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Differential effects of H2 receptor antagonists on male reproductive function and hepatic enzymes in Wistar rats

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Abstract
Histamine 2 (H_2) receptor antagonists are clinically very useful drugs for the treatment of peptic ulcers. We undertook this study to investigate the effects of therapeutic and double therapeutic dose levels of H_2 antagonists, cimetidine and ranitidine, on reproductive parameters and serum levels of hepatic enzymes in the Wistar rat. Cimetidine (30 or 60mg/kg/day), ranitidine (8 or 16mg/kg/day) and vehicle (1ml/kg/day) were administered to different animal groups (n=8) by gastric gavage for 14days. Animals were sacrificed and blood serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were measured. Epidydimal sperm and histological analyses of the testis were also performed using standard methods. Cimetidine and ranitidine had no significant ($p>0.05$) effects on ALP, AST and ALT levels. Ranitidine had no significant effect on all sperm and histological parameters evaluated. Cimetidine causedsignificant ($p<0.05$) and dose-dependent decreases in sperm count and motility, without an effect on sperm morphology and viability. Cimetidine also caused alterations in the histology of the testis. We conclude that subchronic administration of clinical dose levels of cimetidine may alter testicular function, possibly via direct adverse effects on the seminiferous tubules, while ranitidine may not. Cimetidine and ranitidine may not impair normal hepatic biochemical function.

Keywords: Cimetidine, histamine, spermatogenesis, spermatozoa.

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INTRODUCTION

Histamine is a monoamine synthesized from histidine exclusively by L-histidine decarboxylase in most mammalian tissues. The amine is widely distributed in the body and plays vital roles in several physiological processes. As described by Hough [1] and Falus et al., [2] most of the actions of histamine are mediated by histamine 1 (H_1), histamine 2 (H_2), and histamine 3 (H_3) receptors. Histamine 2 receptor antagonists bind competitively with H_2 receptors and inhibit most H_2 receptor-mediated histamine actions in the body, particularly parietal acid secretion in the stomach. Excessive parietal acid (HCl) secretion results in peptic ulcer disease- gastric and duodenal ulcers.

Peptic ulcer disease is a chronic disease and its treatment requires long period, in some conditions it may be lifelong. The disease is also highly prevalent in most parts of the world and affects a wide range of the population. [3,4] Histamine 2 receptor antagonists, which include cimetidine, ranitidine and famotidine, are clinically very important drugs. Histamine 2 receptor antagonists, especially cimetidine and ranitidine are widely prescribed for the treatment of peptic ulcer disease and they are also readily available without prescriptions. The drugs are also used to alleviate the symptoms of a number of other gastrointestinal diseases including, oesophagitis, dyspepsia, gastroesophageal reflux disease and Zollinger-Ellison syndrome. [5,6] Because of the high prevalence and chronic nature of peptic ulcer and other gastrointestinal diseases, H_2 receptor antagonists are widely and frequently used and over long periods of time. As a result of this, there is need for a regular evaluation of their safety profiles in biological systems. Additionally, because of the high sensitivity of the male reproductive system to therapeutic agents, [7-9] and the increasing rates of infertility among couples, [10,11] H_2 receptor antagonists become potential candidates for screening for reproductive toxicity in the male.

In earlier studies, cimetidine has been shown to cause testicular dysfunction, [12-15] however, most of such studies used relatively high doses over long periods of exposure, and not much is documented on such effects at clinical dose levels. In addition, most previous studies neither showed dose-related effects nor did comprehensive evaluation of sperm parameters. There is also a dearth of information on the reproductive effects of other H_2 receptor antagonists prior to this study. The present study investigated the effects of therapeutic and double therapeutic dose equivalents of cimetidine and its structural analogue, ranitidine on sperm parameters (sperm count, motility, morphology and viability) and histology of the rat testis. The study also evaluated the effects of both drugs on serum phosphatase and transaminase levels to assess their possible effects on liver function.

MATERIALS AND METHODS

Drugs

Cimetidine tablets (Medrel Pharmacy, India) and ranitidine tablets (Alpha Laboratory Limited, India) were purchased from the Department of Pharmacy, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. The drugs were administered as aqueous suspensions by oral gavage and continuously agitated during administration.

