala Lake, Damietta, Egypt. Fishes, apparently healthy, were transported immediately to the lab and acclimatized for two weeks in glass aquaria (40* 35* 70 cm) with a capacity of 60 L and were fed on a basal fish diet.

After acclimation, 100 fishes were divided into ten groups (10 fishes/ group) for determination of LC₅₀, each group was exposed to a different concentrations of CdCl₂ (10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, 60 mg/l, 70 mg/l, 80 mg/l,) at constant temperature 25°C. Fishes were kept under observation for 96 hours and numbers of dead fishes were recorded daily. The LC₅₀ was statistically determined by using dose-effect analysis using XLSTAT software.

2 Experimental fishes model:

After determination of LC₅₀, 72 fishes were divided randomly into equal three groups with 8 fishes in all aquaria, each group was represented by triplets (24 fishes for each treatment); Control, Cd 10% of LC₅₀ and Cd 30% of LC₅₀ for 60 days.

3 Test Compound

Cadmium as CdCl₂.H₂O (monohydrate, molecular weight 201.32 g, purity 98%), manufactured by Oxford Lab Chem, India.

4 Blood sampling

Fishes fasted for 24 hours before sampling; blood samples were obtained from the caudal vein using 3 ml syringe within less than 3 minutes to minimize handling stress. The collected blood samples were divided into two tubes, one containing heparin as anticoagulant agent for haematological assessment and the other was anticoagulant free for biochemical estimation.

Haematological measurements were assayed within one hour of sampling using blood cell automated counter. Red blood cells count (RBCs), haemoglobin content (Hb), thrombocytes count (TC), and white blood cells count (WBCs) were evaluated. Different blood indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and

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mean corpuscular hemoglobin concentration (MCHC) were calculated.

Anticoagulant free samples were used for serum preparation by centrifugation for 20 minutes at 1207 g within one hour of sampling, the sera samples were used for determination of malondialdehyde (MDA), Catalase (CAT), Sodium (Na^+), Potassium (K^+), Calcium (Ca), Glucose (GLU), Total protein (TP), Albumin (ALB), Glutamic – Oxaloacetic Transaminase (AST), Glutamic – Pyruvic Transaminase (ALT), Ammonia (AMM), Urea (UREA), Uric Acid (UR AC), Total Bilirubin (TB), Creatinine,(CREA), Total lipids (TL), Cholesterol(CHOL), Triglycerides(TRI)and Cortisol (COR), while superoxide dismutase (SOD) was determined in RBCs lysate later on the same day with commercial kits. Absorbance values of samples and standards were measured using a UV spectrophotometer.

5 Statistical analysis

Data were expressed as mean \pm SEM of different treated groups compared to control ones. Normal distribution of all parameters was tested. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey (HSD) test to compare groups with each

other and Dennett two-sided test for comparisons with the control group. $P < 0.05$ was considered significant. All statistical analyses were performed using XLSTAT program.

RESULTS

As shown in Table (1), Hb and TC were significantly lower in Cd10% group compared to control group. On the other hand, MCV was significantly lower in control group compared with Cd 10 % and Cd 30% group. Eosinophils also were significantly higher in Cd10% group compared to control group. For other haematological parameters no significant changes were found between different groups.

Glutathione reduced (GSH) concentration was significantly lower in Cd 10% and Cd 30% groups compared to control group. On the other hand catalase (CAT) activity was significantly higher in Cd 30% group compared to control group.

Lipid peroxide product (Malondialdehyde, MDA) was significantly higher in Cd 10% group compared to control group, while superoxide dismutase (SOD) activity was significantly lower in Cd 10% group compared to control group.

Table 1: Means \pm SEM of haematological parameters of African catfish, *clarias gariepinus* treated with different Cd concentrations (10%, 30% LC_{50}) for 60 days.

