ACTA BIOSCIENTIA
Journal of Biomedical and Biological Sciences

Acta Bioscientia **2025**:1(3);153-160 **OPEN ACCESS**
https://doi.org/10.71181/actabioscientia12300

An Assessment of the Effects of Vitamin A on Biochemical Indices, Sperm Parameters and Testicular Morphology in 5-Fluorouracil-treated Male Rats

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ABSTRACT

Chemotherapeutic agents like 5-Fluorouracil (5-FU) are useful in cancer management, but they are known to be associated with adverse effects, including subfertility in male patients. Such adverse effects may limit clinical application, necessitating the search for agents that can reduce them. This study evaluated the possible protective effect of Vitamin A against 5-FU-induced fertility impairment in male rats. Sixty male Wistar rats (150–170 g each) were randomly assigned to six groups (n=10). Group A received normal saline orally at 10 ml/kg, while Groups B and C received Vitamin A at 500 IU/kg and 1000 IU/kg, respectively. Group D received a single dose of 5-FU (50 mg/kg) intraperitoneally. Groups E and F were co-treated with 5-FU and Vitamin A at 500 IU/kg and 1000 IU/kg, respectively, for 14 days. After treatment, rats were euthanised, and blood samples were collected for the estimation of interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), total antioxidant capacity (TAC), and malondialdehyde (MDA). Body weight, feed intake, and sperm parameters (count, motility, morphology) were assessed. Testes and epididymes were harvested for histological evaluation. Data were analysed using One-way ANOVA and Tukey's Honestly Significant Difference (HSD) test; with statistical significance set at $p<0.05$. Results indicated that Vitamin A, especially at 1000 IU/kg, attenuated 5-FU-induced oxidative stress and reproductive toxicity, highlighting its potential as a protective agent against chemotherapy-induced subfertility. The 5-FU group had lower body weight, reduced food intake, decreased total antioxidant capacity, and elevated lipid peroxidation. Co-treatment with Vitamin A improved these parameters, restored IL-10 levels, and reduced TNF-α expression. Sperm quality and testicular structure were also better preserved in the Vitamin A-treated groups. However, further studies are needed to establish this.

KEYWORDS: Antioxidants, Beta-carotene, Chemotherapy, Infertility, Oxidative Stress, Retinol

1. Introduction

Among the various treatment modalities for cancers, chemotherapy is widely employed due to its ability to target rapidly dividing cancer cells [1,2]. One of the significant and often under-addressed side-effects of chemotherapy is its impact on fertility, particularly in male patients [3]. 5-Fluorouracil (5-FU) is an antimetabolite that interferes with DNA synthesis, and it is often used in treating various cancers, including colorectal, breast, and head/neck cancers

[4, 5] Chemotherapy-induced subfertility has garnered attention, as increasing numbers of cancer survivors face reproductive challenges post-treatment [6-8]. Agents like 5-FU, while effective against malignant cells, can damage normal cells, particularly those involved in spermatogenesis, leading to fertility issues [9]. Studies have shown that 5-FU induces testicular toxicity, oxidative stress, and germ cell apoptosis, leading to subfertility in male cancer patients [5]. The damage caused by 5-FU is not limited to the spermatozoa alone as histopathological studies had revealed that 5-FU treatment results in severe damage to the seminiferous tubules, which are critical for sperm production [5]; the structural integrity of these tubules is essential for maintaining spermatogenesis, and their destruction can result in permanent infertility if the damage is extensive. Long-term follow-up studies of cancer survivors treated with 5-FU indicate that fertility does not always recover post-chemotherapy; with some patients experiencing persistent azoospermia (complete absence of sperm) years after treatment, suggesting irreversible testicular damage [10]. Others may see partial recovery, but with compromised sperm quality, reducing the chances of natural conception. Considering these challenges, recent research has been exploring the potential use of antioxidants and other protective agents to mitigate the toxic effects of chemotherapy on reproductive organs [11, 12].

Vitamins including vitamin A are essential organic compounds required in small quantities for the maintenance of health and wellbeing and prevention of disease [13, 14]. Vitamin A is a fat-soluble micronutrient essential for reproductive health, immune function, vision, and cellular processes; mainly through its active form, retinoic acid, which regulates gene expression [15-17] and supports spermatogenesis by promoting germ cell differentiation and maintaining Sertoli cell function [18]. It also acts as a powerful antioxidant that counters oxidative stress, enhances antioxidant enzyme activity, stabilises cell membranes, and reduces lipid peroxidation; thereby protecting testicular tissue and sperm quality [19, 20]. Recent findings suggest that Vitamin A may offer protective benefits against 5-Fluorouracil (5-FU)-induced reproductive toxicity [15]

2. **Methodology**

2.1 Drugs and Reagents

5-Fluorouracil (Flurasted™ 500 ml), Vitamin A (Jesdol Vitamin A 25000 IU), dimethyl sulfoxide, assay kits, and saline solution were procured from African Biosciences Ltd, Lagos, Nigeria. Rodent chow was sourced from Top Feed, Ogbomoso Oyo State, Nigeria.

