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Full Length Research Paper

Effects of vitamins C and E pretreatments on cadmium-induced serum levels of some biochemical and hormonal parameters in the female guinea pig

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Cadmium is a widely distributed environmental pollutant and toxicant. The present study was carried out to evaluate the effects of vitamins C and E on cadmium-induced serum levels of alkaline phosphatase (ALP), urea, creatinine, progesterone, LH and FSH in the female guinea pig. Animals were given single doses of vitamins C (1.5 mg/kg) and E (50 mg/kg) per oral and (0 - 8 mg Cd/kg ip) for 24 h. Animals were sacrificed and the serum levels of the above parameters were measured. Also, the effects of pretreatments with vitamins C and E on Cd-induced serum levels of the parameters were determined. Serum levels of all parameters were significantly ($p \leq 0.05$) decreased in a dose-dependent manner in vitamins-treated animals, while they were increased in cadmium-treated animals, compared to the control animals. Furthermore, pretreatments with vitamins C, E and combination of both vitamins reduced the cadmium-induced serum levels of all parameters, which was most pronounced in animals pretreated with a combination of both vitamins, especially on ALP and progesterone levels. These results may be due to the oxidative and anti-oxidative properties of cadmium and the vitamins (C and E) respectively, acting through calcium and protein kinase C signal transduction pathways.

Key words: Antioxidants, cadmium, pretreatment and vitamins.

INTRODUCTION

Vitamins C and E are potent antioxidative compounds (Padayatty et al., 2003; Valko et al., 2006). These agents reduce the production of reactive oxygen species (ROS), via their “scavenging”, “quenching” and “mopping up” properties (Tanaka et al., 1997; Padayatty et al., 2003; Valko et al., 2006). In addition, vitamin C causes regeneration of oxidized vitamin E molecules and both vitamins have sparing effects on one another, resulting in potentiation of the individual antioxidant effects of the agents when administered concurrently (Traber and Atkinson, 2007).

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Abreviations: ALP, Alkaline phosphatase; **Cd,** cadmium; **Vit C,** vitamin C; **Vit E,** vitamin E; **LH,** luteinizing hormone; **FSH,** follicle- stimulating hormone; **ip,** intra-peritoneally; **ROS,** reactive oxygen species; **PKC,** protein kinase C; **ALP,** alkaline phosphatase; **SEM,** standard error of mean.

Cadmium is a non-biodegradable transition heavy metal. It is widely distributed in our environment as a pollutant and toxicant. It is highly present in nickel-battery factories, tobacco smoke and pigment industries among other industrial activities (Järup, 2003; Mannino et al., 2004). Furthermore, cadmium, which is widely present in most industrialized towns and cities has also been shown to be a major product of petroleum refining activities (*Stigter* et al., 2002), making the toxicity of cadmium to be particularly high in areas involved with oil production such as the Niger delta areas in Nigeria. Exposure of experimental animals to cadmium has resulted in various medical abnormalities including, hepatotoxicity, nephrotoxicity, mutagenesis, teratogenesis, carcinogenesis etc (Waalkes, 2003; Huff et al., 2007). The metal has also been associated with reproductive dysfunctions in both male and female experimental animals (Zhang et al., 2008; Aprioku et al., 2009). Cadmium causes alterations in the serum levels of androgens and other reproductive hormones and histopathological damages to reproductive organs (Massányi et al., 2007; Zhang et al., 2008; Aprioku

Table 1. The effects of single dose administration of vitamins C, E and a combination of vitamins C and E over 24 h on the basal (control) serum levels of some biochemical and hormonal parameters of the female guinea pig.

Drug	ALP (IU/L)	Urea (g/L)	Creatinine (µmol/L)	LH (g/L)	FSH (g/ml)	Progesterone (ng/ml)
Control	12.55±0.8	2.80±0.02	35.61±1.3	2.50±0.06	2.15±0.02	2.14±0.04
Vitamin C (1.5 mg/kg)	11.0±1.3	2.0±0.03*	25.6±1.6*	2.3±0.04	2.0±0.0	2.1±0.04
Vitamin E (50 mg/kg)	8.6±1.0*	1.9±0.05*	20.0±0.8*	1.9±0.04*	1.8±0.03*	2.0±0.11
Vitamin C (1.5 mg/kg) + Vitamin E (50 g/kg)	5.9±0.7*#	1.3±0.08*	15.8±1.2*	1.8±0.05*	1.95±0.06	1.62±0.02*#

