THE EFFECTS OF AMMONIUM METAVANADATE ON BIOCHEMICAL HORMONAL, HAEMATOLOGICAL AND HISTOPATHOLOGICAL PARAMETERS OF THE FEMALE WISTAR RATS

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Summary: The effects of different doses of Ammonium metavanadate on the biochemical, haematological, hormonal and histopathological parameters of stilbesterol treated female Wistar rats were investigated. Ammonium metavanadate in the dose- range 0-6mg/kg caused a bi-phasic and time-dependent response on the acid (total and prostate) phosphatase.. Furthermore ammonium metavanadate caused a dose-dependent inhibition of the serum alkaline phosphatases. The maximal inhibitory response at 5mg/kg of ammonium metavanadate was 40.0 ± 1.69 compared to 65.0 ± 0.94 control values. Ammonium metavanadate also caused a positively correlated biphasic response in the serum female hormonal concentrations with an initial increase, followed by a time-dependent decrease in the serum values of luteinizing (LH), follicle stimulating hormone (FSH), prolactin . Furthermore ammonium metavanadate also caused time- and dose- dependent effects on the haematological parameters. The effects were biphasic-increase within 72 hours and a reduction in the values of haemoglobin and packed cell volume within 7-28 days. The white blood count and lymphocyte counts were also reduced significantly at $P \leq 0.05$. However the neutrophil counts were increased dose- and time-dependently. Finally, ammonium metavanadate caused a dose-dependent destruction of the liver and female reproductive organs namely the uterus, ovary and fallopian tubes. These were characterized by necrosis, oedema, eosinophilic deposits and vacuolation. These results may be explained by the oxidative effects caused by the free oxygen (O_2) radical generated by the metavanadate ions.
Key words: vanadium, female hormones, blood count, histology, uterus

Introduction

Vanadium is a transitional metal that exists in different oxidational states (Barceloux & Barceloux 1999) in fossil fuels, industrial and environmentally degraded areas (Osuji & Adesiyan 2005). It is also a trace element found in the catalytic distillation of crude oil. Absorption of vanadium compounds depends on the chemical composition of the compound as well as species and the route of exposure (Conklin *et al* 1982). The toxicity of vanadium varies with the chemical form and the oxidational states (Waters *et al* 1974).

Animal studies with vanadate show that the compound of vanadate caused haemorrhagic exudates from the nose, marked diarrhea, respiratory distress and convulsion (Gossellin *et al* 1984). The pathological effects include diffuse desquamative enteritis of lungs, liver, kidneys, adrenal cortex, brain, spinal cord and bone marrow (Gosellin *et al* 1984). Higher concentrations may cause irreversible kidney damage (Kumar & Corder 1980). The compounds also caused vasoconstriction of vessels in the lungs (Erdmann *et al* 1984). Sodium metavanadate has also been shown to cause increase in blood Urea, Uric acid and histopathological lesions in the kidneys and spleen (Domingo *et al* 1985), decreases in erythrocyte count and haemoglobin level (Zaprowska and Wasilewski 1991), inhibition of mating and reproductive toxicity (Domingo, 1996), pulmonary Oedema (Sjoberg 1950), increased leucocytes in bronchiolar lavage (Knecht *et al* 1992), increased alveolar macrophages (Lee and gillies 1986), reduced oxyhaemoglobin and pathological changes in the lungs (pazyniah 1966), increased fatty changes in the liver of rats, reduced fertilization of females mated to exposed males (Hacket and Kalman 1983).However, most of these reports were single doses of the metavanadate and thus not qualitative. There were no correlation studies between the phosphatase increases, tissue organ toxicities and dysfunctions in addition to histopathological effects on the blood cells and hormonal dysfunction.

It is therefore in that light that we seek to investigate the qualitative pharmacological effects of ammonium metavanadate on some biochemical (phosphatases), histological (the liver, blood cells, kidney, ovary, oviduct and uterus) and the female hormonal parameters of the female wister rats. In other words, what is the relationship between detailed biochemical responses and histological structure and function of the female wister rats traumatised with ammonium metavanadate. Also, what is the correlation with the hormones studies?

Finally in this study, we have also investigated the dose- and time- dependency of ammonium metavanadate on phosphatases, hormones, haematological and histological changes on tissues. What are the various forms of correlations of these parameters if any?