2.2 Animals

Healthy male Wistar rats, (weighing 150-170g each) used in this study were obtained from Empire Breeders, Osogbo, Osun State. The rats were acclimatised to the experimental conditions for the first week and housed in wooden cages measuring 20 x 10 x 12 inches. They were allowed free access to food and water. All procedures were conducted in accordance with the approved protocols of the Research Ethical Committee of the Faculty of Basic Medical Sciences, LAUTECH; and as prescribed by the European Council Directive (EU2010/63) for the use and care of laboratory animals.

2.3 Experimental Methods

Sixty male rats, each weighing 150-170g, were randomly allocated into six groups of ten (n=10) animals each. Group A was given normal saline orally at 10ml/kg. Groups B and C received 500 IU/kg and 1000 IU/kg of vitamin A, respectively, for 14 days. Group D was administered a single dose of 5-FU at 50 mg/kg body weight intraperitoneally [18]. Groups E and F received a single dose of 5-FU at 50 mg/kg body weight intraperitoneally, plus 500 IU/kg and 1000 IU/kg of vitamin A, respectively, over 14 days. On the 14th day, the animals were euthanised by cervical dislocation, and blood was collected via intracardiac puncture to measure inflammatory cytokines (interleukin-10 and TNF-α), lipid peroxidation (measured as malondialdehyde (MDA) concentration), and antioxidant status (total antioxidant capacity (TAC)). The testes were excised, examined grossly, and fixed in 10% neutral buffered formalin. Sections of the testes were then processed for paraffin embedding, cut at 5µm, and prepared for general histological study.

2.4 Assessment of body weight and food intake

Body weight of animals in all groups was measured weekly using an electronic weighing scale (Mettler Toledo Type BD6000, Switzerland), while food consumption was assessed using a weighing balance as previously described-[21]. The percentage change in body weight or food intake for each animal was calculated using the following equation, and the results for all animals were computed to find the statistical mean:

$$\frac{\text{Final body weight/feed intake} - \text{Initial body weight/feed intake}}{\text{Initial body weight or food intake}} \times 100$$

2.5 Biochemical Assays

2.5.1 Estimation of malondialdehyde (MDA) level

Measurement of malondialdehyde level (MDA) was performed following previously described protocols [22, 23]. 200µl of serum was incubated with 500µl of Thio barbituric acid (TBA) reagent for 20 minutes in a boiling water bath; then cooled under running tap water for 13 minutes. After

cooling, the solution was centrifuged at 3,000 rpm for 10 minutes, and the precipitate was removed. The absorbance of the supernatant was determined spectrophotometrically at 532 nm against a blank containing all the reagents minus the serum, using a Shimadzu UV-VIS Recording 2401 PC®. The concentration of MDA was calculated using the following equation:

MDA (mol/ml) = (Absorbance of sample / Absorbance of standard) ×100

2.5.2 Total Antioxidant Status

Total antioxidant capacity was measured as described by [24, 25]. To 240µl of the FRAP reagent, 8µl of serum was added and incubated for a few minutes at 37°C. The absorbance of each sample solution was measured at 532nm against the blank using a Shimadzu UV-VIS Recording 2401 PC®. The results were expressed as mmol/L.

2.5.3 Tumour necrosis factor-α and Interleukin 10

Tumour necrosis factor-α (TNF-α) and interleukin-10 (IL-10) levels were assessed using enzyme-linked immunosorbent assay (ELISA) techniques. We utilised commercially available kits from Enzo Life Sciences Inc., NY, USA, designed to measure the total quantities (both bound and unbound) of these cytokines. This methodology was based on the procedures described by [26].

2.6 Semen Analysis

Sodium bicarbonate was used to maintain the epididymis in its active state. Sperm count, motility, and morphology were assessed using a haemocytometer.

2.7 Tissue Histology

Formalin-fixed samples of the testes and epididymis were embedded in paraffin and sectioned at 5µm thickness. The sections were then dewaxed and rehydrated before being mounted on slides and stained with haematoxylin-eosin (H&E) for general histological study. Histopathological assessment of the connective tissue of the testes was performed using the Verhoeff-Van Gieson stain. Histological changes in the structure of the testes were also examined. The definitions for the different stages included: primordial spermatogonia characterized by small, rounded cells with a single layer of follicular epithelial cells; primary spermatocytes, surrounded by a monolayer or multilayer of prismatic or highly prismatic follicular epithelial cells; secondary spermatocytes, covered by more than two layers of granulosa cells where the formation of the antrum begins; mature (Graafian) spermatocytes, fully developed cells with a single large antrum filled with follicular fluid, surrounded by granulosa and cumulus cells; and atretic spermatocytes, degenerated cells marked by pyknosis in granulosa cells and debris within the antral cavity. The thickness of the epithelial layer of the testes was also measured. The sections of the testes and epididymis were examined microscopically using a Sellon-Olympus trinocular microscope (XSZ-107E, China) with a digital camera (Canon PowerShot 2500), and photomicrographs were taken. Histopathological examination was conducted by a technician blinded to the groupings.

