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VITAMIN C PROTECTS SPERMATIC AND TESTICULAR HISTOPATHOLOGICAL CHANGES BY ETHANOL IN WISTAR RATS

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ABSTRACT

Alcohol is a known psychoactive compound and its consumption has been associated with several adverse effects, including testicular toxicity. Vitamin C is a potent antioxidant. We investigated the protective effect of vitamin C against alterations in sperm parameters and testis histology by ethanol in Wistar rats. The rats were divided into five equal groups: group 1, control (given 0.5 ml/kg distilled water), group 2 (given 1 g/kg ethanol, 30%v/v), group 3 (given 2 g/kg ethanol, 30%v/v), group 4 (given vitamin C and 1 g/kg ethanol), and group 5 (given vitamin C and 2 g/kg ethanol). Administrations were done by gastric gavage and lasted for 21 days. Ethanol treatment resulted in reduction of sperm motility ($p < 0.001$) and count ($p < 0.01$), while percentage of sperm with abnormal morphology was increased dose-dependently ($p < 0.05$,$p < 0.01$) compared to control. Vitamin C treatment inhibited these effects of ethanol, as sperm motility and count were increased, and abnormal morphology was decreased in vitamin C treated groups when compared to those that received only ethanol. Testes of ethanol treated rats showed altered histology, while those of vitamin C + ethanol treated animals showed improved or normal histology. Results indicate that vitamin C is capable of protecting ethanol induced impairments in sperm parameters and testis histology in Wistar rats.

KEYWORDS: Alcohol, Vitamin C, Antioxidant, Sperm parameters, Testicular function.

INTRODUCTION

Alcohol (ethanol) is a psychoactive substance, and is widely and commonly used for recreational purposes. Alcohol affects the central nervous system, causing mood changes and other behavioral effects, including euphoria, reduced anxiety, elevated sociability, sedation, impaired cognition and memory, and generalized depression of central nervous system function.[1,2] It is also considered as a drug of addiction which can result in dependence and withdrawal symptoms.[3] Functionally, alcohol primarily enhances gamma aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, resulting in suppression of the central nervous system, but can also affect the actions of other neurotransmitter systems such as glutamate, glycine, acetylcholine, dopamine and serotonin in brain.[4] Alcohol ingestion produces increased levels of dopamine and endogenous opioids in the reward pathway of the brain, which accounts for its euphoric effect.[5] Following ingestion, ethanol is distributed rapidly throughout the body, and is metabolized mainly in the liver to acetaldehyde which is toxic.[6,7] Chronic consumption of alcohol has been reported to cause liver damage,[8] brain damage, and increased risk of cancer.[9] Studies have also shown that alcohol consumption can cause reduction of testosterone, alteration of spermatogenesis and testicular function including, the hypothalamic-pituitary-testicular axis function.[10,11]

Vitamin C (ascorbic acid), which was discovered since 1912 and isolated in 1928, is a water soluble vitamin with potent antioxidant properties. Present naturally in some food, vitamin C is routinely added in dietary supplements and widely used in human health care for prevention of diseases (e.g., scurvy) and maintenance of tissue functions, including collagen synthesis and bone.[12,13] Vitamin C is available over the counter and is used widely by many. As an antioxidant, vitamin C inhibits the oxidation of cell molecules. Oxidation is a chemical process that generates free radicals (unpaired electrons that are highly reactive) leading to a chain of reactions that damage cells.[14,15] Vitamin C interferes with this reaction by donating electron to the unpaired radical to stabilize it and prevent or terminate the free radical-mediated chain reaction.[16,17] In the process, vitamin C loses an electron and is oxidized forming a radical (ascorbyl radical), which is very stable and less likely to attract electrons from other molecules in its environment.[15,16] Generally, while lipid soluble antioxidants protect cell membrane from lipid peroxidation, water soluble antioxidants like vitamin C reacts with oxidants in the cell cytosol and the blood plasma.[15,18]

Increased release of highly reactive oxidative species in biological systems by compounds readily interferes with cell function and chronic exposure to such compounds leads to cell function depletion and death. Some alcohols like methanol and isopropyl alcohol are very toxic and are not consumed, but ethanol is added in several food products and drinks including, beverages, beer, wine and distilled spirits.[19] Thus, increased human alcohol consumption and its potential to induce oxidative stress and testicular toxicity remains a major concern. The present work was intended to investigate the preventive role of vitamin C in ethanol induced alterations in sperm parameters and histology of the testis in Wistar rats.

