

The Synergistic Effect of Ascorbic Acid and Selenium on Serum Testosterone in Restraint Stressed Rats

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Abstract

The present study aimed to determine the synergistic effects of ascorbic acid and selenium on serum testosterone level in male rats exposed to restraint stress . Wistar rats weighting 175 ± 25 grams were divided randomly into four equal groups (n=10), some were supplemented with ascorbic acid (50 mg/kg bw/day) orally, the second group was supplemented with selenium (0.02 μ g/kg bw/day) orally, third group was orally supplemented with both ascorbic acid and selenium , the control included ten rats .Blood was collected on 20th days of experiment, and serum testosterone, and luteinizing hormone (LH) were measured by enzyme-linked immunosorbent assay (ELISA). The results for 20th days showed that supplement of rats with ascorbic acid has highly significantly ($P < 0.001$) increased serum testosterone to be (5.18 ± 0.1 ng/ml) compared to stressed rats which recorded (2.37 ± 0.16 ng/ml) and showed that supplement of rats with selenium has highly significantly ($P < 0.001$) increased serum testosterone level to be (5.28 ± 0.15 ng/ml) compared to stressed rats, and showed that supplement of rats with (ascorbic acid+ Selenium) has highly significantly ($P < 0.001$) increased serum testosterone level to be (5.78 ± 0.09 ng/ml) compared to stressed rats which recorded (2.37 ± 0.16 ng/ml). It is probable that there is a synergistic effect of ascorbic acid and selenium in relieving effect on restraint stress on male rats and enhancing testosterone levels .

Key Words— *restraint stress, testosterone, ascorbic acid, selenium*

Introduction

Stress is becoming an inescapable part of modern life, and has been dubbed the Health Epidemic of the 21st Century ⁽¹⁾ . It is defined as any response that elicit physiological and behavioral adaptations to maintain homeostasis ⁽²⁾ . Events that confront the organism's environment activate central stress response system, which is mainly mediated by the (HPA) axis ⁽³⁾ ; the regulatory functions of the HPA axis control the behavior , reproduction, cardiovascular, immune functions, , and metabolic system activation of the HPA axis by several stressors mainly inhibits reproductive function ⁽⁴⁾ ; several studies have shown of stress along

with depression and anxiety may lead to infertility ⁽⁵⁾ . Infertility is regarded as the public and clinical trouble because it affects the health system and social life, it is a reasonably common condition with psychological, medical, and financial consequences ⁽⁶⁾ . 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⁾ , disrupts both spermatogenesis and the production of testosterone caused by lack of vitamin C leads to a state of oxidative stress in the testes ⁽⁸⁾ . Selenium is an essential micronutrient , it acts as a cofactor for enzymes antioxidant, for example glutathione peroxidase ⁽⁹⁾ .

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Selenium importance was for successful in male reproduction due to its role in testosterone biosynthesis and subsequently in the typical development and

formation of spermatozoa⁽¹⁰⁾, thus selenium is a major factor sperm formation and male fertility, selenium deficiency has been found to be associated with reduced or impaired reproduction⁽¹¹⁾.

Methods

Forty fertile adult's albino male rats (*Rattus norvegicus*) were used in the present study, their averages ages (10-12) weeks, with a weight range (100-200) gm, the animals were housed in the animal house, the animals housed in well ventilated wire-plastic cages with dimensions design cages (50 × 35 × 15 cm) with metal covers and containing bedding of wood shaving which was changed once per daily.

The animals were maintained under controlled environment about 12-hour light and 12-hour dark with (degree of temperature 22-26 °C) and exact circumstances to the normal laboratory nutrition with profitable diet (pellets) and water provided to animals except during the experimental period, for two weeks before the experiment started, to adapt rats to the new environment none of the rats had any clinically obvious contagions, the rats were divided into four groups with five rats for each cage, for animal were ethics research procedures approval by our institutional animal care and care committee (IACUC).

Rats were classified into four groups: the first group: included ten rats exposed to restraint stress for six hours a day, and supplemented with vitamin C in a dose of (7.5 mg /day) orally by gavage; the second group: included ten rats exposed to restraint stress for six hours a day, and supplemented with selenium in a dose of (3 µg /day) orally by gavage; the third group: included ten rats exposed to restraint stress for five hours, and supplemented with vitamin C in a dose of (7.5 mg/ day), then with Selenium in a dose of (3 µg / day) orally by gavage; the fourth group: included (five)

rats exposed to restraint stress for five hours a day and given 2 ml normal saline orally by gavage and served as positive control (PC), and (five) rats without exposed to restraint stress, and given 2 ml normal saline orally by gavage and served as negative control (NC).

Rat were placed in the restraint cage used to produce restrain stress in a glass container (12×5 cm), for six-hour a day⁽¹²⁾, 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 14:30 PM for twenty days of the experiment. Animals weights have been recorded before the dosage by using electrical balance Blood samples were collected via cardiac puncture. Serum was obtained by centrifugation at 3000rpm for 20min. Serum was used for hormonal assay.

Results

All animals in the study were monitored for their feed and well-being and found healthy and active throughout the study period.

The result showed a high significant decrease ($P < 0.001$) in serum concentrations of testosterone at the twentieth day of experiment in positive control (stressed rat) (2.37 ± 0.16) compared to those which were not exposed to stress (3.6 ± 0.25) which constitute the negative control.

On other hand in same table was highly significant increase ($P < 0.001$) in serum concentrations of testosterone level in rats supplemented with vitamin C (50 mg/kg/day), Selenium (0.02 mg/kg / day) and both vitamin C plus Selenium, to be (5.18 ± 0.1), (5.28 ± 0.15) and (5.78 ± 0.09) respectively compared to positive and negative control.