Parameter	Groups			P
	Control	Cd 10% LC_{50}	Cd 30% LC_{50}	
PCV (%)	29.43 \pm 0.57	29.33 \pm 0.83	29.11 \pm 0.45	NS
Hb (g/dl)	11.97 \pm 0.48 ^a	10.35 \pm 0.28 ^b	10.88 \pm 0.56 ^a	0.01
MCV (fl)	81.90 \pm 2.61 ^a	90.39 \pm 2.50 ^b	87.47 \pm 3.11 ^b	0.01
MCH (Pg)	31.24 \pm 0.56	31.6 \pm 0.75	32.37 \pm 1	NS
MCHC (g/dl)	38.91 \pm 1.01	35.15 \pm 0.95	37.13 \pm 1.66	NS
Erythrocytes ($10^6/\mu\text{l}$)	3.77 \pm 0.14	3.28 \pm 0.09	3.37 \pm 0.14	NS
Thrombocytes ($10^3/\mu\text{l}$)	62.43 \pm 12.8 ^a	52.75 \pm 9.2 ^b	61.89 \pm 14.8 ^a	0.04
Leucocytes ($10^3/\mu\text{l}$)	3.40 \pm 0.23	3.78 \pm 0.15	3.57 \pm 0.32	NS
Neutrophils (%)	7 \pm 0.58	6.33 \pm 1.21	6.56 \pm 1.08	NS
Eosinophil (%)	2.57 \pm 0.20 ^a	3.17 \pm 0.21 ^b	2.89 \pm 0.39 ^a	0.01
Lymphocyte (%)	86 \pm 0.76	83.50 \pm 0.89	85.11 \pm 1.26	NS

Values different superscript letters within each row are significantly different ($P < 0.05$).

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As shown in Table (2) there were significant lower in Cd10 % and Cd30 % groups comparing to control group in potassium, total protein, and cholesterol, while Cd 30% group was significantly lower than control group in sodium. On the other hand, Cd 30% group was significantly higher in ALT, ammonia and cholesterol level comparing to control group but in triglycerides, Cd10 % group was higher than control group, while Cd

10% and Cd30% groups were significantly higher comparing to control group in both uric acid and creatinine.

No significant differences were found between different concentrations of treated and control groups in other parameters such as calcium, glucose, albumin, AST, urea and total lipids (Table 3).

Table 2: Means± SEM of Lipid peroxidation and oxidative system of African catfish, *Clarias gariepinus* treated with different Cd concentrations (10%, 30% LC₅₀) for 60 days.

Parameter	Groups			P
	Control	Cd 10% of LC ₅₀	Cd 30% of LC ₅₀	
GSH(U/L)	15.69± 1.59 ^a	10.65± 0.60 ^b	11.19± 0.96 ^b	0.041
CAT (U/l)	86.98± 29.19 ^a	97.61± 20.59 ^a	206.62± 27.38 ^b	0.015
MDA(nmol/ml)	40.89± 5.49 ^a	45.32± 6.91 ^b	39.60± 3.29 ^a	0.009
SOD (U/gm)	267.18± 527.9 ^a	197.84± 191.8 ^b	233.33± 350.5 ^{ab}	0.018

Values different superscript letters within each row are significantly different ($P<0.05$).

Table 3: Means± SEM of biochemical parameters of Catfish *Clarias gariepinus* treated with different Cd (10%, 30%) for 60 days.

Parameter	Groups			P
	Control	Cd10% of LC ₅₀	Cd 30% of LC ₅₀	
Sodium (mEq/L)	228.3± 10.8 ^a	199.2± 4.4 ^{ab}	183.2± 7.19 ^b	0.008
Potassium (mEq/L)	4.21± 0.21 ^a	3.92± 0.12 ^b	3.95± 0.18 ^b	0.08
Calcium(mg/dl)	11.29± 0.10	11.41± 0.17	11.22± 0.31	NS
Glucose (mg/dl)	51.6± 3.03	54.41± 3.80	58.23± 4.69	NS
T. protein (g/dl)	5.75± 0.23 ^a	4.79± 0.31 ^b	4.25± 0.24 ^b	0.001
Albumin(g/dl)	2.47± 0.08	2.68± 0.10	2.51± 0.06	NS
AST(g/dl)	35.83± 7.35	37.3± 8.02	39.6± 12.23	NS
ALT(g/dl)	16.20± 2.13 ^a	17.11± 2.25 ^a	18.00± 1.59 ^b	0.0006
Ammonia(mg/dl)	164.1± 13.5 ^a	242.2± 7.9 ^{ab}	266.3± 25.4 ^b	0.008
Urea(mg/dl)	8.30± 0.22	9.43± 0.40	8.14± 0.52	NS
Uric Acid(mg/dl)	3.46± 0.33 ^a	10.58± 1.1 ^b	9.49± 1.25 ^b	0.0001
Bilirubin(mg/dl)	1.34± 0.0 ^a	2.09± 0.19 ^b	2.09± 0.25 ^b	0.0004
Creatinine(mg/dl)	0.18± 0.04 ^a	0.26± 0.06 ^b	0.31± 0.05 ^b	0.03
T. lipids(mg/dl)	765.9± 51.2	572.5± 68.25	607.4± 52.09	NS
Cholesterol(mg/dl)	455.9± 38.5 ^a	308.7± 28.0 ^b	211.1± 18.8 ^b	0.03
Triglycerides(mg/dl)	240± 13.78 ^a	406.4± 69.6 ^b	237.7± 31.1 ^a	0.02
Cortisol (ng/ml)	100± 6.7 ^a	278.9± 102.9 ^a	718± 84.0 ^b	0.0001

