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The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats

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Abstract

This study was conducted to investigate the effects of ascorbic acid supplementation in drinking water on semen quality, lipid peroxidation and plasma testosterone level of male rats. In this investigation, 24 male Wistar rats were used. The animals were divided into three groups, and 500, 250 and 0 (control) mg/kg/day ascorbic acid were supplemented with drinking water of rats in Groups A, B and C during 8 weeks, respectively. Ascorbic acid supplementation did not increase in the body weight and weights of the testis, epididymis, seminal vesicles and ventral prostate. Exogenous supplementation with ascorbic acid significantly increased ($P < 0.05$) the concentration of ascorbic acid in the testes and blood plasma, and the level of lipid peroxidation significantly decreased ($P < 0.05$) in these locations. There was no significant difference in spermatozoon motility among the three groups. However, epididymal sperm concentration and plasma testosterone level significantly increased ($P < 0.05$) in the ascorbic acid treated animals when compared to the control animals. The results suggest that ascorbic acid supplementation improves reproductive traits of male rats that are associated with high fertility.

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Keywords: Ascorbic acid; Rat; Testosterone; Epididymal sperm concentration

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1. Introduction

Ascorbic acid, also known as Vitamin C, is a water-soluble vitamin which is essential for normal functioning of the body. It has been associated with fertility for many years and may have evolutionary significance [1,2]. But its precise physiological role in reproduction has been uncertain.

The production of reactive oxygen species (ROS) is a normal physiological event in various organs including the testis. Overproduction of ROS can be detrimental to sperm being associated with male infertility. The sperm plasma membrane contains a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage. The lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and the defects of membrane integrity [3,4].

Epididymal fluid contains appreciable amounts of antioxidants that balance lipid peroxidation and prevent excessive peroxide formation [5]. Ascorbic acid is an antioxidant substance. It is present at a high concentration in the epididymal fluid and seminal plasma of several species compared to blood plasma, and it is a protective vitamin in the epididymis [6,7]. High concentrations of ascorbic acid in seminal plasma may play a role in protecting sperm from ROS and in maintaining the genetic integrity of sperm cells by preventing oxidative damage to sperm DNA [8]. Testes and seminal plasma are extremely sensitive to a decrease in body levels of ascorbic acid. Moreover, ascorbic acid deficiency caused a reduction in reproductive performance [9]. On the other hand, the excessive intake of ascorbic acid has been reported to cause reproductive failure in the male [10].

The objective of this study was to investigate the effects of ascorbic acid supplementation in drinking water on semen quality, lipid peroxidation and plasma testosterone level of male Wistar rats.

2. Materials and methods

2.1. Animals and treatment

All the chemicals were purchased from the MERCK Chemical Co. In this investigation, 24 healthy adult male Wistar rats, 4 months of age and weighting 170–190 g were used. The animals were obtained from Firat University, Medical School, Experimental Research Centre, Elazığ, TURKEY. The rats were individually housed in plastic cages. The animals were kept under standard laboratory conditions (12 h light:12 h dark and 24 ± 3 °C) during experimentation period. Body weight and water intake were assessed weekly throughout 8-week period and the dose was adjusted accordingly. The rats were fed standard commercial laboratory chow [(pellet form), in the sack (50 kg), Elazığ Food Company]. Compound of this pellet form was given in Table 1. Feed and water were provided ad libitum. The rats were divided into three groups of eight rats each. The animals in Groups A and B were administered orally with ascorbic acid in drinking water at 500 and 250 mg/kg/day, respectively, for 8 weeks. The animals

Table 1
Compound of standard commercial laboratory chow

Contents	Percent
Wheat	10
Corn	22
Barley	15
Bran	8
Soybean meal	26
Fish meal	8
Meat-bone meal	5
Molasses	5
Salt	5
Inorganic substances ^a	1.25
Vitamin compound ^b	1.25

^a Inorganic substances: zinc basitrasin (100,000 mg), manganese (80,000 mg), iron (80,000 mg), zinc (60,000 mg), copper (8000 mg), iodine (500 mg), cobalt (200 mg), selenium (150 mg), calcium (8000 mg).

