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

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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, or psychological and environmental stress. Some researchers showed that ascorbic acid has been related to fertility, it has 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 many biochemical and physiological functions. Thus, the present study aimed to determine the protective effects of vitamin C and selenium against restraint stress levels that cause a decrease in sperm quality in rats. 40 adult male Wistar rats were divided randomly into four equal groups (n=10); first group was exposed to restraint stress for 6 h a day, and supplemented with vitamin C in a dose of (50 mg/kg B.W/day) orally by gavage and served as vit C group, second group exposed to restraint stress for 6 h a day and supplemented with selenium in a dose of (0.02 µg /kg B.W/day) orally by gavage and served as Se group, third group exposed to restraint stress for 6 h a day and given 2 ml normal saline orally by gavage and served as negative control (NC), group 4th considered as control group without exposure to restraint stress, and given 2 ml normal saline orally by gavage and served as Positive Control (PC). The results showed that all the sperm parameters such as total and progressive motility, and sperm viability increased significantly (p≤0.05) in vit C and Se groups compare with NC group. The rate
of acrosome defects in vit C, Se, and PC groups were significantly (p≤0.05) reduced compare to the NC group. Results showed no significant differences among all the 4 groups. The results of the current study confirm the ameliorated effect of vit C and selenium on semen quality and sperm parameters such as; motility, viability, morphology and concentration against adverse effect of stress.

**Keywords:** Sperm, Infertility, Ascorbic Acid, Selenium, Stress

1. **Introduction**

Stress is becoming an inescapable part of modern life; it 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 the 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 is to control the behavior, reproduction, spermatogenesis, cardiovascular, immune functions, and metabolic system. Activation of the HPA axis by several stressors mainly inhibits reproductive function (5) (Joseph and Whirledg, 2017). Several studies have shown that stress along with depression and anxiety may lead to dramatic decrease in the sperm quality and hence lead to some degrees of infertility (6). Infertility is regarded as public and clinical problem because it affects the health system and social life.

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

The positive influence of selenium in male reproduction has been approved. This importance was due to its role in testosterone biosynthesis and subsequently in the typical development and formation of spermatozoa (9). Relatively, there were few studies showed the effect of ascorbic acid and selenium on male reproductive hormones such as LH, FSH, and testosterone (10, 11). Several studies have been conducted by many researches showed the strong capacity of vitamin C and selenium to reduce the stress effects in the living creatures (12, 13).
Thus, the current study was designed to evaluate the protective effects of vitamin C and selenium against restraint stress stimuli that cause 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, their weight averages between 250-350 grams and housed in clean cages kept in the animal house at the Faculty of Science, University of Kufa. Animals had ad-libitum axes to food and water during the experiment. The animals were maintained about two weeks for adaptation before starting the experiment.

2.1.1. Ethics

The animals were divided into 4 groups (n=10). All the procedures during the current study were approval by the institutional animal care and ethics committee.

2.2. Study design

Forty adult male lab rats were divided randomly into four equal groups (n=10) as following; the first 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 B.W/day orally by gavage, this group named Vit C group; the second group exposed to restraint stress for a period of 6 h a day, and received selenium in a dose of 0.02 µg/kg B.W/day orally by gavage and served as Se group; the third group exposed to restraint stress for a period of 6 h a day and given 2 ml normal saline orally by gavage this group considered as Negative Control (NC); the fourth group of animals did not exposed to restraint stress, they onle given 2 ml normal saline orally by gavage and served as Positive Control (PC).

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 six-hour a day (14), narrow enough to prevent the rats from moving freely but wide enough to cause no real physical discomfort, pain or impairment of respiratory movements, the rats were exposed to stress between 08:30 AM and 2:30 PM for twenty days of the experiment. During the experiment movement of the rats was highly restricted as they are in the restraint container; a negative control 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 sacrificed at different intervals from starting the experiment as described below: The animals were anesthetized using intraperitoneally injection of Ketamine 90 mg/kg B.W and Xylazine 40 mg /kg B.W., then by using sterile surgical instrument the testis and epididymis were dissected from the animal's body.

2.5. Epididymal spermatozoa sampling
The left tail epididymis was rinsed and 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 400× magnifications using a Computer Assisted Sperm Analysis (CASA; Genex laboratories; Florida, USA).

2.7. Sperms concentration (SPM/MI) evaluation
Ten μl of semen suspension was added to 999 μl of holding solution, so the dilution factor was 1:1000. The holding solution contains: normal saline 95%, formaldehyde 4%, 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
Values of sperm viability and normal sperm morphology were examined with eosin-nigrosin (EN) dye (18). Briefly the EN staining involved the following procedure: a 10 μL drop of raw sperm was added to 30 μL of EN, and stir for 10 seconds, then, the mixture was smeared on a dry warmed slide and left to air on slides warmer at temperature 45°C (19). Then, the slides were read under a microscope at 40× magnifications; either 200 spermatozoa or five microscopic fields were calculated. Furthermore, in viability, the pink-stained spermatozoa were considered dead while unstained spermatozoa were being alive. Furthermore, for acrosome evaluated which intact acrosome recorded as integrated acrosome while distortion acrosome of sperm recorded as damaged acrosome.
2.9. Statistical analysis
Statistical analysis of the experimental results was conducted according to Graphpad prism 8. was T-test and (one-way ANOVA) was used to assess the significance of differences between groups and within times. The data were expressed as mean ± standard errors (SE) and (P value < 0.05) was considered statistically significant LSD was carried out to test the significant level among means of treatment (Prism, 2019).

