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Research Article

Impairment of sperm characteristics unrelated to hormonal alterations in rats treated with cimetidine

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ABSTRACT

Background: Cimetidine has been reported to possess clinically significant anti androgen activity at high dosage. This study was therefore designed to investigate the effect of medium and high dosages of cimetidine on fertility parameters including hormonal components in male wistar albino rats.
Methods: Eighteen male wistar albino rats were used, and were randomly separated into three groups of 6 rats each. Group one served as control and was gavaged 5ml of distilled water, group II (medium dose) received 475 mg/kg and group III (high dose) received 950 mg/kg of cimetidine respectively twice daily for 21 days. On the 22nd day blood samples were obtained by cardiac puncture; blood serum was obtained for hormonal assay and the testes were harvested for sperm analysis.
Results: FSH level was significantly higher in the medium dose group when compared to the control group. Sperm motility was significantly lower in both medium dose and high dose group when compared to the control group. Percentage sperm concentration was significantly lower in both medium dose and high dose group, this decrease was not dose dependent. Percentage normal sperm morphology was significantly lower in both medium dose and high dose group. Normal sperm morphology was significantly lower in high dose group when compared to medium dose group.
Conclusions: This result is suggestive of a deleterious effect of cimetidine on sperm characteristics with minimal impact on the hormonal component.

Keywords: Cimetidine, Sperm characteristics, Male fertility hormones

INTRODUCTION

Cimetidine is an H_2-receptor antagonist that inhibits the action of histamine on the acid producing cells of the gastric mucosa, thus reducing hydrochloric acid secretion. Cimetidine has been found to possess clinically significant anti androgen properties at high dosage.[1] It is reported to directly antagonize the binding of testosterone and dihydrotestosterone to the androgen receptors in animals.[1]

Cimetidine is commonly used in the treatment of duodenal and gastric ulcers, gastroesophageal reflux disease (GERD), other pathological hypersecretory condition (e.g. Zollinger-Ellison disease), heart burn and in the prevention of upper gastrointestinal bleeding. A study has shown that after 12 months administration of 150-950mg/kg of cimetidine on experimental animals, there was a significant reduction in the size of the prostate, testes and seminal vesicles especially in the high dosage groups.[2] Twelve months treatment of dogs with cimetidine at a dosage of 41-50 mg/kg resulted in reduction in prostate weight.

Both human and animal studies have shown that cimetidine crosses the feto-placental barrier, with high

risk of teratogenicity.[2] Prolonged and regular use of cimetidine has been reported to increase prolactin levels. Increase plasma prolactin levels may cause a decrease in Luteinizing Hormone (LH) and testosterone plasma levels with consequential complications including lower sperm count, decrease libido all of which may contribute to fertility compromise.[3] Also, in the testing that preceded registration of cimetidine with food and drug administration, testicular atrophy was indicated as an adverse effect but no mechanism of cimetidine action was demonstrated.[1]

Infertility rate seems to be on the increase globally. Male factors are thought to be the major cause of infertility in 30% of cases and to contribute to infertility in another 20%.[4] Acute infections in the reproductive tract of the male have long been recognized to interfere with sperm productivity, transport and longevity. Post pubertal mumps, for example, can result in testicular atrophy and fibrosis. The significance of chronic genital tract infections as causes of semen abnormalities and infertility, however, has not yet been resolved. There are still questions concerning symptoms, etiology and interpretation of positive and negative semen cultures.[5]

Several factors such as drugs, chemotherapy, toxins, air pollutants and stress have harmful effects on fertility.[6] Parameters commonly estimated during spermanalysis include sperm count, sperm morphology, sperm volume or concentration, fructose level and pH. Apart from infertility diagnosis, these analyses could also be employed to assess the success of contraceptive treatment in males. For instance, a vasectomy is considered successful if the subject sample is azospermic.[7] Obstructive azoospermia is ruled out by ensuring that up to at least 8 hours has lapsed since last ejaculation to time of sample collection.[8]

Fertility physiology in males as in females is initiated and executed by a profound hormonal orchestra. GnRH, FSH, LH, prolactin and testosterone exert a well-coordinated and regulated action to bring about the production and delivery of the male gamete, spermatozoa that is the key to a successful male reproductive accomplishment.

