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Vitamin C (ascorbic acid) intake and its effect on steroid hormones in mice

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Abstract

Vitamin C is an antioxidant, composed of ascorbic acid (AA), monohydro-ascorbic acid (MDHAA), and dihydro-ascorbic acid (DHAA). These three forms are interconvertible. This study aimed to evaluate the relationship between the effects of vitamin C (ascorbic acid) on steroid hormone levels in male white rats and its antioxidant properties. Eighteen adult male Wistar white rats, aged 3–4 months, were used in this study. The mice were randomly divided into three equal groups (6 mice per group): a control group, a medium dose of 250, and a high dose of 400. Vitamin C was administered orally for 21 days. The results showed an increase in testosterone concentration in both the medium and high dose groups. Elevated FSH levels were observed in the high-dose group, while LH levels decreased in the medium-dose group, attributed to increased negative feedback resulting from elevated testosterone. Statistically significant improvements in the enzymatic activity of the two key enzymes in the steroid biosynthesis pathway, 3β-HSD and 17β-HSD, were also observed in both treatment groups. Antioxidant results following vitamin C treatment showed a marked decrease in malondialdehyde (MDA) levels, along with a high increase in the activity of the antioxidant-enzymes SOD, CAT, and GSH. A significant increase in vitamin C concentration within testicular tissue was also observed, indicating effective protection against oxidative stress. The study demonstrated that vitamin C-treated mice exhibited statistically significant increases in sperm concentration, motility, and the percentage of normal sperm morphology, reflecting the positive effect of vitamin C on testicular and sperm function in general.

Keywords: *Vitamin C, Ascorbic acid , Oxidative stress; Reactive oxygen species (ROS), sperm.*

Introduction

Vitamin C, also known as ascorbic acid, is a micro-nutrient with a clear role in maintaining overall body health. Its found in two basic forms ascorbic-acid (AA) and dehydro-ascorbic-acid (DHA), vitamin C is a powerful anti-oxidant and a co-factor for lots of enzymes, it participates in many biological- functions, such as immune-system, iron absorption from food, carnitine and catecholamine metabolism, and collagen synthesis, the body can't synthesize it for a mutations in the glunolactone oxidase (GLO) gene, which is in charge of its synthesis from gluno-1,4- lactons, vitamin C is affluent in foods rich in ascorbic-acid (such as potatoes, tomatoes, green leafy vegetables, citrus fruits, and berries), its able to accumulate in various tissue (such as the liver, skeletal muscles, brain, corneal epithelium, stem cells, and neutrophils) by specialized trans-porters, and its able to promote cell functions and activity (**Alberts *et al*., 2025**).

Vitamin C is a powerful anti-oxidant and co-enzyme in the human body, it is essential for many enzymes and bodily functions, it neutralizes re-active oxygen-species (ROS) generated by normal metabolic processes, helping to prevent oxidative stress and cell damage when taken in high doses, vitamin C can act as a powerful and effective antioxidant, and scientific studies are currently investigating it as a potential drug in cancer treatment (**Pawlowska et al., 2019**).

Vitamin C also plays a crucial role in collagen hydroxylation, the synthesis of carnitine, catecholamine's, folic acid, tryptophan, and tyrosine metabolism, it is involved in the hydroxylation of proline, glycine, and lysine, contributing to the stability of the triple-helical structure of collagen, furthermore, it plays a role in the changing the cholesterol to bile-acids, helping cholesterol to be balanced and reduce the danger of hyper-cholesterolemia, consequently, gallstone formation (**Maharana, 2025**).

Vitamin C stimulates the absorption of iron from the intestines by reducing Fe^{3+} to Fe^{2+}. Here, it stabilizes the iron element and thus contributes to the treatment of anemia. It also contributes in energy metabolic, as it participates in the forationn of (carnitine), a natural compound derived from the amino acids (methionine and lysine). It is produced in the body by the liver and kidneys and plays a vital role in transporting fatty acids to the mitochondria (the power plants of cells) to be burned and produce energy. It also has the ability to act as a cofactor for the enzyme (dopamine beta-hydroxylase), which converts the neurotransmitter dopamine into norepinephrine (noradrenaline) within noradrenergic neurons. It is an essential and important neuro-transmitter for the nervous-system (**Hardiansyah & Sugiyanti, 2024**).

