ORIGINAL ARTICLE

Ascorbic acid treatment elevates follicle stimulating hormone and testosterone plasma levels and enhances sperm quality in albino Wistar rats

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ABSTRACT

Background: Infertility issues have been linked to the effect of oxidative reaction in the reproductive system. **Aim:** The effect of ascorbic acid, a naturally occurring antioxidant, on fertility parameters of male albino Wistar rats was studied. **Materials and Methods:** Eighteen albino Wistar rats weighed between 178 g and 241 g were used, randomly assigned into three groups. Group 1 was the control group; oral gavaged 5 ml of distilled water; Groups 2 and 3 were administered medium dose (250 mg/kg) and high dose of ascorbic acid (400 mg/kg), respectively; twice daily for 21 days. Blood samples were obtained by cardiac puncture, and blood serum was obtained for hormonal assay, and the testes were harvested for sperm analysis. **Results:** Follicle stimulating hormone levels significantly increased in the high-dose group as compared to both the control and medium dose groups. Luteinizing hormone levels in the medium dose group decreased significantly as compared to the control group. Testosterone significantly increased in both the medium- and high-dose groups as compared to the control group. Sperm motility increased significantly in the high-dose group as compared to both control and medium-dose groups. Percentage sperm concentration decreased significantly in the medium-dose group when compared to the control and increased significantly in the high-dose group as compared to the medium-dose group. For percentage normal morphology, there was a dose-dependent increase in the test groups when compared to control group. **Conclusion:** These results are indicative of a positive influence of ascorbic acid on male fertility modulators and may therefore, serve as a potential adjuvant treatment for male infertility cases.

Key words: Ascorbic acid, male reproductive hormones, sperm characteristics

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INTRODUCTION

The rising interest in fertility research is occasioned by the rising incidence of infertility cases, especially those attributed to male factors.[1,2] Several factors have been implicated as responsible for this adverse occurrence. Among the suspected factors include zenobiotics, chemical preservatives in food and beverages, and nutrient deficiency or overdosage.[3] A wide variety of foods are known to contain ascorbic acid.[3] Those notably rich in it include varieties of citrus fruits (orange and lemon), peaches, strawberries, banana, and grapefruits.[3] Other edibles that contain sufficient quantities of Vitamin C are cabbage, broccoli, cauliflower, leaf lettuce, tomatoes, potatoes, and beans. The estimated content is between 7 mg/100 g and 163 mg/100 g.[4] Considering the multiple and diverse sources of ascorbic acid, it implies that the chances of wide consumption across the regional or geographical divide are very high.

Dehydroascorbate is the primary form of Vitamin C that cross the basolateral membrane of the enterocytes and

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other cells.[5] Ascorbic acid is concentrated in isolated nerve terminals and synaptic vesicles concentrate it by active transport mechanism.[6,7] It is one of the essential vitamins in humans and primates as they lack L-glucono-γ-lactone oxidase, the enzyme, required in the last step of its synthetic pathway.[8] Ascorbic acid is a six carbon ketolactone synthesized from glucose.[9,10] It is reported to be abundant in many endocrine tissues[11,12] and plays a cardinal role in the regulation of adrenal and gonadal steroidogenesis.[13] High concentration of Vitamin C in the adenohypophysis has also been documented[14-17] because Vitamin C is present in high concentration in the pituitary gland, it has been hypothesized that it may play a significant role in the secretion of anterior pituitary hormones including follicle stimulating hormone (FSH) and luteinizing hormone (LH).[18] This release is said to be mediated by nitric oxide (NO) and is Ca^{2+} dependent phenomenon.[18]

The reproductive process in the males is pivoted by the development of normal and mature spermatozoa in the first instance, and FSH and LH are the key facilitators of these process. It therefore follows that, any substance that could alter their synthesis and secretion could also alter the process of spermatogenesis in the same direction.

Testosterone is also known to play a major role in spermatogenesis, and its production by the Leydig cells is mainly regulated by LH. It can therefore be logically inferred that, both FSH and LH may play an indispensable role in the production and maturation of spermatozoa and by implication could serve as a key determinant of both quantity and quality of sperm.