This study was conducted to assess the effect of cimetidine on the functional status of sperm and the accompanying hormonal changes in the same group of experimental animal with a view to determine any possible correlation between the findings in sperm status and hormonal variations. This may offer some insight to the possible mechanism by which cimetidine exerts its anti-fertility effect.

METHODS

Collection and identification of drug

The brand of cimetidine drug used in the cause of this research work was CIMEC 400 mg: cimetidine tablets B.P. 400 mg. manufactured by ZIM laboratories LTD. B-21/22 MIDC area Kalmes Kalmeshwar, Nagpur 441501. Manufacturing license no. 1224. Sole agent - Climax Pharmchem Ltd, Nigeria.

Animal preparation, experimental groupings and treatment

Eighteen male albino wistar rats weighing between 150-200 g were used for this study. The animals were randomly assigned to one of three groups such that each group had six (6) animals. After fourteen days of acclimatization, oral administration of cimetidine 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 cimetidine (475 mg/kg). Group 3 was treated orally with a high dose of cimetidine (950 mg/kg). With an oral cannula, these doses were administered twice daily for 21 days. All animals had access to water ad libitum. The animals were sacrificed on day 22. All experiments were examined and approved by the ethical committee of the University of Uyo on animal research and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

Sperm cell concentration

Testes were mashed into pieces, diluted in 5 ml of normal saline and allowed for 5-10 minutes to enable the spermatozoa to spread out into the diluents solution. 1ml of supernatant was diluted in 100 ml solution e.g. 0.1 of solution 1 + 5 ml of solution. 0.01 of the suspension was loaded into a charged Neubauer counting chamber and cover slipped. It was allowed to stand for 10 minutes and observed microscopically. Number of cells was counted in millions/ml.

Sperm morphology

1 ml of seminal fluid 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 following were observed: tail defect, neck defect, mid-piece defect, head defect and percentage of normal morphology was determined.

Sperm motility

1 ml 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 cover slip and observed microscopically.

Serum FSH, LH and prolactin measurements

The FSH-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 are washed with washing solution to remove unburned labeled antibodies. Tetra methyl benzidine substrate is added and incubated, resulting in the development of a blue colour. The colour development is stopped with addition of stopping reagent, changing the colour to yellow. The concentration of FSH, LH and prolactin is directly proportional to the colour intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Serum testosterone measurement

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 an 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 colour change in tetramethylbenzidine substrate solution.

Statistical analysis

All results were presented as mean ± standard error of mean. Three sets of data were analyzed using one way ANOVA, followed by the Least Significant Difference (LSD) procedure for significant F values, (P = 0.05) was considered significant. Computer software SPSS and excel analyzer was used for the analysis.

RESULTS

Comparison of FSH levels in experimental groups

Comparison of FSH levels in different experimental groups treated with cimetidine. Mean values of FSH levels in control, medium dose and high dose were, Mean ± SEM: 0.10 ± 0.00, 0.15 ± 0.02 and 0.13 ± 0.02 respectively. FSH level was significantly higher in the medium dose group (*P <0.05) when compared to control (Figure 1).

Comparison of LH levels in experimental groups

Comparison of LH levels in different experimental groups treated with cimetidine. Mean values of LH levels in control, medium dose and high dose were, Mean ± SEM: 0.18 ± 0.00, 0.18 ± 0.02 and 0.13 ± 0.02 respectively. There was no significant difference in LH level between the experimental groups (Figure 2).

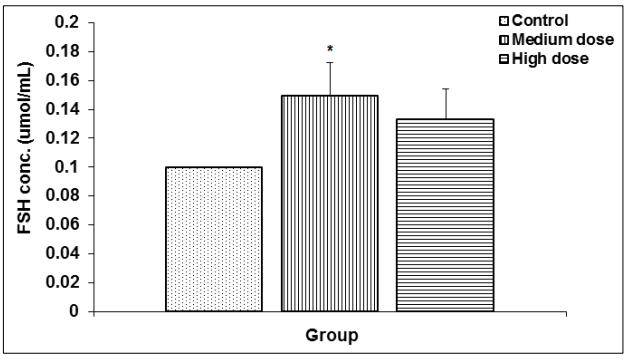

Figure 1: Comparison of follicle stimulating hormone (FSH) levels in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6. *P <0.05 vs. control