The values are expressed in means ± SEM. * Significantly different from control at $p \leq 0.05$; # synergistic response.

et al., 2009). Furthermore, cadmium-induced toxicity is mostly mediated through the formation of reactive free radicals, known as reactive oxygen species (e.g. S_2O_2, H_2O_2 and OH), resulting in increased oxidative stress that may cause lipid peroxidation of cell membranes and altered Ca^{2+} and sulfhyhdryl homeostasis (Joseph, 2009).

The present study was designed to investigate the effects of vitamins C and E on the basal and cadmium-induced serum levels of some biochemical (alkaline phosphatase, urea and creatinine) and hormonal (progesterone, leutinizing hormone and follicle stimulating hormone) parameters in the female guinea pig.

MATERIALS AND METHODS

Materials

Cadmium chloride (BDH Chemicals Lab, England), urethane and formalin (BDH Chemicals Lab, England) were obtained from the Department of Pharmacology, University of Port Harcourt, Port Harcourt, Nigeria. Vitamin C tablets (EmVit C, Emzor Pharmaceuticals Ltd, Lagos, Nigeria) and vitamin E softgels (Strides Arcolab Ltd, Indiavadi Cross, Bangalore-862 106, India) were purchased from the University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. All other chemicals were of analytical grade. Vitamins C and E were administered orally, while cadmium chloride was given intra-peritoneally (ip).

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 as promulgated 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 given tap water *ad libitum*.

Experimental design

Animals investigated were in their proestrus state during the period of the experiment. The experiment was divided into two categories: "vitamins treatment" and "cadmium treatment". For the "vitamins treatment", the animals were divided into 3 groups (n = 5) and administered orally with single doses of 1.5 mg/kg of vitamin C, 50 mg/kg of vitamin E and a combination of 1.5 and 50 mg/kg of vitamins C and E, respectively. The animals for "cadmium treatment" were grouped into four (A, B, C and D). Each of these groups was further subdivided into 7 groups containing 5 animals per group. Animals in group A were given single ip injection of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 8.4 mg/kg of $CdCl_2$. 'Group B animals were pretreated with1.5 mg/kg of vitamin C orally for 1 h before ip injection of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 8.4 mg/kg of $CdCl_2$. Group C animals were pretreated with 50 mg/kg of vitamin E orally for 1 h before being given ip injection of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 8.4 mg/kg of $CdCl_2$. Group D was orally given 1.5 mg/kg of vitamin C and 50 mg/kg vitamin E in combination for 1 h before ip injection of 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 8.4 mg/kg of $CdCl_2$. A fifth group, which served as the control for the experiment, was given normal saline ip. All animals were observed for 24 h and sacrificed under pentobarbital anaesthesia (37 mg/kg ip) (Flecknell, 1996). Blood was collected into lithium haparinized bottle, 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 (Kaplan, 2007). Also the serum levels of FSH and LH were measured by enzyme-linked immunoassay (EIA) method (Amballi et al., 2007).

Statistical analysis

The data were expressed as mean values ± SEM. Analysis of data was performed with one-way analysis of variance (ANOVA). Statistical significance was set at $p \leq 0.05$.

RESULTS

Biochemical parameters

The biochemical parameters investigated were alkaline phosphatase (ALP), urea and creatinine. Vitamins C, E and a combination of both vitamins caused significant ($p \leq 0.05$) decreases in ALP, urea and creatinine (Table 1). The serum levels of ALP, urea and creatinine in animals given a combination of vitamins C and E were 5.9 ± 0.7 IU/L, 1.3 ± 0.08 g/L and 15.8 ± 1.2 µmol/L, respectively. These values were lower compared to the serum levels obtained in the control animals group: 12.55 ± 0.8 IU/L, 2.80 ± 0.02 g/L and 35.61 ± 1.3 µmol/L, respectively (Table 1). Furthermore, the serum levels of these parameters in animal groups that received the combination of

Table 2a. Effects of pretreatments with single doses of vitamins C, E and a combination of vitamins C and E on Cd-induced serum levels of alkaline phosphatase (ALP) in the female guinea pig.