^b Vitamin compound: Vitamin A (12,000,000 IU), D₃ (2,400,000 IU), E (30,000 mg), K₃ (2500 mg), B₁ (3000 mg), B₂ (7000 mg), B₆ (4000 mg), B₁₂ (15 mg), nicotinic acid (40,000 mg), folic acid (1000 mg), biotine (45 mg) and choline chloride (125,000 mg).

in Group C served as control. Rat in each cage received water from a separate glass container. Containers were checked each day and protected from light. Animals were observed twice daily.

2.2. Necropsy

The rats were weighed and sacrificed using ether anaesthesia at the end of 8 weeks. Blood was collected into heparinized tubes and centrifuged at 3000 rpm for 10 min. Plasma was stored at -20°C for analysis.

The testes, epididymides, seminal vesicles and ventral prostate were removed, cleared of the adhering connective tissue and weighed.

2.3. Epididymal sperm concentration and motility

Spermatozoa in the right epididymis were counted by a modified method of Yokoi et al. [11]. Briefly, the epididymis was finely minced with anatomical scissors in 5 mL of physiological saline, placed in a rocker for 10 min then allowed to sit at room temperature for 2 min. After incubation, supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate, 1 mL formalin (35%) and 25 mg eosine per 100 mL of water. Total sperm number was determined by using a hemocytometer. Approximately 10 μL of the diluted sperm suspension was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 min. The cells settled during this time were counted with the help of light microscope (Magnification, 200 \times). Progressive sperm motility was evaluated using the standard method [12]. The fluid obtained from cauda epididymis with a pipette was diluted to 2 mL with Tris buffer solution. A slide was placed on phase contrast microscope and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 \times . Motility

estimations were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were kept at 35 °C.

2.4. Biochemical studies

The testes tissue (0.5 g) in each male rat was dissected and homogenized in physiological saline solution, and then centrifuged at 10,000 rpm for 20 min. The supernatants were used for biochemical analyses.

2.4.1. Ascorbic acid

Ascorbic acid concentration in testis and blood plasma was determined at spectrophotometrically at 700 nm using acid phosphotungstate [13]. Briefly, one volume of the sample in a centrifuge tube was added slowly one volume of colour reagent (Solution A: a mixture of 20 g of sodium tungstate and 10 g of disodium hydrogen phosphate was suspended in 30 mL of water and warmed to dissolve. Solution B: to 15 mL of water, 5 mL of sulphuric acid was added. Solution B was poured slowly into warm Solution A. The content was boiled gently for 2 h under reflux, and then cooled to room temperature). The mix was allowed to stand for 30 min at room temperature, and then centrifuged at 3000 rpm for 15 min. The blue-coloured supernatant was placed in a spectrophotometer cuvette and read at 700 nm against a blank. The concentration of ascorbic acid was expressed as mg/100 mL.

2.4.2. Lipid peroxidation

Lipid peroxidation (LPO) concentration in testis and blood plasma was measured according to the concentration of thiobarbituric acid reactive species [14]. Briefly, one volume of the test sample and two volumes of stock reagent (15%, w/v trichloroacetic acid in 0.25 N HCl and 0.375%, w/v thiobarbituric acid in 0.25 N HCl) were mixed in a centrifuge tube. The solution was vortexed and heated for 15 min in a boiling water. After cooling, the precipitate was removed by centrifugation at 2500 rpm 10 min and absorbance of the supernatant was measured at 532 nm against a blank. The concentration of lipid peroxidation was expressed as nmol/100 mL.

2.4.3. Testosterone

The plasma testosterone level was assayed using Coat-a-Count Radioimmunoassay kits (Active[®] Testosterone RIA DSL-4000, Diagnostic System Laboratories Inc., Texas, USA). The amount of testosterone was expressed as ng/mL.