3. Results and Discussion
In the present study effects of vitamin C and selenium supplementation against restraint stress levels that cause a decrease in sperm quality in rats was investigated. Results showed that sperm motility has markedly decreased (P<0.001) in stressed rats compared to those with no stress immobilization or restraint stress. On the other hand, the induced stress lead to a significant decrease in plasma testosterone concentrations and blunts the plasma testosterone in rats that lead to depression in spermatogenesis (Table 1). These results are in agreement with previous study conducted by Nowicka-Bauer and Nixon (20) and Baiee, Al-Wahab (19). Recorded data in the current study in case of sperm concentration showed significant reduction in this parameter in the stressed animals similar to the results of previously published works (16, 21) as shown in figure 1. Recent study conducted by Choudhury, Rivero (22) proved that levels of generation of reactive oxygen species (ROS) were increased in the exposure to stress, therefore, causes decreased in sperm quality, viability and acrosome integrity. In agreement with the results of Choudhury, Rivero (22) the recorded data in the current study which figured out in table 1 showed significant (p ≤ 0.05) decreased in the sperm motility and viability as well increments in acrosome defect. Moreover, 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, as one of the prominent products of lipid peroxidation is malondialdehyde (MDA), therefore MDA is considered as the indicator of stress-induced damage in terms of lipid peroxidation (23) as proved in our study. On the other hand, the result illustrated the ameliorated effect of the vitamin-c and selenium on all groups compared with negative control group which showed significant increase in sperm parameters (general motility, progressive and viability) as well as decreased in the acrosomal defect.
Selenium, which accumulates in the pituitary gland and stimulates the gonadotropin releasing hormone (GnRH) receptor has been suggested to increase the production of LH (24), LH stimulates the production of testosterone from Leydig cells which are necessary for normal sperm formation (19, 25) that was proved in the results of the current study which figured out in table 1. Vitamin C and Selenium are essential trace elements effective as antioxidants (26) prevent oxidative damage which ability of these compounds to prevent oxidative stress and therefore regulation of defect body (27), we determined depicted a high significant increase in sperm parameters as well as in the sperm concentration. The researcher proved the positives effect of vitamin c and selenium in decreased the ROS in testis and decreased the adverse effect of stress on testes and sperm parameters quality as showed in our results (28).

Table 1. Effect of Vitamin C and Selenium on sperm parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Motility</th>
<th>Progressive Motility</th>
<th>Viability</th>
<th>Acrosome Defect</th>
<th>Morphology integrity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>88.00 ± 1.53 a</td>
<td>83.67 ± 0.67 a</td>
<td>85.33 ± 0.60 a</td>
<td>0.83 ± 0.17 a</td>
<td>95.17 ± 1.01 a</td>
</tr>
<tr>
<td>Vit.C</td>
<td></td>
<td>83.33 ± 1.67 a</td>
<td>78.33 ± 1.67 a</td>
<td>81.00 ± 2.65 ac</td>
<td>1.23 ± 0.17 a</td>
<td>95.50 ± 0.50 a</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>45.00 ± 4.00 b</td>
<td>40.00 ± 4.00 b</td>
<td>26.67 ± 6.69 b</td>
<td>1.88 ± 0.17 b</td>
<td>95.83 ± 0.73 a</td>
</tr>
<tr>
<td>NC</td>
<td></td>
<td>73.33 ± 3.33 a</td>
<td>68.33 ± 3.33 a</td>
<td>64.33 ± 1.36 c</td>
<td>0.83 ± 0.17 a</td>
<td>95.67 ± 0.17 a</td>
</tr>
<tr>
<td>Pc</td>
<td></td>
<td>73.33 ± 3.33 a</td>
<td>68.33 ± 3.33 a</td>
<td>64.33 ± 1.36 c</td>
<td>0.83 ± 0.17 a</td>
<td>95.67 ± 0.17 a</td>
</tr>
</tbody>
</table>

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

The results of the current study depicted in figure 1 illustrated the effect of vitamin c and selenium on the sperm production between different groups of the experiment. It is showed the positive effect of vitamin c and selenium on the increase in sperm concentration due to decrease in the amount of ROS raised from stress effect that loaded on rats (29), which previous studies proved that stress decreased the activity of spermatogenesis as showed on the other study (30). The result
in figure 1 proved ameliorated effect of vitamin C and selenium against the stress effect which illustrated increases the sperm concentration significantly of vitamin c and selenium groups among control and negative groups. In a previous study (Alahmar (31)) explains the positive effect of vitamin c and selenium on the sperm quality and sperm concentration similar to the results of the current study.

![Bar chart showing sperm concentration](image)

**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 significant different with column have (**) Denote)

Based on the result, the results are proven ameliorated effect of ascorbic acid and selenium on the sperm parameters: motility, viability, morphology and concentration against adverse effect of stress. Thus, we suggest that ascorbic acid and selenium play as productive antioxidant against harmful effect of stress on tastes and therefore sperm parameters and fertility.

**References**


23. Lodhi GM, Latif R, Hussain MM, Naveed AK, Aslam M. Effect of ascorbic acid and alpha tocopherol supplementation on acute restraint stress induced changes in testosterone, corticosterone and nor


