

Icacinia manni (False Yam)-Induced Reproductive Toxicity in Male Rats: Comparative Effects of Vitamins C and E.

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Abstract

Icacinia manni or false yam grows in both tropical and temperate regions. Use of its tuber as a replacement for energy content in animal and bird feeds is being advocated. Studies show it impairs reproductive function in male rats. Possible ameliorating effect of vitamins C and E is considered in this study since oxidative stress is common metabolic pathway for many tissue dysfunctions. Twenty five male wistar rats were randomly placed into five groups of five/rats each viz control, low dose Icacinia manni, high dose Icacinia manni, high dose Icacinia manni + vitamin C and high dose Icacinia manni +vitamin E groups. Rats had free access to rat feeds and water. Animals were anaesthetized, blood samples collected and testes harvested for evaluation of relevant parameters after 28 days of treatment. P-values < 0.05 were considered statistically significant. Results showed decreased testosterone in low dose, high dose and high dose + vitamin C groups compared with control (p<0.05 each) but increased in high dose + vitamin E compared with high dose groups (p<0.05). Total sperm counts were reduced in all extract treated groups (p<0.05 each) compared with control. Sperm motility was decreased in all extract administered groups (p<0.05 each) compared with control but higher in high dose + vitamin C (p<0.05) compared with low dose group and in the high dose + vitamin C and high dose + vitamin E groups compared with the high dose extracts group (p<0.05 each). Wobbling rate increased in all extract-administered groups (p<0.05 each) compared with control and decreased in the high dose + vitamin C (p<0.05) compared with high dose group. Beat cilia frequencies decreased in all extract-administered (p<0.05) compared with control. Sperm linearity was decreased in all extract-treated groups (p<0.05 each) compared with control and reduced in the high dose, high dose + vitamin C and high dose + Vitamin E (p<0.05) compared with low dose groups but increased in the high dose + vitamin C and high dose + vitamin E (p<0.05) compared with high dose groups. Velocity of active path reduced in both low and high dose extract groups (p<0.05 each) compared with control but increased in high dose + vitamin C and high dose + vitamin E (p>0.05 each) compared with the low and high dose groups. Normomorphology was reduced in the low and high dose groups (p<0.05 each) compared with control but increased in high dose + vitamin C and high dose + vitamin E (p<0.05 each) compared with low and high dose extracts groups. In conclusion, ethanolic extract of Icacinia manni impairs male reproductive function in a dose-related matter with co-administration of vitamin C or E ameliorating some of the parameters almost at the same rate.

Keywords: Icacinia manni, ethanolic extract, vitamin E, vitamin C, reproductive toxicity.

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INTRODUCTION

Icacinia manni grows as a wild shrub in both tropical and subtropical regions. It is known by different names in different regions example “false yam”, “earth ball” etc. Though a shrub or a small plant on the surface, the underground part is tuberous and develops into large size tubers mainly made up of carbohydrate (Agyakwa and Akobunta, 1998). It is an all season plant, locally abundant in the humid tropics of Cross River State, Nigeria and is not commonly and

directly consumed by humans (Agyakwa and Akobundu, 1998).

The interest of researchers has been drawn to Icacinia manni tuber because of the prevailing economic situation and the high exchange rate which has made it difficult to produce animal and bird feeds with a hundred percent imported constituents. Attempts have therefore been made to increase the use of locally available and cheap alternatives. The tuber of Icacinia manni is one of such alternative replacements of energy

source being evaluated for the production of animal and poultry feeds (Umoren *et al.* 2007, Umoren *et al.* 2008, Effiong and Jimmy, 2019).

The *Icacinia manni* tuber is mainly made up of carbohydrates and little proteins (Agyakwa and Akobundu, 1998). Its phytochemical screening shows it contains among other things, oxalates alkaloids, hydrogen cyanide, phytic acid and tannins (Antai and Obong, 1992).

Previous Studies on the effect of this tuber on biological systems have shown it to improve packed cell volume and total white blood cell (Solomon *et al.* 2011), increased dressed weights, as well as increased kidney, lungs, gizzard, liver shank, intestine and neck weights (Umoren *et al.* 2007) as well as heart weights. Yet other studies have reported negative effect of the tuber on reproductive indices. According to Asuquo and Udedibie (2012), the tuber causes damage to gonadal tissues in birds with resultant reduction in number of eggs laid, haugh unit as well albumin and yolk indices. In another study, Udokang *et al.* (2019) reported decreases in sperm concentration, motility, testosterone and increase in morphologically defective sperms while in another Udokang *et al.*, (2020), observed reduction in number of litre and reduced birth weight.