2.8 Statistical Analysis

Data were analysed using Chris Roden's ezANOVA for Windows with one-way ANOVA. One-way ANOVA was used to test the hypothesis, and Tukey's HSD test was employed for post-hoc analysis. Results were expressed as mean ± S.E.M., and $p<0.05$ was considered significant.

3. Results

3.1 Effect of Vitamin A on body weight

Figure 1 shows the effect of vitamin A on relative change in body weight in rats exposed to 5-fluorouracil. There was a significant ($p<0.05$) decrease with VIT. A500, VIT.A1000, 5-FU, 5-FU/VIT-A500, 5-FU/VIT.A1000) when compared to the control. Compared with 5-FU, body weight decreased with 5-FU/VIT.A500 and 5-FU/VIT.A1000.

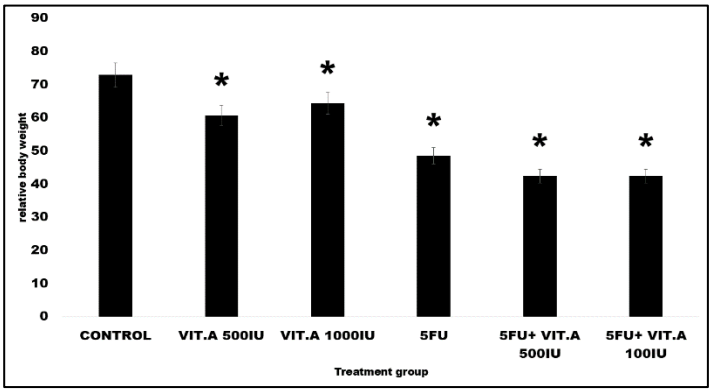

Figure 1: Effect of Vitamin A on Body Weight. Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. *$p<0.05$ vs control. VIT.A: Vitamin A, 5-FU :5-Fluorouracil

3.2 Effect of Vitamin A on feed intake

Figure 2 shows the effect of vitamin A on relative percentage change in feed intake in 5-fluorouracil treated rats. There was a significant ($p<0.05$) decrease with VIT A500, VIT.A1000, 5-FU, 5-FU/VIT-A500, 5FU/VIT.A1000) when compared to the control. Compared with 5-FU, feed intake significantly increased with 5-FU/VIT.A 500 and 5-FU/VIT.A1000.

3.3 Effect of Vitamin A on Biochemical parameters

Table 1 shows the effects of vitamin A on various inflammatory and oxidative stress biomarkers in 5-fluorouracil-treated rats. Results of interleukin -10 showed a significant decrease ($p<0.005$) in with VIT.A 500, VIT.A 1000, 5FU, 5-FU/VIT A500 and 5FU/VIT.A 1000 compared to control. Compared to 5-FU, IL-10 levels increased significantly with 5-FU/VIT A500 and 5FU/VIT.A 1000 respectively. Similarly, TNF-α levels, decreased significantly with VIT.A500 and increased with 5FU compared to control. Compared to 5FU, TNF-α levels decreased significantly with 5-FU/VIT A500 and 5FU/VIT.A 1000 respectively. Lipid peroxidation measured as MDA levels, increased significantly with 5-FU compared to the control, however, compared to 5FU, MDA levels decreased with 5-FU/VIT A500 and 5FU/VIT.A1000 respectively. Total antioxidant capacity (TAC) significantly increased in the VIT A 500 and 5fu /VIT.A 1000 and decreased with 5FU compared to the control, while compared to 5FU, TAC levels increased significantly with 5-FU/VIT A500 and 5FU/VIT.A1000 respectively.

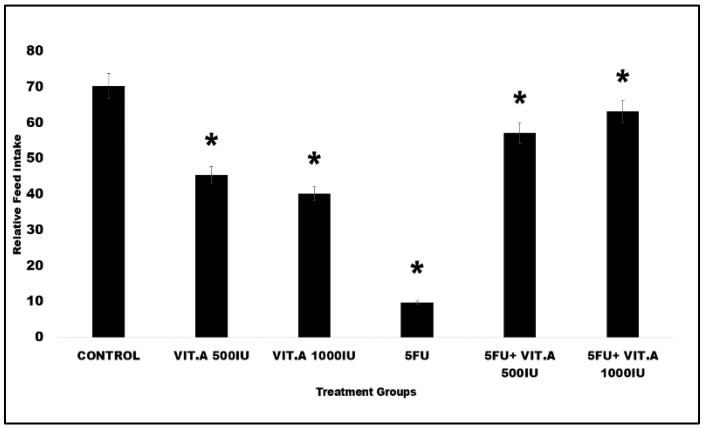

Figure 2: Effect of Vitamin A on feed intake in 5-fluorouracil-induced subfertility Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. 5p<0.05 significant difference from control, VIT.A: Vitamin A, 5-FU :5-Fluorouracil.