Experimental design

Forty male Wistar rats aged 20-21 weeks, weighing 200-300 g, obtained from the animal house of the University of Port Harcourt, Nigeria were used for all experiments. Animals were grouped into five (n=8), housed (12 hr light/dark cycle) and given access to rodent chow and tap water *ad libitum*. The animals were maintained in accordance with National Institutes of Health laboratory care standards. The experimental protocol was approved by the Committee for Ethics in Animal Experimentation of the University of Port Harcourt, Nigeria, which conforms with international standards. Drugs were administered according to standard regimens: [5] cimetidine, 30 and 60 mg/kg/day (in 2 divided doses) for 14 days; ranitidine, 8 and 16 mg/kg/day (in 2 divided doses) for 14 days; vehicle (distilled water), 1 ml/kg/day (in 2 divided doses) for 14 days by oral gavage. The animals were sacrificed by cervical dislocation under deep diethyl ether anesthesia and blood was collected by cardiac puncture for biochemical analysis. Testis was also removed from the animals for sperm and histopathological analyses.

Biochemical analysis

Blood samples were centrifuged for 15 min at 3,000 rpm and clear sera were separated from the cells and stored at −80°C. Serum was assayed for alkaline phosphatase (ALP) using phenolphthalein method. [16] Aspartate transaminase (AST) and alanine transaminase (ALT) levels were measured according to the method described by Reitman and Frankel. [17]

Sperm analysis

The testis was excised and the caudal epididymis was carefully isolated and placed in a Petri dish containing 3 ml of $NaHCO_3$ buffered Tyrodes's Lactate solution. Several (1 mm) incisions were made on it and sperm was gently drawn into a plastic transfer pipette and transferred into 5 mL test tubes and was then vigorously shaken for homogeneity and dispersal of sperm cells. Sperm was then analyzed to determine sperm motility, sperm count, percentage of abnormal

sperm cells (sperm morphology) and percentage of viable sperm cells (sperm viability) following standard procedures. [18]

Histopathological analysis

The testis was fixed in 10 % buffered formalin. The testicular tissues were embedded in paraffin and tissue sections (5 µm) were stained with hematoxylin and eosin (H&E), and examined with light microscope (Nikon Eclipse E400). All alterations from the normal structure were registered and histopathological changes between control and experimental animals were noted. The images were photographed with an Olympus Model BX51 microscope at magnification of 100x.

Statistical analysis

Data were expressed as mean±SEM. ANOVA tests were performed on data with GraphPad Prism 5 Software for comparisons. Statistical significance was set at $p < 0.05$.

Results

Biochemical parameters

The biochemical parameters measured were: alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT). There was no significant ($p > 0.05$) difference between serum levels of ALP, AST and ALT in cimetidine-treated animals and control (Table 1).

Dose (mg/kg)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Control	44.80±5.14	111.20±8.00	38.50±3.50
30	51.07±5.50	112.75±6.70	32.75±5.80
60	43.60±7.16	116.82±8.80	32.75±5.80

Table 1: Effects of 14 days administration of cimetidine (30, 60 mg/kg) on serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) in wistar rats.

Data expressed as mean ± SEM

Similarly, ranitidine-induced serum levels of ALP, AST and ALT were not significantly ($p > 0.05$) different from control (Table 2).

Dose (mg/kg)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
Control	44.80±5.14	111.20±8.00	38.50±3.50
8	50.00±6.61	102.50±7.85	33.20±4.13
16	45.50±4.16	106.00±9.34	39.82±5.00

Table 2: Effects of 14 days administration of ranitidine (8, 16 mg/kg) on serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) in wistar rats. Data expressed as mean ± SEM

Sperm parameters

The sperm parameters measured were: sperm count, sperm motility, percentage of abnormal sperm cells (sperm morphology) and percentage of viable sperm cells (sperm viability).

There was a significant ($p < 0.05$) and dose-dependent decrease in epididymal sperm count and motility in cimetidine-treated animal groups, compared to controls (Figures 1A and 1B). Total sperm counts obtained in animal groups that were given 30 and 60 mg/kg of cimetidine were 285.00±9.88 $x10^6$ and 235.20±10.50 $x10^6$, respectively, while the value in control group was 366.00±8.45 $x10^6$ (Figure 1A). These values were equivalent to 22.13 and 35.74 % decreases, respectively, compared to the control. Sperm motility obtained in cimetidine-administered animals was 82.50±4.86 and 63.10±4.40 %, respectively, while control motility was 85.50±7.20 % (Figure 1B). These values corresponded to 3.51 and 26.2 % decreases, respectively, compared to the control, but only the 60 mg/kg-induced sperm motility was significant ($p < 0.05$). In addition, the percentage of abnormal sperm cells (sperm morphology) obtained in animal groups that received cimetidine (23.20±5.50 and 29.80±5.21 %, respectively) were higher compared to the value obtained in control animals- 18.10±6.43 % (Figure 1C). These values were however, not significantly ($p > 0.05$) different from the control. There was also no significant ($p > 0.05$) difference between the percentage of viable sperm cells (sperm viability) obtained in cimetidine-treated animal groups and control (Figure 1D).