Values with different superscript letters within each row are significantly different ($P<0.05$).

DISCUSSION

1 Haematological parameters:

In the current study, Hb and thrombocytes were significantly lower in Cd 10% and Cd 30% groups compared to

control group, while eosinophil was significantly higher Cd10% compared to control group and MCV was significantly higher in Cd10% and Cd 30% groups comparing to control. Similar results were

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reported by where there were increase in Hb and thrombocytes and decrease in MCV and eosinophil after exposure to cadmium. This changes may be due to the defects that occurred in both hemopoietic and metabolic states of the treated fishes where exposure to Sublethal concentrations of Cd cause hemopoietic organs dysfunction resulting in low Hb concentrations due to destructive effect of Cd (Sharma and Langer, 2014).

2. Oxidative Stress Enzymes

In the present study, GSH concentration was significantly lower in Cd 10% and Cd 30%, respectively, compared to the control group. Similar results were found in *Clarias graiepinus* where GSH concentration decreased after 21 days of exposure to different concentrations of cadmium (Amaeze *et al.*, 2015). Oxidative stress may be initiated by a decline in the antioxidative defense system, GSH loss in fishes reflects the ability of metals in oxidizing sulfhydryl group resulting in inactivation of oxidative enzymes by free radicals.

CAT activity increased in Cd 30% compared to the control group. Some investigators have suggested that severe oxidative stress may suppress the activity of antioxidant defense enzymes due to oxidative damage and a loss of the compensatory mechanisms (Atli *et al.*, 2006; Liu *et al.*, 2006). Similar results on *Clarias graiepinus*, showed that cadmium increased CAT activity (Bakare *et al.*, 2013). This increase in CAT activity is due to the degradation of H₂O₂, a potent oxidant at high cellular concentration is affected by CAT due to its induction against increased oxidative stress.

MDA, is used as an effective biomarker of toxic pollutants in fish exposed to cadmium, MDA level, in the current study, increased in Cd 10% group compared to the control group. Similar results were found in *Clarias graiepinus* where MDA level increased after

exposure to varying concentrations of cadmium (Farombi *et al.*, 2007), the production of MDA is a marker of lipid peroxidation results from the decomposition of polyunsaturated fatty acid due to oxidative stress (Alfanie *et al.*, 2015). SOD activity decreased significantly in Cd 10% compared to the control group and then increased in Cd30% group. This pattern of change was found in similar results were found in *Clarias graiepinus* where SOD activity decreased after exposure to cadmium and increased near to control values (Asagba *et al.*, 2010), these pattern indicate the activation of protective mechanisms required for removing O₂- radical in RBCs (Zikić *et al.*, 2001).

3 Biochemical parameters:

In the current work, sodium level reduced significantly in Cd 30% compared to the control group. Similar results were found in *Clarias graiepinus* where sodium level decreased in African catfish after exposure to 10 mg/L cadmium (El-Boshy *et al.*, 2014a). Similarly, potassium level reduced in Cd 10% and Cd 30% compared to the control group. Similar result was found in *Clarias graiepinus* where exposure to cadmium reduced potassium level (El-Boshy *et al.*, 2014a).

