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Ocimum gratissimum Linn., Cadmium and Testicular Structure Of The Male Guinea-pig

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ABSTRACT

The effects of the aqueous crude extracts of *Ocimum gratissimum* Linn. leaf and cadmium (Cd) on basal serum phosphatase levels of the male guinea-pig were investigated. Furthermore, the effects of the aqueous crude extracts of *Ocimum gratissimum* Linn. leaf and Vitamin C on Cd-induced toxicity of the testicular/sperm cell structure of the male guinea-pig (GP) were also investigated. *Ocimum gratissimum* (0-160mg) caused a significant inhibition/decrease ($p<0.05$) in basal serum phosphatase levels of the male guinea-pig. In contrast, cadmium (0.5-8mg/kg) caused a significant stimulation/increase ($p<0.05$) in basal serum phosphatase levels, which was positively correlated with a dose-dependent destruction of the testes and sperm cell histology/structure. Pretreatments with 5mg of *O. gratissimum* and 1.25mg/kg of vitamin C, with subsequent administration of cadmium, blocked/reversed the Cd-induced toxicities of the testes/sperm cell histology. These results may be explained in terms of the antioxidative properties and the post-receptor biochemical and molecular (transduction) events of *O. gratissimum* on Cd-induced responses.

Keywords: Histology; Sperm cell; *Ocimum gratissimum* leaf, testes

Ocimum gratissimum Linn. is a herb used as spice in most West African dishes. It is locally called "Scent Leaf" in Nigeria because of its dominant pungent flavor. The plant, which belongs to the genus- *Ocimum* and family- Lamiaceae (formally Labiatae), is widely distributed over the tropics of Africa and Asia (Darrah 1980).

The leaf of *Ocimum gratissimum* Linn. contains essential oils which contains mainly eugenol, terpenoids, thymol and linalool, (Jedlickova et al 1992, Sulistiarini 1999, Leal et al 2006). These special oils have been shown to possess several pharmacological properties (Nakamura et al 1999, Aziba et al 1999, Afolabi et al 2007).

O. gratissimum L. is used as a medicinal plant to treat several medical conditions in folk medicine (Sofowora 1993) including infertility in men. It is also known to be a powerful antioxidant (Afolabi et al 2007). Currently however, there is no data of this plant on male reproductive/histological dysfunction. It is therefore in that light that this work was carried out to investigate the effect of the aqueous crude extracts of *O. gratissimum* leaf on toxicities in the testicular/sperm cell histology using cadmium as the toxicant. This is a follow-up to our earlier report on the effects of the crude extract of the plant on testicular/spermatic function.

MATERIALS AND METHOD

Materials

Solutions of Cadmium Chloride ($CdCl_2$) 99.5% (Chadwell Heath Essex, England) were made with 0.9% normal saline (Dana Pharmaceuticals Ltd, Nigeria), while Vitamin C tablets (Emzor Pharm Industries Ltd, Lagos, Nigeria) were dissolved in distilled water obtained from the Department of Chemistry in the University of Port Harcourt.

Adult male guinea-pigs of average weight 450±5g were obtained from the Animal House of the University of Port Harcourt, Nigeria and allowed to acclimatize for 14 days. The guinea-pigs were fed with fresh elephant grass daily *ad libitum* at a room temperature of 22°C with 12-hour light/dark cycle.

Methods

Extraction of *Ocimum gratissimum* Linn. leaf

The plant, *O. gratissimum* L. was identified by Dr. Goodie Uzo Obute- a senior

botanist in the botanical garden of the University of Port Harcourt and some fresh leaves were collected and dried in the oven at 60°C to a constant weight. They were then ground to fine powder. Forty grams (40g) weight of the powdered herb was added to 400ml of boiling distilled water and allowed to boil for five minutes. The mixture was allowed to cool for 45 minutes and filtered to obtain a solution of 200mg *O. gratissimum*/ml. The extract was stored in the refrigerator at 4°C and used for the experiments.

The effects of cadmium on the histology of the sperm cell and testes of the male GP.

In n=30 animals, adult male guinea-pigs were divided into seven groups of five each. The animals were administered single doses of 0, 0.5, 1, 2, 4 and 8mg/kg of Cd intraperitoneally and observed for 24 hours. The animals were then anaesthetized with 25% urethane solution and semen was carefully extracted from the epididymis of the testes and stored in sodium bicarbonate solution for sperm cell histology. The testes was also collected and fixed with 10% formalin, routinely stained with haematoxylin and eosin (H&E) for histological study.

.The effects of O. gratissimum pretreatments on Cd-induced serum levels of Phosphatases in the male GP.