Hyperprolactinaemia inhibits the pulsatile secretion of the gonadotropin-releasing hormone that causes decreased pulsatile release of the FSH, LH, and testosterone which in turn causes spermatogenic arrest, impaired sperm motility, and altered sperm quality.[19] There are many studies suggesting that hyperprolactinemia has a definite role in male infertility and is one of the reversible causes of infertility.[8,10-12]

Procedures for the assessment of male fertility basically revolve around the estimation of sperm quantity and quality.[20] Parameters usually assayed for male fertility status, therefore, include hormonal assay (FSH, LH, prolactin, and testosterone), the volume of sperm, sperm concentration, sperm motility, and morphology. All these parameters are assessed based on an approved standard ranges.[20] The new normal values are based on men who took 12 months or less to help conceive a child.[21]

In view of the observations that Vitamin C forms a major dietary component globally and its proposed influence on gonadotropin biosynthesis and secretion, this study was, therefore, designed to further investigate if there could exist a possible correlation between the reproductive hormonal effect of ascorbic acid and sperm characteristic vis-à-vis their key role in male fertility.

MATERIALS AND METHODS

Eighteen male albino Wistar rats were used for this study. The animals were randomly assigned to one of three groups such that each group had six animals. After 14 days of acclimatization, oral administration of ascorbic acid extract to Groups 2 and 3 commenced. Group 1 served as the control group fed with normal rat chow (feed) and 10 ml/kg of distilled water. Group 2 was treated orally with a medium dose of ascorbic acid (250 mg/kg). Group 3 was treated orally with a high-dose of ascorbic acid (400 mg/kg)). With an oral cannula, these doses were administered twice daily for 21 days to the animals. All animals had access to water *ad libitum*. The animals were sacrificed after 21 days. All experiments were examined and approved by the Ethical Committee of the University of Uyo on Animal Research and had therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

The harvested testes were crushed into pieces, diluted in 5 ml of normal saline, and allowed for 5–10 min to enable the spermatozoa to spread out into the diluents solution. A volume of 1 ml of supernatant was diluted in 100 ml solution, about 0.01ml of the suspension was loaded into a charged Neubauer counting chamber and coverslipped. It was allowed to rest for 10 min and observed microscopically. The number of cells was counted in millions/ml.

A volume of 1 ml of seminal fluid about was diluted with 20 ml of buffered formol saline, and then 0.01 ml of the solution was loaded on a grease free slide with cover slip and viewed under a microscope and the followings were observed: Tail defect, neck defect, mid-piece defect, head defect, and percentage normal morphology were determined.

A volume of 1ml of seminal fluid was diluted with 20 ml of buffered formol saline, and 0.01 ml of the solution was loaded on a grease free slide and covered with a coverslip and observed microscopically.

The FSH-reverse transcriptase (RT), LH-RT, and prolactin-RT each is a one-step immunoassay, based on the principle of sandwich method. The assay system utilizes a high affinity and specificity monoclonal antibody (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact FSH, LH, and prolactin molecule. The test sample is allowed to react simultaneously with two antibodies, resulting in the FSH, LH, and prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies.

After incubation, the wells were washed with washing solution to remove unburned labeled antibodies. Tetramethylbenzidine substrate is added and incubated, resulting in the development of a blue color. The color development is stopped with the addition of stopping reagent, changing the color to yellow. The concentration of FSH, LH, and prolactin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Testosterone level is determined using competitive microplate enzyme immunoassay. Plates are coated with anti-testosterone antibodies. Calibrator specimen is first added to microplate well. Enzyme-testosterone conjugate is added. Testosterone present in the sample competes with enzyme-testosterone conjugate for building with anti-testosterone counted microplate to form antigen-antibody complex. Unbound conjugate is removed by washing. The enzyme activity in the antibody-bound fraction is inversely proportional to the native testosterone concentration. The enzyme activity is revealed by color change in tetramethylbenzidine substrate solution.

All results were presented as mean ± standard error of mean (SEM). Three sets of data were analyzed using one-way ANOVA, followed by the least significant difference procedure for significant F values $P < 0.05$ were considered significant. Data analysis was done using the statistical software package SPSS for windows version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Mean values of FSH levels in control, medium- and high-dose groups were mean ± SEM: 0.10 ± 0.00, ±0.10, ±0.00, and ± 0.37, ±0.11, respectively. There was a significant increase in FSH level in high-dose group as compared to control and medium-dose group ($P < 0.05$) [Figure 1].

