

Full Research Paper

Biochemical and hormonal effects of cadmium in female guinea pigs

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Cadmium is a heavy metal toxicant, present widely in our environment and workplaces. In this study, the effects of cadmium on some biochemical and hormonal parameters of female guinea pigs were investigated. Single Intraperitoneal injection (ip) injection of 0.2 to 8.4 mg/kg of CdCl₂ was given to female guinea pigs in proestrus state and observed for 24 h. Also, single ip injection of 3 mg/kg of CdCl₂ was given to another set of animals that were observed for 24, 48, 72, 96 and 120 h. The animals were sacrificed at the end of each treatment period and blood samples were assayed for alkaline phosphatase (ALP), creatinine, urea, progesterone, Follicle Stimulating Hormone (FSH) and Lutenising Hormone (LH). Cadmium caused significant ($p \leq 0.05$) dose- and time-dependent increases in the serum levels of ALP, urea, creatinine, progesterone, FSH and LH. The results indicate that cadmium affects female reproductive function in guinea pigs, which may be due to interruption of the endocrine function.

Key words: Cadmium, endocrine, hormones, proestrus.

INTRODUCTION

Cadmium is an inorganic toxicant, occurring naturally in ores containing copper, zinc or iron. It is distributed widely in our environment and workplaces and is of great concern as an environmental and occupational toxicant, especially with increasing industrialization (Järup, 2003). A very significant source of Cd is in the production of nickel-cadmium batteries, while other sources include tobacco smoke, pigment plants, cadmium-contaminated food (e.g. rice and vegetable), soldering activities and petroleum refining processes (Nordberg et al., 2007; Wood et al., 2001).

Cadmium has been classified by the International Agency for Research on Cancer as class I carcinogen in humans and shown to cause various kinds of tumors including pulmonary, testicular and prostatic tumors (IARC, 1993; Joseph, 2009). Acute exposure to cadmium is associated with hepatic toxicity, while the kidney is the major target organ in chronic exposure and both conditions are dependent on the dose and route of

exposure (Koyuturk et al., 2007; Robert et al., 2002). Furthermore, recent studies had shown that cadmium administration is associated with alteration in reproductive functions in experimental animals (Aprioku et al., 2009; Massányi et al., 2007). In addition, previous studies had shown that cadmium alters the release of female reproductive hormones and induces cancer in rats (Waalkes et al., 1999; Zhang et al., 2008).

In the present study, we investigated the dose- and time-dependent effects of ip administration of cadmium on some biochemical (alkaline phosphatase (ALP), urea and creatinine) and hormonal (progesterone, FSH and LH) parameters in the female guinea pig. The biochemical and hormonal parameters were used to assess the levels of cadmium-induced toxicity in specific tissues/organs in the female guinea pig.

MATERIALS AND METHODS

Materials

Cadmium chloride was from BDH Chemicals Laboratory, England and formalin from BDH Chemicals Laboratory England. Cadmium solutions were prepared by dissolving pure CdCl₂ in 0.9% saline.

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Table 1. The effects of CdCl₂ (0.2 to 8.4 mg/kg) exposure, given ip for 24 h, on the serum levels of some biochemical parameters in the female guinea pig.

Dose (mg/kg)	ALP (IU/L)	Urea (g/L)	Creatinine (μmol/L)
Control	12.55 ± 0.8	2.80 ± 0.02	35.61 ± 1.3
0.2	13.28 ± 0.8	2.95 ± 0.04	37.33 ± 0.9
0.4	18.05 ± 0.6*	3.10 ± 0.06	39.14 ± 1.5*
0.8	26.60 ± 1.3*	3.54 ± 0.10*	44.30 ± 2.3*
1.6	28.25 ± 1.19*	4.34 ± 0.13*	53.72 ± 1.1*
3.2	33.65 ± 0.2*	5.82 ± 0.15*	71.13 ± 3.2*
6.4	43.75 ± 0.4*	8.04 ± 0.21*	103.24 ± 2.4*
8.4	42.5 ± 0.58*	8.40 ± 0.23*	100.50 ± 1.0*

Data are mean ± SEM. *Significantly different from control at p ≤ 0.05. ALP: Alkaline phosphatase.

All other chemicals were of analytical grades.

Animals

All animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals in Biomedical Research outlined by the Canadian Council of Animal Care (2009).

Outbred strains of adult female guinea-pigs (*Cavia porcellus*) of an average body weight of 550 ± 5 grams were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Port Harcourt, Nigeria. The animals were randomly distributed into separate cages (4 per cage) and allowed to acclimatize for 2 weeks in a well ventilated room, maintained at a room temperature of 28 ± 2°C, under natural lighting condition. They were fed with standard rodents chow and water was given *ad libitum*. The pigs used were in their proestrus state (determined by cervicovaginal smear cytological analyzes).