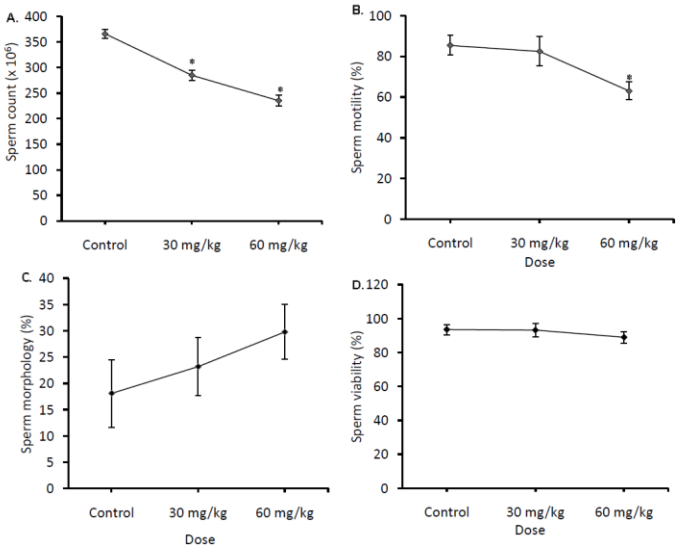


Figure 1: Effects of 14 days administration of cimetidine (30, 60 mg/kg) on:(A) sperm count,(B) sperm motility, (C) percentage of abnormal sperm cells (sperm morphology), and (D) percentage of viable sperm cells (sperm viability) in Wistar rats.

Data expressed as mean ± SEM. * $P < 0.05$.

Furthermore, sperm count, motility, morphology and viability values obtained in animal groups that were treated with ranitidine (4, 8 mg/kg) were not significantly ($p > 0.05$) different from controls (Figures 2A, 2B, 2C and 2D).

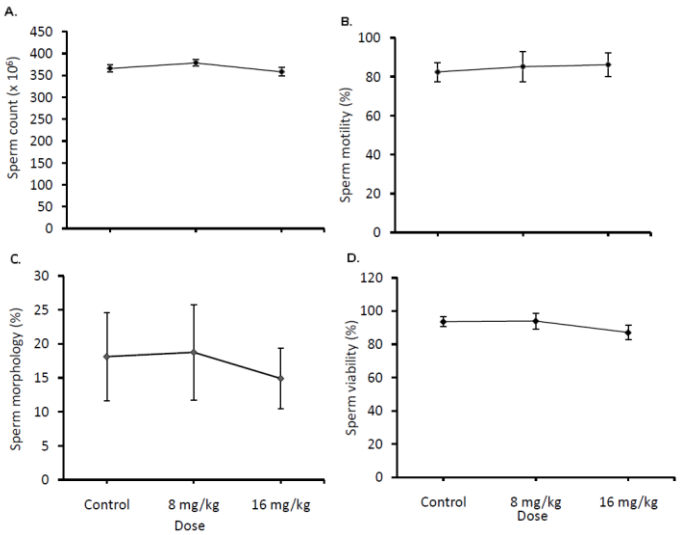
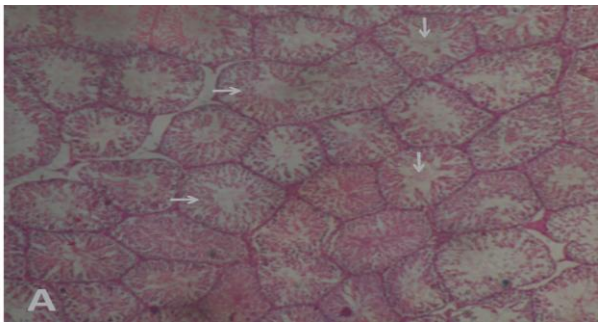


Figure 2: Effects of 14 days administration of ranitidine (8, 16 mg/kg) on:(A) sperm count,(B) sperm motility, (C) percentage of abnormal sperm cells (sperm morphology), and (D) percentage of viable sperm cells (sperm viability) in Wistar rats.