2.5. Statistical analyses

All values were presented as mean \pm S.D. Differences were considered to be significant at <0.05 against control group. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by an unpaired Student's *t*-test using the SPSS/PC computer program.

Table 2

The effect of ascorbic acid supplementation on body weight and weights of testis, epididymis, seminal vesicles and ventral prostate of rats

	Group A (500 mg/kg/day)	Group B (250 mg/kg/day)	Group C (control)
Initial body weight (g)	181.6 ± 4.6	184.5 ± 3.7	179.1 ± 4.7
Final body weight (g)	228.5 ± 5.7	225.6 ± 7.1	221.8 ± 6.1
Testis weight (mg)	1327.5 ± 76.8	1298.9 ± 68.7	1266.3 ± 77.2
Epididymis (mg)	451.3 ± 20.8	421.4 ± 61.3	418.8 ± 32.1
Seminal vesicles (mg)	264.5 ± 31.3	242.0 ± 28.6	237.4 ± 27.2
Ventral prostate (mg)	142.5 ± 39.1	122.8 ± 30.2	124.6 ± 20.2

The data are expressed in mean ± S.D. for eight animals per group.

Table 3

The effect of ascorbic acid supplementation on plasma testosterone level, epididymal sperm concentration and sperm motility of rats

	Group A (500 mg/kg/day)	Group B (250 mg/kg/day)	Group C (control)
Sperm motility (%)	62.5 ± 7.6	65.8 ± 4.9	67.6 ± 6.4
Epididymal sperm concentration (10 ⁶ /g)	342.8 ± 47.3a	319.7 ± 38.4a	224.7 ± 24.7b
Testosterone (ng/mL)	3.72 ± 0.86a	3.58 ± 0.45a	2.76 ± 0.27b

The data are expressed in mean ± S.D. for eight animals per group. Within rows, means with different letters (a and b) are significantly different ($P < 0.05$).

3. Results

During the 8 weeks of ascorbic acid treatment, the rats remained healthy, growing at a normal rate. The body weight and weights of the testes, epididymis, seminal vesicles and ventral prostate did not show any significant changes in the rats supplemented with ascorbic acid as compared to those of control animals (Table 2).

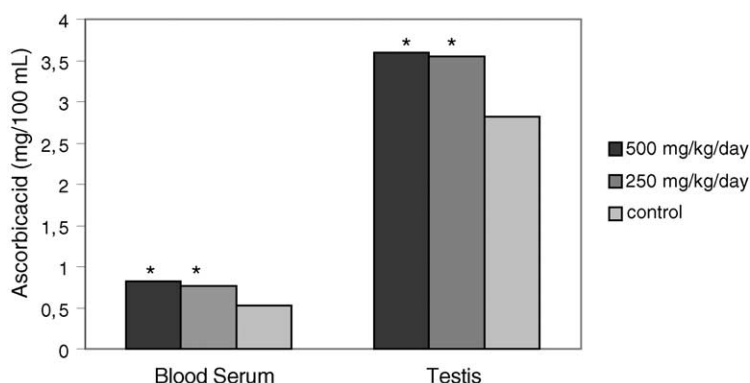


Fig. 1. The ascorbic acid concentrations in blood serum and testis of control and ascorbic acid supplemented groups. * $P < 0.05$ compared to control.

