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Original Article

Ameliorated Effect of Ascorbic Acid and Selenium against the Stress Effect on Sperm Quality of Rats

Baqir Al-Dhalimy, A. M[1], Alabsawy, S. K[2], Al-Mousaw, M[3], Al-Dhalemi, D. M[4] *

1. Department of Physiology and Pharmacology, College of Nursing, Altoosi University College, Najaf, Iraq
2. Department of Physiology and Pharmacology, University of Kufa, Najaf, Iraq
3. Department of Medical Laboratory Techniques, College of Medical and Health Technology, University of Alkafeel, Najaf, Iraq
4. College of Pharmacy, Islamic University, Najaf, Iraq

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Corresponding Author: aldhalimy.a@altoosi.edu.iq

Abstract

Stress is defined as physical and/or psychological modifications that disrupt homeostasis in living organisms. The stimuli that confront homeostasis are determined as stressors; these external factors may be physical, chemical, psychological, and environmental. The results of some studies have shown that ascorbic acid is related to fertility and has an evolutionary significant role as an essential nutrient for humans and other animal species. Selenium is the most important mineral element in protecting health and growth and performing various biochemical and physiological functions. Therefore, the present study aimed to determine the protective effects of vitamin C and selenium against restraint stress levels that caused a decrease in sperm quality in rats. This study was conducted on40 adult male Wistar rats that were randomly divided into 4equal groups (n=10 each). The first group (vitamin C group)was exposed to restraint stress for 6 h a day and supplemented with vitamin C (50 mg/kg bw/day) orally by gavage; the second group (Se group)was exposed to restraint stress for 6 h a day and supplemented with selenium (0.02 µg /kg bw/day) orally by gavage; the third group (negative control[NC] group)was exposed to restraint stress for 6 h a day and given normal saline (2 ml)orally by gavage; the fourth group (positive control [PC] group)was not exposed to restraint stress and given normal saline (2 ml)orally by gavage. The results showed that all the sperm parameters, such as total and progressive motility, and sperm viability increased significantly ($P \leq 0.05$) in vitamin C and Se groups, compared to the NC group. The rate of acrosome defects in vitamin C, Se, and PC groups was significantly reduced ($P \leq 0.05$), compared to the NC group. Moreover, the findings showed no significant differences among all the four groups. The results of the current study confirmed the ameliorated effect of vitamin C and selenium on semen quality and sperm parameters, such as motility, viability, morphology, and concentration, against the adverse effect of stress.

Keywords: Sperm, Infertility, Ascorbic acid, Selenium, Stress

1. Introduction

Stress is becoming an inseparable part of modern life and has been dubbed the Health Epidemic of the 21st Century (1, 2). Stress is defined as any stimulus that causes a stress response, which relies on physiological and behavioral adaptations to maintain homeostasis (3). Events that confront the organism's environment activate the central stress response system, which is mainly mediated by the Hypothalamic Pituitary Adrenal (HPA) axis (4). The regulatory functions of the HPA axis are controlling the behavior, reproduction, spermatogenesis, cardiovascular system, immune functions, and metabolic system. The activation of the HPA axis by several stressors mainly inhibits reproductive function (5). The results of several studies have shown that stress, along with depression and

anxiety, may lead to a dramatic decrease in sperm quality, and therefore, result in some degrees of infertility (6). Infertility is regarded as a public and clinical problem because it affects the health system and social life.

Ascorbic acid (Vitamin C) has been considered an essential nutrient for animal species and linked with fertility for many years. The effect of ascorbic acid on fertility is mostly considered to be related to these principal functions: its role in hormone production, promotion of collagen synthesis, and prevention or protection against oxidation (7). It acts as a cofactor for enzymes and antioxidants, such as glutathione peroxidase (8).

