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Ocimum gratissimum Linn. Reverses Cadmium-induced Toxicity of Spermatic Parameters of the Male Guinea-pig.

***J. S. APRIOKU[1], A. W. OBIANIME[1] AND M. GOGO-ABITE[2]**
[1]Department of Pharmacology. [2]Department of Anatomical Pathology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.
*Author for correspondence

ABSTRACT

The influence of the aqueous crude extracts of *Ocimum gratissimum* Linn. leaf on cadmium (Cd)-induced toxic effects on spermatic parameters of the male guinea-pig (GP) was investigated. In n=5, Cd (0-8mg/kg) caused a dose-dependent inhibition or reduction of various spermatogenic parameters namely- number of normal sperm cells: 55.75±2.02 $\times 10^6$ to 7.50±1.19 $\times 10^6$/ml; number of abnormal sperm cells: 2.25±0.25 $\times 10^6$ to 8.25±2.18 $\times 10^6$/ml and total sperm count: 58.00±1.96 $\times 10^6$ to 15.75±2.63 $\times 10^6$/ml; motility: 64.25±2.39 % to 26.50±1.71%; morphology: 5.75±0.75 % to 38.25±2.72 %; and a significant increase ($P<0.05$) in particulate and primordial sperm cell counts in the male GP. However, injection of *Ocimum gratissimum* Linn. extract after Cd administration had little or no significant effect on the above mentioned parameters. Pretreatment with 5mg of *O. gratissimum*, with subsequent administration of cadmium, blocked or reversed the Cd-induced toxicities on the various spermatogenic parameters- motility**:** 26.50±1.71% to 53.25±2.14%; morphology: 38.25±2.72% to 8.75±1.25%; number of normal sperm cells: 7.50±1.19 $\times 10^6$ to 27.25±1.60 $\times 10^6$/ml; number of abnormal sperm cells: 8.25±2.18 $\times 10^6$ to 5.25±0.63 $\times 10^6$/ml and total sperm count: 15.75±2.63$\times 10^6$ to 32.50±1.85 $\times 10^6$/ml. The observed influence of *O. gratissimum* on Cd-induced toxicity may be the consequence of the antioxidant action of the plant extract on the spermatogenic apparatus of the organism.

Keywords: Spermatic parameters, *Ocimum gratissimum* leaf, Cadmium, Pretreatment

Ocimum gratissimum Linn., locally called "Scent Leaf" in the southern part of Nigeria is commonly used as food additive in most West African dishes as a spice due to its volatile oil content. The plant belongs to the genus, *Ocimum* and the family, Lamiaceae (formally Labiatae). The genus *Ocimum* is collectively called BASIL and contains between 50-150 species of herbs and shrubs, distributed over the tropics of Africa and Asia (Darrah 1980).

O. gratissimum L. contains essential oils which possess pharmacological actions. The important phytochemicals in *O. gratissimum* L. include, eugenol, terpenoids, thymol, linalool, 1, 8 cineol, citral, ethyl cinnamate, β-selinene, 1, 8 cineole, trans-caryophyllene alkaloids, tannins and flavonoids (Jedlickova et al 1992, Sulistiarini 1999, Leal *et al* 2006)

O. gratissimum L. is commonly used in folk medicine to treat different diseases which include- upper respiratory tract infections, diarrhoeae, ophthalmic conditions, skin diseases, rheumatism, paralysis, epilepsy, influenza, gonorrhea and mental illness (Sofowora 1993, Sulistiarini 1999). Pharmacological studies on the plant have shown that it has chemotherapeutic (Nwosu and Okafor 1995, Ilori et al 1996, Nakamura et al 1999, Dubey et al 2000), anthelmintic (Fakae et al 2000, Chitwood 2003), antimutagenic (Obaseiki-Ebor et al 1993) antispasmodic (Aziba et al 1999), analgesic (Rabelo et al 2003), antioxidant (Odukoya et al 2005, Leal et al 2006, Afolabi et al 2007) and insecticidal (Pessoa et al 2003) properties.

There is however little or no information in the literature on the effect of *O. gratissimum* on the male fertility profile, such as its effects on the testicular, ovarian and uterine histology as well as influence on hormonal and spermatic parameters. It is therefore in that light that we decided to investigate the effects of *O. gratissimum* on the semen parameters of the male guinea-pig. The effect of *O. gratissimum* was compared with that of ascorbic acid, a known hydrophilic antioxidant (Niki et al 1995).