Mean values of LH levels in control, medium- and high-dose groups were mean ± SEM: 0.18 ± 0.02, 0.12 ± 0.02, and 0.17 ± 0.02, respectively. There was a significant increase in LH level in medium-dose group when compared with the control group ($P < 0.05$) [Figure 2].

Mean values of prolactin levels in control, medium- and high-dose groups were mean ± SEM: 0.12 ± 0.02, 0.11 ± 0.01, and 0.12 ± 0.01, respectively. There was no significant difference in prolactin level any of the groups [Figure 3].

Mean values of testosterone levels in control, medium- and high-dose groups were mean ± SEM: 6.45 ± 1.10, 25.27 ± 7.99, and 30.93 ± 8.12, respectively. There was a significant dose-dependent increase in both medium and high doses ($P < 0.05$) [Figure 4].

Mean values of fast progressive movements (motility) in control, medium- and high-dose groups were mean ± SEM: 71.67 ± 2.79, 74.33 ± 1.48, and 78.33 ± 1.05, respectively. There was a significant increase in the high-dose group when compared to the control and medium-dose group ($P < 0.05$) [Figure 5].

Mean values of percentage sperm concentration in control, medium- and high-dose groups were mean ± SEM:

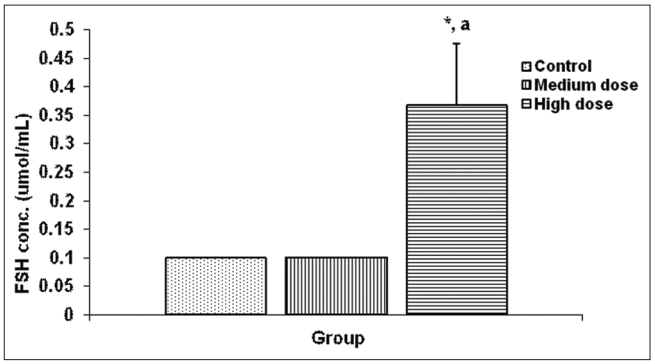

Figure 1: Comparison of follicle stimulating hormone levels in different experimental groups treated with ascorbic acid

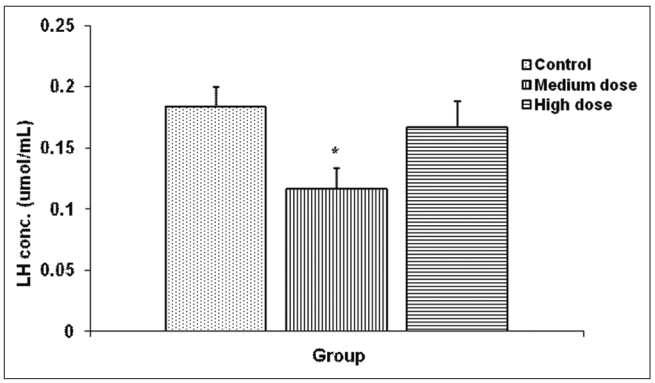

Figure 2: Comparison of luteinizing hormone levels in different experimental groups treated with ascorbic acid

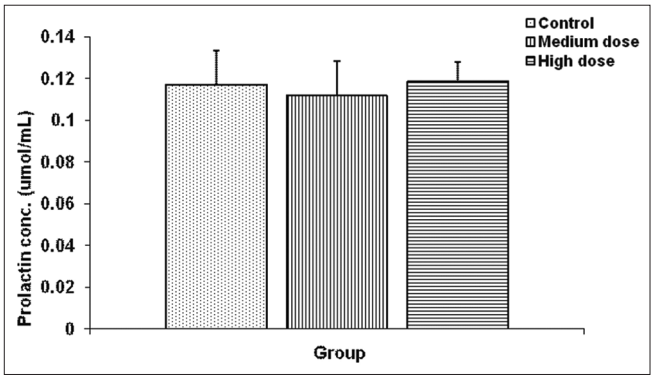

Figure 3: Comparison of prolactin levels in different experimental groups treated with ascorbic acid