In n=50 animals, guinea-pigs were divided into ten (10) groups of five (5) animals each and pretreated with 5 mg and 1.25mg/kg of *O. gratissimum* and vitamin C respectively, given orally for two hours, before administering 0.5, 1, 2, 4, and 8mg/kg of $CdCl_2$ intraperitoneally. Animals were observed for 24 hours and sacrificed. Semen and testes were collected and analyzed for testicular/sperm cell histology as above.

Statistical Analysis

Data were expressed as means ± standard errors of mean. Comparisons between control and treated groups of guinea-pigs were performed with one-way analysis of variance (ANOVA), followed by Duncan's multiple comparison test. Statistical significance was set at $p<0.05$.

RESULTS

In n=5 experiments, *O. gratissimum* (5-160mg) caused significant decrease ($p<0.05$) in basal serum levels of phosphatase enzymes (Fig. 1a). In contrast, cadmium (0.5-8mg/kg) caused significant increase ($p<0.05$) in the basal serum levels of phosphatase enzymes (Fig. 1b).

In n=5 experiments, cadmium (0.5-8mg/kg) caused a dose-dependent histopathological damage of the testes in the male guinea-pig. These damages were characterized by destruction of germinal cells, oedema, vascular congestion and vacuolation. Furthermore, cadmium caused significant and dose-dependent damage to the histology of the sperm cell of the male GP, with increase in structural abnormalities, primordial/premature cells and particulate debris (Fig. 2).

In n=5 experiments, pretreatments with *O. gratissimum*/or vitamin C, with subsequent administration of cadmium, caused a reversal of the toxic effects of cadmium on the sperm cells histology (Figs. 3 and 4) and testes. Sperm cells of animals pretreated with *O. gratissimum*/or Vitamin C showed better morphology, fewer premature cells and debris compared to spermatozoa of the Cd-treated animals (Figs. 3 and 4).

DISCUSSION

The serum level of phosphatases is used as an index of toxicity in the body, with an increase indicating toxicity (Gary and Michael, 2002). In this study, while *O. gratissimum* decreased basal serum phosphatase activities, cadmium, a non biodegradable environmental toxicant, caused elevation of serum phosphatases, thus inducing toxicity. In our previous study, *O. gratissimum* caused a reversal of Cd-induced elevations of serum phosphatase levels (Aprioku and Obianime, 2008- In Press). The present study was thus carried out to find out if *O. gratissimum* would produce similar effects on Cd-induced toxicities in the architecture or histology of the testes/sperm cell.