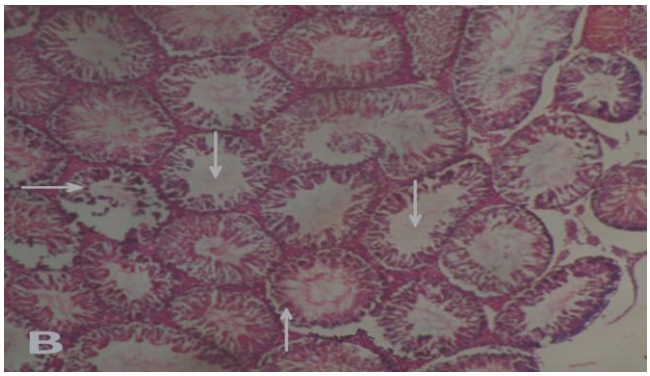
Data expressed as mean ± SEM.

Histopathology

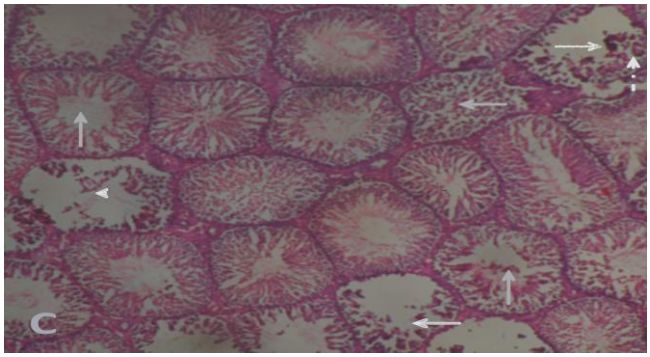
In different groups of animals (n=8), the effects of cimetidine (30, 60 mg/kg) and ranitidine (4, 8 mg/kg) on histopathology of the testis were investigated. Histopathological analysis of testis in the control group showed normal architecture of testis with normal seminiferous epithelium, normal spermatogenic cell differentiation, and numerous spermatozoa in the lumen (Figure 3A). Cimetidine-treated animal groups revealed mild degeneration of seminiferous tubules, vacuolization of spermatogonia, and mild reduction of sperm production at 30 mg/kg (Figure 3B); degeneration of seminiferous tubules with poor differentiation of spermatogenic germ cells, vacuolization of spermatogonia, maturation arrest at the level of secondary spermatocytes, and marked depression of spermatogenesis at 60 mg/kg (Figure 3C). Additionally, histopathological analysis of testis in the ranitidine-treated groups revealed normal architecture of testis with normal seminiferous epithelium and well differentiated germ cells at 4 mg/kg (Figure 3D) and 8 mg/kg (Figure 3E), compared to normal testis in the control group(Figure 3A).



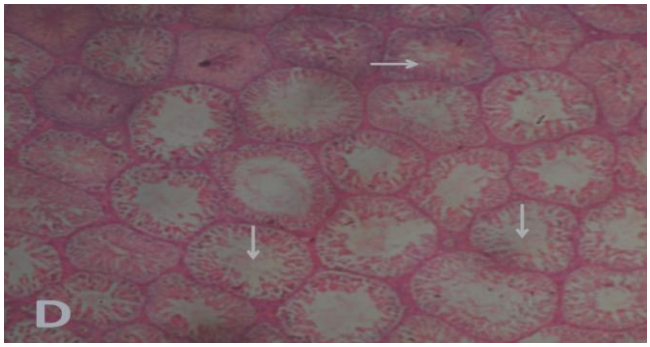
(Figure 3A) Control group



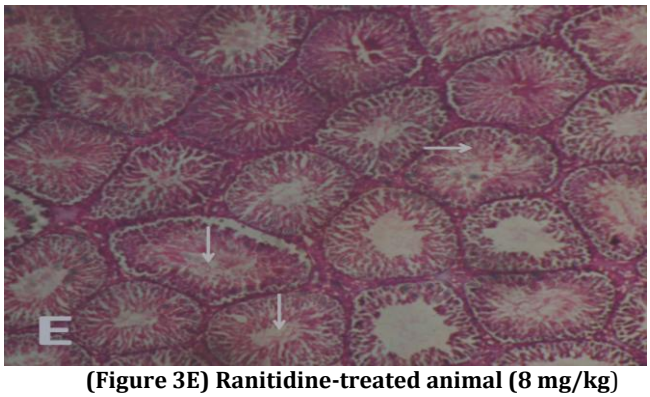
(Figure 3B) Cimetidine-treated animal (30 mg/kg)



(Figure 3C) Cimetidine-treated animal (60 mg/kg)



(Figure 3D) Ranitidine-treated animal (4 mg/kg)



(Figure 3E) Ranitidine-treated animal (8 mg/kg)