Table.1 : Differences in serum testosterone level among rat groups at the twentieth day of experiment

Study Groups	Total Number	Serum Testosterone (ng/ml) M ± SD	F test (P value)
Vitamin C (50 mg/kg orally)	10	5.18 ± 0.1 *	593.19 (0.000)
Selenium (0.2 mg/kg orally)	10	5.28 ± 0.15 *	
Vitamin C + Selenium	10	5.78 ± 0.09 *	
Negative Control (without stress)	5	3.6 ± 0.25 *	
Positive Control (with stress)	5	2.37 ± 0.16 *	

* high significant difference at $P < 0.01$.

Discussion

Previous studies have shown that restraint stress lowers plasma testosterone concentrations and blunts the plasma testosterone in rats ⁽¹³⁾, and in monkeys ⁽¹⁴⁾.

Stress of different origins suppresses male reproductive functions by releasing stress hormones corticosterone levels are increased in psychological and physical stress, as in experimentally induced restraint stress in rats, therefore, levels of serum corticosterone may serve as an appropriate indicator of stress in rats ⁽¹⁵⁾. Stress activates sympathetic-adrenal system with release of catecholamine like epinephrine and norepinephrine along with proportionate release of increased glucocorticoid levels disrupted the male reproductive axis ⁽¹⁶⁾. Epinephrine and norepinephrine are biogenic amines Its secretion from of the adrenal medulla may also affect the testis by affecting flow of blood, since these stimulants are thought to cause vasoconstriction in many mother tissues ⁽¹⁷⁾. It is well-known that monoamine oxidase (MAO) convey several amines into metabolism that involve their MAO final product in testicular maturation and androgen synthesizing ⁽¹⁸⁾, therefore the serum testosterone levels were significantly lowered direct testosterone production inhibitor by Leydig cells, which are responsible for 95% of plasma testosterone levels in males, low testosterone levels follow a reduced regulation of LH signals, which stress directly suppress Leydig cell steroidogenesis consequently testosterone

⁽¹⁹⁾.

Stress is generally thought to generate reactive oxygen species (ROS), when ROS surpass body's natural antioxidant defense, impairment to macro molecules such as DNA, lipids and proteins would occur, during stress, lipid peroxidation is increased in the body, as one of the prominent products for fat oxidation is malondialdehyde (MDA), therefore MDA is considered as an indicator of stress induced damage by lipid peroxidation ⁽¹⁵⁾.

On other hand, there was an evidence that testosterone decreases may be associated activity levels of testicular steroidogenic indicator enzymes 3β -hydroxysteroid dehydrogenase 3β -HSD and 17β -hydroxysteroid dehydrogenase (17β -HSD) were reduced significantly in stress animals ⁽²⁰⁾, since testicular steroidogenesis is organized by these two rate determining enzymes, a decrease in their activities in Leydig cells greatly reduces production and thus level of testosterone ⁽²¹⁾.

Certainly, we observed the stress decreased the testosterone and fertility in the male rat by blocked the receptor of LH in the Leydig cell and Excessive oxidative stress that lowers major enzymatic and non-enzymatic antioxidant levels in Leydig cells, all of which leads to reduced testosterone secretion that conforming ⁽²²⁾.

Orr et al., (1994) reported a suppressive effect of immobilization stress on plasma testosterone level ⁽²³⁾; while Hu et al., (2000) had studied the effect

of restraint stress on testosterone levels in rats and found that long duration of restraint stress (2 hours daily for 2 months) had significantly decreased serum testosterone concentrations with noticeable reduction in spermatogenesis ⁽²⁴⁾ .

On the other hand, the present study has revealed that vitamin C has highly significantly ($P < 0.001$) increased in serum testosterone level (table.1) which indicated the effect of vitamin C supplement in enhancing male fertility ; this come along a previous study that investigated the effect of a twice daily dose (250 mg/kg) of vitamin C on serum testosterone levels in Wistar rats, after 21 days they found increase significantly in serum testosterone concentrations (25) .

Vitamin C is well known to play key roles in testosterone synthesis ⁽²⁶⁾, in extracellular fluids vitamin C is the most essential free radical scavenger that traps and maintains the roots of aqueous biomembranes from peroxidative harm and prevent increased the lipid peroxidation levels resulting from stress and reduce the complication causes of the stress such as increased oxidative stress, lipid peroxidation which is converted to dehydrascorbic acid by free radical reaction is regulated via the glutathione enzyme complex ⁽²⁷⁾ . This may provide additional evidence for the anti-stress effects of vitamin C as stress is an imbalance between the production of reactive oxygen and antioxidant defense ⁽²⁸⁾ .

This study showed that the level of testosterone in plasma increased significantly in animals treated with ascorbic acid When compared with animal controls. This rise could be dependent on the ascorbic acid that activates LH release.

In addition, our result in same exhibited high significant rise ($P < 0.001$) in testosterone serum concentrations level at the twentieth days of experiment in rats with Selenium (3 µg/day) which selenium is required for normal testicular development and spermatogenesis in rats where observed the severe selenium deficient mice reduced testosterone concentrations in the blood ⁽²⁹⁾ .

Previous studies found that selenium may have a positive effect on Leydig cells to increase spermatogenesis ⁽³⁰⁻³¹⁾ , this result is in agreement with present result that

recorded a high increase in testosterone secretion .

In the Our result showed in (vitamin C + Selenium) highly significant group increase ($P < 0.001$) in testosterone serum concentrations for seventh and twentieth days in contrast with negative and positive Control showed in table -1 that conforming the Previous studies and protect our theses of this study show effect of vitamin C and selenium together in male rats in stress.

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

Conflict of Interest : Nil

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