MATERIALS AND METHODS

In this study, cadmium, which has been

shown in several works/literature to be toxic on the testis, through an effect on spermatogenesis (Francavilla et al 1981, Jones et al 1988) was used to cause alteration in spermatic function.

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 in 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^{0}C with 12-hour light/dark cycle.

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^{0}C to a constant weight. The dried leaves were then ground to fine powder. Forty grams (40g) weight of the powdered leaves 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^{0}C and used for the experiments

Twenty-five (25) adult male guinea-pigs were used in this experiment. The animals were divided into five (5) groups of five each and were administered single doses of 0, 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 and analyzed for sperm parameters (sperm count, motility, morphology and debris) using standard laboratory techniques. The experiment was repeated after pretreatment with 5mg and 1.25mg/kg of *O. gratissimum* and vitamin C respectively, given orally for two hrs.

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 $p<0.05$.

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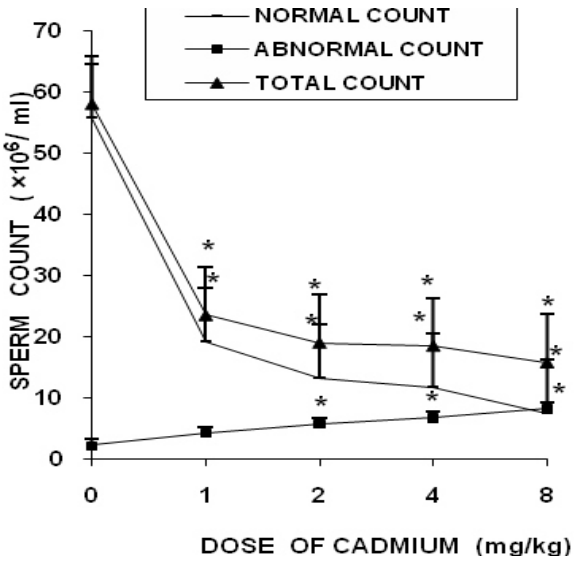
RESULTS

In this study, the effects of *O. gratissimum* and vitamin C on Cd-induced toxicity of the semen parameters- sperm counts, sperm motility and morphology of the male GP were investigated.

In n=5, Cd caused significant ($p<0.05$) and dose-dependent decreases in number of normal sperm cells, total sperm cells and motility (Figs. 1a and b), with increase in the number of abnormal sperm cells, sperm morphology and debris (Fig. 2). The number of normal and total sperm cells were decreased from 55.75±2.02 $\times10^6$ and 58.00±1.96 $\times10^6$ to 7.50 $\times10^6$ and 15.75 $\times10^6$/ml (that is 86.5 and 73% decreases) respectively, while the number of abnormal sperm cells was increased by over 200% (Fig. 1a) at 8mg/kg of cadmium.

Also in this experiment, the sperm motility value changed/decreased from the control value of 64.25±2.39 % to 46.25±0.63 % and 26.50±1.71 % (i.e. 39% and 55% inhibitions) at 1 and 8mg/kg respectively (Fig 1b), showing that Cd is toxic to sperm cells. Cadmium also caused a significant ($p<0.05$) dose-dependent increase in the structural abnormalities of the sperm cells (Fig. 1c). The control morphology value (%) was increased from 5.75±0.75% to 27.00±1.47 % and 38.25±2.72% at 1 and 8mg/kg of Cd respectively (Fig. 1c).

(1a)