METHODS

The experiment was divided into dose-dependent study and time-dependent study. In the dose-response study, animals were divided into 7 groups (n = 5/group) and given single intraperitoneal injection of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 8.4 mg/kg of CdCl₂ and observed for 24 h. The time-dependent study was carried out in 5 animal groups (n = 5/group) animals were injected 3 mg/kg CdCl₂ ip and observed for 24, 48, 72, 96 and 120 h, respectively. At the end of the treatment periods, the animals were sacrificed under pentobarbital anaesthesia (37 mg/kg ip) (Flecknell, 1996). Blood was collected into blood specimen bottles, centrifuged at 3000 rpm for 15 min and serum was separated. Serum was then assayed for ALP using phenolphthalein method (Babson et al., 1966), creatinine by colorimetric method (Junge et al., 2004) and urea by enzyme method (Glick, 2006). Also, the serum levels of Follicle Stimulating Hormone (FSH) and Lutenising Hormone (LH) were measured by enzyme-linked immunoassay (EIA) method (Amballi et al., 2007).

Statistical analysis

The data were expressed as mean values ± SEM. Comparisons between control values and treated groups were performed with one-way analysis of variance (ANOVA) using GraphPad Prism 5 Software. Statistical significance was set at p ≤ 0.05.

RESULTS

Dose-dependent study (biochemical and hormonal parameters)

Intraperitoneal administration of 0.2 to 8.4 mg/kg CdCl₂ over 24 h caused significant (p ≤ 0.05) dose-dependent increases in the serum levels of ALP, urea and creatinine in Cd-treated animal groups, compared to the serum levels in the control group (Table 1). Furthermore, there was a dose-dependent increase in the serum levels of progesterone, FSH and LH in Cd-treated animals (Figure 1). At 8.4 mg/kg CdCl₂ (maximal dose), serum levels of progesterone (6.04 ± 0.12 ng/ml), FSH (6.37 ± 0.09 g/L) and LH (4.78 ± 0.05 g/L) obtained in the experimental group of animals were higher than serum levels of 2.14 ± 0.04 ng/ml, 2.15 ± 0.02 g/L and 2.50 ± 0.06 g/L, respectively in the control animals group (Figures 1a, b and c).

Time-dependent study (biochemical and hormonal parameters)

The time course experiments showed that 3 mg/kg CdCl₂ caused a significant (p ≤ 0.05) time-dependent increase in biochemicals such as ALP, creatinine and urea (Table 2) as well as hormones such as progesterone, FSH and LH (Figures 2a, b and c) in experimental animals compared to serum levels in the control animals. Maximal effects were observed at 120 h post-treatment (Table 2 and Figure 2).

DISCUSSION

In the present study, we determined the dose-dependent (0.2 to 8.4 mg) and time-dependent (24 to 120 h) effects of cadmium on some biochemical and hormonal parameters in the female guinea pig in proestrus state.