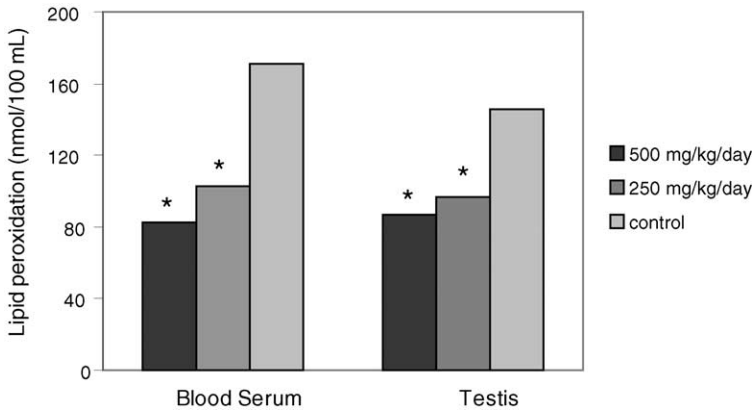


Fig. 2. Lipid peroxidation concentrations in blood serum and testis of control and ascorbic acid supplemented groups. * $P < 0.05$ compared to control.

While ascorbic acid concentration significantly increased ($P < 0.05$) in the ascorbic acid treated animals, the level of lipid peroxidation decreased ($P < 0.05$) in those animals when compared with the control animals (Figs. 1 and 2).

There was no significant difference in spermatozoon motility among the three groups. However, spermatozoa concentration and plasma testosterone level significantly increased ($P < 0.05$) in the ascorbic acid treated animals when compared to the control animals (Table 3).

4. Discussion

Ascorbic acid is an essential component in the diet of humans, nonhuman primates, guinea pigs, and some species of bats. It has been associated with fertility for many years, but its precise physiological role in reproduction has been uncertain.

Sapra et al. [15] observed that ascorbic acid deficiency in guinea pigs caused a decrease in weights of reproductive organs. Other studies [16,17] indicated that the weights of the testis and accessory sex organs were significantly decreased by some toxic substances, and the administration of ascorbic acid reserved this reduction. It has been reported [18,19] that supplementation with ascorbic acid will counteract the deleterious of toxins and metabolic poisons. However, Hsu et al. [20] showed that dietary ascorbic acid supplementation of healthy rats did not increase body weight and weights of reproductive organs which is in agreement with our results. In our study, the body weight, as well as, weights of the testes, epididymis, seminal vesicles and ventral prostate did not show and significant changes in rats supplemented. Ascorbic acid is presence in testis of healthy adults in high concentration, ranging from 1.9 to 12 mg/g, has been reported by various authors [15,21]. The present study showed that ascorbic acid concentrations of testis and blood serum were normal level in control rats although ascorbic acid levels significantly increased in treatment groups when compared to control animals, and there was no observed deficiency of ascorbic acid in control animals.

The concentration of ascorbic acid in semen is 8–10-fold higher than that in blood [22] and the concentration in testes and seminal plasma is extremely sensitive to a decrease in blood levels of ascorbic acid [9]. Fraga et al. [8] reported that an increase in dietary ascorbic acid for 28 days (from 5 to 250 mg/day) caused a doubling in seminal plasma ascorbic acid level and reduced oxidative DNA damage in men. In our study, while ascorbic acid concentration significantly increased in blood serum and testis of the ascorbic acid treated animals, the level of lipid peroxidation decreased in those animals when compared with the control animals. The high concentrations of ascorbic acid are a defense against free radicals. Therefore, the low level of lipid peroxidation was coincident with increase in ascorbic acid.