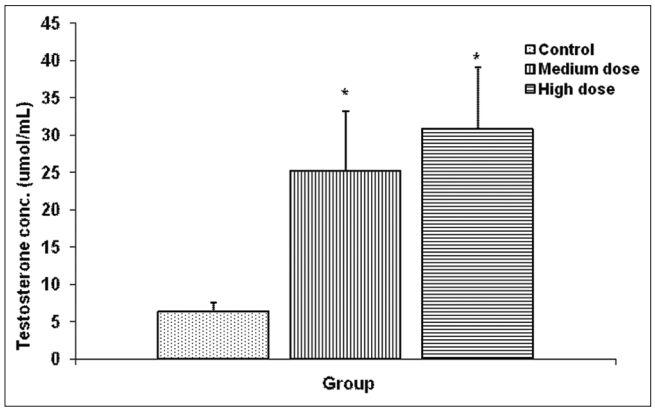

Figure 4: Comparison of testosterone levels in different experimental groups treated with ascorbic acid

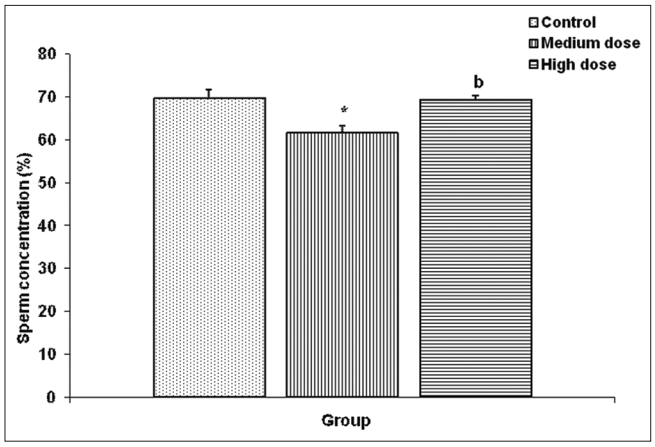

Figure 6: Comparison of percentage sperm concentration in different experimental groups treated with ascorbic acid

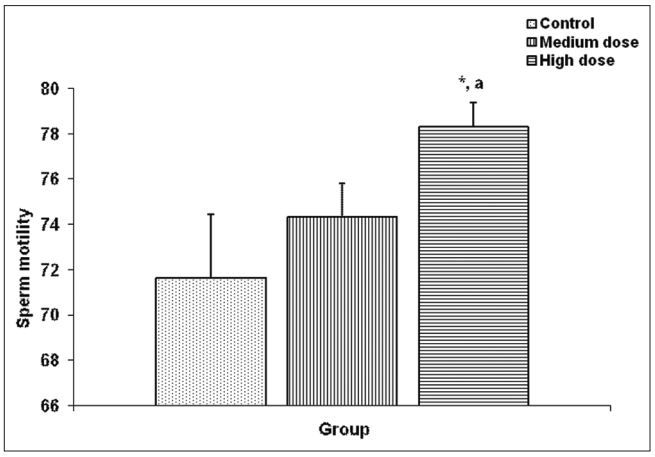

Figure 5: Comparison of fast progressive movement of sperm in different experimental groups treated with ascorbic acid

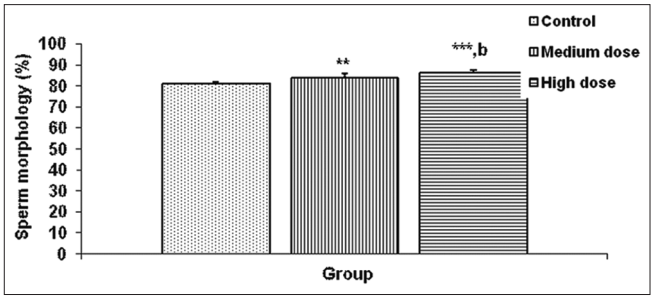

Figure 7: Comparison of percentage normal morphology in different experimental groups treated with ascorbic acid

69.67 ± 2.08, 61.67 ± 1.65, and 69.33 ± 0.92, respectively. There was a significant decrease in the medium-dose group to control ($P < 0.05$) and a significant increase in the high-dose group when compared to the medium-dose group ($P < 0.01$) [Figure 6].

Mean values of percentage normal morphology in control, medium- and high-dose groups were mean ± SEM: 81.33 ± 0.56, 84.33 ± 0.56, and 86.67 ± 0.42, respectively. There was a significant increase in percentage normal morphology in the medium dose group compared to control group ($P < 0.01$), a very significant increase in the high-dose group to control group ($P < 0.001$), and a significant increase in the high-dose group to medium-dose group compared ($P < 0.01$) [Figure 7].