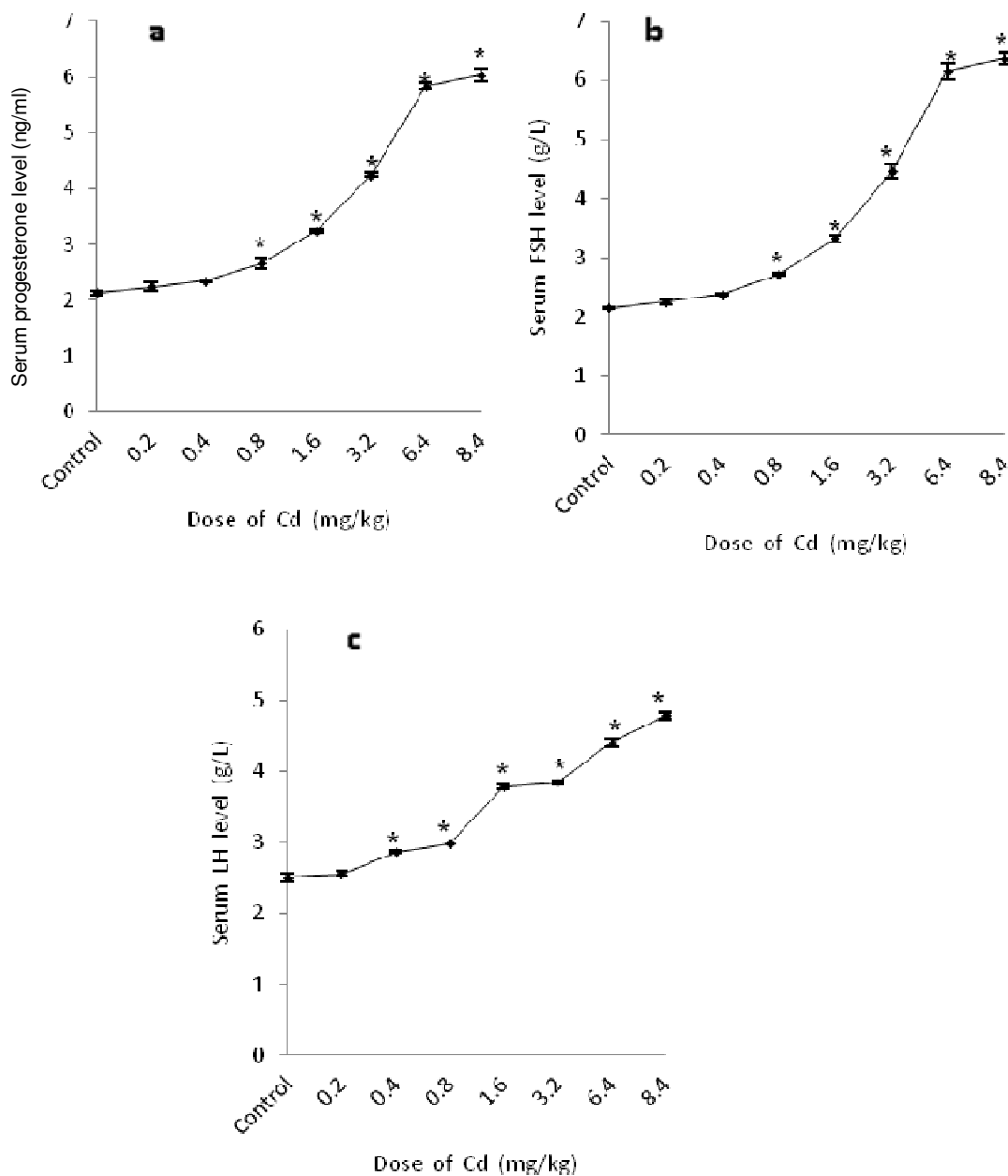


Figure 1. The effects of CdCl₂ (0.2 to 8.4 mg/kg) exposure, given ip for 24 h, on the serum levels of: (a) Progesterone; (b) FSH, and (c) LH in the female guinea pig. Data are mean \pm SEM. * Significantly different from control at $p \leq 0.05$.

Table 2. The effects of single ip administration of 3 mg/kg CdCl₂ over 120 h on the serum levels of some biochemical parameters in the female guinea pig.

Time (h)	ALP (IU/L)	Urea (g/L)	Creatinine (μ mol/L)
Control	12.55 \pm 0.8	2.80 \pm 0.02	35.61 \pm 1.3
24	23.6 \pm 0.05*	4.72 \pm 0.03*	55.52 \pm 0.04*
48	30.10 \pm 0.10*	5.80 \pm 0.12*	70.0 \pm 0.16*
72	36.0 \pm 0.08*	6.41 \pm 0.11*	79.8 \pm 0.02*
96	41.20 \pm 0.08*	7.00 \pm 0.05*	83.10 \pm 0.03*
120	46.30 \pm 0.10*	7.80 \pm 0.05*	86.50 \pm 0.07*

Data are mean \pm SEM. * Significantly different from control at $p \leq 0.05$. ALP: Alkaline phosphatase.