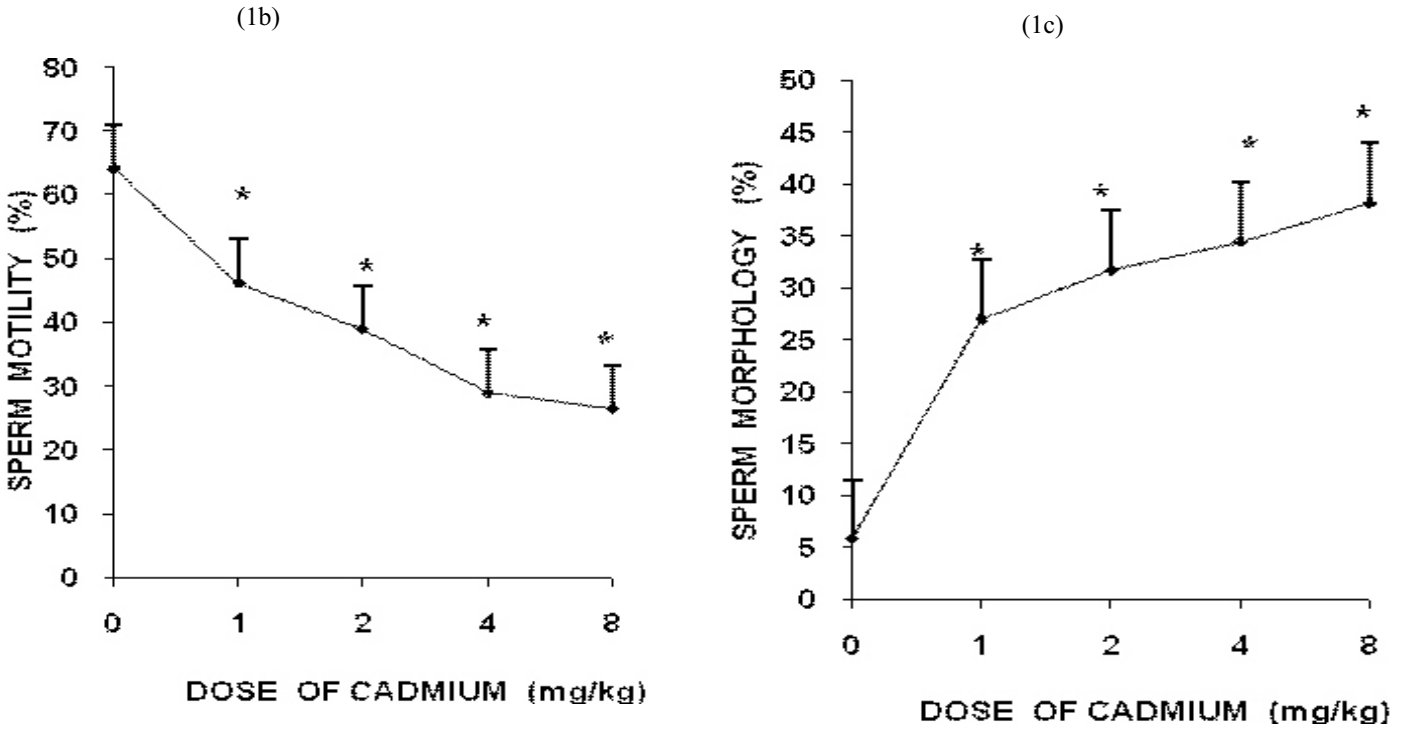
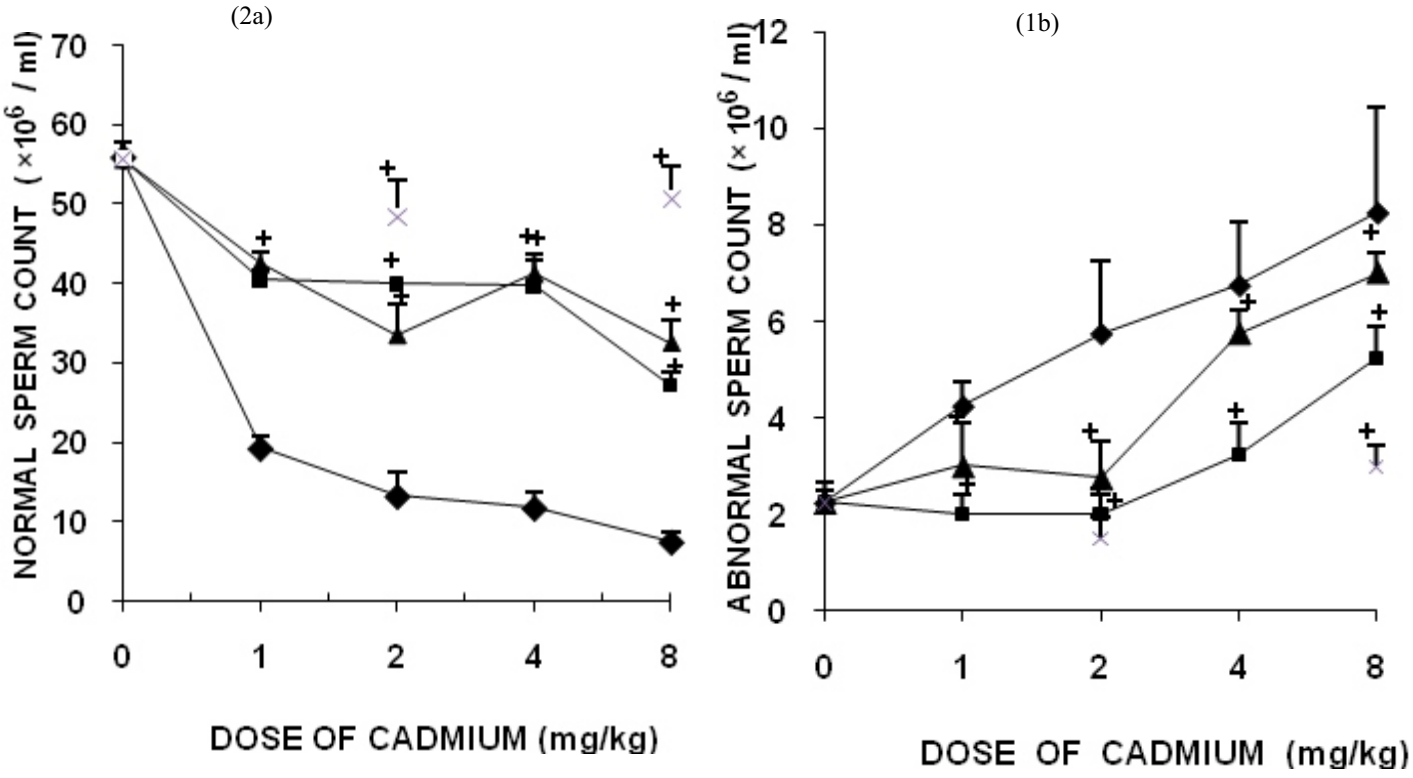
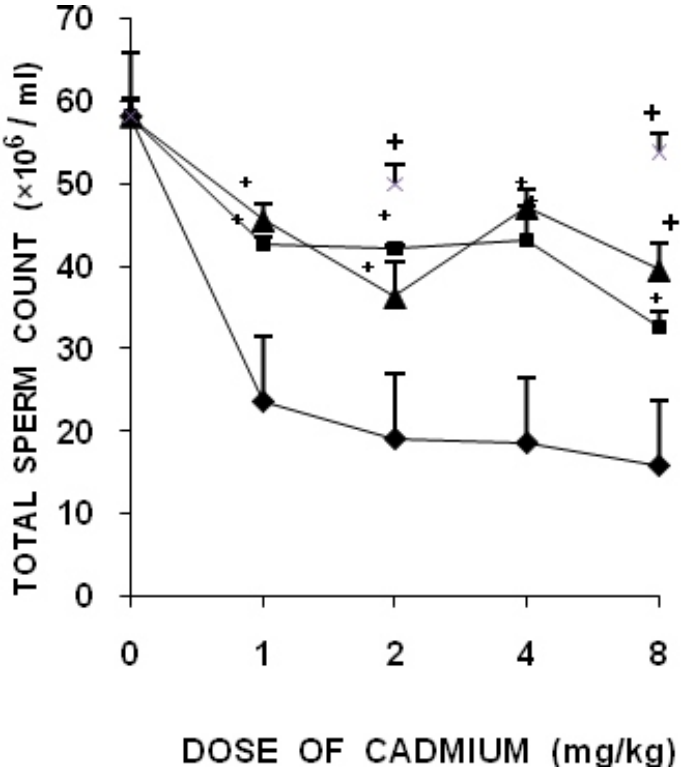