Since freshwater fish are hyperosmotic to the surrounding water, disruption of the gill epithelium that occurs after long-term metal exposures will increase epithelium permeability, water influx, and salt efflux (Kamunde *et al.*, 2002), and thus lead to a final significant decrease in plasma osmolality and ion concentrations such as Na⁺ and K⁺ (Monteiro *et al.*, 2005).

The Total protein level, in the current study, increased in both Cd 10% and Cd 30 % compared to the control group. Similar results were found in *Clarias graiepinus* where total protein level elevated with the presence of high concentrations of cadmium (Gaber *et al.*,

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2013). On contrary, , the exposure to 0.5 mg Cd⁻¹ and 1.0 mg Cd⁻¹ recorded a significant decrease in the level of protein in *Clarias graiepinus* (Kori-Siakpere et al., 2006). Moreover, *Clarias batrachus* exposed to 1 mg of CdSO₄ for 32 days, showed a significant reduction in protein levels (Kumar, 2016). Hyperproteinemia observed in the study groups may be due to liver or kidney damage (El-Boshy et al., 2014b).

The current results showed a significant increase of ALT activity in fishes treated with 30% Cd. A similar result was found in *Clarias graiepinus* where ALT activity was higher after exposure to cadmium (El-Boshy et al., 2014a). This elevation may reflect hepatocytes damage.

Ammonia level increased Cd 30 % compared to the control group. In the same line, *Clarias graiepinus* showed a high level of ammonia in study areas that contained a high concentration of cadmium (Gaber et al., 2013). In another study on the Nile River region from Aswan to Damietta, the concentration of cadmium was at the highest level and above the permissible limit in catfish *Clarias graiepinus*, and ammonia level was very high on the studied fish (Osman and Kloas, 2010). An increase in ammonia production may be a result of metal-induced stress together with an impaired ability to excrete ammonia across the gill which is the typical response to metal exposure in freshwater fish and leads to elevated plasma ammonia levels (Grosell et al., 2004; Patra et al., 2015).

The uric acid level, in the current study, increased in both Cd 10% and Cd 30%, compared to the control group. Similar results were found in *Clarias graiepinus* exposed to 10 mg/L cadmium where uric acid level was higher than the control group (El-Boshy et al., 2014a). This increase in uric acid concentration could be attributed to the action of accumulated recorded pollutants on the

glomerular filtration rate, which caused pathological changes in the kidney. These results may be also supported by the findings that exposure of fish to high concentrations of heavy metals led to the disintegration of the renal epithelial cells, displacement of nuclei, shrinkage of glomeruli, breakdown of Bowman's capsules and heavy infiltration by inflammatory cells (Authman and El-Sehamy, 2007).

In the current study, bilirubin increased in Cd 10% and Cd 30% compared to the control group. A similar result was found in *Clarias batrachus* where bilirubin was higher due to the effect of 1/10th of LC₅₀ cadmium treatment for 4 days . The elevated levels of bilirubin may indicate liver impairment and destruction of RBCs due to cadmium toxicity (Harabawy and Ibrahim, 2014).

Similarly, creatinine level increased in Cd 10% and Cd 30% groups compared to the control one. Similarly, *Clarias batrachus* exhibited elevated creatinine levels when exposed to 2.5 ppm cadmium, this elevated creatinine levels may indicate that cadmium is a nephrotoxic metal to fishes which cause kidney dysfunction..

Cholesterol level, in the current study, decreased in Cd 10% and Cd 30% compared to the control group. Similar results were found with *Clarias garaiepinus* that maintained in cadmium-contaminated water for a period of 14 days (Samuel et al., 2017). Also, *Clarias batrachus* showed decreased level of cholesterol after exposure to 1 mg/l of CdSO₄ compared to the control group (Kumar, 2016). This alteration in cholesterol concentration could be due to hazardous effects of metals on the cell membrane or may be attributed to an increase in lipid utilization for meeting additional energy requirements under stress conditions (Sindhe et al., 2002; Srivastava et al., 2002).