Table 1: Effects of vitamin A on biochemical assays in rats treated with 5-fluorouracil.

Groups	IL-10	TNF-α	MDA	TAC
Control	7.47±0.05	7.73±0.64	0.97±0.13	14.67±0.72
VIT.A 500IU	6.18±0.05*	5.79±0.15*	0.84±0.02	15.52±0.5
VIT.A 1000IU	6.47±0.01*	6.55±0.74	0.96±0.02	19.92±0.17*
5FU	2.72±0.37*	17.34±1.48*	2.01±0.03*	6.5±0.31*
5FU+VIT.A500IU	6.72±0.72*#	6.79±0.42#	1.01±0.09#	15.87±0.01#
5FU+ VIT.A1000 IU	6.26±0.05*#	6.78±0.25#	0.85±0.02#	19.22±2.13*#

Data are represented as mean ± S.E.M., *p<0.05 significant difference from control. #p<0.005 significant difference from 5FU. Numbers of rats per treatment group=10. 5FU: 5-Fluorouracil, VIT.A: Vitamin A. TNF-α and Interleukin-10 levels measured in pg/ml, Malondialdehyde measured in µM, Total antioxidant capacity measured in (mM Trolox equivalent.

3.4 Effect of Vitamin A on Sperm Analyses

Figure 3, 4, 5, 6, 7 and 8 shows the effects of vitamin A on different sperm parameters. Total sperm count decreased significantly ($p<0.05$) with VIT A.1000, 5-FU, 5-FU/VITA.500 and 5FU/VITA.1000 compared to control. Compared to 5FU, total sperm count increased significantly with 5-FU/VITA.500 and 5FU/VITA.1000. Percentage normal sperm decreased significantly with 5-FU, 5-FU/VITA.500 and 5FU/VITA.1000 compared to control. compared to 5FU, normal sperm increased with 5-FU/VITA.500 and 5FU/VITA.1000. Percentage of sperms with rapid progressive motility decreased significantly ($p<0.05$) with VIT A.1000, 5-FU, 5-FU/VITA.500 and 5FU/VITA.1000 compared to control. Compared to 5FU, rapidly progressing sperms increased significantly with 5-FU/VITA.500 and 5FU/VITA.1000. Percentage dead sperm increased significantly with 5-FU, 5-FU/VITA.500 and 5FU/VITA.1000 compared to control. compared to 5FU, dead sperm decreased with 5-FU/VITA.500 and 5FU/VITA.1000. Percentage of sperms with nonprogressive progressive motility increased significantly ($p<0.05$) with VIT A.1000, 5-FU, 5-FU/VITA.500 and 5FU/VITA.1000 compared to control. Compared to 5FU, non-progressing sperms decreased significantly with 5-FU/VITA.500 and 5FU/VITA.1000. Animals in the 5FU group had a significant number of head, tail and mid piece defects compared to control, while compared to 5FU, there was a decrease in head, tail and mid piece defect with 5-FU/VITA.500 and 5FU/VITA.1000.

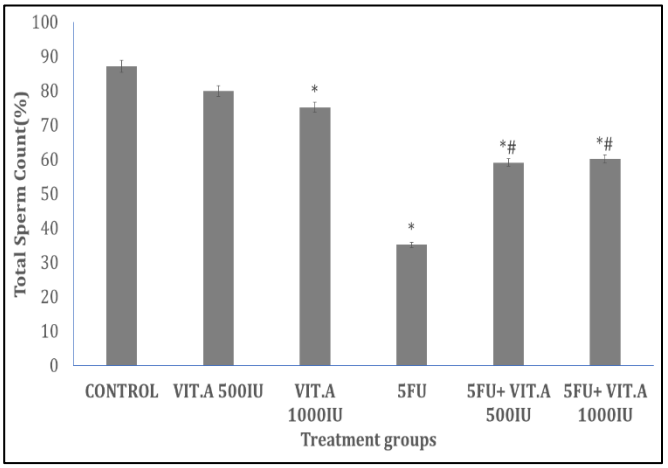

Figure 3: Effect of Vitamin A on sperm total sperm count**.** Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. 5-Fluorouracil *p<0.05 significant difference from control. VIT.A-Vitamin A, 5-FU-Fluorouracil.