Dose (mg/kg)	Alkaline phosphatase (ALP) activity			
	Cd	Vit C + Cd	Vit E + Cd	Vit C+Vit E + Cd
Control	12.55±0.8	12.55± 0.8	12.55± 0.8	12.55± 0.8
0.2	13.28±0.8	9.32± 0.3*	5.07± 0.54*	1.33± 0.5*
0.4	18.05±0.6	10.17±0.16*	5.75± 1.3*	1.67± 0.21*
0.8	26.60±1.3	11.53± 0.21	6.77± 0.12*	2.52± 0.42*
1.6	28.25±1.19	15.27±0.10*	9.83± 0.4*	6.09± 0.6*
3.2	33.65±0.2	17.05±0.2*	11.57±0.15*	7.05± 0.19*
6.4	43.75±0.4	27.17±0.23*	17.15±0.40*	11.19±1.16*
8.4	42.5±0.58	32.95±0.53*	24.65±0.17*	17.65±0.3*

Vit C, Vitamin C; Vit E, vitamin E; values are expressed in means ± SEM. * Significantly different from Cd-induced serum levels at $p \leq 0.05$.

Table 2b. Effects of pretreatments with single doses of vitamins C, E and a combination of vitamins C and E on Cd-induced serum levels of urea (g/L) in the female guinea pig.

Dose (mg/kg)	Serum level of urea (g/L)			
	Cd	Vit C + Cd	Vit E + Cd	Vit C+Vit E + Cd
Control	2.80±0.02	2.80±0.02	2.80±0.02	2.80± 0.02
0.2	2.95±0.04	2.15±0.03	1.30±0.04*	0.56± 0.03*
0.4	3.10±0.06	2.30±0.06*	1.44±0.08*	0.62± 0.04*
0.8	3.54±0.10	2.94±0.08	1.92±0.06*	1.134± 0.10*
1.6	4.34±0.13	3.30±0.10*	2.30±0.08*	1.51± 0.08*
3.2	5.82±0.15	3.70±0.05*	2.61±0.08*	1.70± 0.11*
6.4	8.04±0.21	5.70±0.07*	3.73±0.10*	2.53± 0.16*
8.4	8.40±0.23	6.90±0.13*	5.23±0.12*	3.10± 0.16*

Vit C, Vitamin C; Vit E, vitamin E; values are expressed in means ± SEM. * Significantly different from Cd-induced serum levels at $p \leq 0.05$.

vitamins C and E were lower than the serum levels in animals that were given either of vitamin C or E, showing synergistic effects in ALP (Table 1).

In contrast, cadmium caused a significant dose-dependent increase in ALP, urea and creatinine. Maximal effects were obtained at 8.4 mg Cd/kg, with serum levels of ALP (42.5 ± 0.58 IU/L), urea (8.40 ± 0.23 g/L) and creatinine (100.50 ± 1.0 µmol/ml) in Cd-treated animals (Tables 2a, b and c). Pretreatments with single doses of 1.5 and 50 mg/kg of vitamins C and E individually before administration of 0.2 to 8.4 mg Cd/kg, caused a decrease in the serum levels of all the biochemical parameters, compared to the serum levels obtained in only Cd administration. Furthermore, pretreatment with a combination of vitamins C and E resulted in more reduced serum levels of ALP, urea and creatinine compared to the results obtained with only vitamin C or vitamin E pretreatment (Tables 2a, b and c).

Hormonal parameters

The reproductive hormones investigated were progesterone, LH and FSH. Serum levels of progesterone, LH and FSH were significantly ($p \leq 0.05$) decreased in a dose-dependent manner in vitamins C and E- treated animals and increased in Cd-treated animals when compared to the serum levels in the control animals group. The serum levels of progesterone in animals given vitamins C, E and a combination of both vitamins were: 2.1 ± 0.04, 2.0 ± 0.11 and 1.62 ± 0.02 ng/ml, respectively (Table 1). These values when compared to the basal control serum level (2.14 ± 0.04 ng/ml) shows that administration of vitamins C and E together resulted in a synergistic potentiation in the reduction of serum progesterone levels by vitamin C and vitamin E individually (Table 1). Furthermore, the serum levels of LH and FSH in animal groups that were given a combination of vitamins C and E were also lower, compared to serum levels in animals given only vitamin C or E, although these were not synergistic (Table 1).