Materials and methods

Dose-dependent Studies.

The effect of intraperitoneal administration of different doses of ammonium metavanadate on the biochemical, haematological, hormonal and histopathological parameters of the female stilbesterol treated albino rats were investigated. Thirty-five albino rats of average weight 250±6gms were divided into seven cages of five each. The first was administered with distilled water as control, the second to the sixth cages were administered with different doses of ammonium metavanadate in the dose range 0-6mg/kg. The rats were anaethesized, blood and tissue samples were removed and subjected to various forms of analysis.

Time-dependent Studies:

The effects of time of administration of a single dose of ammonium metavanadate on various responses of the female albino rats were investigated. Twenty 25mg stilbesterol-treated female rats, divided into four cages of five rats each were administered with single doses of ammonium metavanadated intraperitoneally. The blood and tissue samples were harvested from the first cage on the first day after four (4) hours, the rest were harvested after 24 hours, 4 days and seven days respectively. These were then analysed for biochemical, hormonal and histopathological parameters.

Animals

The average weight of animals used in this study was 250±6gms. All animals used in this study were handled with the international, natural and institutional guidelines for care and use of laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care (1984). Outbred strains of the female Wister rats of average weight 250mg±6gms aged between 15-20 weeks were obtained from the animal house of the University of Port Harcourt and allowed to acclimatise for 14 days. They were housed in cages with wire bar lids used to hold the water bottle and feed to prevent contamination with urine or faeces. Bedding was placed directly into the shoe box cage to allow the absorption of urine. They were kept well ventilated room at ambient temperature of 28.0 ± 2.0^{o}C under 12hr light/dark cycle and well provided with food and tap water *ad libitum.* Generally the study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in care and use of animals.

Phosphatase Analysis

Determination of serum alkaline phosphatase (ALP), serum acid phosphatase tartrate labile (AcP_T) and the prostatic acid phosphatases (AcP_P) was carried out using the hydrolysed phenol method (Kind & King 1954).

Hormonal Analysis

Blood samples were collected in a 10 ml plastic syringe. The peptide hormones namely follicle stimulating hormone, Luteinizing hormone and Prolactin were measured by radioimmunoassay as described by Banu *et al* (2002) and concentration expressed as ng/ml. while the steroid hormone oestrogen was measured by radioimmunoassay as described by Banu *et al* (2002) in ng/ml.

Histological analysis

The tissues were isolated and preserved in 10% formalin and routinely processed for histopathological examination using H&E stain technique for paraffin embedded tissue sections as described by Benjamin (2001). The various tissue sections were viewed and photographed with the DMLS camera and digital microscope (LEICA).

Haematological Analysis

In the morning hours, about 5 ml of blood was collected in standing position from right jugular vein of the female Wister rats and placed in citrated vials. Blood samples were analyzed for Haemoglobin (Hb) by acid heamatin (Sahali's haemoglobinometer) method and Packed Cell Volume (PCV) by Wintrobes method. These methods are standard methods used for haematological parameters (Schalm, 1986).

Analysis of Urea and Creatinine

Urea measurements were performed using the diacetyl monoxime, a Total urinary excretion method (Toro & Ackermann 1975), creatinine was assayed using the Jaffe alkaline picrate method (Annino & Giese 1979).

Statistical analysis:

All values were represented as mean ± SEM of n=5 and the values were taken as significant at $p \leq 0.05$ ANOVA (analysis of variance).

Results:

The effects of ammonium metavanadate on the biochemical, hormonal, haematological and histopathological parameters of the female Wistar rats were investigated.
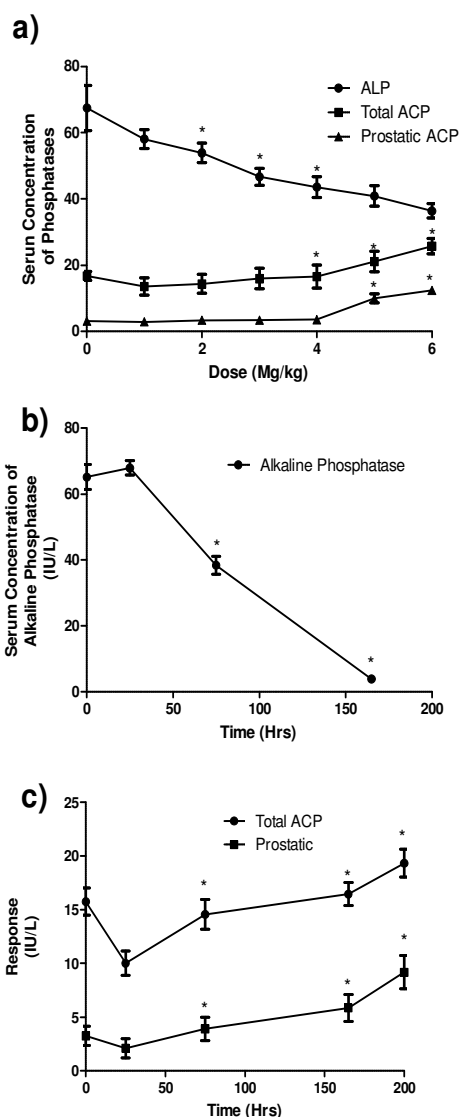
Fig 1. The effects of (a) different doses of the ammonium metavanadate-induced responses on acid, alkaline and prostatic phosphatases (b) Time on a single dose of Ammonium metavanadate on alkaline phosphatases (ALP) and (c) Total and prostatic phosphatases (AcP_T & AcP_P) Data is presented as mean ± SEM * shows significance at $P \leq 0.05$ (ANOVA).

Biochemical effects:

Ammonium metavanadate caused a dose-dependent decrease in the alkaline phosphatase levels (fig 1a) and biphasic responses in the total and prostatic acid phosphatases (fig 1b&c). Ammonium metavanadate at the lower doses caused a decrease which was followed at higher doses by a stimulation or increase in phosphatase levels (fig 1b&c). The responses showed that the basal values of ALP changed from 65.0 ± 0.94 to 40 ± 1.89 IU/L.
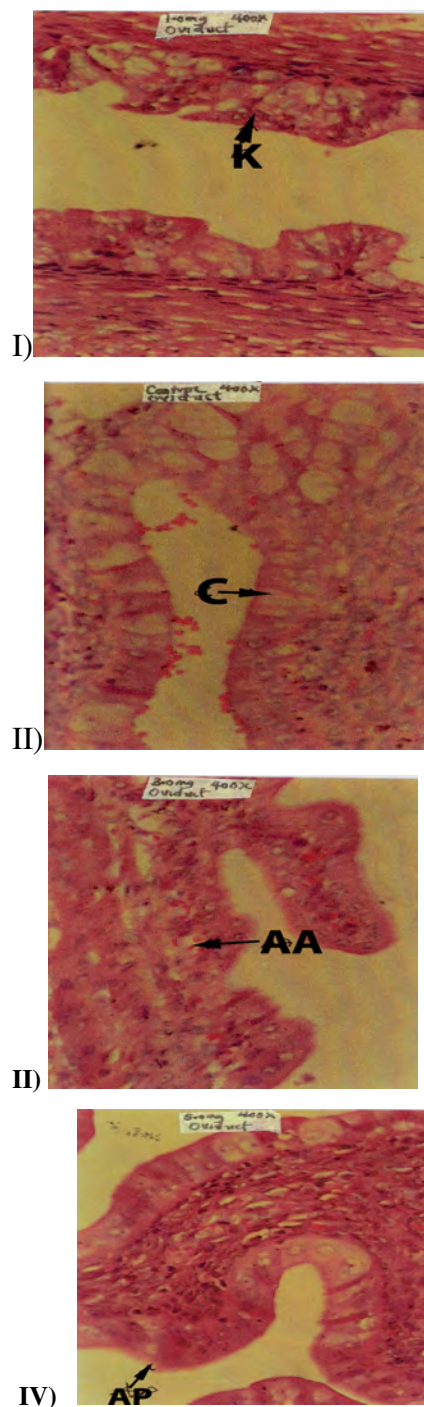

Fig 2. Photomicrographs of the effects of different doses of ammonium metavanadate on the histology of the oviduct of the female rats fixed by immersion in Bouin.s solution and stained with hematoxylin and eosin. Magnification is 400x. (i) The control shows areas of normal epithelium of the oviduct (**C**), while (ii) with 1mg metavanadate shows there was increased proliferation of the epithelial lining of the oviduct (**K**), (iii) at 3mg metavanadate showed areas of vascular congestion in the wall (**AA**) and (iv) with 5mg metavanadate showed gradual loss of surface eosinophilic band (**AP**).