MATERIALS AND METHODS

Materials

Ascorbic acid, 99.8% (Kermel Ltd., China), formalin (May & Baker Ltd., England), ethanol (Guanhua Sci. Techco., China), normal saline (Agary Pharmaceutical Ltd., Nigeria), Eosin & Hematoxylin (Organo Biotech Laboratories Private Ltd., India).

Methods

Experimental animals

A total of 25 adult male Wistar rats weighing 180-220 g were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, Niger Delta University, Nigeria. They were housed five per cage avoiding overcrowding, and fed with standard rat feeds and tap water was given *ad libitum* under natural lighting condition with proper ventilation. Study protocol was approved by the local Niger Delta University Research Ethics Committee and animal handling and care was in accordance with the International Guidelines for Animal Care.[20]

Experimental design

The rats were divided into five equal groups. Group I (control group) animals were given 0.5 ml/kg distilled water once a day for 21 days. Group II animals were given 1 g/kg ethanol (30%v/v) once daily for 21 days. Group III animals were given 2 g/kg ethanol (30%v/v) once daily for 21 days. Group IV animals received 1.25 mg/kg vitamin C plus 1 g/kg ethanol (30%v/v) once daily for 21 days. Group V animals received 1.25 mg/kg vitamin C plus 2 g/kg ethanol (30%v/v) once daily for 21 days. Vitamin C was administered 30 min before ethanol, and all agents were administered by gastric gavage. At the end of the administrations, the animals were anesthetized with ether and sacrificed by cervical dislocation. The testis and epididymis were removed and sperm was expressed from the

epididymis by maceration for sperm analysis, while testis was fixed in 10% formalin and processed for histological analysis.

Sperm analysis

For the evaluation of sperm motility, one drop of liquefied and properly mixed sperm was placed on a clean slide, covered with a clean slip, and then examined microscopically to determine sperm motility, count and morphology. Sperm motility was determined by counting both motile and non-motile spermatozoa in not less than 10 randomly selected fields under the microscope using 400× magnification. The number of rapid progressively motile, slow progressively motile, and nonmotile spermatozoa were estimated. Sperm count was performed with the Neubauer counting chamber (hemocytometer). The Neubauer counting chamber was prepared, charged with diluted sperm and allowed to stand in a moist chamber for 20 min. The specimen was then viewed under the microscope and sperm cells were counted using 400× magnification. Sperm morphology was analyzed by preparing a smear with a drop of well mixed sperm on a clean slide and quickly fixed with a cytological fixative (95% alcohol). The smear was then stained with 5% aqueous eosin, and slide was viewed under the light microscope with a 1000× magnification. The sperm cells were categorized based on the presence of one or more abnormal features, such as tail defect, neck and middle piece defects and head defect. Findings were expressed as percentage of morphologically abnormal sperm.

Testis histology

Testis tissue was embedded in paraffin, and sectioned using a rotary microtome set at 5 μm thickness. The tissue was then stained with hematoxylin and eosin (H&E). Finally, the stained tissue was examined microscopically under a light microscope (BX-51, Olympus Corporation, Tokyo, Japan), and histological features were noted and photographed.

Statistical analysis

Data were expressed as mean±SEM. Data were analyzed using GraphPad Prism 5 Software, and comparison between experimental and control groups was performed by one way analysis of variance (ANOVA) followed by Dunnet's post-test. Values were considered significant at $p < 0.05$.