Furthermore, the serum levels of progesterone (6.04 ± 0.12 ng/ml), LH (4.78 ± 0.05 g/L) and FSH (6.37 ± 0.09 g/L) in Cd-treated (8.4 mg/kg) animals were higher than the serum levels in control animals: (2.14 ± 0.04 ng/ml), (2.50 ± 0.06 g/L) and (2.15 ± 0.02 g/L), respectively

Table 2c. Effects of pretreatments with single doses of vitamins C, E and a combination of vitamins C and E on Cd-induced serum levels of creatinine (µmol/ml) in the female guinea pig.

Dose (mg/kg)	Serum level of creatinine (µmol/ml)			
	Cd	Vit C + Cd	Vit E + Cd	Vit C+Vit E + Cd
Control	35.61± 1.3	35.61± 1.3	35.61± 1.3	35.61± 1.3
0.2	37.33± 0.9	28.01± 1.3*	19.01± 0.9*	09.21± 1.3*
0.4	39.14± 1.5	30.01± 1.6*	20.41± 0.8*	10.01± 3.04*
0.8	44.30± 2.3	37.21±2.08*	25.21± 1.6*	16.01± 2.1*
1.6	53.72± 1.1	42.01±2.10*	29.24± 1.8*	20.41± 0.8*
3.2	71.13± 3.2	46.21± 1.5*	33.35± 1.5*	22.67± 1.8*
6.4	103.24 2.4	70.01± 3.1*	46.52± 2.1*	32.41± 2.5*
8.4	100.50±1.0	83.61± 1.6*	64.23± 0.8*	40.21± 2.16*

Vit C, Vitamin C; Vit E, vitamin E; values are expressed in means ± SEM. * Significantly different from Cd-induced serum levels at $p \leq 0.05$.

Table 3a. Effects of pretreatments with single doses of vitamins C, E and a combination of vitamins C and E on Cd-induced serum level of progesterone (ng/ml) in the female guinea pig.

Dose (mg/kg)	Serum level of progesterone (ng/ml)			
	Cd	Vit C + Cd	Vit E + Cd	Vit C+Vit E + Cd
Control	2.14± 0.04	2.14± 0.04	2.14± 0.04	2.14± 0.04
0.2	2.24± 0.08	1.68± 0.02*	1.08±0.08*	0.56± 0.01*
0.4	2.35± 0.02	1.76± 0.06*	1.18±0.04*	0.60± 0.05*
0.8	2.66± 0.1	2.24± 1.03*	1.52±0.05*	0.96± 0.09*
1.6	3.24± 0.02	2.54± 0.08*	1.76±0.02*	1.23± 0.09*
3.2	4.24± 0.04	2.78± 0.08*	2.00±0.08*	1.36± 0.05*
6.4	5.84± 0.06	4.24± 0.07*	2.75±0.11*	1.95± 0.1*
8.4	6.04± 0.12	5.04± 0.03	3.74± 0.17	2.19± 0.04*

Vit C, Vitamin C; Vit E, vitamin E; values are expressed in means ± SEM. * Significantly different from Cd-induced serum levels at $p \leq 0.05$.

(Tables 3a, b and c). However, pretreatment of animals with single doses of vitamins C, E and a combination of both vitamins, before ip injection of 0.2 - 8.4 mg Cd/kg resulted in significant reductions in the serum levels of all the reproductive hormones investigated, when compared to the Cd-induced serum levels (Tables 3a, b and c).

DISCUSSION

In this work, the effects of single doses of vitamins C, E and a combination of vitamins C and E on the basal serum levels of some biochemical (ALP, urea and creatinine) and hormonal (progesterone, LH and FSH) parameters in the female guinea pig (in proestrus state) were investigated. In addition, the effects of vitamins C, E and a combination of vitamins C and E pretreatments on Cd-induced serum levels of the above parameters were investigated.