Dehydroascorbate is the primary form of vitamin C that crosses the lateral basement membrane of intestinal cells and other cells, ascorbic acid is concentrated in isolated nerve endings, and synaptic vesicles concentrate it via mechanisms such as active transport. A six-carbon ketolactone is synthesized from glucose (**Okon& Utuk, 2016**). Dehydroascorbate is reported to be abundant in many endocrine tissues and plays a pivotal role in regulating steroidogenesis in the adrenal and gonadal glands (**Patani et al., 2023**). High concentrations of vitamin C have been recorded in the anterior pituitary gland (**Alwan, 2025**). Researchers have hypothesized that vitamin C may play an important role in the secretion of anterior pituitary hormones, as luteinizing hormone (LH) and follicle stimulating hormone (FSH) ,due to the high concentration of vitamin C in the pituitary gland. This secretion is mediated by nitric oxide (NO), a phenomenon dependent on calcium ions (Ca^{2+}). (**Qazzaz et al., 2021**).

Testosterone plays a key role in spermatogenesis, and its production from Leydig cells is primarily regulated by luteinizing hormone (LH). Both follicle-stimulating hormone (FSH) and LH may play a significant role in sperm production and maturation, and thus may be a determining factor in sperm quantity and quality. Studies indicate that vitamin C acts as a vitamin-enhancing messenger, stimulating the secretion of FSH and LH from the anterior pituitary gland (adenohypophysis).

It also enhances the activity of the key steroid genic enzymes 3β-HSD and 17β-HSD in testicular Leydig cells. Vitamin C is stored in the secretory granules of the pituitary cells that produce FSH and LH, and is released together via exocytosis, thus stimulating their secretion through the vitamin-enhancing messenger mechanism (**Santi et al., 2020**). Vitamin C is an essential nutrient that influences the synthesis and secretion of reproductive hormones. This study investigated the potential relationship between the reproductive hormonal effects of ascorbic acid and sperm characteristics (male fertility).

Materials and Methods

2-1: Study design and animal handling

Eighteen adult male Wistar Albino Rats, aged 3–4 months and weighing 170–241 g, were used in this study. The animals were acclimated to a specific environment for 14 days prior to the start of the experiment (12 hours light/12 hours' dark, temperature 24±3°C). The male mice were randomly divided into three equal groups (6 mice per group).

Table (1): Experimental Groups

Duration of treatment	Method of administration	Dosage (Vitamin C)	Number (n)	Group
21 days	**Oral (gavage)**	**5 mL distilled water**	**6 mice**	**Control (1)**
21 days	**Oral (gavage)**	**250 mg/kg**	**6 mice**	**(2) Group**
21 days	**Oral (gavage)**	**400 mg/kg**	**6 mice**	**(3) Group**

* Ascorbic acid was administered via oral gavage twice daily for 21 days.

2.2 Sample Collection and Hormonal Analysis

After the dosing was completed, the animals were sacrificed, and blood samples were collected by cardiac puncture under anesthesia. The serum was centrifuged and stored at -20°C until hormonal analysis was performed using radioimmunoassay (RIA) and ELISA.

The testes were immediately removed and weighed. One was used for histological examination, and the other for measuring sex enzyme activity and redox markers.

ELISA tests for sex hormones were performed according to the instructions of the Elabscience China kit (Rat FSH "E-EL-R0391" and (Rat LH "E-EL-R0026"), and antioxidants were measured according to the instructions of the Elabscience China kit (MDA "E-BC-K025-M", T-SOD "E-BC-K020-M"(, and)CAT "E-BC-F006"). And (3β-HSD , 17β-HSD) were measured using the RIA test kit from the American company Mybiosource.

3-3: Statistical Analysis

SPSS version 25 was used, and the data were analyzed according to descriptive statistics (mean ± SEM), inferential statistics using one-way ANOVA, and post-hoc tests using Tukey's test and Dunnett's test (significance level: $p < 0.05$).