DISCUSSION

Results obtained from this study indicate a positive influence of ascorbic acid oral administration on male fertility modulators. A dose-dependent increase was observed for FSH and testosterone. There was also a dose-dependent improvement in sperm motility and morphology. Previous reports have postulated an acute effect of ascorbic acid on the secretion of FSH and LH from the adenohypophysis.[18] However, in our study, only the FSH level was increased, but previous evaluation tends to equate LH secretion with that of FSH. For instance, the effect of ascorbic acid on basal and stimulated release of luteinizing hormone-releasing hormone (LH-RH) from medial basal hypothalamic explants produced by high potassium medium and N-methyl-D-aspartic acid has been reported.[22] It was noted that sodium nitroprusside, which spontaneously releases Nitric oxide (NO) stimulated LH-RH release and inhibition of NO release by a competitive inhibitor of NO synthase (NOS), N-monoethyl-L-arginine (NMMA), inhibited release of LH-RH. This result demonstrated that ascorbic acid had no effect on basal release of LH-RH-induced by the stimulants. Ascorbic acid was then proposed to act as a cotransmitter, released with classical transmitters from synaptic vesicles that act to reduce the NO formed, thereby providing feed-forward inhibitory control over LH-RH release.

Our results present the possibilities that there may exist different isoforms of LH-RH and FSH-releasing

hormone (FSH-RH) on the one hand. An alternative hypothesis may be that since testosterone level was significantly increased; this could trigger a feedback signal to the LH-gonadotrophs that inhibited further LH secretion. We may then propose that both FSH and LH were equally elevated via different mediators, only for the negative feedback signal of rising testosterone to oppose further LH increase. This imposes a challenge for us to now find the missing link which we propose to be an FSH-RH, quite different from LH-RH and their specific receptors. It has been documented that NO stimulates cAMP production from Gs protein coupled guanylate cyclase in the pituitary.[23] It is therefore possible that, ascorbic acid induced LH-RH and FSH-RH release with consequential gonadotropin (FSH and LH) secretion is mediated by NO. NO is formed from L-arginine by the enzyme NOS.[24-28] There are two forms of NOS, a calcium-independent inducible form induced by bacterial lipopolysaccharide and cytokines such as interleukin-1 and two calcium-dependent constitutive forms found in vascular endothelial cells and neurons.[24,26-28] Both forms of NOS require L-arginine as the substrate and are inhibited by L-arginine analogs such as NMMA.[26,27]

Karanth *et al*.[18] had described ascorbic acid as a vitaminergic transmitter that activates the release of both FSH and LH from the adenohypophysis on the presumption that ascorbic acid is stored in the secretory granules that also contain LH and FSH and is co-released with FSH and LH by exocytosis. Once ascorbic acid is outside the cell membrane, it is transmitted by means of ascorbic acid transporter into the cells.[29] Once inside the cell, it induces an increase in intracellular Calcium (Ca^{2+}) that combines with calmodulin and activates NOS which in turn releases NO.

The enhanced sperm motility in our study is consistent with earlier report by FertilAid (2010).[30] It was reported that ascorbic acid prevents clumping of semen, thereby increasing the ability of the sperm to swim. Ascorbic acid is thought to prevent this clumping by preventing the oxidation of nonspecific sperm agglutinin (NSSA) and the reduced form of NSSA, which binds to sperm to act as a nonstick coating,[31] thus increasing sperm motility. The reduction in sperm concentration in the medium-dose group of experimental rats is a rather "curious" occurrence as every related index assessed in this study favors the reversed. The closest explanation is the rare FSH-receptor gene mutation theory with subsequence impairment of spermatogenesis.

There was improved percentage sperm morphology attributed to the antioxidant activity of ascorbic acid. The reactive oxygen species that would otherwise cause spermatocyte DNA fragmentation[32] was effectively mobbed up by ascorbic acid. Ascorbic acid treatment within the duration and dosage applicable in this study did not appear to have a significant activity on all the different cell types of the adenohypophysis as seen in prolactin.

CONCLUSION

Ascorbic acid treatment appears to improve key fertility hormones in male rats and also enhance the sperm quality. The underlying mechanism seems to be mutually re-enforcing for both indices, with FSH-RH as a missing element to be identified and isolated in the nearest future. There is a need for clinical trial of ascorbic acid as adjuvant therapy in the cases of male infertility if this result in rats could be extrapolated to human bearing every other limitation of this study.

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Conflicts of interest
There are no conflicts of interest.

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