Selenium has been demonstrated to positively influence male reproduction. This importance is due to its role in testosterone biosynthesis, and consequently, in the typical development and formation of spermatozoa (9). To the best of our knowledge, few studies have been conducted to investigate the effect of ascorbic acid and selenium on male reproductive hormones, such as luteinizing hormone (LH), follicle-stimulating hormone, and testosterone (10, 11). The findings of several studies conducted by numerous researchers showed the strong capacity of vitamin C and selenium to reduce the stress effects in living creatures (12, 13). Therefore, the current study aimed to evaluate the protective effects of vitamin C and selenium against restraint stress stimuli causing a decrease in sperm quality in rats as an animal model.

2. Material and Methods

2.1. Animals

A total of 40 fertile adult Wistar male rats were used in the present study with an average weight of 250-350 grams. The rats were housed in clean cages kept in the animal house at the Faculty of Science, University of Kufa, Najaf, Iraq. The rats had ad-libitum access to food and water during the experiment and were maintained for about 2weeks for adaptation before starting the experiment.

2.2. Study Design

The population of this study consisted of 40 adult male lab rats that were divided randomly into 4 equal groups (n=10) as follows: 1) the first group (Vit C group) was exposed to restraint stress for a period of 6 h a day and received vitamin C in a dose of 50 mg/kg bw/day orally by gavage; 2) the second group (Se group) exposed to restraint stress for a period of 6 h a day, and received selenium in a dose of 0.02 µg/kg bw/day orally by gavage; 3) the third group (Negative Control group) was exposed to restraint stress for a period of 6 h a day and given 2 ml normal saline orally by gavage; and 4) the fourth group of animals (Positive Control group) were not exposed to restraint stress and only given 2 ml normal saline orally by gavage.

2.3. Preparation of Stressors and Stress Protocol

The rat was placed in the restraint cage used to produce restrain stress in a glass container (12×5 cm)for 6 hours a day (14). The glass container was narrow enough to prevent the rats from moving freely, however, wide enough to cause no real physical discomfort, pain, or respiratory movement impairment. The rats were exposed to stress between 08:30 AM and 2:30 PM for 20days of the experiment. During the experiment, the movements of the rats were highly restricted as they were in the restraint container; nevertheless, the negative control group was not put in the restraint container during the experiment time.

2.4. Sample Preparation

To get access to the testis and epididymis for sperm sampling and evaluations, the rats were sacrificed at different intervals from starting the experiment. Accordingly, the animals were anesthetized using intraperitoneal injection of Ketamine 90 mg/kg/b.w. and Xylazine 40 mg/kg b.w. Subsequently, the testis and epididymis were dissected from the animal's body using a sterile surgical instrument.

2.5. Epididymal Spermatozoa Sampling

The left tail epididymis was rinsed, incubated in 2 ml of normal saline at 37°C, and cut into about 200 pieces

using an anatomical micro- scissor to leak the spermatozoa from the epididymal tubules for further tests (15).

2.6. Sperms Motility Evaluation

To evaluate the percentages of the sperm's total and progressive motilities, 10µl of the sperm suspension was placed on a dry and warm slide and examined at 400x magnifications using a Computer Assisted Sperm Analysis (CASA; Genex laboratories; Florida, USA).

2.7. Sperm Concentration (SPM/Ml) Evaluation

In this stage, 10 µl of semen suspension was added to 999 µl of holding solution, resulting in the dilution factor of1:1,000. The holding solution contained normal saline 95%, formaldehyde 4%, and eosin stain 1% (16). The sperm concentration was determined using a Neubauer Hemocytometer as previously mentioned by Yokoi, Uthus (17).

2.8. Sperms Viability, Acrosomal Integrity, and Sperm Morphology Evaluation

The values of sperm viability and normal sperm morphology were examined with eosin-nigrosin (EN) dye (18).The procedure of EN staining involved the following steps: 1) a 10 µL drop of raw sperm was added to 30 µL of EN and stir for 10 sec; 2) the mixture was smeared on a dry warmed slide and left to arid on warmer slides at 45°C (19). Afterward, the slides were read under a microscope at 40× magnifications; either 200 spermatozoa or 5microscopic fields were calculated. Furthermore, in viability, the pink-stained spermatozoa were considered dead, while unstained spermatozoa were alive. Regarding the acrosome evaluation, the intact and distorted acrosomes were recorded as integrated and damaged ones.