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Triglycerides level increased in Cd10% group compared to the control group. These results in line with those of *Clarias batrachus* that exposed to different doses of cadmium (0, 70.0, 75.0, 82.5, 92.5 and 95.0 mg/l) (Dhara *et al.*, 2014). There are a number of reports indicating that cadmium influence thyroid function. Since decreases thyroid secretion (hypothyroidism) greatly increases triglycerides level in the blood, the observed hypertriglyceridemia may due to hypothyroidism induced by cadmium and/or liver dysfunction because the liver is the principal center of lipid metabolism (Gaber *et al.*, 2013)

Cortisol level increased in Cd 30% compared to the control group. Similar results were found in *Clarias gariepinus* where cortisol level increased significantly after exposure to 0.25 and 0.50 ppm cadmium (Karaytug *et al.*, 2010). Stress factors such as exposure to cadmium affect cortisol concentration in fishes, hormonal response is the 1st response to varying environmental condition, cortisol is a key hormone in maintaining homeostatis (Karaytug *et al.*, 2010).

Conclusion

This study shows that different concentration of Cd leads to stress in the metabolic and antioxidant enzyme activities and other biomarkers of oxidative stress in *Clarias gariepinus* which may cause biochemical dysfunction in this species. In addition, the results provide evidence that enzymatic and non-enzymatic biomarkers of oxidative stress can be used as sensitive indicators of aquatic pollution.

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تأثيرات الكاديوم على بعض المعايير الدموية والبيوكيميائية لسماك القرموط الأفريقي، *Clarias graiepinus*

نجلاء العرباني¹ ومحمد بهنساوي¹ ومنى الفاخري²

1 - قسم علم الحيوان- كلية العلوم- جامعة دمياط- مصر

2 - قسم علوم الحياة- كلية العلوم- جامعة سيها- ليبيا

المستخلص

هدفت الدراسة الحالية الي تقييم اثار التركيزات تحت المميئة للكاديوم ($CdCl_2$) على بعض معايير الدم والمعايير البيوكيميائية في 172 قرموط افريقي. تم صيد الأسماك من بحيرة المنزلة ، مصر، وتم أقلمتها وتغذيتها لمدة شهر قبل بدء التجربة. بعد تعيين التركيز القاتل لنصف المجتمع LC_{50} . قسمت الأسماك الى ثلاثة مجموعات، تم معالجة مجموعتين بتركيزات 10% و 30% من LC_{50} لكلوريد الكاديوم بالاضافة للمجموعة الضابطة لمدة ستين يوماً. بنهاية التجربة، تم قياس بعض المعايير الدموية المختلفة مثل عدد كريات الدم الحمراء و حجم الكريات المضغوط والهيموجلوبين و عدد الصفائح الدموية و عدد خلايا الدم البيضاء والعد النوعي لخلايا الدم البيضاء. كما تم قياس بعض المعايير البيوكيميائية مثل الصوديوم والبوتاسيوم والكالسيوم والجلوكوز والبروتين الكلي والألبومين و AST و ALT والأمونيا واليوريا وحمض اليوريك والبيليروبين الكلي والكرياتينين و الدهون الكلية والكوليسترول والدهون الثلاثية والكورتيزول ومعايير الأوكسدة مثل مثل الجلوتاثيون ، الكاتاليز ، SOD، و المالون داي الدهيد (MDA). اظهرت نتائج المجموعة التي عولجت بـ 10% Cd تركيزات أقل بشكل ملحوظ من الهيموجلوبين والصفائح الدموية و انخفاض معنوي لنشاط SOD و تركيزات أعلى بكثير في عدد خلايا الدم البيضاء الحامضية و MDA والدهون الثلاثية. ومن ناحية أخرى ، أظهرت المجموعة التي عولجت بـ 30 % Cd تركيز أقل بكثير من الصوديوم ونشاط مرتفع معنويا لكلا من ALT و CAT و تركيز الأمونيا. أظهرت كلتا المجموعتين 10% Cd و 30% نشاطاً أقل بكثير من GSH و تركيزات أقل من البوتاسيوم والكوليسترول ، وأظهروا تركيزات أعلى بكثير من حمض اليوريك والكرياتينين. على صعيد اخر لم تظهر اى اختلافات معنوية بين المجموعات في تركيزات الكالسيوم ، الجلوكوز ، الألبومين ، AST، اليوريا والدهون الكلية.