3.5 Effect of Vitamin A on Testicular histomorphology

Plates 1and Plate 2 are representative photomicrographs of haematoxylin and eosin (1A-F) and Van Gieson (2 A-F) stained section of the rat testis. Examination of the testis in the control group (Plate 1A, 2A) and in the groups administered vitamin A at 500 (1B, 2B) and 1000 (1C, 2C) revealed normal seminiferous tubules lined by completely developed germinal cells. Normal Leydig cells, were also

observed in the interstitial spaces. Myoid cells, and Sertoli cells were also observed. The lumen of the seminiferous tubules contained spermatozoa at various levels of maturity; features which are all in keeping with normal architecture of the testes. In the 5FU group (1D, 2D) the seminiferous tubules showed significant structural distortion of the germ cells, Sertoli cells, with occlusion of the lumen. In the 5FU/VIT.A 500(1E, 2E) and 1000 (1F, 2F) preservation of testicular architecture was observed with numerous Sertoli cells, germ cells, myoid cells and Leydig cell. The Van Gieson-stained slides, the interstitial connective tissue appeared more prominent and distinctly stained, with collagen fibres showing a characteristic red coloration. This provided a clearer demarcation between seminiferous tubules and the surrounding stroma.

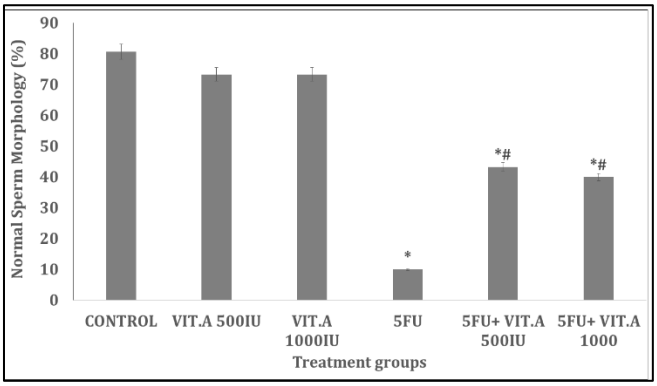

Figure 4: Effect of Vitamin A on normal sperm morphology. Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. 5-Fluorouracil *p<0.05 significant difference from control. VIT.A: Vitamin A, 5-FU :5-Fluorouracil.

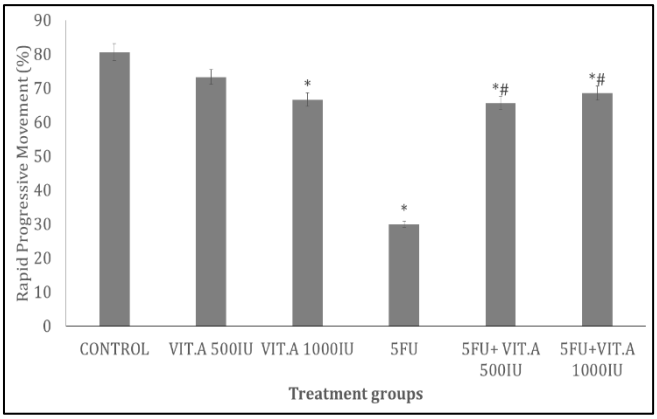

Figure 5: Effect of Vitamin A on sperm motility (Rapid progressive sperm cells)**.** Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. 5-Fluorouracil *p<0.05 significant difference from control. VIT.A: Vitamin A, 5-FU :5-Fluorouracil.

3.6 Effect of Vitamin A on Epididymal histomorphology

Plates 3 and Plate 4 are representative photomicrographs of haematoxylin and eosin (3A-F) and Van Gieson (4A-F) stained section of the rat epididymis. Photomicrographs of rats in the control group (3A, 4A), and in the groups administered vitamin A at 500 (3B, 4B) and 1000 (3C, 4C) revealed the presence of the basal cells, stereocilia, spermatozoa and a well-shaped basement membrane; features which are in keeping with normal architecture of the epididymis. In the 5-FU group (3D, 4D) showed damage to the epididymis, disrupting basal cells, stereocilia, and sperm presence when compared to control. In the 5FU/VIT.A 500(3E, 4E) and 1000(3F, 4F) significant improvement in stereocilia and basal cell morphology was observed. Sperm were more apparent.

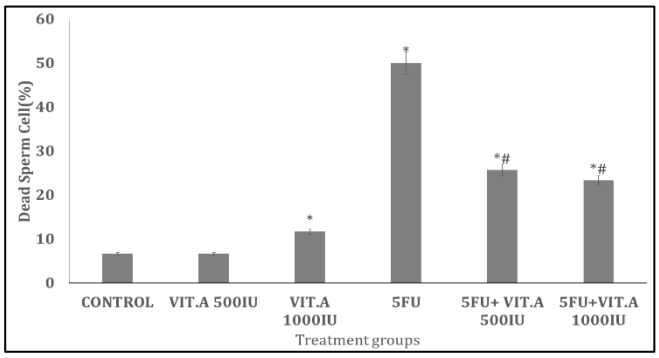

Figure 6: Effect of Vitamin A on sperm morphology (Dead sperm cells)**.** Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. 5-Fluorouracil *p<0.05 significant difference from control. VIT.A: Vitamin A, 5-FU :5-Fluorouracil.