The results show that vitamins C and E, given individually caused a significant ($p \leq 0.05$) decrease in the basal serum levels of ALP, urea, creatinine, progesterone, LH and FSH. Furthermore, while causing synergistic effects in the reductions of the serum levels of ALP and progesterone, administration of a combination of vitamins C and E caused additive effects in the reductions of the serum levels of the other parameters by vitamins C and E. This is consistent with our previous study (Obianime and Aprioku, 2009). In addition, ip injection of 0.2 - 8.4 Cd mg/kg over 24 h caused significant increases in both biochemical (ALP, urea and creatinine) and hormonal (progesterone, LH and FSH) parameters. This is also consistent with the previous works of Swarup et al. (2006) and Aprioku and Obianime (2009). However, pretreatment of animals with vitamins C and E individually decreased the Cd-induced serum levels of these parameters, while pretreatment with both vitamins combined, lowered Cd-induced serum levels more than either of the individual vitamins.

Damages to certain tissues and organs result in the elevation of serum concentrations of specific biochemical parameters as a result of their release or secretions from the damaged tissues/organs (Robert et al., 2002). The Cd-induced increases in serum levels of ALP, urea and

Table 3b. Effects of pretreatments with single doses of vitamins C, E and a combination of vitamins C and E on Cd-induced serum level of follicle stimulating hormone (FSH) in the female guinea pig.

Dose (mg/kg)	Serum level of follicle stimulating hormone (g/L)			
	Cd	Vit C + Cd	Vit E + Cd	Vit C+Vit E + Cd
Control	2.50±0.06	2.50±0.06	2.50±0.06	2.50±0.06
0.2	2.55±0.04	2.23±0.02	2.02±1.03	1.88±1.03*
0.4	2.87±0.01	2.50±0.03	2.16±0.08*	1.97±0.09*
0.8	2.98±0.0	2.59±0.04	2.22±1.0*	2.00±0.10*
1.6	3.78±0.02	2.68±0.12*	2.31±0.06*	2.08±0.3*
3.2	3.84±0.03	2.82±0.05*	2.38±0.2*	2.11±0.1*
6.4	4.40±0.06	3.08±0.06*	2.51±0.15*	2.22±0.06*
8.4	4.78±0.05	3.20±1.1*	2.58±0.07*	2.28±0.07*

Vit C, Vitamin C; Vit E, vitamin E; values are expressed in means ± SEM. * Significantly different from Cd-induced serum levels at $p \leq 0.05$.

Table 3c. Effects of pretreatments with single doses of vitamins C, E and a combination of vitamins C and E on Cd-induced serum level of luteinizing hormone (g/L) in the female guinea pig.

Dose (mg/kg)	Luteinizing hormone (g/L) in the female guinea pig			
	Cd	Vit C + Cd	Vit E + Cd	Vit C+Vit E + Cd
Control	2.15± 0.02	2.15± 0.02	2.15± 0.02	2.15± 0.02
0.2	2.26± 0.05	1.66± 0.05	1.01± 1.0*	0.43± 0.03*
0.4	2.38± 0.02	1.79± 0.03*	1.11±0.02*	0.49± 0.03*
0.8	2.72± 0.02	2.25± 0.12	1.47±0.06*	0.88± 0.10*
1.6	3.33± 0.06	2.57± 0.15	1.74±0.05*	1.16± 0.05*
3.2	4.46± 0.11	2.84± 0.03*	2.00±0.05*	1.31± 0.1*
6.4	6.16± 0.14	4.39± 0.08*	2.85± 0.1*	1.94± 0.04*
8.4	6.37± 0.09	5.27± 0.12*	4.05± 1.1*	2.20± 0.02*

Vit C, Vitamin C; Vit E, vitamin E; values are expressed in means ± SEM. * Significantly different from Cd-induced serum levels at $p \leq 0.05$.

creatinine suggest signs of hepatic and renal toxicity which is also consistent with previous works of Robert et al. (2002) and Kara et al. (2005). This may be due to the oxidative effects of cadmium which is a sulfhydryl-active and powerful oxidative metal (Ikediobi et al., 2004). Cadmium is also known to cause various forms of damages and dysfunction to different tissues and organs, including the reproductive organs, by inducing oxidative stress on the affected tissues/organs. The hormonal result of this study suggests that Cd may affect the endocrine function in the testes, which is in agreement with earlier works on Cd which also show interference of the hypothalamo-pituitary-gonadal function in different experimental animals (Massányi et al., 2007; Zhang et al., 2008).