2.9. Statistical Analysis

The statistical analysis of the experimental results was conducted in Graphpad prism (version 8) using T-test and one-way ANOVA (to assess the significance of differences between groups and within times). The data were expressed as mean ± standard errors (SE) and a p-value of<0.05 was considered statistically significant. The least significant difference test was carried out to test the significance level among the means of treatment (Prism, 2019).

3. Results and Discussion

In the present study, the effects of vitamin C and selenium supplementation were investigated against restraint stress levels causing a decrease in sperm quality among rats. The results showed that sperm motility markedly decreased in stressed rats ($P<0.001$), compared to those with no stress immobilization or restraint stress. On the other hand, the induced stress led to a significant decrease in plasma testosterone concentrations and reduced the plasma testosterone in rats resulting in depression in spermatogenesis (Table 1). These results were in agreement with those of previous studies conducted by Nowicka-Bauer and Nixon (20) and Baiee, Al-Wahab (19).

Considering the sperm concentration, the recorded data in the current study showed a significant reduction in this parameter in the stressed animals, which was similar to the results of previously published studies (16, 21) as shown in Figure 1. Based on the findings of a recent study conducted by Choudhury, Rivero (22), the generation levels of reactive oxygen species (ROS) were increased in the exposure to stress, and therefore, causes a decrease in sperm quality, viability, and acrosome integrity. In agreement with the results of the study performed by Choudhury, Rivero (22), the recorded data in the current study showed a significant decrease in sperm motility and viability and an increase in acrosome defect ($P\leq0.05$) (Table 1).

Stress is generally thought to generate ROS; when ROS exceeds the body's natural antioxidant defense, impairment to macromolecules, such as DNA, lipids, and proteins, would occur. During stress, lipid peroxidation is increased in the body and since one of the prominent products of lipidperoxidation is malondialdehyde (MDA), MDA is considered the indicator of stress-induced damage in terms of lipid peroxidation (23), which was demonstrated in our study. On the other hand, the results indicated the

ameliorated effect of the vitamin C and selenium on all groups, compared to the negative control group, which showed a significant increase in sperm parameters (i.e., general motility, progression, and viability) and a decrease in the acrosomal defect. Selenium, which accumulates in the pituitary gland and stimulates the gonadotropin-releasing hormone receptor, has been suggested to increase the production of LH (24), which in turn, stimulates the production of testosterone from Leydig cells that are necessary for normal sperm formation (19, 25) (Table 1). Vitamin C and selenium, as essential trace elements, are effective as antioxidants (26) and prevent oxidative damage; these compounds can also hinderoxidative stress, and consequently, the regulation of defective bodies (27). The results of the current study showed a highly significant increase in sperm parameters and concentration. The results of a previously published research demonstrated the positive effects of vitamin C and selenium in decreasing the ROS in testis and the adverse effect of stress on testes and sperm parameters quality (28). Figure 1 illustrated the effect of vitamin C and selenium on sperm production between different groups of the experiment. It shows the positive effect of vitamin C and selenium on an increase in sperm concentration due to a decrease in the amount of ROS raised from the stress effect loaded on rats (29). The findings of previous studies indicated that stress decreased the activity of spermatogenesis (30). The results in Figure 1 showed the ameliorated effect of vitamin C and selenium against the stress effect, which increased the sperm concentration significantly in vitamin C and selenium groups, compared to the control and negative groups. Alahmar (31) reported the positive effect of vitamin C and selenium on sperm quality and concentration, which was in line with the results of the current study.