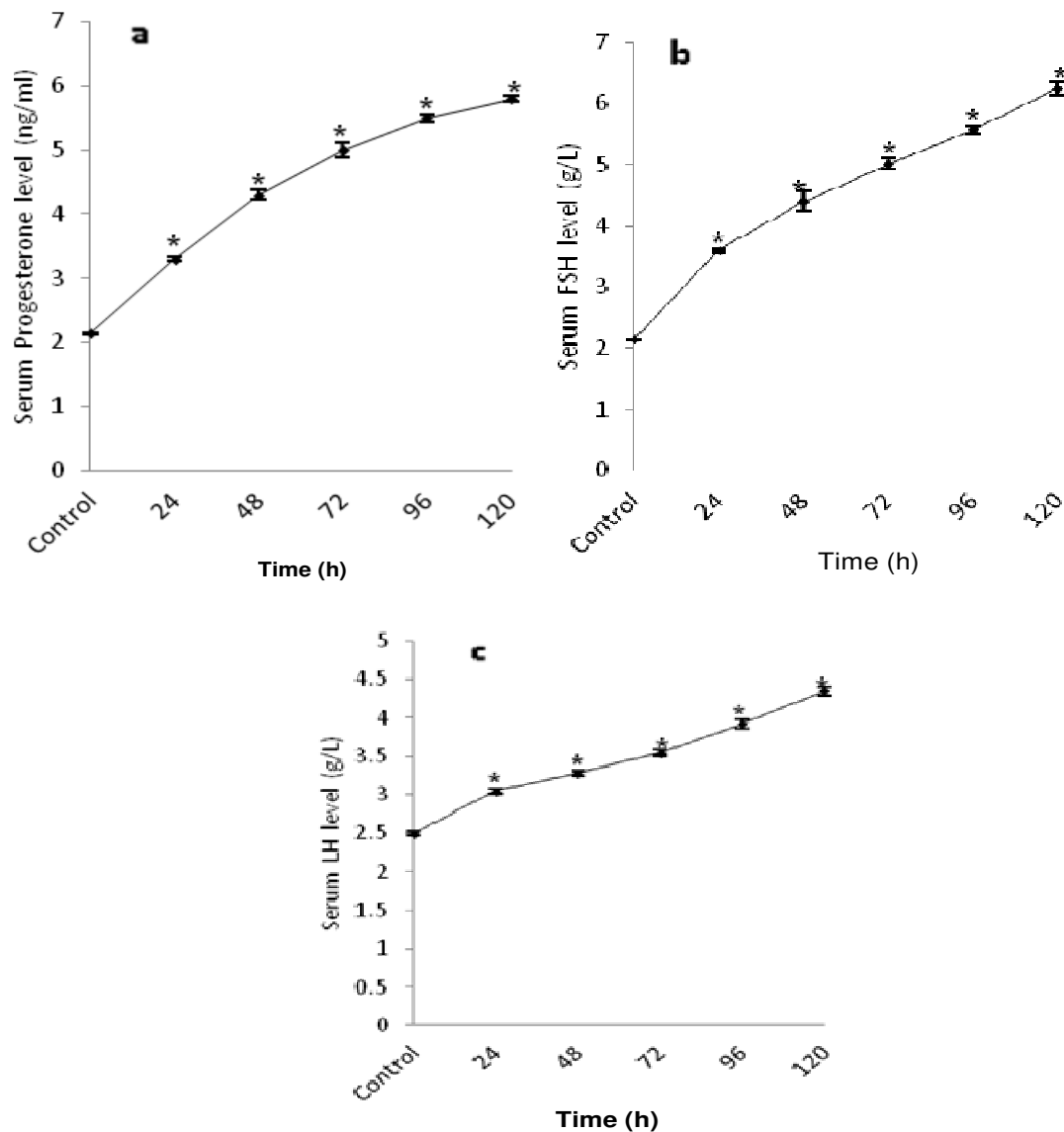


Figure 2. The effects of single ip administration of 3 mg/kg of CdCl₂ over 120 h on the serum levels of: (a) Progesterone, (b) FSH and (c) LH in the female guinea pig. Data are mean \pm SEM. *Significantly different from control at $p \leq 0.05$.

Single ip administration of 0.2 - 8.4 mg/kg CdCl₂ for 24 h caused significant ($p \leq 0.05$) dose-dependent increases in the serum levels of alkaline phosphatase (ALP), creatinine, urea, progesterone, LH and FSH. Furthermore, single ip injection of 3 mg/kg CdCl₂ over a period of 24 to 120 h also caused time-dependent increases in serum levels of ALP, creatinine, urea, progesterone, LH and FSH. Although cadmium has been shown to cause alterations in biochemical and hormonal profiles in mice and rats, there has been no similar study in the guinea pig prior to this study. The hormonal results correlated positively with the damaging effects of Cd on the ovary and uterus as reported in previous studies, where Cd caused vacuolation, congestion and necrosis

of the tissues (Massányi et al., 2007).

Serum levels of hepatic enzymes (e.g. ALP, alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase) are used as surrogate markers of hepatic toxicity (Jeong et al., 2000), while creatinine and urea are used as indices of renal toxicity (Traynor et al., 2006). In this study, the Cd-induced increases in ALP and urea, suggests hepatic and renal toxicities respectively. This is in agreement with previous studies (Harstad and Klaassen, 2000; Aprioku and Obianime, 2008).

The androgen (progesterone) is secreted by the ovary, while LH and FSH are secreted by the anterior pituitary. The secretion of these pituitary hormones is closely regulated by the hypothalamus, mainly through a

negative feedback mechanism (Guyton, 2006). It thus suggests that the hormonal effect of cadmium in this study is likely due to a direct stimulation of the anterior pituitary and inhibition of the negative feedback mechanism of the hypothalamo-pituitary system. Cd-induced serum elevation of LH will stimulate progesterone synthesis by the ovary, resulting in a corresponding increase in plasma progesterone level.

Conclusion

This study shows that cadmium may have adverse affect on the reproductive system in the female guinea pig, with consequences of alterations in biochemical components and endocrine function. These effects may be due to disruption (interruption) of the hypothalamo-pituitary-gonadal axis by Cd.

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