Pretreatments with vitamins C and E reduced the Cd-induced increases in serum levels of ALP, urea, creatinine, progesterone, LH and FSH, which may be due to the antioxidant effects of the vitamins. This is consistent with previous pretreatment studies using vitamins C and E and other antioxidative agents (Valko et al., 2006; Obianime and Aprioku, 2009). Vitamins C and E are water and lipid soluble vitamins respectively, and they have been established as very effective antioxidants (Tanaka et al., 1997; Padayatty et al., 2003; Valko et al., 2006). They scavenge free radicals by readily donating electrons to these unstable and highly reactive molecules (ROS) during biological reactions in the body and become oxidized themselves. As scavengers of free radicals, they therefore reduce the production of ROS and oxidative stress. This will in turn prevent or reduce lipid peroxidation and tissue injury or damage that may be induced by oxidative trauma/stress. Furthermore, the serum levels of the biochemical and hormonal parameters in animal groups (basal and pretreatment studies) that were given a combination of both vitamins were lower, compared to serum levels in animals given single vitamin. This result shows that combined administration of vitamins C and E will cause a potentiation of the individual effects of either vitamin on basal and Cd-induced serum levels of ALP, urea, creatinine, progesterone, LH and FSH. This is consistent with previous works that showed synergistic antioxidative effects of the two vitamins in decreasing lipid peroxidation (Tanaka et al., 1997; Obianime and Aprioku, 2009).

The oxidative effects of cadmium are mediated through activation of protein kinase C (PKC) during signal transduction. PKC activity is in turn dependent on cytosolic calcium ion concentration (Gregory, 1997; Stohs et al., 2001). Furthermore, the action of vitamin C is mediated through inhibition of calcium ion mobilization or utility (Huang et al., 2000), while vitamin E causes inhibition of PKC (Freedman et al., 1996). These mechanisms may also explain the opposite (increasing and decreasing) effects of cadmium and the vitamins (C and E) respectively on basal serum levels of the parameters investigated in this study, or their oxidative and antioxidative activities. Further-more, it had been shown that a simultaneous activation of PKC and calcium ion will result in a synergistic response of cellular activity in signal transduction (Nishizuka, 2001). In this study, inhibition of both pathways (calcium ion and PKC) by co-administration of vitamins C and E also resulted in a potentiation of the individual inhibitory effects of vitamin C and E on the parameters investi-gated.

Conclusion

The results of this study show that cadmium alters the serum levels of some biochemical parameters and reproductive hormones in the female guinea-pig, which can be biochemically antagonized by vitamins C and E. The mechanism of the cadmium's effects is suggested to be via an increased oxidative stress on the liver, kidney and reproductive organs with impairment of the hypothalamic-pituitary function in the female guinea pig. However, the results obtained with vitamins C and E are likely due to their antioxidant activities-scavenging of ROS, prevention of ROS formation and inhibition of lipid peroxidation.

REFERENCES

Amballi AA, Dada OA, Adeleye AO, Jide S (2007). Evaluation of the determination of reference ranges for reproductive hormones (prolactin, FSH, LH, and testosterone) using enzyme immunoassay method. Sci. Res. Essay, 2: p. 135.

Aprioku JS, Obianime AW, Didia BC (2009). *Ocimum gratissimum* Linn., cadmium and testicular structure of the male guinea-pig. JECA. 8(1): 8-12.

Aprioku JS, Obianime AW (2009). Effects of the aqueous leaf extract of *Ocimum gratissimum* Linn. (Lamiaceae) on some cadmium-induced biochemical changes in male guinea-pigs. W. Afr. J. Pharmacol. Drug Res. p. 24.

Babson LA, Greeley SJ, Coleman CM, Phillips GD (1966). Phenolphthalein Monophosphate as a Substrate for Serum Alkaline Phosphatase. Clin. Chem. 12: p. 482.

Flecknell PA (1996). Laboratory Animal Anaesthesia. An Introduction for research workers and technicians. Academic Press, New York.

Freedman JE, Farhat JH, Loscalzo J, Keaney JF (1996). Alpha-tocopherol inhibits aggregatin of human platelets by a protein kinase C-dependent mechanism, Circulation, 94: p. 2434.

Gregory JL (1997). The Effect of Cadmium on Cytosolic Free Calcium, Protein Kinase C and Collagen Synthesis in Rat Osteosarcoma (ROS 17/2.8) Cells. TAP. 143: p. 189.