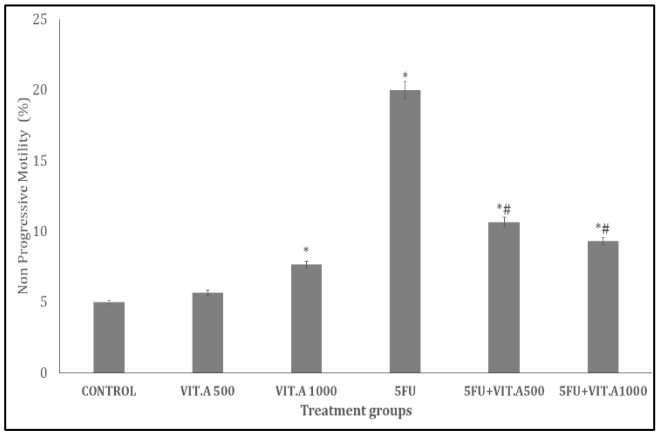

Figure 7: Effect of Vitamin A on sperm motility (Non progressive sperm cells)**.** Each bar represents Mean ± S.E.M, number of rats per treatment group = 10. 5-Fluorouracil *p<0.05 significant difference from control. VIT.A: Vitamin A, 5-FU :5-Fluorouracil.

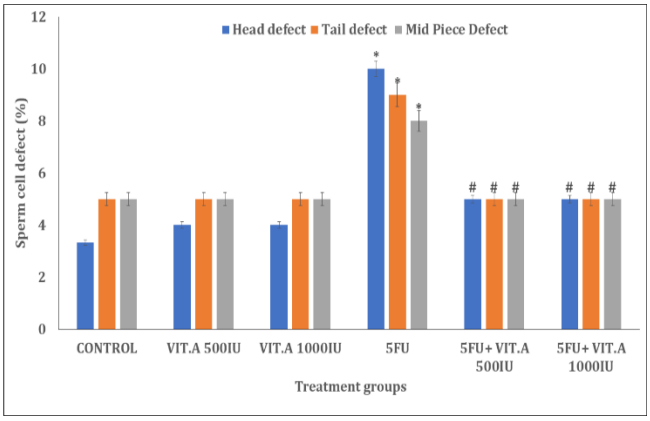

Figure 8: Effect of Vitamin A on % sperm with structural defects (**.** Each bar represents Mean ± S.E.M, number of rats per treatment

group = 10. 5-Fluorouracil *p<0.05 significant difference from control. VIT.A: Vitamin A, 5-FU :5-Fluorouracil.

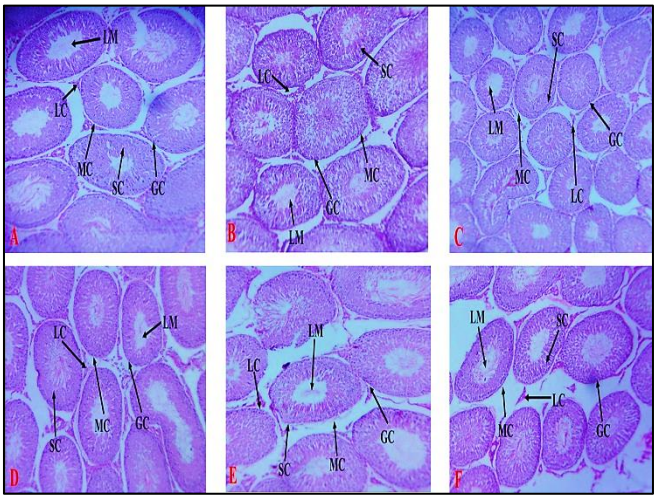

Plate 1: Effect of Vitamin A on histomorphology of the testis of control (A), VIT. A 500IU (B), VIT.A 1000IU (C). 5-fluorouracil (D), VIT.A (500,1000IU)/5-FU (E and F). LC: Leydig cells, MC: myoid cells, SC: Sertoli cells, GC: germ cell, LM: lumen. (Magnification,100x), Haematoxylin and Eosin stain.