Though the precise mechanism of tissue injury Is not found in literature, oxidative stress is reported to be a common metabolic pathway for tissue injury in many diseases. Vitamin C and E are potent antioxidants (Padayatty *et al.* 2003, Traber and Stevens, 2012, Bendich *et al.* 2007) which have been used to combat or ameliorate several oxidative stress-induced tissue dysfunctions (Lee *et al.*, 2007, Ebuehi *et al.* 2012). In this study we therefore wanted to find out the possible ameliorating roles and the degree of amelioration by vitamins C and E.

MATERIALS AND METHODS

Ethical approval

Ethical approval for the animal study was obtained from the Ethics and Research Committee of the Faculty of Basic Medical Sciences, University of Uyo, Uyo.

Collection and authentication of Plant

Tubers of *Icacinia manni* were harvested from the bush at Uyo where it grows as a wild plant. The Plant was then taken to the Department of Botany, University of Uyo for authentication.

Preparation of ethanolic extract of *Icacinia manni*

The plant tubers were washed with water to remove sand, cut into pieces and sun-dried before it was blended to powder after two weeks of drying. The powder was then macerated in 80% ethanol and the

solution so formed evaporated at 40°C to dryness using a rotary evaporator. The extract was then preserved in a refrigerator till used.

Determination of LD₅₀

The LD₅₀ of the extract was assessed by intraperitoneal injection of the extract using Lorke's method (1983). It was found to be 894.42 mg/kg.

Experimental animals

Twenty five pre-pubertal male wistar rats were used for the study. The rats were obtained from the Department of Pharmacology, University of Uyo and housed in wooden cages at the Animal House of the Faculty of Basic Medical Sciences, University of Uyo. They were given free access to potable water and rat chow for the duration of experimentation (twenty eight days).

Experimental Protocol

The twenty five male wistar rats were randomly assigned into five (5) groups of 5 rats each. Group 1 served as control, group 2 was given low dose extract (89.44mg/kg), group 3 was given high dose extract (268.33mg/kg), group 4 was treated with high dose extract + vitamin C at a dose of 2.8mg/kg while group 5 was treated with high dose extract plus vitamin E at 2.85mg/kg. Both drugs and extracts were administered daily by gavaging for twenty eight days.

Sample collection

At the end of the experimentation period, animals were anaesthetized, blood samples taken and testes harvested for determination of relevant parameters.

Evaluation of Serum concentration of testosterone

This was done using ELISA method with testosterone kit.

Evaluation of Sperm Parameters

The cauda epididymis of each testis was dissected out and several small incisions of about 1mm made on it and the tissue suspended in 1ml of semen buffer solution to allow for spermatozoa swim up the solution. Sperm analysis was then carried out in accordance with WHO (2015) guideline and a computer-assisted sperm analysis (CASA) technique (Tilley, 2007). In brief, a freshly prepared semen solution from each epididymis was drawn and diluted approximately in mixed agglutination reaction (MAR) test buffer. The diluted sample was pipetted into a Mackler chamber placed on a heated (37°C) microscope stage. Video recordings were made from four different fields of the chamber using a magnification objective. Analysis was carried out based on capturing sequences of 64 frames per field and counting a minimum of one hundred spermatozoa. The following measurements Were assessed, sperm concentration ($\times 10^6/\text{ml}$); total

motility (%); progressivity (PR); velocity of active path (VAP); amplitude of lateral head (ALH); wobbling rate (WOB) (%); beat cilia frequency (BCF); Linearity (LIN) (%); curvilinear velocity; straight forward line travel; immotility, non-progressivity, velocity of straight line travelled and morphological abnormalities.

STATISTICAL ANALYSIS

Data were expressed as mean \pm SEM. Differences between mean values were evaluated by analysis of variance (ANOVA) followed by Tukeys post hoc test for pairwise comparisons. Values of $p < 0.05$ were considered statistically significant. Graph Pad Prism 7.0 software (GraphPad, Inc USA) was used for the statistical analysis.