RESULTS

Sperm parameters

Number of sperm with rapid progressive motility was reduced ($p < 0.001$) in the rats that received 1 and 2 g/kg ethanol, whereas nonmotile sperms number was elevated compared to the control group (Figures 1A and 1C). The number of rapid progressively motile sperm in vitamin C + 1 g/kg ethanol group was higher compared to the group that received only 1 g/kg ethanol ($p < 0.001$), and was not significantly different from the control (Figure 1A). Number of rapid progressively motile sperm in vitamin C + 2 g/kg ethanol group was higher ($p < 0.001$) compared to the group that received only 2 g/kg ethanol, but the number was lower ($p < 0.01$) compared to the control (Figure 1A). Furthermore, the nonmotile sperms in vitamin C treated groups were lower than the groups that received only ethanol ($p < 0.01$,$p < 0.001$), and the values were not significantly different compared to control (Figure 1C). Number of sperm with slow progressive motility was not altered in all groups (Figure 1B).

Sperm count was decreased in ethanol treated groups compared to control ($p < 0.01$), but vitamin C treatment inhibited the effects of ethanol (Figure 2A). Percentage of sperm with abnormal morphology was increased in ethanol treated groups dose-dependently ($p < 0.05$,$p < 0.01$) compared to control, but vitamin C treatment also inhibited the effects of ethanol (Figure 2B).

Testis histology

The testis histology of control rats (Group I) showed normal features with normal seminiferous tubules, spermatogenic cells, and numerous spermatozoa (Figure 3A). The groups that were administered only ethanol (Groups II and III) showed altered histology characterized by numerous adipocytes, few spermatogenic cells, eroded seminiferous tubules and hemorrhage compared to control (Figures 3B and 3C). Vitamin C + 1 g/kg ethanol (Group IV) showed normal histology similar to control (Figure 3D), while vitamin C + 2 g/kg ethanol (Group V) showed mild changes compared to the control (Figure 3E).

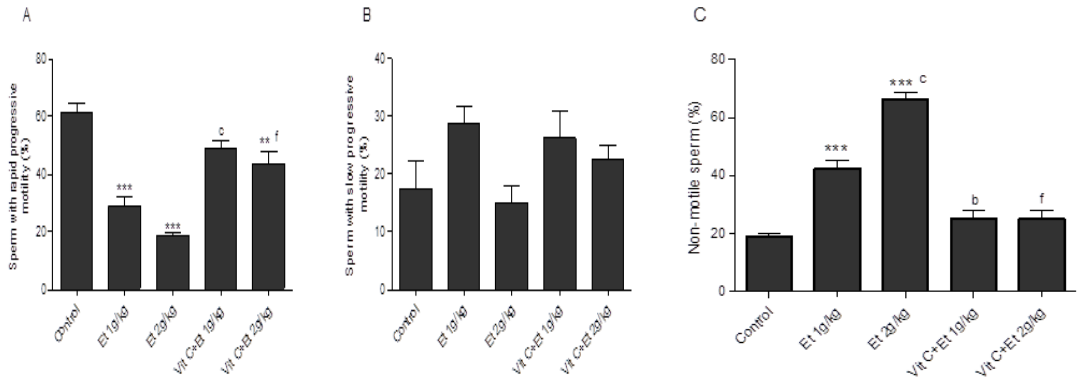

Figure 1: Vitamin C (1.25 mg/kg) pretreatment inhibits negative effects on sperm motility by ethanol in Wistar rats.

**p < 0.01, ***p < 0.001, compared to control

[b]p < 0.01, [c]p < 0.001, compared to Et 1 g/kg

[f]p < 0.001, compared to Et 2 g/kg

Vit C: vitamin C; Et: ethanol.