Similarly the basal values of the total acid phospatase (ACP) changed from 15.4 ± 2.2 to 21.0

± 1.1 IU/L and 3.3 ± 0.1 to 9.4 ± 0.3 IU/L for prostatic acid phosphatase (fig 1a). These values were significant at $p \leq 0.005$ ANOVA. These effects on the biochemical phosphatases parameters were also time- dependent maximizing at 7 (seven) days of treatment (figs 1b&c).

Hormonal effects:

Stilbesterol-treated female Wister rats, ammonium metavanadate in the dose range 0-6mg caused a positively correlated dose-dependent biphasic responses in the serum concentration of the hormonal profiles of the female wister rats studied (Table 1 & 2). Ammonium metavanadate at the initial lower doses caused an increase (a spike) in the hormonal concentrations measured. This was followed by a dose-dependent decrease in the basal serum hormonal levels (Table 1). The maximal inhibitory responses at 5mg/kg of ammonium metavanadate were 2.7± 0.34, 2.1 ± 0.06, 72.0 ± 2.0 and 0.14 ± 0.01 for LH, FSH, PROL and Oest respectively (Table 1). These effects were also time-dependent (Table 2) maximizing at 7 days of treatment. The effects were characterized by an initial surge in hormonal effects within 48 hours followed by a time-dependent decrease in seven days.

Haematological parameters:

Ammonium metavanadate in the dose-range 0-10mg/kg caused both dose- and time – dependent effects on the haemoglobin and packed cell volume (Table 3). In the short term (24-72 hours), vanadate caused a transient increase in the neutrophils, Hb and PCV values (Table 3). However after 7 to 28 days of persistent treatment with Vanadate, ammonium metavanadate caused a dose-dependent decrease in Hb, PCV, lymphocytes and white blood cell values (Table 3). Finally the histological examination of the red blood cells under the microscope revealed that the red blood cells were characterized by the presence of target cells, acanthocytes, segmented cells of stave forms, increased anisocytosis and segmented leukocytes largely made up of band and juvenile forms when viewed under the light electron microscope fitted with DMLS camera. Showing that the agent caused anaemic responses.

Histopathological effects:

Ammonium metavanadate in the dose range 0-10mg/kg caused a dose-dependent destruction of the female reproductive tract viz the uterus, ovaries, and fallopian tubes (Figs 2, 3 & 4). These results were positively correlated with the results of the biochemical, haematological and hormonal parameters. This was characterized by oedema of the ovarian tissue, reduction in follicular cells, abortive follicles, complete ablation of the ovaries and para-ovarian tissue, congestion, small vessel

proliferation, thrombosis and increase in inflammatory cells (figs 2, 3 & 4). These toxic/destructive effects were dose-and time-dependent and were completely moderated/inhibited by vitamin E, vitamin C, selenium and their combination (not shown).

Table 1: The effect of different doses (0 – 5mg/kg of ammonium metadanadate on the serum concentration of various female hormones in the female Wistar rats

Dose of Cadmium (mg/kg)	Prolactin	LH	FSH	Oestrogen
0	95.35 ±12.73	6.32 ±1.37	5.32± 1.23	0.21 ±0.09
1	145.74 ±18.93*	9.15 ±1.89 *	5.59 ±1.01	0.26 ±0.07
2	141.92 ±13.83*	8.27 ±1.8*	5.89 ±1.08	0.23 ±0.01
3	99.29 ±3.27	6.38 ±1.1	4.98 ±1.42	0.25 ±0.11
4	81.97 ±4.5*	6.09 ±1.32	4.36± 0.97*	0.27 ±0.06
5	90.23 ±2.41	5.19 ±0.99	3.95± 1.02*	0.25 ±0.04

N=5 * Significance at $P \leq 0.05$ (ANOVA).

