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# COMPARATIVE EFFECT OF VITAMINS A AND E ON SPERM MORPHOLOGY OF NICOTINE-TREATED WISTAR RATS

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## ABSTRACT

This study compared the effect of vitamins A and E on sperm morphology of nicotine-treated rats. Thirty male wistar rats were randomly assigned into six groups (n=5) thus: control received 0.2mL/kg of normal saline; Nicotine (N) group received 1mg/kg of nicotine; Vit A group received 1µg/kg of vitamin A; Vit E group received 50mg/kg of vitamin E; N+Vit A group received both nicotine and vitamin A and N+Vit E group received both nicotine and vitamin E. All groups had access to rat feed and water throughout the study period (3 weeks treatment period and 1 week acclimatization period). Semen were collected for evaluation of sperm morphology. Total sperm defect was significantly increased ($p<0.05$) in Nicotine group compared with control and decreased ($p<0.05$) in Vit A, Vit E, N+Vit A and N+Vit E groups compared with Nicotine group. Sperm head defect was significantly ($p<0.05$) decreased in Vit E and N+Vit E groups compared with Nicotine group. Sperm tail defect was significantly increased ($p<0.05$) in Nicotine and N+Vit A groups compared with control and decreased ($p<0.05$) in Vit A and Vit E groups compared with Nicotine group. Normal sperm morphology was significantly decreased ($p<0.05$) in Nicotine group compared with control and increased ($p<0.05$) in other treatment groups compared with Nicotine group. Nicotine thus exhibited deleterious effect on sperm morphology but both vitamins A and E were effective in protecting sperm cells from nicotine-induced damage. Vitamin E exhibited a slightly greater protective effect than vitamin A.

**KEYWORDS:** *Morphology; Nicotine; Sperm; Vitamin A; Vitamin E.*

## INTRODUCTION

Infertility is the inability to achieve pregnancy after a year of unprotected intercourse. More than seventy million people suffer from infertility worldwide.[1] Globally, male factors account for at least 50% of all infertility cases.[2] Cigarette smoking is one of the factors that contribute to male infertility.[3,4] Tobacco smoke contains nicotine, carbon monoxide, carcinogens and irritant substances[5] and nicotine is considered the main chemical in tobacco that is responsible for engendering tobacco use and dependence.[6] Studies have shown that nicotine is detrimental to the male reproductive system[7,9] and these deleterious effects of nicotine have been linked to oxidative stress.[10,12]

Vitamin E (α tocopherol) is a powerful lipid-soluble antioxidant and its antioxidant effect has been widely reported including its protective effect on the testis. Vitamin E has been reported to protect the testis from oxidative damage caused by nicotine[7,9] and other toxic substances like cadmium and lead acetate.[13,14]

Vitamin A (retinol) is another antioxidant that is used therapeutically in cases of dermatological disturbances, immunodeficiency, weight gain of preterm infants and leukemia[15,16] and is required for normal functioning of the immune system[17] and normal vision.[18] Very few studies have investigated the effects of vitamin A on sperm parameters.[19] Therefore, the present study sought to investigate the effect of vitamin A on sperm morphology of nicotine-treated male wistar rats since sperm morphology is one of the key indices of male fertility[20] and compare this effect with that of vitamin E on sperm morphology of male wistar rats also treated with nicotine.

## MATERIALS AND METHODS

### Experimental Animals

Thirty (30) male wistar rats weighing 150-200g were employed in the study. The rats were bought from Department of Agriculture, University of Calabar, Nigeria and handled in line with standard principles for handling of animals[21] after gaining approval of the research work from the Ethics Committee of Faculty of Basic Medical Sciences, University of Calabar. They were kept in well ventilated plastic cages in the animal house of Physiology Department, University of Calabar for the entire duration (3 weeks) of the study. They were first allowed to acclimatize for seven days before the 3 weeks treatment period. The animals were given rat feed and water *ad libitum* and exposed to 12/12 hours light/dark cycle.

**Purchase of Drugs**

Nicotine and vitamins A and E of Sigma-Aldrich, St. Louis, MO, USA were purchased from Bez Pharmacy, Calabar, Nigeria.

**Experimental Design and Drug Administration**

The rats were randomly assigned into six (6) groups of five (5) animals each thus: Control group - received 0.2mL/kg of normal saline; Nicotine (N) group – received nicotine (1mg/kg)[8]; Vitamin A (Vit A) group – received vitamin A (1µg/kg); Vitamin E (Vit E) group – received vitamin E (50mg/kg); N+Vit A group – received both nicotine and vitamin A (same doses as received separately) and N+Vit E group received both nicotine and vitamin E. All the groups had access to rat feed and water throughout the study period and drug administration was done daily via oral route.

**Semen Collection and Determination of Sperm Morphology**

The left testis and epididymis were removed with the caudal part of the epididymis separated from the testis and lacerated to collect semen on a microscope slide for evaluation of semen characteristics as described by Raji *et al.*[22] Sperm morphology was evaluated by staining the sperm smears on the microscope slides with two drops of Walls and Ewas stain. This was then air-dried and the slides examined under a microscope using x100 objectives under oil immersion. The abnormal sperm cells were then counted and the percentage was computed by the method described by Wyrobek and Bruce.[23]

**Statistical Analysis**

Results are presented as mean ± standard error of mean (SEM). Data were analyzed using Statistical Package for Social Sciences (Version 21). Statistical measure used was one way analysis of variance (ANOVA) along with post hoc multiple comparison test (least square difference). $p<0.05$ was the criterion for statistical significance.