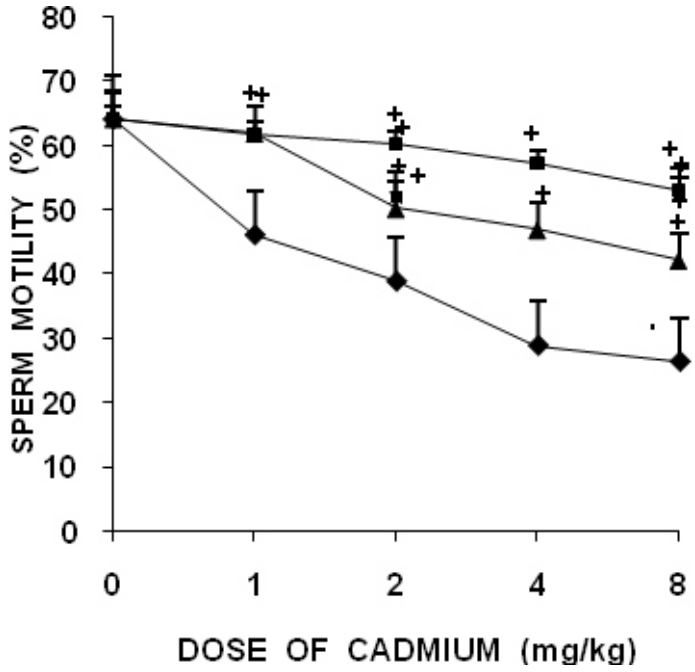
FIGURE 1: The dose-dependent effects of cadmium on spermatic parameters (a) normal cell number, abnormal cell number and total sperm counts; (b) - sperm motility and (c) - sperm morphology of the male GP. Data are mean ± SEM, n=5. * Data significantly different from control at P< 0.05 ANOVA.