Results

3-1: Effect of Vitamin C on Steroid Hormone Levels

As shown in Table 2, the study results revealed a statistically significant increase in testosterone concentration in both the medium-dose (250 mg/kg) and high-dose (400 mg/kg) groups compared to the control group. We also observed elevated FSH levels in the high-dose group, while LH levels decreased in the medium-dose group—this is explained by increased negative feedback resulting from elevated testosterone. We also observed a statistically significant improvement in the enzymatic activity of the two main enzymes in the steroid biosynthesis pathway, 3β-HSD and 17β-HSD, in treatment groups 250 and 400.

Table (2): Steroid hormone levels and steroid enzyme activity (mean ± standard error)

Hormone	Control Group	Medium dose (250)	High dose (400)	P-value
Testosterone (nanomoles/liter)	**0.9±8.2**	**11.4±1.1***	***1.4± 13.8**	**0.05<**
LH - Luteinizing Hormone (units/liter)	**0.5 ±4.6**	***0.4 ± 3.1**	**0.6 ± 4.2**	**0.05<**
FSH - Follicle-Stimulating Hormone (units/liter)	**0.6 ± 3.8**	**0.7 ± 4.1**	*** 0.8 ± 6.2**	**0.05<**
3β-HSD (units/mg protein)	**0.04 ± 0.42**	***0.05 ± 0.56**	*** 0.05 ±0.64**	**0.05<**
17β-HSD (units/mg protein)	**0.03**	***0.04 ± 0.51**	*** 0.05 ±0.4**	**0.05<**

* The mark indicates that there are statistically significant differences compared to the control group (P < 0.05).

3-2: Oxidative Stress Markers

As shown in Table 3, treatment of mice with vitamin C resulted in a marked decrease in the levels of malondialdehyde (MDA), which is considered an indicator of lipid peroxidation, with a marked increase in the activity levels of the antioxidant enzymes CAT, GSH, and SOD. A marked increase in the concentration of vitamin C within testicular tissue was also observed, indicating the role of vitamin C as an effective protection against oxidation within the testis.

Table (3): Oxidative stress parameters in testicular tissue (mean ± standard error)

Parameter	P-value	High dose (400)	Medium dose (250)	Control Group
MDA - Malondialdehyde (nmol/g)	**0.3 ± 2.8**	*** 0.2 ± 1.9**	***0.2 ±1.4**	0.05<
SOD - Superoxide Dismutase (units/mg)	**1.2 ± 18.4**	*** 1.8 ± 24.6**	***2.1 ±29.1**	0.05<

CAT - Catalase (units/mg)	**1.1 ± 14.2**	*** 1.4 ± 19.8**	*** 1.9 ± 24.3**	0.05<
GSH - Glutathione (micromoles/g)	**1.8 ± 22.1**	*** 2.0 ± 28.7**	*** 2.4 ± 34.5**	0.05<
Ascorbic acid in the testis (micromoles/g)	**0.7 ± 8.4**	***1.0 ± 14.3**	*** 1.5 ± 19.8**	0.05<