The results revealed the ameliorated effect of ascorbic acid and selenium on the sperm parameters, namely motility, viability, morphology, and concentration, against the adverse effect of stress. It is concluded that ascorbic acid and selenium play a productive antioxidant role against the harmful effect of stress on tastes, and therefore, sperm parameters and fertility.

Table 1. Effect of vitamin C and selenium on sperm parameters

Groups	Parameters				
	Total motility	**Progressive motility**	**Viability**	**Acrosome defect**	**Morphology integrity**
Vitamin C	88.00±1.53 [a]	83.67±0.67 [a]	85.33±0.60 [a]	0.83±0.17 [a]	95.17±1.01 [a]
Selenium	83.33±1.67 [a]	78.33±1.67 [a]	81.00±2.65 [ac]	1.23±0.17 [a]	95.50±0.50 [a]
Negative control group	45.00±4.00 [b]	40.00±4.00 [b]	26.67±6.69 [b]	1.88±0.17 [b]	95.83±0.73 [a]
Positive control group	73.33±3.33 [a]	68.33±3.33 [a]	64.33±1.36 [c]	0.83±0.17 [a]	95.67±0.17 [a]

Means with the same letters in the same column are not significantly different.

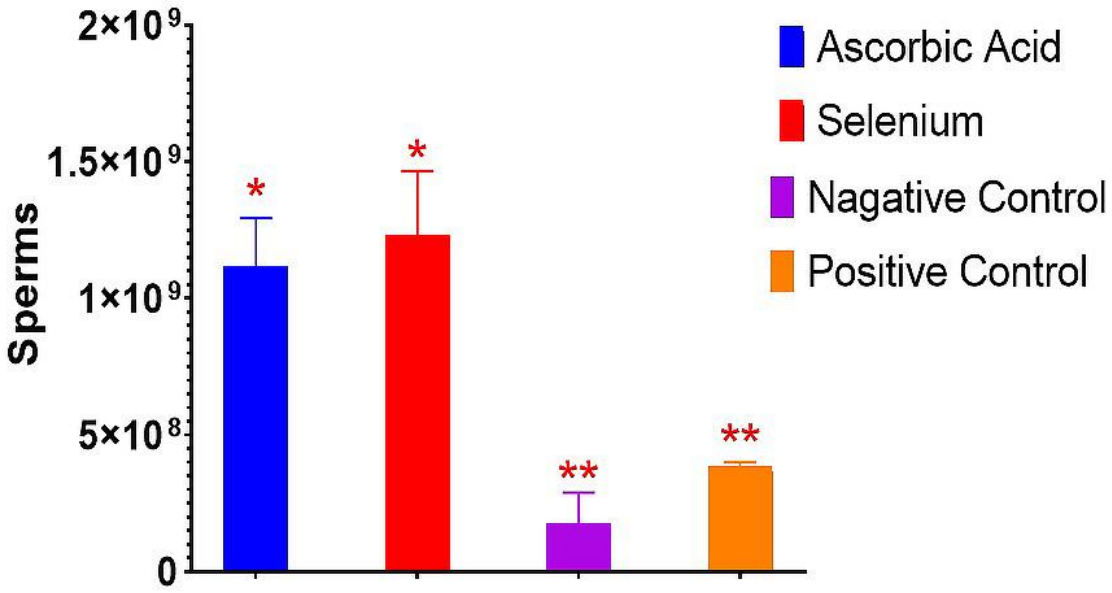

Figure 1. Effect of vitamin C and selenium on sperm concentration of 40 rats
* Denote in the column with same Denote are not significantly different P<0.05, while they have a significant difference with column having ** Denote

Authors' Contribution

Study concept and design: A. M. B. A.
Acquisition of data: S. K. A.
Analysis and interpretation of data: D. M. A.
Drafting of the manuscript: M. A.
Critical revision of the manuscript for important intellectual content: D. M. A.
Statistical analysis: A. M. B. A.
Administrative, technical, and material support: D. M. A.

Ethics

All the procedures during the current study were approved by the institutional animal care and ethics committee.

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