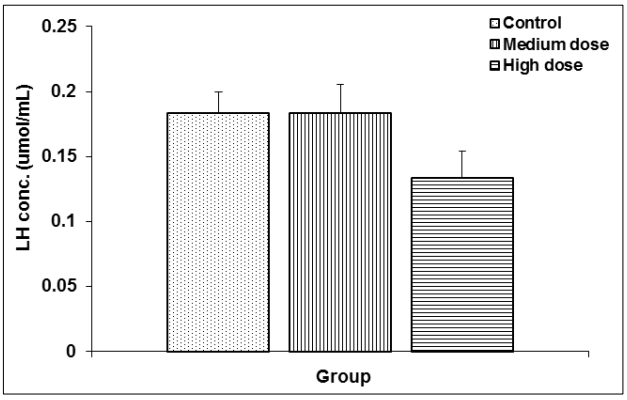

Figure 2: Comparison of luteinizing hormone (LH) levels in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6.

Comparison of prolactin levels in experimental groups

Comparison of prolactin levels in different experimental groups treated with cimetidine. Mean values of prolactin levels in control, medium dose and high dose were, Mean ± SEM: 0.12 ± 0.02, 0.17 ± 0.02 and 0.15 ± 0.02 respectively. There was no significant difference in prolactin level between the experimental groups, though a marginal increase in prolactin level was observed in the medium dose group (Figure 3).

Comparison of testosterone levels in experimental groups

Comparison of testosterone levels in different experimental groups treated with cimetidine. Mean values of testosterone levels in control, medium dose and

high dose were, Mean ± SEM: 6.45 ± 1.10, 9.77 ± 1.59 and 7.30 ± 0.62 respectively. There was no significant difference in testosterone level between the experimental groups (Figure 4).

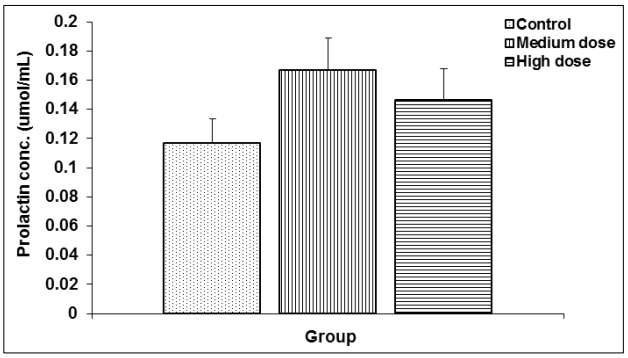

Figure 3: Comparison of prolactin levels in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6.

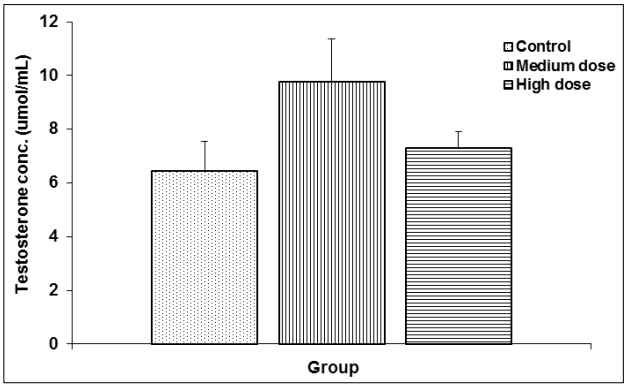

Figure 4: Comparison of testosterone levels in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6.

Comparison of sperm motility in experimental groups

Comparison of fast progressive movement of sperm (sperm motility) in different experimental groups treated with cimetidine. Mean values of fast progressive movement of sperm (sperm motility) in control, medium dose and high dose were, mean ± SEM: 71.67 ± 2.79, 55.00 ± 1.83 and 50.00 ± 1.83 respectively. Fast progressive movement of sperm (sperm motility) was significantly lower in both medium dose and high dose group (P <0.001) when compared to the control group (Figure 5).