*** The mark indicates that there are statistically significant differences compared to the control group (P < 0.05).**

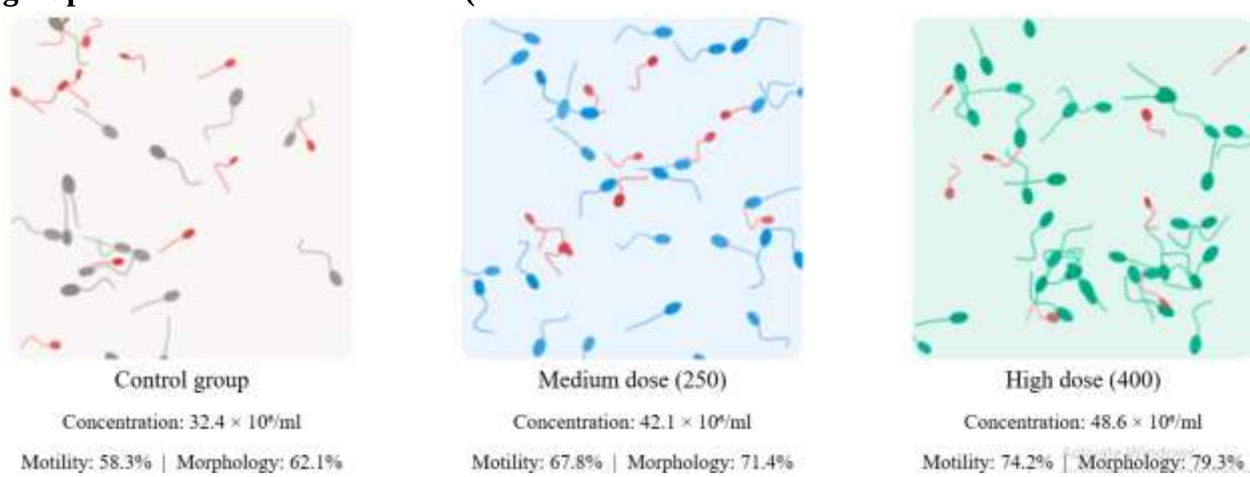
3-3: Sperm Quality Parameters

As shown in Table (4), mice treated with vitamin C exhibited a statistically significant increase in sperm concentration, motility, and the percentage of normal forms, reflecting the positive effect of vitamin C on testicular and sperm function in general.

Table (4): Effect of Vitamin C on Sperm Quality Parameters (Mean ± Standard Error)

Parameter	P-value	High dose (400)	Medium dose (250)	Control Group
Sperm concentration ($\times 10^6$/mL)	**2.6 ± 32.4**	***3.1 ± 42.1**	*** 3.7 ± 48.6**	0.05<
Sperm motility (%)	**3.4 ± 58.3**	*** 4.2 ± 67.8**	*** 4.6 ± 74.2**	0.05<
Morphologically normal sperm (%)	**4.1 ±62.1**	*** 4.8 ± 71.4**	*** 5.2 ± 79.3**	0.05<

***The mark indicates that there are statistically significant differences compared to the control group (P < 0.05).**



Sperm density: The number of sperm reflects the concentration (approximately 28 → 38 → 46 cells(.
Sperm motility: The percentage of motile cells, with increased clarity as the dose increases.
Normal sperm morphology: Normally shaped cells are shown in their original color, while abnormal cells are shown in red.
Sperm tail: Normal sperm have a longer tail and more regular wave-like motion.

Discussion

The results of our study, based on the data above, showed the following:

4-1: First: The effect of vitamin C on reproductive hormones and androgen levels

The results showed a significant increase in testosterone concentration following vitamin C treatment in both the medium- and high-dose groups, a finding consistent with a study by)**Okon & Utuk, (2016** which reported that administration of ascorbic acid (vitamin C) at a dose of 200 mg/kg (medium-dose group) and 400 mg/kg (high-dose group) twice daily for 21 days to white mice led to an increase in total testosterone concentration in both groups (medium-dose and high-dose groups) compared to the control group. Our study was consistent with a study by **Behairy et al., (2020)**, Studies have shown that taking vitamin C supplements restores sperm concentration in the epididymis and improves seminal fluid quality, which contributes to restoring sperm concentration in the epididymis. Many studies have indicated that vitamin C improves sperm motility, protects sperm formation and production, and plays a vital role in seminal fluid health and increased fertility. The recorded effects of vitamin C on sperm motility are related to its cellular enzymatic activity, as an antioxidant and cofactor (**Shabanian et al., 2017**).

Vitamin C is essential for sperm DNA production, as it acts as a cofactor in DNA methylation. Healthy DNA leads to healthy sperm (**Hamidian et al., 2020**). It is worth noting that sperm motility depends heavily on the quantity and quality of mitochondria in the tail. In particular, it has been observed that vitamin C acts as a cofactor to reduce the oxidized forms of copper (Cu2+) and iron (Fe2+) iron (Fe^{2+}) bound to alpha-ketoglutarate in the body also acts as a kinase inhibitor; this action of vitamin C may, in one way or another, play a role in improving sperm motility (**Jiang et al., 2023**).