**RESULTS**

Table 1 shows the sperm morphology in the different experimental groups. Total sperm defect was significantly ($p<0.05$) increased in Nicotine group compared with control. It was however significantly ($p<0.05$) decreased in Vit A, Vit E, N+Vit A and N+Vit E groups compared with Nicotine group. Sperm head defect was significantly ($p<0.05$) decreased in Vit E and N+Vit E groups compared with Nicotine group. Sperm tail defect was significantly ($p<0.05$) increased in Nicotine and N+Vit A groups compared with control. It was

significantly ($p<0.05$) decreased in Vit A and Vit E groups compared with Nicotine group. Sperm tail defect was significantly ($p<0.05$) increased in N+Vit A group compared with Vit A and Vit E groups. Middle piece defect was significantly ($p<0.05$) increased in Nicotine group compared with control. However, it was significantly ($p<0.05$) decreased in Vit A, Vit E, N+Vit A and N+Vit E groups compared with Nicotine group. Percentage normal sperm was significantly ($p<0.05$) decreased in Nicotine group compared with control and increased ($p<0.05$) in Vit A, Vit E, N+Vit A and N+Vit E groups compared with Nicotine group.

**Table 1: Comparison of sperm morphology in the different experimental groups.**

| Parameter | Control | Nicotine | Vit A | Vit E | N+Vit A | N+Vit E |
|---|---|---|---|---|---|---|
| **Total defect (%)** | 2.00±0.58 | 7.00±1.22* | 2.75±0.75a | 1.50±0.50a | 3.75±0.48a | 3.50±0.65a |
| **Head defect (%)** | 1.00±0.00 | 2.00±0.71 | 1.00±0.41 | 0.00±0.00a | 1.25±0.48 | 0.25±0.25a |
| **Tail defect (%)** | 1.50±0.29 | 3.25±0.85* | 1.50±0.29a | 1.50±0.50a | 3.25±0.25*x,m | 2.75±0.75 |
| **Middle Piece defect (%)** | 0.00±0.00 | 1.75±0.48* | 0.50±0.29a | 0.00±0.00a | 0.00±0.00a | 0.50±0.50a |
| **Normal Sperm (%)** | 98.00±0.58 | 93.00±1.22* | 96.50±0.87a | 98.25±0.63a | 96.00±0.58a | 96.50±0.65a |

*Values are expressed as mean ± SEM, n = 5.*

**p<0.05 vs control; a = p<0.05 vs Nicotine; x = p<0.05 vs Vit A; m = p<0.05 vs Vit E*

## DISCUSSION

Nicotine is the main component of tobacco smoke and has been reported to impact negatively on sperm parameters via production of reactive oxygen species. Vitamins A and E are antioxidants and have demonstrated several beneficial effects on body systems and organs. This study investigated and compared the effect of vitamins A and E on sperm morphology of nicotine-treated male wistar rats.

Results from the present study show that nicotine greatly impacted negatively on sperm morphology at all levels as the head, middle piece and tail of the spermatozoa were defective following nicotine treatment. Sperm morphology is one of the key indices of male fertility as it is a prime marker of testicular spermatogenesis.[20] The percentage of normal sperm was significantly decreased in the Nicotine group compared with control implying that consumption of nicotine poses threat to male fertility. This is consistent with Oyeyipo *et al.*[8] and Mahanem *et al.*[9] who reported that administration of nicotine significantly reduced the percentage of sperm with normal morphology in rats. Nicotine had also been previously reported to significantly reduce sperm count, sperm motility, sperm viability and normal sperm cells.[7] The significant decrease in sperm morphology associated with nicotine intake may be due to impairment of spermatogenesis which may be a consequence of reduction in

testosterone secretion caused by nicotine. Nicotine has been previously reported to decrease testosterone secretion.[24] High levels of testosterone is essential for normal spermatogenesis and maintenance of sperm morphology.[25] This negative effect of nicotine is probably due to the action of nicotine in causing oxidative stress.[11,12]

However, both vitamins A and E were effective in protecting sperm from damage associated with nicotine administration. Percentage of normal sperm was significantly increased in Vit A, Vit E, N+Vit A and N+Vit E groups compared with Nicotine group. Except tail defect in N+Vit A group, total defect and the defects at the head, middle piece and tail were reduced in Vit A, Vit E, N+Vit A and N+Vit E groups compared with Nicotine group. Our result is consistent with previous report which shows that vitamin E alleviated the reduction in sperm morphology caused by nicotine and improved testosterone level in nicotine treated rats.[7] Mahanem *et al*[9] also reported that vitamin E improved sperm morphology in nicotine-treated rats. This protective role of vitamin E may be due to its androgenic activity[7] or its ability to mop up reactive oxygen species and prevent lipid peroxidation of the sperm cells.[26] These may also be the possible mechanisms by which vitamin A protects sperm cells from nicotine-induced damage. From the results obtained, vitamin E seems to be a little more effective in protecting sperm from nicotine-induced damage. This is thought to be so because as observed in the N+Vit E group, total sperm defect and defects at the head, middle piece and tail decreased although not significant compared with N+Vit A group. This is also the case when Vit A and Vit E groups are compared. Population of normal sperm also increased although non-significant in Vit E group compared with Vit A group.

## CONCLUSION

This study shows that nicotine exhibited deleterious effect on sperm morphology in rats and both vitamins A and E were effective in protecting sperm cells from reproductive damage caused by nicotine. The effect of vitamin E was a bit greater than that of vitamin A. Smokers and other people consuming nicotine are therefore encouraged to use vitamin A or vitamin E to reduce their chances of developing fertility problems.

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