(2c)



(2d)



(2e)

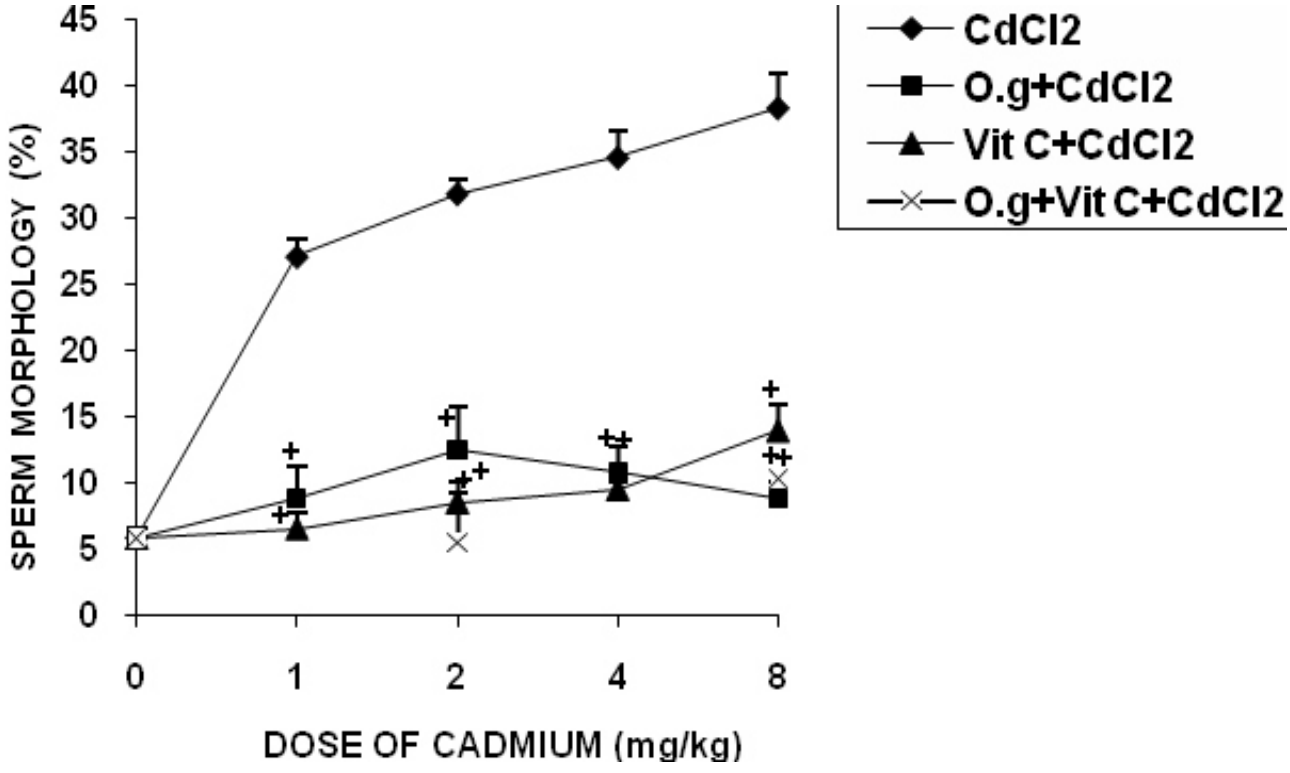


FIGURE 2: The effects of pretreatments with *O. gratissimum*; vitamin C; and a combination of *O. gratissimum* and vitamin C pretreatments on Cd-induced effects on (a) number of normal sperm cells; (b) number of abnormal sperm cells; (c) - total sperm count; (d) - sperm motility and (e) - sperm morphology. Data are mean ± SEM, n=5; $^{+}$Pretreated GPs significantly different from Cd-treated GPs at $P < 0.05$ ANOVA. (O.g represents- *O. gratissimum* and Vit C represents- vitamin C)