4-2: Second: The Role of Vitamin C in Reducing Testicular Oxidative Stress

The results of our study showed that vitamin C reduced total oxidative stress and eliminated free radicals and reactive oxygen species (ROS) in the testes in the medium- and high-dose treatment groups. Our study was consistent with **Jiang et al. (2023),** who demonstrated that vitamin C acts as a protective factor for sperm production within the male reproductive system, shielding it from oxidative stress damage, as it significantly affects semen quality and fertility in both human males and animals, and that this intervention effectively increases testosterone levels and prevents sperm agglutination. This antioxidant, which breaks the chain reaction, is essential because it accounts for up to 65% of the total antioxidant capacity in seminal plasma, as this antioxidant is present both inside and outside cells, and water-soluble vitamin C has a limited ability to resist lipid-loving free radicals found in the fatty parts of membranes and lipoproteins due to its presence in an aqueous medium; vitamin C react rapidly with)O2- and HO2•(, even more rapidly with)OH•(, leading to the formation of hydroascorbate (**Pahune et al., 2013**). Vitamin C acts as an antioxidant through synergistic interaction with tocopherol, even in the presence of continuous oxidation, and it is believed that vitamin C plays a crucial role in replenishing the body's vitamin E stores through interaction with tocopherol radicals (**Strain & Mulholland, 1992**). It has been conclusively demonstrated that vitamins C and E are highly effective in counteracting the effects of free radicals, and it is widely known that vitamins E and C have a synergistic relationship, vitamin E, as the primary antioxidant, produces radicals that interact with vitamin C to convert NADP-dehydroascorbate to ascorbate (**Caruso et al., 2024**).

4-3: Third: The Effect of Vitamin C on Oxidative Stress Markers (MDA, SOD, CAT, GSH)

Our study showed a significant improvement in antioxidant levels after vitamin C treatment in both the medium-dose (250) and high-dose (400) groups. This is consistent with **Yin et al. (2018)**, who found that taking vitamin C supplements at a dose of 500 mg daily for 8 weeks increased serum vitamin C concentration by approximately 50%. It was observed that lymphocytes in individuals who took vitamin C supplements showed increased activity of SOD and catalase enzymes, and there was no significant increase in the activity of SOD and catalase enzymes, or in the levels of HSP60 and HSP70 proteins in the lymphocytes of these individuals in response to hydrogen peroxide. After taking vitamin C supplements, the level of HSP70 protein in the muscle rose to baseline levels, with no further increase after exercise. We conclude that the adaptive responses to oxidants are less effective in individuals taking vitamin C supplements, but this may reflect an increase in the baseline expression of potential protective systems against oxidative stress (SOD, catalase, and heat shock proteins). The study was consistent with (**Aly et al., 2010**) treating mice with chlorpyrifos poisoning with vitamin C resulted in a decrease in lipid peroxide levels and GST activity, a decrease in catalase activity, superoxide dismutase, and glucose-6-phosphate dehydrogenase levels, while reduced glutathione (GSH) levels increased, it was concluded that vitamin C significantly reduces oxidative stress caused by the accumulation of chlorpyrifos in the livers of mice, and that the protective effect of pretreatment with vitamin C was greater than the effect of subsequent treatment.

4-4: Fourth: The Effect of Vitamin C on Sperm Quality

Our study demonstrated that vitamin C intake plays a role in restoring sperm vitality in mice treated with vitamin C. Our findings are consistent with those of **Bouhadana et al. (2025)**, as vitamin C improves sperm quality by reducing oxidative stress and minimizing DNA damage, It also significantly increases sperm count, motility, and morphology, while protecting sperm cells from damage, taking daily vitamin supplements (such as 1000 mg) can improve semen characteristics. The study agreed with **Shabanian et al., (2017)** A study has shown that vitamin C, an antioxidant, positively affects the weight of the reproductive organs (testes and epididymis) and sperm characteristics such as count, morphology, motility, viability, and maturity, fanny and colleagues investigated the effects of vitamin C on men with infertility and found a significant reduction in the number of abnormally shaped sperm (**Vani et al., 2012**). Indicated **Aruwa et al., (2024)** that taking vitamin C, at high doses, led to a significant increase in testicular weight and levels of antioxidant enzymes (glutathione peroxidase and catalase), with improved fertility indicators such as sperm count, viability and motility in treated mice, histological analysis also showed an improvement in testicular structure in the vitamin C-treated groups.