RESULT

Our results showed significant differences in serum testosterone, sperm concentration and indices of sperm motility and morphology. Serum testosterone levels (ng/ml) were decreased in all extract-treated groups ($p < 0.05$ each) compared with control but increased in the extract + vitamin E ($p < 0.05$) compared with other extract administered groups (fig1). Sperm concentration ($\times 10^6/\text{ml}$) was decreased in all extract-administered groups compared with control ($p < 0.05$ each) as in fig 2. Total percentage of normal sperm cells was reduced in the low and high dose groups compared with control ($p < 0.05$ each) but increased in the high dose + vitamin C compared with high and low dose extract groups ($p < 0.05$ each) and reduced in high dose + vitamin E compared with the high dose + vitamin C groups ($p < 0.05$) as in fig 3. Total abnormality rate (%) was increased in all extract treated groups ($p < 0.05$ each) compared with control and also in the high dose and high dose + vitamin E ($p < 0.05$) compared with low dose extract-treated groups (fig 4).

Total motility (%) was reduced in all extract treated groups ($p < 0.05$ each) compared with control but increased in extract+ vitamin E ($p < 0.05$ each) compared with low dose and high extract treated groups ($p < 0.05$ each) as in fig 5. Wobbling rate was higher in all extract-administered groups compared with control ($p < 0.05$ each) but lower in the extract+ vitamin C groups compared to the high dose extract groups ($p < 0.05$) in fig 6. Beat cilia frequency (Hz) was reduced in all extract-administered groups ($P < 0.05$ each) compared with control (fig 7). Linearity compared with control ($p < 0.05$ each), lower in all high dose extract treated groups compared with low dose extract treated group ($p < 0.05$ each) but increased in the high dose extract + vitamin C compared with high dose extract group ($p < 0.05$) as in fig 8. Straight forward line travelled by sperms (%) was decreased in both low and high dose + vitamin compared with low and high dose extract groups ($p < 0.05$) but reduced in the high dose +

vitamin E ($p < 0.05$) compared with high dose + vitamin C (fig 9).

Also from the results, progressivity was decreased in the low and high dose extract treated groups ($p < 0.05$) compared with control but increased in the high extract + vitamin C and high extract + vitamin E ($p < 0.05$ each) compared with high dose extract-treated groups (fig 10). Non-progressivity was increased in all extract- treated groups compared with control ($p < 0.05$ each) as in fig 11. Velocity of active path travelled ($\mu\text{m/s}$) was reduced in both low and high extract-treated groups compared with both low and high extract-treated groups compared with control ($p < 0.05$ each) but increased in high dose + vitamin C and high dose + vitamin E groups ($p < 0.05$ each) compared with both low and high dose groups (fig 12). Curvilinear velocity ($\mu\text{m/s}$) was reduced in low and high dose extract treated compared with control groups ($P < 0.05$) but elevated in high dose + vitamin C and high dose + vitamin E groups ($p < 0.05$) compared with both high and low dose extract treated groups (fig 13). Velocity of straight line travelled ($\mu\text{m/s}$) was decreased in low and high dose extract ($p < 0.05$ each) compared with control but increased in high dose + vitamin C and high dose + vitamin E groups ($P < 0.05$ each) compared with low and high dose extract-treated groups (fig 14).

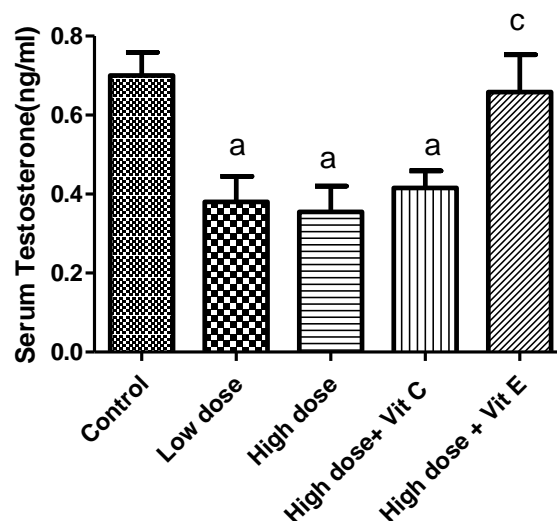


Fig-1: Comparison of serum testosterone levels in experimental groups. n = 5. ^a $p < 0.05$ vs control group. ^c $p < 0.05$ vs high dose group.