In n=5, pretreatments with *O. gratissimum*, vitamin C and their combination, with subsequent administration of Cd, significantly ($P<0.05$) increased Cd-induced normal cells and total sperm counts at all doses of Cd (Figs. 2a and c). The normal cell count was increased from 7.50 to 32.5, 27.25 and 50.75 $\times 10^6$/ml, while total count was increased from 15.75 to 32.5, 39.50 and 53.75 $\times 10^6$/ml respectively at 8mg/kg of Cd, Similarly, sperm motility was increased from 26.50±1.71 to 53.25±2.14, 42.25±0.85 and 52.50±2.22 % by the agents respectively (Fig 2d), showing that the effects of pretreatment with combination of *O. gratissimum* and vitamin C> *O. gratissimum*>> vitamin C.

Conversely, in n=5, pretreatments with the same agents dose-dependently decreased cadmium-induced increases in abnormal morphology and abnormal sperm cell number (Figs. 2b and e). These effects were also most pronounced in pretreatments with the combination of *O. gratissimum* and vitamin C (Figs. 2b and e).

DISCUSSION

O. gratissimum leaf is used locally in some areas in Nigeria as a fertility drug by men. Furthermore, reviewing the ethnopharmacology of *O. gratissimum*, it was observed that no study of the effects of *O. gratissimum* on semen parameters and indeed on the reproductive function had been done. This study was therefore designed to investigate the effect of *O. gratissimum* spermatic parameters.

The fertility of men depends on the sperm count, proportion of motile and morphologically normal sperm cells, among other factors (Chia et al 1998). WHO has recommended the normal values for these parameters as- total sperm concentration (40 million/mL), motility (50%) and normal morphology (30%). In this study, Cd caused a significant decrease ($P<0.05$) in the number of normal sperm cells, total sperm count and sperm motility of the male GP, while it caused a significant increase ($P<0.05$) in the number of the abnormal sperm cells and morphology. This is in agreement with previous toxicity studies with Cd (Jones et al 1988). Furthermore, previous studies had shown that Cd caused dose-and-time-dependent reductions in the serum concentrations of testosterone, prolactin, and follicle stimulating hormone (Waalkes et al 1999), which are necessary for sperm production and maturation (Gonzales et al 1989). These toxic effects were due to marked necrosis of seminiferous tubules, interstitial hemorrhage, edema, decreased blood flow and ischaemia (Francavilla et al 1981, Jones et al 1988). The results obtained in this study showed that the Cd-induced levels of sperm parameters were lower than the WHO recommended levels and this can cause infertility in the male GP.

Also in this study, *O. gratissimum*, vitamin C and their combination reversed the toxic effects of all sperm parameters- sperm counts, motility and morphology. These effects were higher with *O. gratissimum* pretreatment than vitamin C. The percentages of inhibition of Cd on total sperm count at 2mg/kg of Cd were 200, 77 and 333 respectively for *O. gratissimum*, vitamin C and a combination of *O. gratissimum* and vitamin C pretreatments, while at 8mg/kg of Cd, it was 260, 266 and 433 respectively. Thus, these agents reversed the Cd-induced levels of sperm concentrations towards normal values, thus improving spermatic function. The result also shows that *O. gratissimum* and vitamin C interact positively to cause additive or synergistic reversal of Cd action. *O. gratissimum* also countered the effects of Cd on sperm motility and morphology by over 100% at 8mg/kg, a property that will also enhance the reproductive function.

The crude extract of *O. gratissimum* has been shown to contain essential oils that possess several pharmacological actions (Nakamura et al 1999, Leal et al 2006, Rabelo et al 2003). There is however no information in the literature on its effect on reproductive function. The ability of *O. gratissimum* to reverse these toxicities towards the normal therefore makes this work novel.

The action of Cd may be due to oxidative damage to the integrity of the basement membrane of the spermatic cell wall, leading to cell death (Dally and Hartwig 1997, Ikediobi et al 2004). Cd also interferes with calcium metabolism (Kjellstrom 1992, Carlsson and Lundholm, 1996). *O. gratissimum* and vitamin C on the other hand, had been shown to inhibit calcium mobilization or utility (Huang et al 2000, Heller et al 2001, Leyllian et al 2007) and protect the integrity of the cell membrane, by preventing lipid peroxidation.

CONCLUSION

Thus, the action of *O. gratissimum* may be due to the reversal of the oxidative responses of cadmium through an inhibitory effect on the transductional mechanisms for calcium utility and may account for its usefulness in testicular dysfunction.

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