Conclusion

This study revealed that vitamin C at therapeutic doses has a role in improving steroid hormone levels and the antioxidant system in the testes of mice, through mechanisms such as raising testosterone levels by increasing stimulation of the steroidogenesis pathway, activating FSH secretion from the pituitary gland, improving sperm motility and concentration, reducing oxidative stress in testicular tissues, protecting Leydig cells, and improving sperm quality, morphology, and motility, thus opening the door for broader studies using vitamin C for treatment and improving fertility.

Reference

Pawlowska, E., Szczepanska, J., & Blasiak, J. (2019). Pro-and antioxidant effects of vitamin C in cancer in correspondence to its dietary and pharmacological concentrations. Oxidative Medicine and Cellular Longevity, 2019(1), 7286737.

Alberts, A., Moldoveanu, E. T., Niculescu, A. G., & Grumezescu, A. M. (2025). Vitamin C: A Comprehensive Review of Its Role in Health, Disease Prevention, and Therapeutic Potential. Molecules (Basel, Switzerland), 30(3), 748.

Maharana, P. (2025). The Impact of Vitamin C on Collagen Integrity and Tissue Function. MED CAL SCIENCES, 125, 91.

Li, N., Zhao, G., Wu, W., Zhang, M., Liu, W., Chen, Q., & Wang, X. (2020). The Efficacy and Safety of Vitamin C for Iron Supplementation in Adult Patients With Iron Deficiency Anemia: A Randomized Clinical Trial. JAMA network open, 3(11), e2023644. xAa.

Okon, U. A., & Utuk, I. I. (2016). Ascorbic acid treatment elevates follicle stimulating hormone and testosterone plasma levels and enhances sperm quality in albino Wistar rats. Nigerian medical journal : journal of the Nigeria Medical Association, 57(1), 31–36.

Patani, A., Balram, D., Yadav, V. K., Lian, K. Y., Patel, A., & Sahoo, D. K. (2023). Harnessing the power of nutritional antioxidants against adrenal hormone imbalance-associated oxidative stress. Frontiers in Endocrinology, 14, 1271521.

Qazzaz, M. E., Abed, M. N., Alassaf, F. A., Jasim, M. H., & Alfahad, M. (2021). Insights into the perspective correlation between vitamin D and regulation of hormones: sex hormones and prolactin. Current Issues in Pharmacy and Medical Sciences, 34(4), 192-200.

Alwan, N. A. (2025). Acrylamide, Melatonin, Vit. C and Combination of its Effect on Adrenal Gland Functions in Mature Male Rats.

Santi, D., Crépieux, P., Reiter, E., Spaggiari, G., Brigante, G., Casarini, L., Rochira, V., & Simoni, M. (2020). Follicle-stimulating Hormone (FSH) Action on Spermatogenesis: A Focus on Physiological and Therapeutic Roles. Journal of clinical medicine, 9(4), 1014.

Vani, K., Kurakula, M., Syed, R., & Alharbi, K. (2012). Clinical relevance of vitamin C among lead-exposed infertile men. Genetic testing and molecular biomarkers, 16(9), 1001-1006.

Shabanian, S., Farahbod, F., Rafieian, M., Ganji, F., & Adib, A. (2017). The effects of Vitamin C on sperm quality parameters in laboratory rats following long-term exposure to cyclophosphamide. Journal of advanced pharmaceutical technology & research, 8(2), 73–79.

Aruwa, J. O., Bisong, S. A., Obeten, K., Etukudo, E. M., Timothy, N., Kureh, T. G., ... & Usman, I. M. (2024). The potential protective role of ascorbic acid against testicular toxicity induced by Fluoxetine in male Wistar rats. Journal of experimental pharmacology, 441-453.