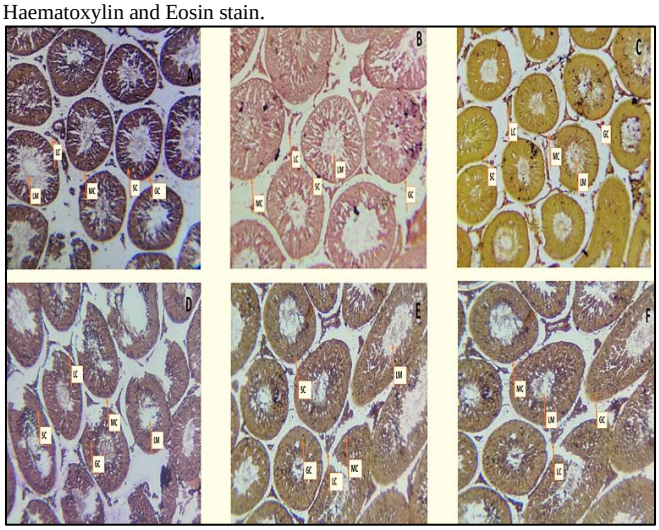

Plate 2: Effect of Vitamin A on histomorphology of the testis control (A), Vitamin A 500IU (B), VIT.A 1000IU (C). 5-fluorouracil (D), VIT.A (500,1000IU)/5-FU (E and F). LC: Leydig cells, MC: myoid cells, SC: Sertoli cells, GC: germ cell, LM: lumen. (Magnification,100x), Van Gieson's stain.

4. Discussion

Chemotherapeutic agents like 5-Fluorouracil (5-FU), while indispensable in cancer management, are often associated with unintended systemic toxicity resulting in subfertility or infertility [8, 27]. This is primarily attributed to oxidative stress and cytotoxic effects on germ cells [8 25, 27]. Studies have shown that 5-FU generates excessive reactive oxygen species (ROS), leading to lipid peroxidation, DNA damage, and apoptosis in spermatogenic cells, thus impairing male fertility [4, 5]. Oxidative stress disrupts the testicular microenvironment, reducing sperm count, motility, and morphology, as observed in animal models [9, 27] In this study, subfertility was induced in rat models using 5-Fluorouracil (5-FU).

Vitamin A supplementation has been identified as a potential therapeutic intervention for male infertility, particularly in oxidative stress conditions. Vitamin A plays a critical role in spermatogenesis, functioning as an antioxidant that neutralises ROS and maintains Sertoli cell, essential for germ cell differentiation [28, 29]. Vitamin A 's ability in improving sperm parameters, reducing lipid peroxidation, and enhancing antioxidant defence status has been previously reported [30].

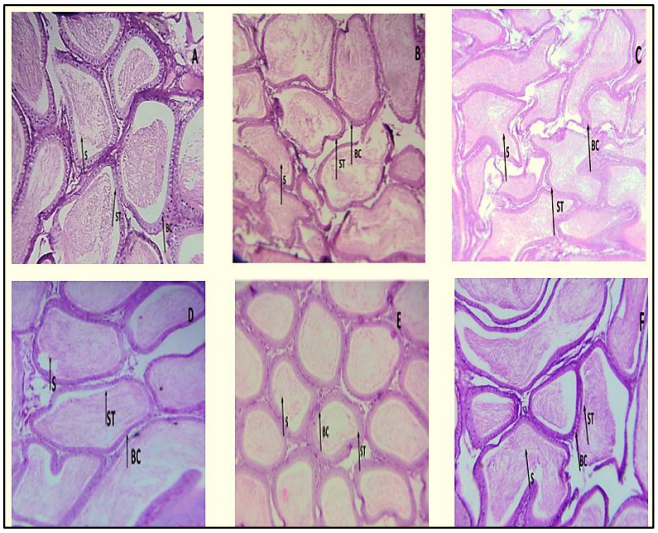

Plate 3: Effect of Vitamin A on histomorphology of the Epididymis control (A), Vitamin A 500IU (B), VIT.A 1000IU (C). 5-fluorouracil (D), VIT.A (500,1000IU)/5-FU (E and F). Leydig cells (LC), Basal cells (BC), Stereocilia (ST), Spermatozoa (S). (Magnification,100x), Haematoxylin and Eosin stain.

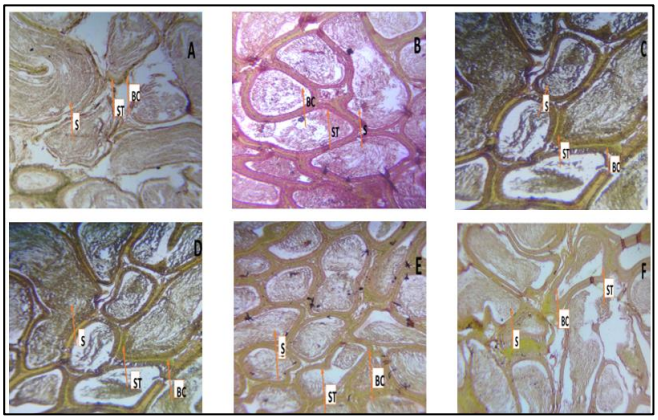

Plate 4: Effect of Vitamin A on histomorphology of the Epididymis. control (A), Vitamin A 500IU (B), VIT.A 1000IU (C). 5-fluorouracil (D), VIT.A (500,1000IU)/5-FU (E and F). Leydig cells (LC), Basal cells (BC), Stereocilia (ST), Spermatozoa (S). (Magnification,100x), Van Gieson's stain.