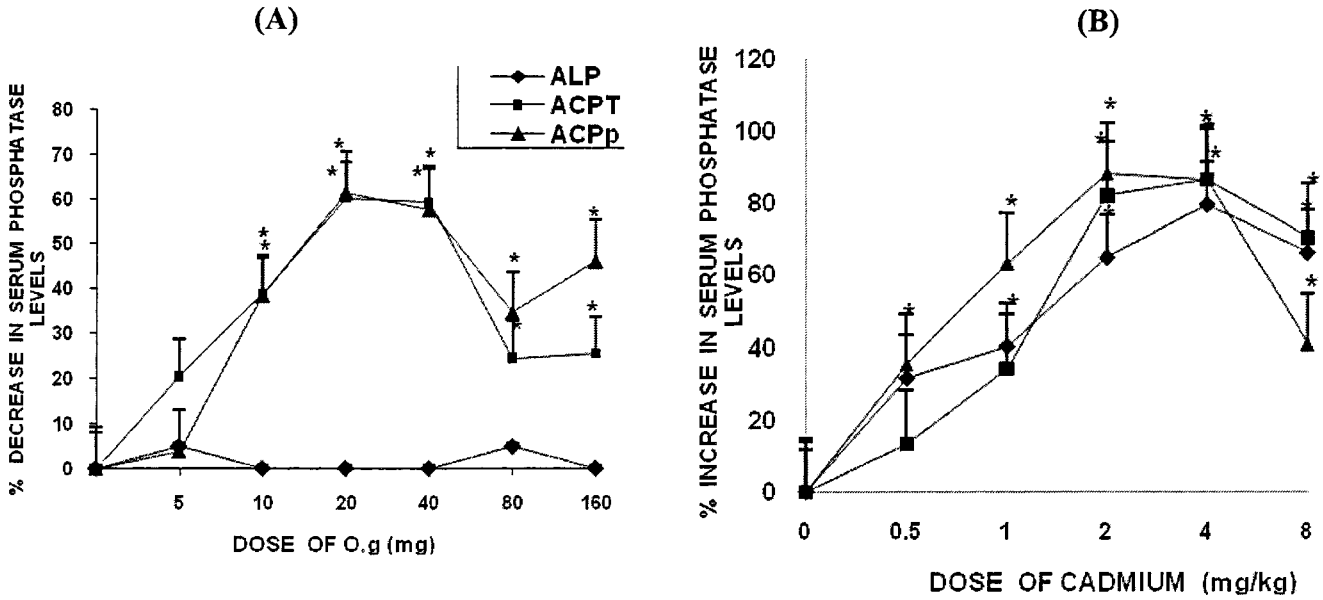

FIGURE 1: The dose-dependent effects of (a) - *O. gratissimum* (O.g) and (b) - Cadmium on the basal serum phosphatase levels of the male guinea-pig. Data are mean ± SEM, n=5.

*Treated GPs significantly different from control GPs at P<0.05 ANOVA. (ALP- alkaline phosphatase; ACPT- Total acid phosphatase and ACP_P- prosptatic acid phosphatase)

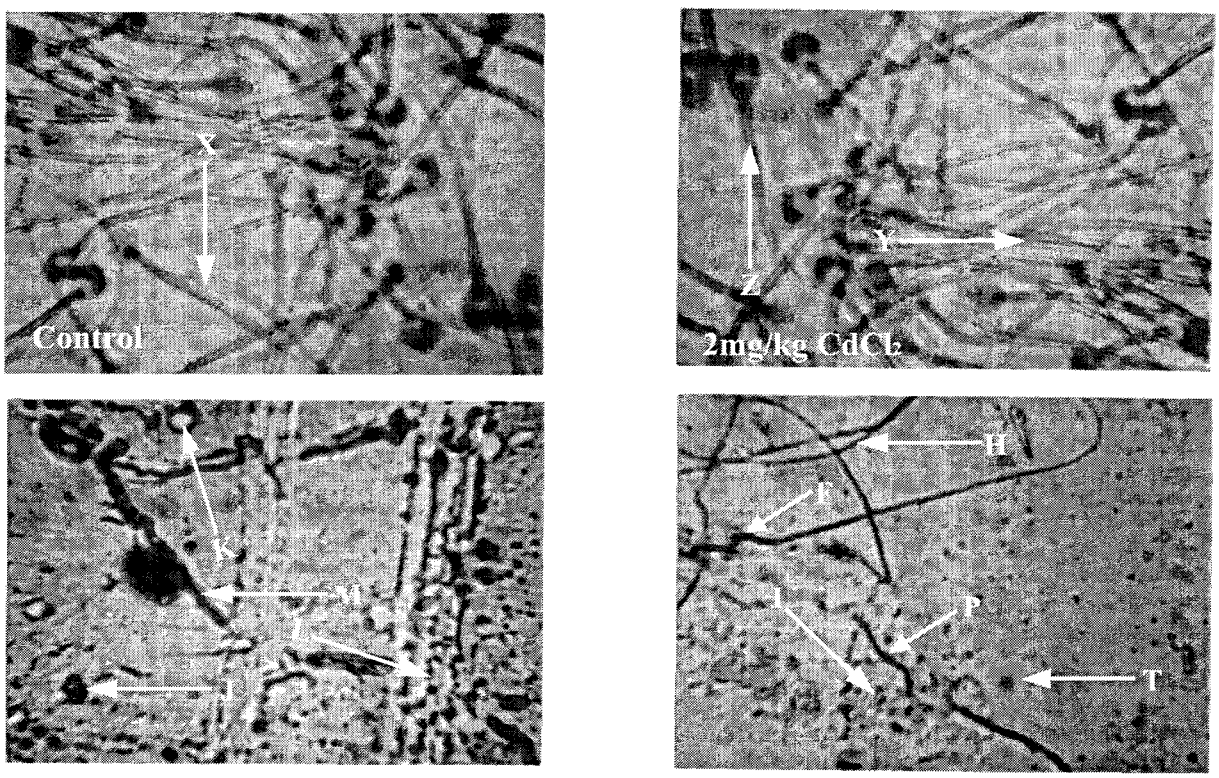

FIGURE 2: The dose-dependent effects of cadmium on the histology of sperm cells of the male GP: Control, showing several spermatozoa with normal morphology- complete head, middle piece (Neck) and tail (X), without particulate debris. 2mg/kg $CdCl_2$, showing clusters of sperm cells (Y); many premature cells; and large particulate debris (Z). 4mg/kg $CdCl_2$, showing spermatozoa with distorted morphology- broken heads, necks and tails (M); many premature cells (J); fatty droplets/granules (K); and many particulate debris (L). 8mg/kg $CdCl_2$, showing spermatozoa with incomplete structures (P); premature cells (T); coiled necks (F); double tails (H); and many particulate debris (I)