Figure 3: The effects of cimetidine (30, 60 mg/kg) and ranitidine (4, 8 mg/kg) on histopathology of the testis different groups of animals (n=8), (Figure 3A) control group; (Figure 3B) Cimetidine-treated animal (30 mg/kg); (Figure 3C) Cimetidine-treated animal (60 mg/kg); (Figure 3D) ranitidine-treated animal (4 mg/kg); (Figure 3E) ranitidine-treated animal (8 mg/kg);

Discussion

Cimetidine and ranitidine are H_2-receptor antagonists which are used in the treatment of gastric and duodenal ulcers. They treat ulcers by blocking the actions of histamine on H_2-receptors in the parietal cells in the stomach, thus inhibiting gastric acid production. Previous works have reported alteration of testicular function by chronic use of high doses (> standard dose levels) of cimetidine but little is known about its reproductive profile at therapeutic dose levels. The present study reports the effects of subchronic administration of normal therapeutic and double therapeutic dose equivalents of cimetidine and ranitidine on sperm parameters and histology of the testisin the rat, which is commonly used in reproductive toxicological studies.[19,20](Velez de la Calle et al., 1989; Morakinyo et al., 2009). The study also reports their effects on serum levels of phosphatase and transaminase enzymes.

Sperm is produced by the process of spermatogenesis in the seminiferous tubules in the testes and sperm parameters are usually the indices used to assess the functionality of spermatozoa. Concentration of sperm or sperm count is a vital property of sperm and male fecundity decreases progressively with reduction in sperm concentrations, especially below 40 million per mL in humans. [21] Sperm motility, on the other hand is a critical indicator of sperm quality and fertility potential. [18,22] The reduction in sperm count and sperm motility by cimetidine in this study thus suggests that cimetidine may cause impairment of tubular functions. Our result is consistent with previous reports. [14,23] Sperm morphology and viability are also important sperm parameters for the evaluation of male reproductive function. [24,25] Most previous investigators evaluated effects on sperm count and motility, without measuring sperm morphology and viability, making such studies not comprehensive. Over the dose range and duration used in this study, cimetidine may not affect spermatozoon's structural and membrane integrity as it caused an increase, but non significant effect on sperm morphology and viability. However, these effects may be significant at higher dose levels and/or longer durations of administration of cimetidine.

In addition, our observation of the alteration of seminiferous epithelium by cimetidine and non significant effect on testicular histology by ranitidine correlate positively with their effects on sperm parameters. The histological effects of cimetidine revealed suppression of spermatogenesis which may account for the low sperm count obtained. Similar results have been reported in previous studies but with higher doses of the drug. [15,26] Our observations were also dose-dependent, which most studies have not shown.

Serum levels of phosphatase and transaminase enzymes are commonly used as markers of hepatic function and increase in their levels is suggestive of hepatic damage. [27] In the present study, serum levels of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were unaffected by cimetidine or ranitidine administration. This indicates that cimetidine, ranitidine and probably other H_2 receptor antagonists may not affect normal biochemical functions of the liver over the dose range and duration used in this study. However, cimetidine inhibits hepatic microsomal enzymes (cytochrome p450) and results in the alteration of metabolism and plasma concentrations of most drugs, but this is not common with other H_2 receptor antagonists, including ranitidine. [28] Thus, there may be need for caution in the use of cimetidine, particularly in conditions of renal insufficiency and during coadministration with other drugs to avoid therapeutic failure or toxicity.

From our results, cimetidine alters male reproductive function, while, ranitidine has no adverse effect on the testis at clinical dose levels. Both drugs produce their pharmacological actions via competitive inhibition of H_2 receptor-mediated acid secretion in the stomach. [1, 2]. Recent studies have identified histamine as a paracrine testicular regulatory molecule in experimental animals, [29] and its inhibition is suspected to affect testicular function. In the present study, however, the testicular effects of cimetidine may not be H_2 receptor mediated, since ranitidine, which is a more potent H_2 receptor antagonist, [30,31] had no significant effect on the testis. This observation indicates that, although, cimetidine and ranitidine have same mechanism of antiulcer action, the drugs may have different mechanisms of toxicity. We suggest that the effects of cimetidine in this study may be due to direct adverse effects to tubular cells of the testis, which results in impairment of normal spermatogenesis.

CONCLUSION

Previous works have shown that chronic administration of high doses of cimetidine induces testicular toxicity. The present study shows that, even at clinical dose levels, cimetidine may cause alteration of testicular function, while ranitidine may have no effect on the testis in rats. In addition, both drugs may not affect hepatic enzymes activity. The testicular effects of cimetidine may be due to direct adverse effects on seminiferous tubules.

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