In this present study, there was a significant reduction in body weight and food intake in the 5-FU-treated group compared with control. This aligns with reports of chemotherapy-induced metabolic dysregulation and appetite suppression [31]. These effects are primarily attributable to the cytotoxicity and gastrointestinal toxicity caused by 5-FU

[32], which disrupt nutrient absorption and energy metabolism. The ability of Vitamin A to counteract these effects, as observed suggests it has a role in stabilising metabolic functions. Vitamin A's influence on appetite and weight maintenance it's linked to its antioxidant properties, which reduce systemic inflammation and oxidative damage, thereby promoting better physiological homeostasis [33].

Among the critical findings of this study were the significant reduction in TAC and the elevation of MDA levels with 5-FU. These changes underscore the oxidative stress induced by 5-FU, which has been well-documented in chemotherapy models [31]. Elevated MDA levels are indicative of lipid membrane damage, a hallmark of oxidative injury. The enhancement of TAC and the reduction of MDA levels in the Vitamin A-treated groups highlight its potent antioxidant activity. Vitamin A antioxidative mechanism is mediated through its ability to scavenge free radicals and upregulate endogenous antioxidant enzymes such as superoxide dismutase and glutathione peroxidase. This is consistent with findings by [17], which demonstrated that retinoids improve oxidative balance in models of drug-induced toxicity. The more pronounced effects observed with the 1000 IU/kg dose of Vitamin A suggest a dose-dependent enhancement of antioxidant defence systems.

The cytokine profile in this study provides insights into the inflammatory milieu associated with 5-FU and Vitamin A. The reduction in IL-10 levels and the elevation of TNF-α in the 5-FU group reflect a pro-inflammatory state, consistent with prior findings [32]. Tumour necrosis factor-α is a key mediator of inflammation that exacerbates oxidative damage, while IL-10 serves as an anti-inflammatory cytokine that modulates immune responses.

Vitamin A supplementation restored IL-10 levels and moderated TNF-α expression, with the 1000 IU/kg dose showing superior effects. These results align with studies by [33], which suggested that retinoids exert immunomodulatory effects by enhancing anti-inflammatory cytokine production and suppressing pro-inflammatory pathways. The combined treatment groups (E and F) further underscore the effect of Vitamin A in counteracting 5-FU-induced immune dysregulation.

The adverse effects of 5-FU on sperm parameters, including reduced count, motility, and abnormal morphology, are indicative of direct gonadotoxicity. These findings are consistent with the literature, where 5-FU has been shown to induce apoptosis in germ cells and disrupt spermatogenesis through oxidative and inflammatory mechanisms [5]. Vitamin A supplementation mitigated these effects, with the 1000 IU/kg dose demonstrating the most significant effects. Enhanced sperm motility and morphology in the Vitamin A groups suggest a protective effect on the structural integrity and functionality of spermatozoa. This could be attributed to Vitamin A's role in maintaining Sertoli cell function and its involvement in the differentiation of spermatogonia, as reported by [20].

Histological analysis of the testes further corroborated these findings, revealing less pronounced degeneration in the Vitamin A-treated groups compared to the 5-FU group. Vitamin A's ability to preserve testicular architecture aligns with its established role in supporting germinal epithelium integrity and reducing testicular oxidative damage.

Conclusion

This study shows that Vitamin A has a remarkable ability to counteract the toxic effects of 5-FU. Improvements in body weight, food intake, TAC, cytokine balance, and sperm parameters suggest Vitamin A's antioxidative and anti-inflammatory properties and its ability to restore physiological balance disrupted by chemotherapy. However, human studies are necessary to validate the efficacy and safety of Vitamin A supplementation in preventing subfertility in cancer patients.

Funding.

None received

Declaration of Ethics approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences LAUTECH with identification number ERC/FBMS/055/2024

Competing interests

The authors affirm that they have no known conflict of interest that would have seemed to affect the work reported in this current study.

Received 5th May, 2025; Revised 11th May, 2025; Accepted 20th May, 2025, **Published online: 25/05/2025**

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Cite as: Oladipupo BM, Ajao JA, Akinsehinwa AF, Famuyide OO, Onaolapo AY, Onaolapo OJ. An Assessment of the Effects of Vitamin A on Biochemical Indices, Sperm Parameters and Testicular Morphology in 5-Fluorouracil-treated Male Rats Acta Bioscientia **2025**:1(3);153-160 10.71181/actabioscientia12300