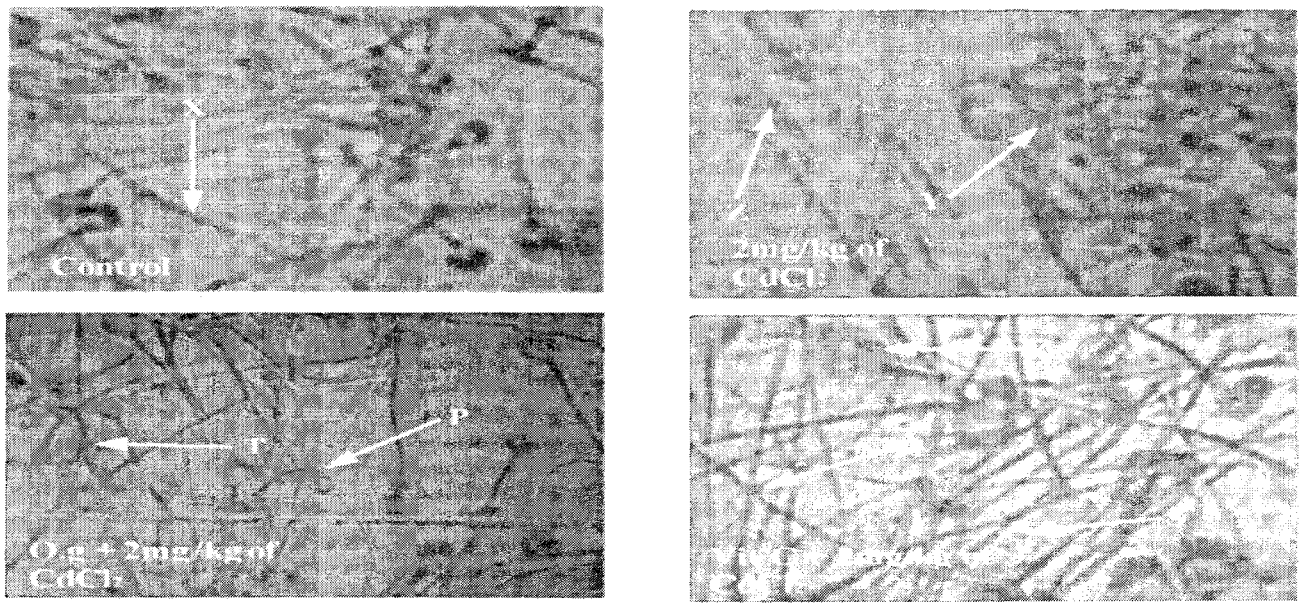

FIGURE 3: The comparative effects of 2mg/kg of Cadmium and *O. gratissimum* and vit c pretreatments on the histology of sperm cells of the male guinea-pig: Control, showing several spermatozoa with normal morphology (X). 2mg/kg $CdCl_2$, showing clusters of sperm cells (Y); many premature cells; and large particulate debris (Z). O.g+2mg/kg $CdCl_2$, showing several normal spermatozoa; few coiled tails (T); and few particulate debris (A). Vit C+2mg/kg $CdCl_2$, showing spermatozoa with enlarged heads (K); few granules on tails (A); and few particulate debris (S).