TABLE 2: The Effects of Time on the Ammonium Metavanadate Induced Hormonal Responses of the Female Wistar Rats

Time (Hrs)	Luteinising hormone	Follicle stimulating hormone	Oestrogen
0	4.35±0.31	3.31±0.35	0.111±0.02
24	6.54±0.24*	4.01±0.29*	0.340±0.09
75	1.72±0.09*	1.25±0.12*	0.380±0.09
165	1.63±0.34*	0.83±0.13*	0.240±0.03

N=5 * Significance at $P \leq 0.05$ (ANOVA).

Discussion

In this study, the quantitative and qualitative effects of ammonium metavanadate on the biochemical, haematological, hormonal and histopathological effects were investigated. Furthermore, 25mg of stilbesterol was given to each female rat to stabilize them at pre-oestrous state to avoid hormonal and cyclic complications in the interpretation of our results.

Ammonium metavanadate in these dose-range measured caused a time-and dose-dependent increase in total acid phosphatase and prostatic acid phosphatase levels. This is consistent with works of Lees *et al*, 2005; Pierce *et al* 1996. However these results were not qualitative.
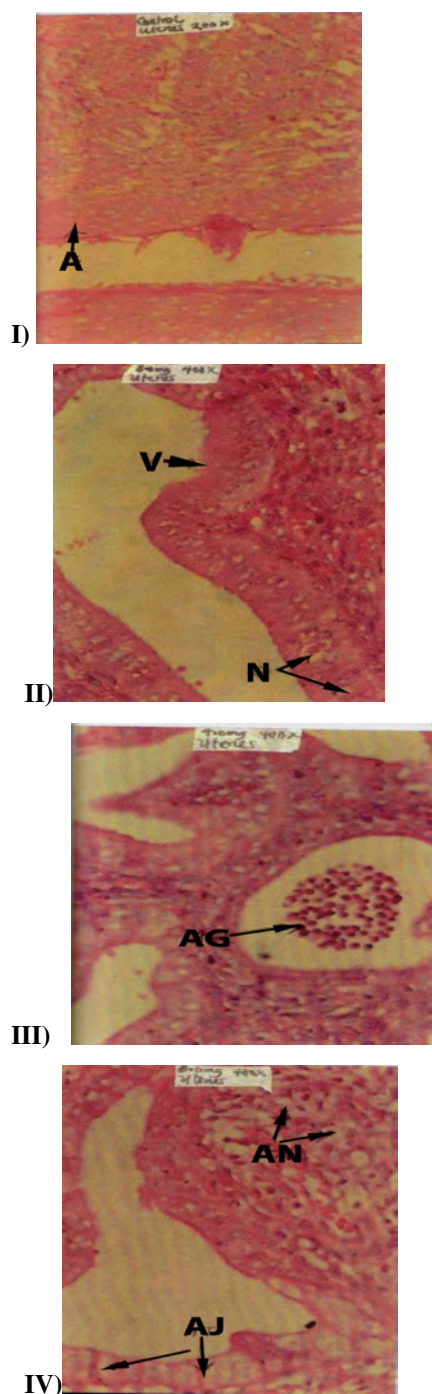
Fig 3 Photomicrographs of the effects of different doses of ammonium metavanadate on the histology of the uterus of the female rats, fixed by immersion in Bouin.s solution and stained with hematoxylin and eosin. Magnification is 400x. (I) Shows the control showing normal endometrial epithelium of the uterus (**A**), (II) while 1.0mg of metavanadate showed loss or absence of eosinophilic band (**V**) and vacuolation of the epithelial lining of the uterus (**N**), (III) at 3. 0mg metavanadate there was marked necrosis of tissues (**AG**) and (IV) at 5.0mg metavanadate showing vacuolation of the epithelium (**AJ**) and marked inflammatory cell infiltrate in the subepithelial tissues of the uterus (**AN**
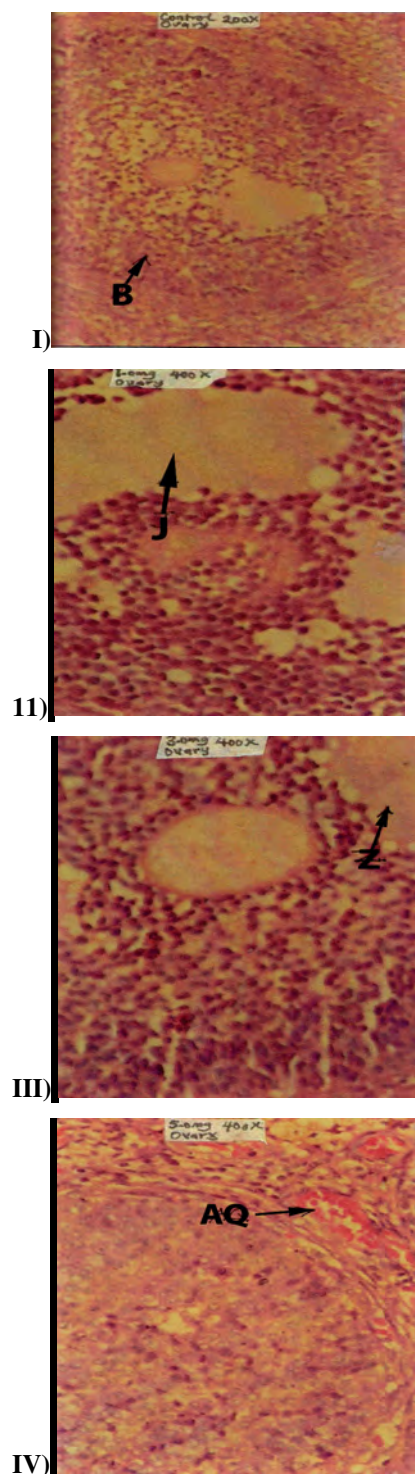
Fig 4. Photomicrographs of the effects of different doses of ammonium metavanadate on the histology of the ovary of the female rats, fixed by immersion in Bouin.s solution and stained with hematoxylin and eosin. Magnification is 400x. (I) The control shows normal graffian follicles at x200 Mag (**B**), (II) at 1.0mg metavanadate shows there are areas of oedema within the ovarian follicle (**J**), (III) 3. 0mg shows areas of marked oedema in ovarian follicle (**Z**) and IV) at 5. 0mg shows areas of congestion of the ovarian cortex (**AQ**)