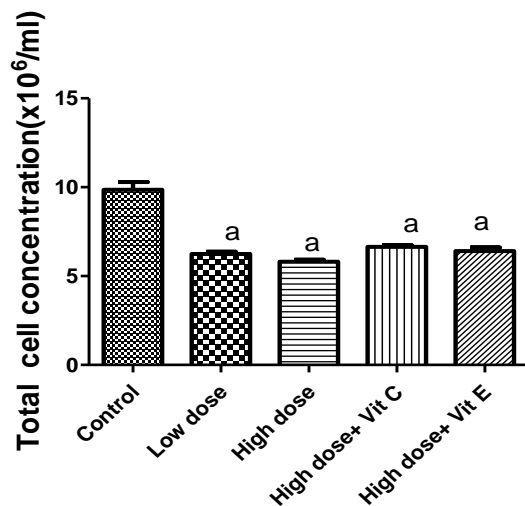


Fig-2: Comparison of sperm concentration in experimental groups. n = 5. ^ap<0.05 vs control group.

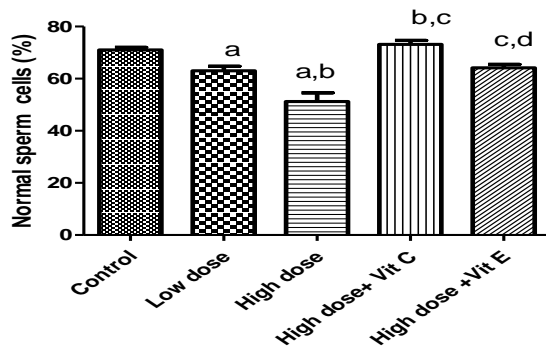


Fig-3: Comparison of normal sperm cells in experimental groups. n = 5. ^ap<0.05 vs control group. ^bp<0.05 vs low dose group. ^cp<0.05 vs high dose group. ^dp<0.05 vs high dose + Vitamin C treated group.

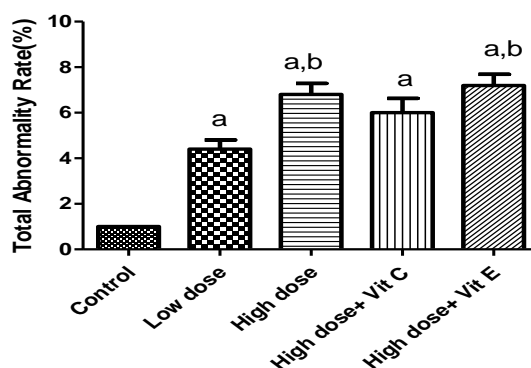


Fig-4: Comparison of total abnormality rate of sperm cells in experimental groups. n = 5. ^ap<0.05 vs control group. ^bp<0.05 vs low dose group.

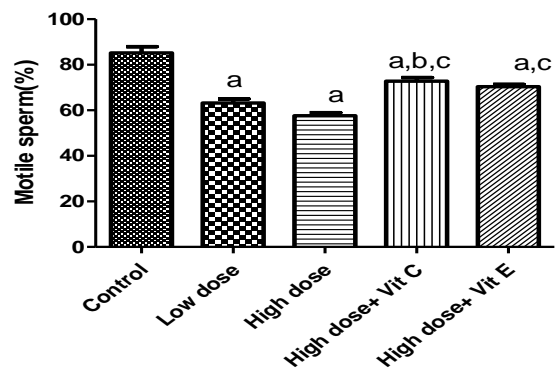


Fig-5: Comparison of percentage of motile sperm in experimental groups. n = 5. ^ap<0.05 vs control group. ^bp<0.05 vs low dose group. ^cp<0.05 vs high dose group.

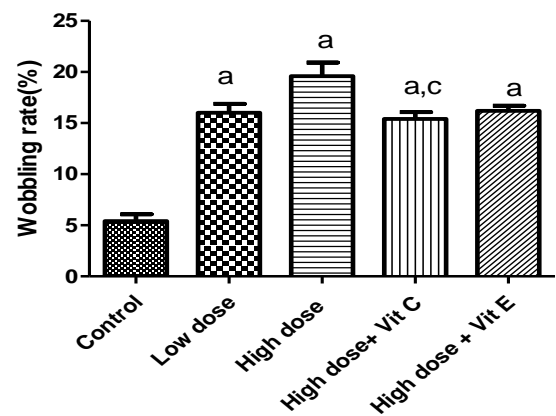


Fig-6: Comparison of wobbling rate of motile sperm in experimental groups. n = 5. ^ap<0.05 vs control group. ^cp<0.05 vs high dose group.