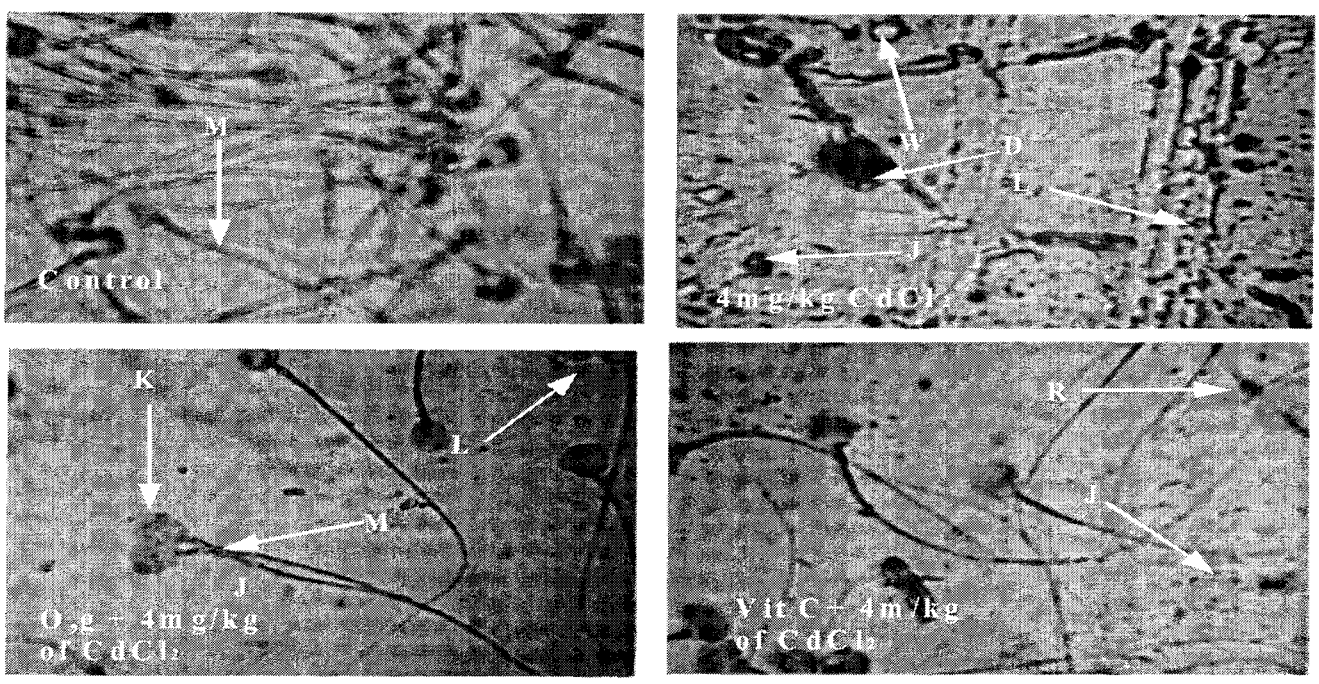

FIGURE 4: The comparative effects of 4mg/kg of cadmium and *O. gratissimum* and Vit C pretreatments on the histology of sperm cells of male guinea-pigs: Control, showing several spermatozoa with normal morphology (X). 4mg/kg $CdCl_2$, showing spermatozoa with distorted morphology- broken heads, necks and tails (D); many premature cells (J); fatty droplets/granules (W); and many particulate debris (L). O.g+4mg/kg $CdCl_2$, showing normal and abnormal sperm cells with double heads (K) fused neck (M) and a few debris (L). Vit C+4mg/kg $CdCl_2$, showing many premature cells (R) and debris (J).

*Note: O.g- *Ocimum gratissimum* and Vit C- Vitamin C)

In this study, the destructive effects of cadmium on testicular architecture/sperm cell histology were reversed following pretreatments with *O. gratissimum*/and or Vitamin C. Spermatozoa of Cd-treated animals showed broken heads, necks and tails; double tails; many premature cells; coiled necks; collection of fatty droplets/granules and much debris, compared to the control animals with several morphologically normal spermatozoa and without debris. These effects were generally ameliorated by pretreatments with *O. gratissimum*/and or vitamin C, showing that *O. gratissimum* could be a useful agent in protecting and treating spermatic cell damage.

Cadmium is a known oxidant (Ikediobi et al 2004) and testicular toxicant (Friberg et al 1986, Waalkes and Rehm 1992, Goering et al 1994). The destructive effects of cadmium on the testicular/sperm cell histology may be due to oxidative trauma on the affected tissues resulting in marked necrosis of seminiferous tubules (Francavilla et al 1981 Jones et al 1988). Necrosis of seminiferous tubules- the cells responsible for spermatogenesis, may be directly responsible for the abnormality in sperm cells histology.

Conversely, the actions of *O. gratissimum* and vitamin C may be due to their antioxidant activities (Niki et al 1995, Afolabi et al 2007). Furthermore, *O. gratissimum* and Vitamin C had been shown to inhibit Ca^{2+} mobilization/utility (Jackson et al., 1998; Huang et al., 2000; Leylian et al., 2007). Thus, the actions of O. *gratissimum* and vitamin C may also be due to a reversal by these agents of the molecular events induced by cadmium i.e. Ca^{2+} mobilization and utility, leading to a blockade and reversal of the toxic effects of cadmium.

CONCLUSION

The present study has shown that, the effects of *O. gratissimum* on serum phosphatases are positively correlated with those on the histology of the testes/sperm cells of the male guinea-pig. Thus, *O. gratissimum* may be a useful prophylactic/therapeutic agent against damage to testicular structure and function of the male guinea-pig.

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