Comparison of sperm counts in experimental groups

Comparison of percentage sperm concentration in different experimental groups treated with cimetidine. Mean values of percentage sperm concentration in control, medium dose and high dose were, Mean ± SEM: 69.67 ± 2.08, 59.33 ± 2.20 and 58.33 ± 1.48 respectively.

Percentage sperm concentration was significantly lower in both medium dose and high dose group (P <0.01) compared to control group (Figure 6).

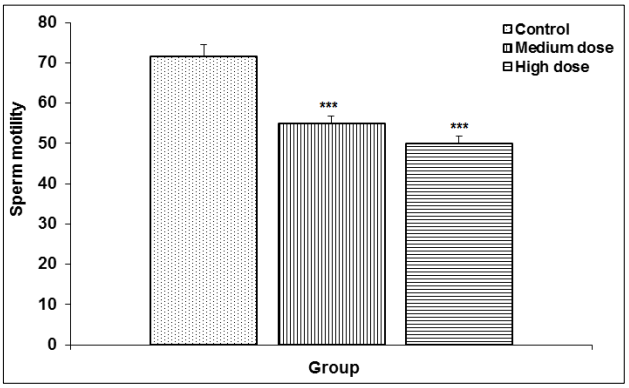

Figure 5: Comparison of fast progressive movement of sperm in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6. ***P <0.001 vs. control

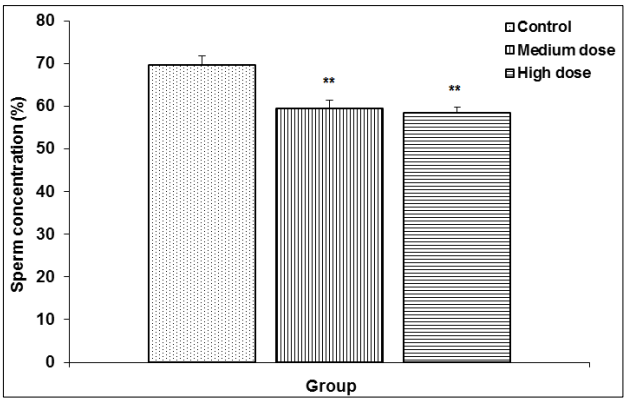

Figure 6: Comparison of percentage sperm concentration in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6. **P <0.01 vs. control

Comparison of sperm morphology in experimental groups

Comparison of percentage normal sperm morphology in different experimental groups treated with cimetidine. Mean values of percentage normal sperm morphology in control, medium dose and high dose were, Mean ± SEM: 81.33 ± 0.56, 62.67 ± 2.43 and 47.00 ± 1.93 respectively. Percentage normal sperm morphology was significantly lower in both medium dose and high dose group (P <0.001) when compared to the control group. Also percentage normal sperm morphology was significantly

lower in high dose group (P <0.001) compared to medium dose group (Figure 7).

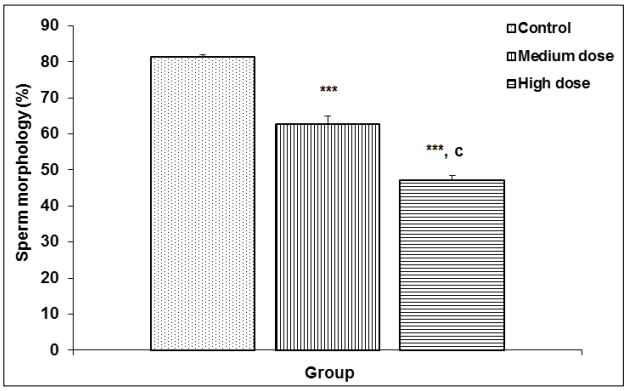

Figure 7: Comparison of percentage normal morphology in different experimental groups treated with cimetidine.

Values are expressed as mean ± SEM, n = 6, ***P <0.001 vs. control; c= P <0.001 vs medium dose

DISCUSSION

The present study showed that the FSH level was significantly higher in the medium dose group when compared to the control group. Fast progressive movement of sperm (sperm motility) was significantly lower in both medium dose and high dose group, this decrease was not dose dependent when compared to the control group. Percentage sperm concentration was significantly lower in both medium dose and high dose groups; this decrease was not dose dependent when compared to the control group. Percentage normal sperm morphology was significantly lower in both medium dose and high dose group, this decrease was not dose dependent when compared to the control group. Also normal sperm morphology was significantly lower in high dose group when compared to medium dose group.