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TABLE 3: The Effect of Dose and Time on the Ammonium Metavanadate-Induced Haematological (Neutrophil, Lymphocyte, White blood cell count, Haemoglobin and Packed Cell Volume) Responses of the Stilbesterol Treated Female Rats.

Dose Cadmium (mg/kg)	Neutrophil count	Lymphocyte count	White Blood Cell	Haemoglobin			Packed Cell Volume		
				24Hrs	7 Days	28 Days	24 Hrs	7 Days	28 Days
0	38.1±1.3	62.20±2.6	5.21 ±0.6	11.25 ±1.2	11.29 ±1.2	11.350 ±1.4	34.76 ±2.6	34.93 ±2.6	34.35 ±2.4
2.5	49.2±1.4*	6.56±0.9*	7.56 ±0.5*	16.25 ±0.9*	9.36 ±1.3*	7.260 ±1.8*	48.57 ±2.9*	33.21 ±2.9*	18.09 ±1.9*
5	56.2±1.4*	40.90±5.5*	3.42 ±0.4*	14.78 ±1.1*	7.14 ±1.1*	5.350 ±1.1*	44.76 ±3.8*	21.73 ±1.9*	10.31 ±1.08*
17.5	60.2±1.2*	24.50±3.3*	2.63 ±0.5*	13.97 ±1.3*	5.67 ±0.9*	4.245 ±1.02*	43.94 ±3.3*	19.37 ±1.9*	9.120 ±1.03*

N=5 * Significance at $P \leq 0.05$ (ANOVA).

Ammonium metavanadate also caused a time- and dose-dependent decrease in the hormonal concentrations studied. This is consistent with the earlier works of Llobert *et al* (1993). These works did not study the time-dependence neither did they show the correlation between the biochemical and hormonal changes. Ammonium metavanadate further caused a biphasic response on the haematological parameters. The agent stimulated an increase in haemoglobin and packed cell volume within 3-days and time-dependently in 30 days, caused a decrease in the same parameters. The stimulatory responses were novel since no works had earlier reported these. However the inhibitory responses (anaemia) recorded were consistent with the earlier works of Knecht *et al* (1985), showing that the results obtained with haematological parameters are dependent on the time of havesting of the blood cells.