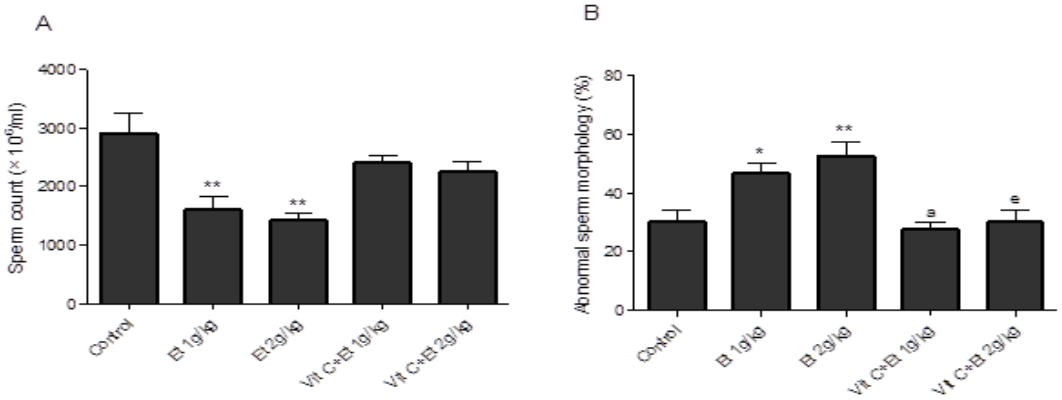

Figure 2: Vitamin C (1.25 mg/kg) pretreatment inhibits negative effects on (A) sperm count, and (B) sperm morphology by ethanol in Wistar rats.

*p < 0.05, **p < 0.01, compared to control

[a]p < 0.05, compared to Et 1 g/kg

[e]p < 0.01, compared to Et 2 g/kg

Vit C: vitamin C; Et: ethanol.