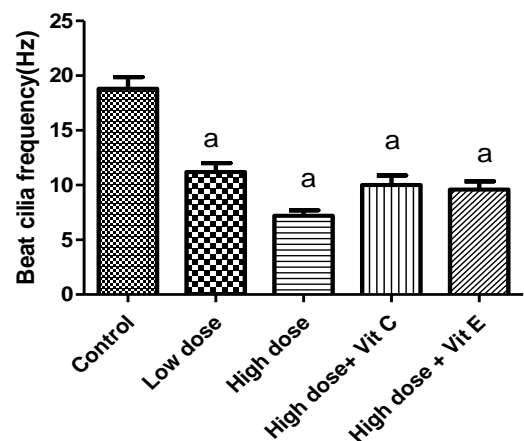


Fig-7: Comparison of beat cilia frequency of motile sperm in experimental groups. n = 5. ^ap<0.05 vs control group.

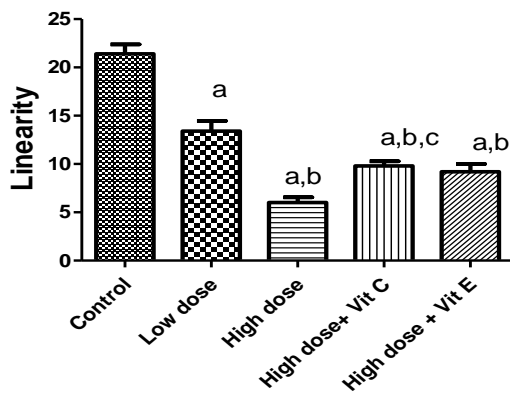


Fig-8: Comparison of linearity of sperm cells in experimental groups. $n = 5$. ^a $p < 0.05$ vs control group. ^b $p < 0.05$ vs low dose group. ^c $p < 0.05$ vs high dose group.

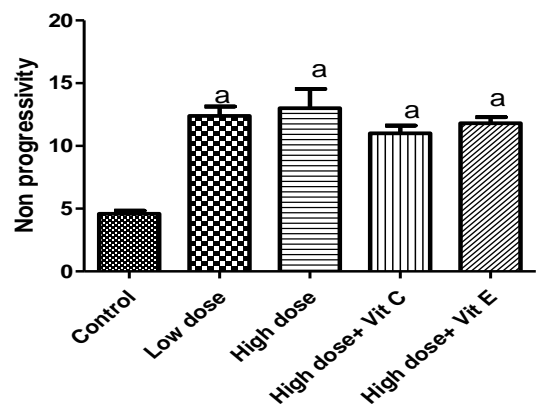


Fig-11: Comparison of non-progressivity of sperm cells in experimental groups. $n = 5$. ^a $p < 0.05$ vs control group.

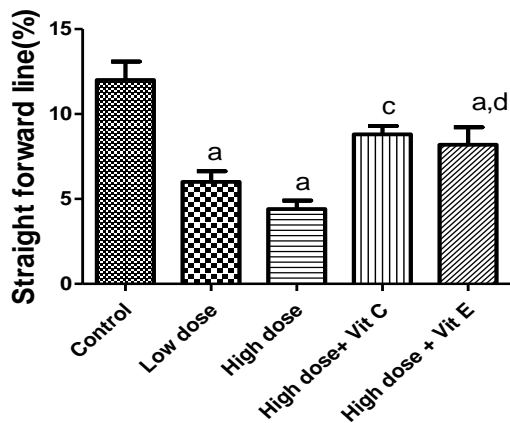


Fig-9: Comparison of straight forward line travelled by sperm cells in experimental groups. $n = 5$. ^a $p < 0.05$ vs control group. ^c $p < 0.05$ vs high dose group. ^d $p < 0.05$ vs high dose + Vitamin C group.