Overproduction of reactive oxygen species (ROS) and free radicals constitutes the oxidative stress in various organs of body including the testis. The oxidative stress can be detrimental to sperm being associated with male fertility [3,4]. Ascorbic acid regenerates melatonin from melatonin radicals [23]. It has been reported [24,25] that melatonin the principle secretory product of the pineal gland, and reduce the oxidative stress together ascorbic acid by scavenging free radicals, but melatonin alters ultrastructure of mouse Leydig cells and possibly influences their secretory activity by inhibiting their capacity to secrete steroids. Cao et al. [26] indicated that excessive oxidative stress reduced levels of key enzymatic and non-enzymatic antioxidants in Leydig cells, and resulted in decline in testosterone secretion. El-Missiry [17] reported that ascorbic acid has a protective effect against oxidative damage by free radicals, and the administration of ascorbic acid after alloxan treatment blunted the increased testicular lipid peroxidation and the decreased plasma testosterone level. The decline in ascorbic acid concentration was correlated with a decrease in steroidogenesis [27]. Gomes et al. [28] indicated that the deficiency of dietary ascorbic acid caused a decrease in plasma testosterone level and degeneration of the spermatogenic epithelium in guinea pigs. Similarly, Chinoy et al. [9] indicated that ascorbic acid deficiency markedly affected the androgen-sensitive parameters of the reproductive glands and caused an “androgen-deprived effect” in these target organs in guinea pigs. Biswas et al. [29] reported that ascorbic acid stimulated testicular steroid dehydrogenase activity and increased in plasma testosterone level. Ito et al. [30] indicated that ascorbic acid deficiency caused no significant change in basal plasma levels of testosterone in adult scorbutic rats. Karanth et al. [31] reported that ascorbic acid is a vitaminergic transmitter that activates the release of both FSH and LH from the anterior pituitary gland by autocrine action by means of nitric oxide. LH causes the release of testosterone from Leydig cells. This study demonstrated that plasma testosterone level significantly increased in the ascorbic acid treated animals when compared to control animals. This increase may dependent that ascorbic acid activates release of the LH.

Dawson et al. [32] reported that ascorbic acid supplementation (excess of 200 mg/day) resulted in improved sperm quality in heavy smokers, and there was a significant relationship between serum and seminal plasma ascorbic acid levels and sperm qualities. Phillips et al. [33] indicated that low levels of ascorbate in bull semen were associated with poor breeding performance, and subcutaneous administration of ascorbic acid increased sperm concentration and fertility rate. Similarly, Sönmez and Demirci [34] reported that intramuscularly administration of ascorbic acid for 30 days increased ascorbic acid level and sperm concentration in semen of ram. On the other hand, Rolf et al. [35] reported that

high dose oral ascorbic acid treatment did not improve semen parameters in infertile men. Our results demonstrated that despite the absence of significant differences in sperm motility among three groups, epididymal sperm concentration was increased in supplemented ascorbic acid groups when compared to those of control groups. These results are in agreement with the findings of Yousef et al. [36] who reported that ascorbic acid supplementation in drinking water for 12 weeks increased sperm concentration of male rabbits. The increase in the epididymal sperm concentration might be explained with activated of both FSH and LH by ascorbic acid. Because of activated FSH and LH stimulate spermatogenesis, and increases the sperm concentration.

High concentration of ascorbic acid in human semen plays a key role in maintaining the genetic integrity of sperm cells by preventing oxidative damage. The decline in ascorbic acid concentration of human semen caused decrease in activities of antioxidant enzymes superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase and increase in levels of hydrogen peroxide and lipid peroxidation in the epididymal sperm [37]. Thiele et al. [7] reported that the decrease in epididymal sperm counts may be due to increased lipid peroxidation. Dabrowski and Ciereszko [38] indicated that the concentration of ascorbic acid in seminal plasma reflected the dietary intake of ascorbic acid, and it caused the increase in sperm quality. Harris et al. [39] reported that dietary supplementation of ascorbic acid reversed nonspecific sperm agglutination and might facilitate deposition of the metals in semen, which associated with improvement in the physical characteristics of the semen in men. On the other hand, Paul and Datta-Gupta [10] reported that excessive intake of ascorbic acid caused reproductive failure in the male. But, ascorbic acid toxic reactions are rare at dosages less than 4 g/day. The huge doses of Vitamin C are well tolerated in body, and the excessive Vitamin C rapidly eliminated in the urine for 24 h [40].

In conclusion, the present study indicated that high ascorbic acid concentration caused to increase in epididymal sperm concentration and serum testosterone level of rats. Therefore, ascorbic acid supplementation can improve semen quality.

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