FSH is known to regulate development, growth, pubertal maturation, and reproductive processes of the body. FSH and LH act synergistically in reproduction. Specifically, an increase in FSH secretion by the anterior pituitary causes follicular maturation. In this study, FSH level showed a significant elevation in the medium dose group. Similar results have been reported by Wang, et al., (1982).[9] Using single blood samples, others have found no significant change in basal LH and FSH[10-12] during cimetidine therapy. Increase in FSH has been reported to occur in patients with severely impaired spermatogenesis.[13-15] Also FSH stimulates primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes. FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes,[16] and is critical for the initiation of spermatogenesis. Therefore cimetidine being a weak anti-androgen may probably competitively block FSH from binding to its receptor and as a result the action of Sertoli cells is inhibited which will in turn adversely affect spermatogenesis. An alternative explanation of the rise of FSH without increase in LH[17] could be related to the slightly inhibition of spermatogenesis as evidenced by decreased sperm counts observed in this study.

Serum LH showed no significant difference between experimental groups, rather a marginal decrease in the high dose group was observed. It has been reported that using either integrated LH or a mean LH determined from multiple samples generally would provide a more accurate reflection of the secretion than single LH measurements.[18]

Marginal increase was observed in prolactin levels of both medium and high dose groups compared to control, there was no significant difference in the comparison of the prolactin levels in this study. However, with inference on the duration of cimetidine oriented therapy, it is generally observed that in most cases treatments lasts for more than a month, but this research work only lasted for 21 days. Probably with a longer duration of treatment, prolactin levels may increase significantly. The amount of prolactin can be an indicator for the amount of sexual satisfaction and relaxation.[19] Unusually high amounts are suspected to be responsible for impotence and loss of libido. Highly elevated levels of prolactin decrease the levels of sex hormones - estrogen in women and testosterone in men.[20] The non-uniformity of response after oral cimetidine can account for the discrepancies in prolactin levels reported in previous studies.[11,12]

The mechanism of action of cimetidine on prolactin secretion is not fully understood, although it has been suggested that it probably acts through mechanisms other than pituitary dopamine receptors.[11] It has been found that compared to un-mated males, fathers and expectant fathers have increased prolactin concentrations Nelson, (2005).[21] Prolactin levels peak during REM sleep, and in the early morning. PRL Levels can rise after exercise, meals, sexual intercourse, or minor surgical procedures - Melmed & Jameson (2005).[22] This fact is in line with the experimental procedures of this research work. How these proponents contributed to the result of our studies were not investigated.

Testosterone level in this study showed marginal increase in both medium dose and high dose compared to control. Similar result has been reported by Wang et al., (1982)[9] and Van Thiel et al., (1979).[10] Cimetidine has been hypothesized as a weak anti-androgen;[23] this anti-androgen activity may cause an increase in gonadotropin levels presumably by antagonizing the negative feedback control of gonadotropin secretion by androgens with subsequent increase in gonadotropin levels, elevations in testosterone concentration may occur.[24] Testosterone is necessary for normal sperm development. It activates genes in Sertoli cells, which promote differentiation of spermatogonia.[25] A second possible mechanism of cimetidine may be the result of chronic antagonism of H_2

receptors. Vascular smooth muscle cells of the body are generally known to be H_2 responsive.[26] And we believe those of the testis are likewise responsive although a report describing that peritubular cells possess H_2 receptors have not been confirmed. Apoptotic cell activities probably took place at the vascular smooth muscles of the testis on administration of cimetidine at high dose as seen in the marginal decrease in testosterone level in the high dose group.

CONCLUSION

Hence, this study reveals that cimetidine caused adverse effect on male fertility parameters (sperm count, motility, and morphology). These effects were not directly related to the hormonal alterations observed in this study. There may be need for caution when administering cimetidine for therapeutic purposes especially in males with or family history of fertility challenges.

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Conflict of interest: None declared
Ethical approval: The study was approved by the University of Uyo on animal research

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