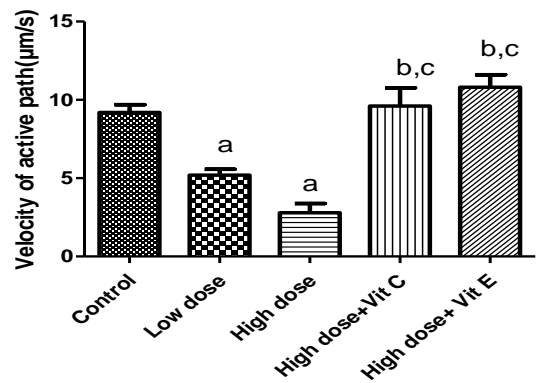


Fig-12: Comparison of velocity of active path travelled by sperm cells in experimental groups. $n = 5$. ^a $p < 0.05$ vs control group. ^b $p < 0.05$ vs low dose group. ^c $p < 0.05$ vs high dose group.

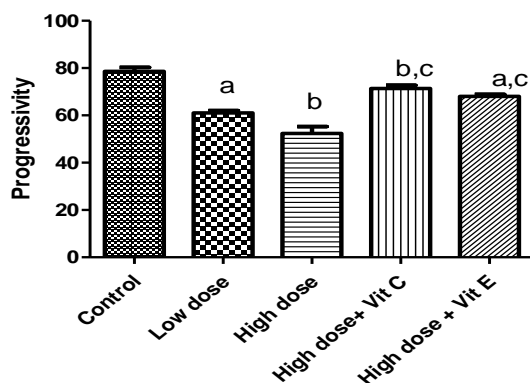


Fig-10: Comparison of progressivity of sperm cells in experimental groups. $n = 5$. ^a $p < 0.05$ vs control group. ^b $p < 0.05$ vs low dose group. ^c $p < 0.05$ vs high dose group.

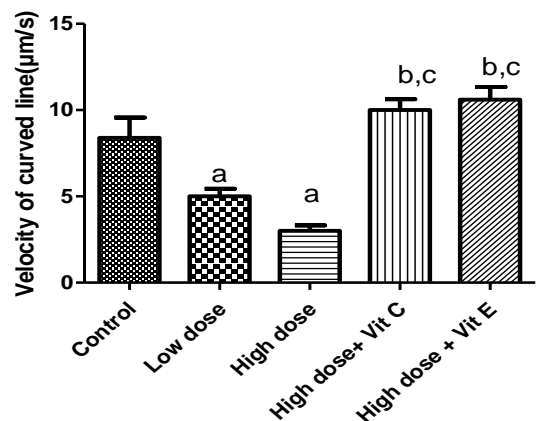


Fig-13: Comparison of curvilinear velocity travelled by sperm cells in experimental groups. $n = 5$. ^a $p < 0.05$ vs control group. ^b $p < 0.05$ vs low dose group. ^c $p < 0.05$ vs high dose group.

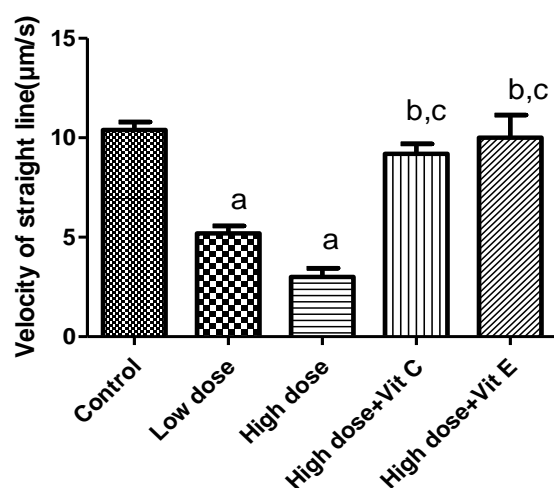


Fig-14: Comparison of velocity of straight line travelled by sperm cells in experimental groups. n = 5. ^ap<0.05 vs control group. ^bp<0.05 vs low dose group. ^cp<0.05 vs high dose group.

DISCUSSION

Previous studies on *Icacinia manni* show it has a deleterious effect on sperm count, morphology, motility and testosterone. We therefore investigated the comparative effects of two common antioxidants (vitamins C and E) on possible alterations in reproductive indices, oxidative stress being a common metabolic pathway in many cellular dysfunctions. Our results are discussed here.