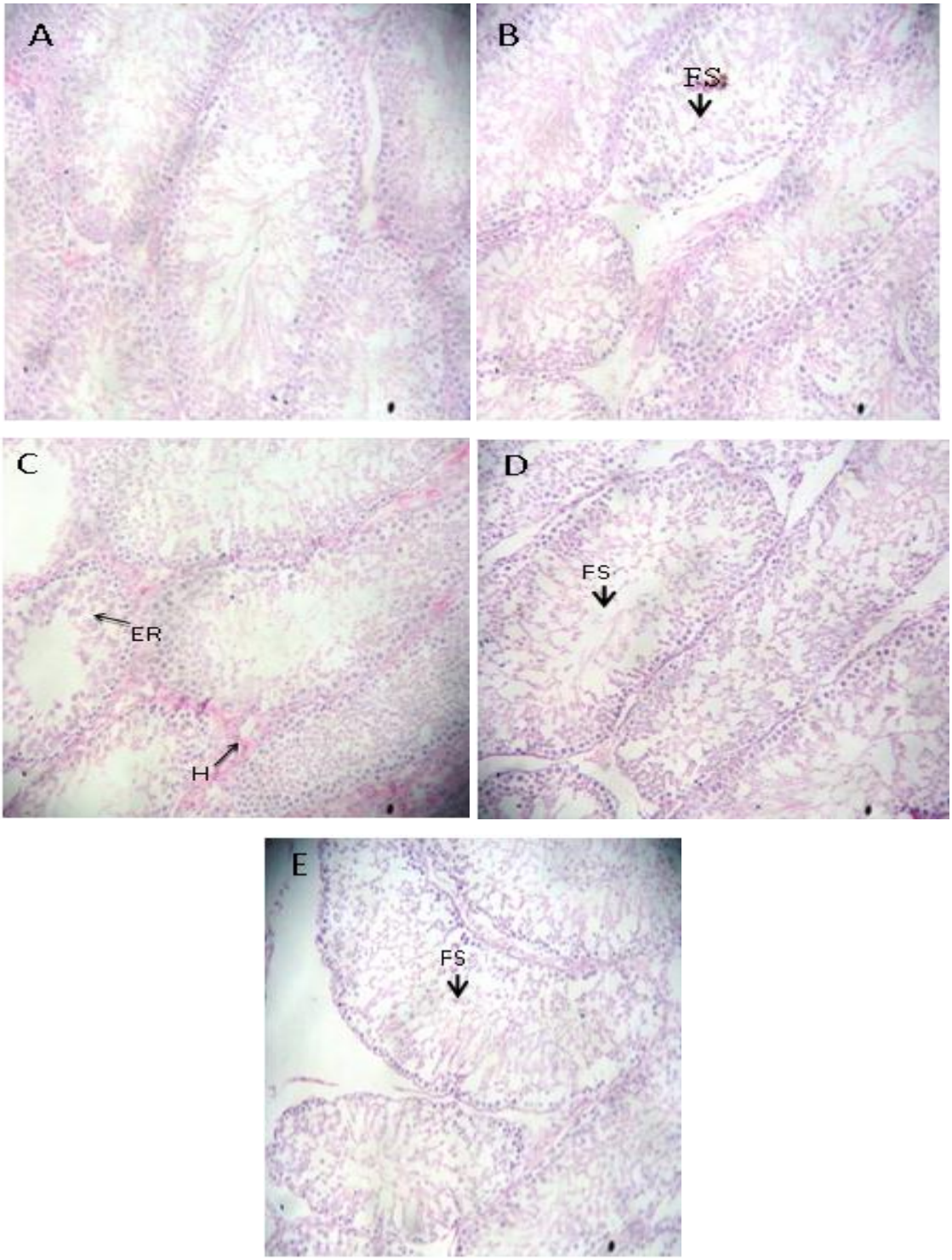

Figure 3: Photomicrographs of Wistar rats' testes after treatment with ethanol and combination of vitamin C and ethanol (H&E staining, 100×).

A- Group I (control): Normal seminiferous tubules with spermatogenic cells, and viable testicular parenchyma.

B- Group II (ethanol, 1 g/kg): Few spermatozoa (FSP)

C- Group III (ethanol, 2 g/kg): Eroded seminiferous tubule (ER) and hemorrhage (H).

D- Group IV (vitamin C, 1.25 mg/kg + ethanol, 2 g/kg): Normal histology

E- Group V (vitamin C, 1.25 mg/kg + ethanol, 2 g/kg): Few spermatozoa (FS)

DISCUSSION

The effect of vitamin C on ethanol induced alterations of sperm parameters (sperm count, motility and morphology) in rats, as well histology of the testis was studied.

From the results, oral treatment with ethanol for 21 days at 1 g/kg and 2 g/kg dose levels inhibited sperm motility by simultaneously reducing the number of active progressively motile cells and increasing the number of nonmotile cells. Ethanol treatment equally caused reduction in the number of sperm cells (sperm count), and increase in morphological abnormalities (broken neck, coiled tails, etc.) of sperm cells dose-dependently. These findings are consistent with earlier reports where alcohol has been shown to adversely alter sperm indices in animals.[21-23] Further, the adverse consequences of ethanol treatment have been shown to be via increase in oxidative stress in the testis.[21,23]

Testicular function is easily compromised by increase in lipid peroxidation or reduction of antioxidant status. Vitamin C is a potent water soluble antioxidant which reduces oxidative stress by mopping up reactive oxygen species.[15,17] This may explain the minimized effects relative to ethanol observed in the vitamin C treated rats. On sperm count, the results obtained indicated that vitamin C completely inhibited the high level of reduction that ethanol caused. The same was observed on sperm morphology. On motility, vitamin C improved the harmful effect of ethanol by increasing motility of the sperm, which was more pronounced in the animals that were administered lower dose of ethanol.

The histological examination of the testis showed varying degree of histological alterations after ethanol treatment, indicating that consumption of alcohol is capable of affecting testicular function. This is also in agreement with the findings of other authors who reported that alcohol caused testicular histopathological toxicity.[24-26] The presence of normal testicular architecture and seminiferous epithelium in the rats that received 1 g/kg ethanol concurrently with vitamin C, and mild histological alterations in the rats that received 2 g/kg ethanol concurrently with vitamin C indicates that the ethanol induced histopathological effects were inhibited. The result shows that vitamin C prevented the toxic effects of ethanol at 1 g/kg, and ameliorated the effects at 2 g/kg, which was consistent with the results of the sperm parameters.

CONCLUSION

Vitamin C is capable of protecting against testicular injury and alterations in sperm parameters by ethanol in Wistar rats.

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