The agent also caused a dose-and time-dependent increase in neutrophils, and inhibition of lymphocytes which is an indication of immunological suppression (Pierce *et al* 1996, Cohen *et al* 1996). Showing that this agent is inflammatory and neutropenic. This is also novel. The histological analysis of the blood cells showed the presence of acanthocytes, anisocytes and segmental leucocytes largely made up of band and juvenile forms. This explains the initial increase of haemoglobin and packed cell volume values, showing that vanadate stimulates mitosis in the short run and inhibit meiosis and maturation in the long run which predisposes the animal to anaemia (Yang *et al* 1986). The results are indicative of maturation arrest of haematological cells. Thus no matter the value of the Hb, the remaining cells are physiologically not useful.

Vanadate caused dose-and time-dependent pathological changes, to the histology of the female reproductive system. Furthermore, it also caused hormonal depressions/inhibitions which were positively correlated to the histological damages to uterus, ovaries and oviduct, showing that the histological destruction resulted in the pathophysiological decreases in hormonal production and function which may lead to infertility (Gupta *et al* 2004, Uche & Obianime 2008 and Lafuente 2000). This aspect of positive correlation between structure and function is novel as no other studies had ever undertaken such. Furthermore, studies with oxidizing agents like cd, oxolinic acid, lansoprazole and procymedone have shown that these agents may in addition have direct effects on the pituitary axis, leydig cell damage, perturbation of testosterone production and overstimulation of luteinising hormone (Fort *et al* 1995, Murakami 1995, Yamada 1994) thus resulting in reduction in normal feedback inhibitory mechanisms which will reduce hormonal production.

Another school of thought also has it that these hormonal disruptions precede tissue and uterine damage and carcinogenesis (Waalkes *et al* 1997). Also, these increases in the oxidative metabolism of cells, may have resulted in the direct destruction of the uterus, ovaries and fallopian tubes. These destructions were characterized by oedema, increase in fat tissue, necrosis of tissues, vacuolation etc (this study). These results were consistent with the earlier works of Obianime and Aprioku (2008). Previous reports did not show the detailed histological and micrographic results, neither did they show correlation between the dose and time of vanadate-induced effects on the phosphatase, haematological and hormonal responses.

Vanadate is known to cause an increase in the serum concentration of free radicals H_2O_2, OH+ (Llobert *et al* 1993). These radicals are known to cause various oxidative pathological destructions (Kerchaert et al 1996, Rojas *et al* 1996, Nangi and Hilter 1996, Lafuente (2001, 2003) which may result in the compromising of the intergrity of the female reproductive system and function.

Also vanadate caused a dose-and time-dependent inhibition of the serum hormonal levels and these results were consistent with previous reports on the effects of vanadate on the animal studies (Uche & Obianime 2008). These results were not qualitative neither were they correlated to histopathological stimulation of various phosphatases, which are indices of general toxicity and an increase which is an index of toxicity of the prostate and testicular tissues (Eyo 2003, Hirano *et al* 1990, Stohs *et al* 2001, Shimizu & Morita. 1990). The prostatic acid-induced toxicity is positively correlated to damage of the ovarian and uterine architecture and function, resulting in the various histological destructions seen in this study. Thus showing that vanadate would induce both biochemical, hormonal and histopathological alterations which are positively correlated to one another.

Finally, pretreatment with individual and combination studies of vitamin C, E and Selenium, showed that the vitamins and selenium individually caused a significant inhibition of the vanadate responses but in combination totally blocked the vanadate-induced toxicity (not shown). Showing that, Ammonium metavanadate caused tissue toxicities through the stimulation of the protein kinase C (PKC) and calcium ion signal transductional pathways (Bonkent *et al* 2007). This is consistent with the results of Bonkent *et al* 2007 in the rat intestine.

Vitamin C, E and selenium are antioxidants with effects on calcium and PKC pathways while selenium is an important agent in the rate limiting step of antioxidation (Niki *et al* 2000).

These antioxidants effect on calcium metabolism and PKC reduced and inhibited the generation of oxidative free radicals and also mop up excessive peroxidation, thus their inhibitory and remediating action.

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