Huang A, Vita JA, Vanema RC, Keaney JF (2000). Ascorbic acid enhances endothelial nitric-oxide synthase activity by increasing intracellular Tetrahydrobiopterin. J. Biol. Chem. 275: p. 17399.

Huff J, Lunn RM, Waalkes MP, Tomatis L Infante PF (2007). Cadmium-induced cancers in animals and in humans. Int. J. Occup. Environ. Health, 13: 202-212.

Ikediobi O, Christopher BL, Veera AT, Lambert LM, Lekan WJ (2004). Response of antioxidant enzymes and redox metabolites to cadmium-induced oxidative stress in CRL-1439 normal rat liver cells. Int. J. Mol. Med. 24: p. 87.

Järup L (2003). Hazards of heavy metal contamination. Br. Med. Bull. 68: 167-82.

Joseph P (2009). Mechanisms of cadmium carcinogenesis. TAP, 238: 272-279.

Junge W, Wilke B, Halabi A, Klein G (2004). Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffé method. Clinica Chimica Acta, 344: 137-148.

Kaplan A (2007). The Determination of Urea, Ammonia and Urease. In: Glick D (ed). Methods of Biochemical Analysis, John Wiley and Sons, California. Vol. 17.

Kara H, Karatas F, Canatan H, Servi K (2005). Effects of exogenous metallothionein on acute cadmium toxicity in rats. Biol. Trace Elem. Res. 104: p. 223.

Mannino DM, Holguin F, Greves HM, Savage-Brown A, Stock AL, Jones RJ (2004). Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey. Thorax, 59: 194-198.

Massányi P, Stawarz R, Lukac N, Kovacik J, Toman R, Pivko J, Rafay J, Uhrin V (2007). Cadmium associated microscopic and ultrastructural alterations in female reproductive organs of rabbits. Acta Microscopica, 16: p. 114.

Nishizuka Y (2001). The protein kinase C family and lipid mediators for transmembrane signaling and cell regulation. Alcohol Clin. Exp. Res. 25: p. 3.

Obianime AW, Aprioku JS (2009). Comparative and interactive studies of aqueous leaf extracts of *Ocimum gratissimum* Linn. (Lamiaeceae), Vitamins C and E on the basal serum phosphatase levels of male guinea-pigs. West Afr. J. Pharmacol. Drug Res. p. 24.

Padayatty S, Katz A, Wang Y, Eck P, Kwon O, Lee J, Chen S, Corpe C, Dutta A, Dutta S Levine M (2003). Vitamin C as an antioxidant: evaluation of its role in disease prevention. J. Am. Coll. Nutr. 22: p. 18.

Robert KK, Michael PW, Peter L, Ben L, Robert SM Glenn SI (2002). Differential Hepatotoxicity Induced by Cadmium in Fischer 344 and Sprague-Dawley Rats. Toxicol. Sci. 65: p. 151.

Stigter JB, De Hean HP, Guirherit R, Dekkers CR Daane ML (2002). Determination of cadmium, zinc, copper, chromium and arsenic in crude oil cargoes. Environ. Pollut. 107: p. 451.

Stohs SJ, Bagchi D, Hassoun E Bagchi M (2001). Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol. 20: 27.

Swarup D, Naresh R, Varshney VP, Balagangatharathilargar M, Kumar P, Nandi D Patra RC (2006). Changes in plasma hormones profile and liver function in cows naturally exposed to lead and cadmium around different industrial areas. Res. Vet. Sci. 3: p. 87.

Tanaka K, Hashimoto T, Tokumaru S, Iguchi H Kojo S (1997). Interactions between Vitamin C and Vitamin E are Observed in Tissues of Inherently Scorbutic Rats. J. Nutr. 127: p. 2060.

Traber MG, Atkinson J (2007). Vitamin E, antioxidant and nothing more. Free Rad. Biol. Med. 43: p. 4.

Valko M, Rhodes CJ, Moncol J, Izakovic M Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. J. Chem. Biol. Interact. 169: p. 1.

Waalkes MP (2003). Cadmium carcinogenesis. Mutat. Res. 533: 107-120.

Zhang W, Pang P, Huang Y, Yan P Lin W (2008). Cadmium exerts toxic effects on ovarian steroid hormone release in rats. Toxicol. Lett. 182: 18-23.