The observed decrease in serum testosterone in the low dose, high dose and high dose + vitamin C group strongly points to a defective steroidogenesis caused by the extract. Udokang *et al.* (2019) had reported a low testosterone level associated with consumption of *Icacinia manni* ethanolic extract in male rats. *Icacinia manni* contains toxic substances and anti-nutrients including hydrocyanide, oxalates and phytates (Asuquo and Udedibie 2012). The decrease in serum testosterone concentration could also be the result of direct toxic effect of the extract on Leydig cells as reported by Udokang *et al.* (2019) essential cells in testosterone synthesis. This decline in testosterone levels might have resulted from toxicity from cyanide which is known to cause apoptosis of cells and damages cytochrome- C oxidase which is important in ATP production and this via oxidative stress (Mills *et al.*, 1996). Vitamin C did not seem to have any effect on the impairment. However, it was increased in the high dose extract + vitamin E group suggesting that vitamin E ameliorated the oxidation process and so restoring the testicular function.

We observed that total sperm concentration was decreased in all extract-treated rats including the high dose + vitamin C and high dose + vitamin E

groups. Spermatogenesis is affected by many factors including genetic, hormonal, environmental and nutritional factors. Oxidative stress which might have been induced by phytochemicals in *Icacinia manni* like hydrocyanides and alkaloids (Mills *et al.* 1996, Lee *et al.*, 2017) might have affected the process of spermatogenesis so much so that the dose of antioxidants vitamins C and E could not make any appreciable impact. Also anti nutrients phytochemicals like oxalates and phytates might have caused impaired calcium and zinc metabolism (Lopez *et al.* 2002), elements which are vital in testicular function (Gallo-Payet and Payet, 1986).

The decrease in the percentage of normal sperm cells which is similar to the findings of Udokang *et al.*, (2019) in the low and high dose extract-treated groups could have been due to the toxic effect of the extract on sperm morphology. The toxic nature of some of the phytochemicals in the extract could have been responsible for this. The increase in the percentage of normal sperms in vitamins C or E co-administered groups compared with plain extract treated groups means that the vitamins ameliorate the negative effect of the extract. Its decrease in extract +vitamin E compared with extract + vitamin C groups suggests that vitamin C was more effective in that regard. E. A comparison of the total sperm abnormality rate shows a similar pattern and suggests the differential effect of the vitamins to ameliorate the impairment.

The decrease in total sperm motility in the low and high dose groups is in line with that observed in the study by Udokang *et al.* (2019). Sperm motility is influenced by its micro environment as well as its structural and functional factors. The observed decrease in motility therefore might have been due to the increased morphological defects (Aribo *et al.*, 2018) extract-treated rats also noted in the study. Co-administration of vitamin C or vitamin E did not have any significant influence on the impaired motility suggesting the doses of vitamins C and E were inadequate or there could be another mechanism of testicular damage other-than oxidative stress.

Characterization of sperm motility indices is important especially in artificial fertilization technology (Franken and Henkel, 2012) since the cells must be screened for maximal results. Characterization of the motility indices showed deranged levels. The increased wobbling rate in all extract-administered rats shows the effect across board. However, co-administration of vitamin C significantly reduced the wobbling rate indicating vitamin C a better ameliorator than vitamin E for this index. Beat cilia frequency which was decreased in all extract-treated groups did not show the ability of vitamins C or E to influence the outcome. Reduction in the linearity of sperm cells showed a dose-dependent effect on linearity. However, vitamins C or E

co-administration similarly increased the linearity. Straight forward line travelled by sperms decreased in low and high dose-treated groups was improved by vitamins C and E with vitamin C showing better result. In the progressivity of sperms, it was decreased I low and high dose groups, effect that was ameliorated by vitamin C or E co-administration though vitamin C appears to be more effective. The decreased velocity of active path travelled by sperms, velocity of straight line travelled and curvilinear velocity in both low and high dose groups were equally reversed by both vitamins.

Sperm motility is influenced by intrinsic and extrinsic factors including genetics, morphology, microenvironment factors. This also might have been due to impaired energy metabolism which the sperm requires for its movement. *Icacinia manni* contains cyanide which can inhibit cytochrome C oxidase thereby blocking ATP synthesis (Mills *et al.*, 1996). It also contains oxalates and phytates which impede calcium metabolism essential for sperm motility (Gallo Payet and Payet, 1986). The observed abnormal sperm kinematics could have therefore been due to the defective morphology in sperms noticed here or other factors which could be caused by the extract as mentioned above.

CONCLUSION

From the foregoing, we conclude that ethanolic extract of “false yam” (*Icacinia manni*) impairs testosterone steroidogenesis, sperm concentration, morphology and kinematics, most of which were ameliorated by vitamins C and E co-administration with vitamin C demonstrating greater effect.

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