Assessment of reproductive toxicity profile of ramipril in male wistar rats

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ABSTRACT

Background: Several adverse effects have been associated with the long-time use of antihypertensive drugs in which caution should be taken when administering them.

Objective: To evaluate the effect of 28-days oral administration of ramipril on reproductive functions of male rats.

Methods: Twenty (20) male rats (120 - 150 g) were randomly divided into 4 groups of 5 rats per group and treated orally with distilled-water, 5 mg/kg, 10 mg/kg and 20 mg/kg of ramipril, respectively for 28-days. On day 29, 2 ml of blood samples were collected from the rats in each group through ocular puncture into clean test-tubes and centrifuged at 2500 rpm for 5 mins to obtain the serum for the analysis of testosterone and follicle stimulating hormone (FSH) levels, using enzyme-linked immunosorbent assay (ELISA) technique. All the male rats were later sacrificed by cervical dislocation and the testes removed along with the epididymis for semen analysis.

Results: Oral administration of ramipril produced a significant ($P<0.05$) decrease in levels of testosterone of the treated rats at all tested doses used and a non-significant ($P>0.05$) increase in Follicle Stimulating Hormone (FSH) at 5 and 10 mg/kg compared to the distilled water group. There was also a decrease in sperm count, sperm motility and sperm morphology of the treated male rats.

Conclusion: Based on the findings from this study, it can be stated that ramipril may adversely affect fertility as seen by decrease in testosterone levels, sperm count, sperm motility and sperm morphology in the treated rats.

Keywords: Reproductive functions, ramipril, semen quality, Follicle Stimulating Hormone, testosterone

Évaluation du profil de toxicité sur la reproduction du ramipril chez les rats Wistar mâles

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RÉSUMÉ

Contexte: Plusieurs effets indésirables ont été associés à l'utilisation prolongée d'antihypertenseurs et il convient de faire preuve de prudence lors de leur administration.

Objectif: Évaluer l'effet de l'administration orale de ramipril pendant 28 jours sur les fonctions reproductives des rats mâles.

Méthode: Vingt (20) rats mâles (120 - 150 g) ont été répartis au hasard en 4 groupes de 5 rats par groupe et traités par voie orale avec de l'eau distillée, 5 mg/kg, 10 mg/kg et 20 mg/kg de ramipril, respectivement pendant 28 jours. Le 29è jour, 2 ml d'échantillons de sang ont été prélevés sur les rats de chaque groupe par ponction oculaire dans des éprouvettes propres et centrifugés à 2 500 tr/min pendant 5 minutes pour obtenir le sérum destiné à l'analyse des niveaux de testostérone et d'hormone folliculo-stimulante (FSH) à l'aide de la technique de test immuno-enzymatique (ELISA). Tous les rats mâles ont ensuite été sacrifiés par luxation cervicale et les testicules ont été retirés ainsi que l'épididyme pour l'analyse du sperme.

Résultats: L'administration orale de ramipril a produit une diminution significative ($P<0,05$) des niveaux de testostérone des rats traités à toutes les doses testées utilisées et une augmentation non significative ($P>0,05$) de l'hormone folliculo-stimulante (FSH) à 5 et 10. mg/kg par rapport au groupe ayant reçu l'eau distillée. On a également constaté une diminution du nombre de spermatozoïdes, de leur motilité et de leur morphologie chez les rats mâles traités.

Conclusion: Sur la base des résultats de cette étude, on peut déduire que le ramipril peut avoir un effet néfaste sur la fertilité, comme en témoigne la diminution des taux de testostérone, du nombre de spermatozoïdes, de leur motilité et de leur morphologie chez les rats traités.

Mots-clés: Fonctions de reproduction, ramipril, qualité de sperme, hormone folliculo-stimulante, testostérone

INTRODUCTION

Hypertension, a critical global health condition is defined as chronic blood pressure elevation of 130/80 mmHg or higher.[1] It is one of the pathological conditions affecting about 1.28 billion adults aged 30 - 79 years worldwide with about 14 % having it under control.[2] It is a major risk factor for brain, kidney and heart diseases like stroke, heart attack, heart failure, aneurysm, renal dysfunction, dementia[3-4] and a major cause of early death.[5]

Management of hypertension is essential for preserving health and minimizing risk of dangerous cardiovascular complications; it can be through lifestyle adjustments and/or the use of antihypertensive medications such as diuretics, beta blockers, alpha blockers, calcium channel blockers, vasodilators, angiotensin receptor blockers, angiotensin converting enzyme (ACE) inhibitors.[6-7]

Angiotensin converting enzyme inhibitor is a class of medicines often prescribed for management of cardiovascular and kidney related diseases including hypertension, heart attack, heart failure and diabetic nephropathy.[8-9] Among the frequently prescribed ACEIs are benazepril, zofenopril, perindopril, trandolapril, captopril, enalapril, lisinopril and ramipril.

Ramipril, a white crystalline substance of the class of medicines called Angiotensin-converting enzyme (ACE) inhibitor is a dipeptide, carboxylic acid monoester that has minimal pharmacological activity until it is metabolized by carboxylesterase 1 via cleavage of its ester group into its active form ramiprilat in the liver.[10-11] It is a 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative soluble in polar organic solvents and buffered aqueous solutions,[12] it was patented in 1981 and approved to be used medically in 1989.[13]

Ramipril is a drug that is documented for the management of cardiovascular diseases. It is a drug of choice for the management of mild to severe hypertension, congestive heart failure and nephropathy and may be used to reduce the development of renal disease in people with hypertension, diabetes mellitus, microalbuminuria or overt nephropathy.[14] It may also be implicated in reducing the frequency of death, myocardial infarction and stroke in individuals prone to cardiovascular diseases.[15, 11]

Ramipril lowers blood pressure by antagonizing the effect of Renin-angiotensin aldosterone system (RAAS) via inhibition of the conversion of angiotensin I to angiotensin II; as a result it reduces inflammation and vasoconstriction, thus producing renoprotective, antihypertensive and cardioprotective effects.[14] It also increases bradykinin concentration which results in dilation of blood vessels and subsequently lowers blood pressure.[16]

In addition to the desired and/or needed effects, ramipril also causes some unwanted effects which include dry or tickly cough, headache, gastrointestinal disorder, postural hypotension, hyperkalemia, angioedema, liver problem and abnormal kidney function.[17, 10]

Toxicities or adverse effects of some antihypertensive drugs are well documented in literature. For instance,[18] reported the adverse effects of beta-adrenergic receptor blocker on spermatogenesis. Use of calcium channel blockers e.g. nifedipine, verapamil and diltiazem have also been reported to cause infertility by reducing sperm count and motility according to.[19] Similarly, a study by[20] revealed that oral administration of amlodipine produced harmful effect on the spermatological parameters of the treated male rats.

Several toxicological studies including tumerogenic, teratogenic and mutagenic activities of Ramipril have been conducted and reported in literature,[11] however, there is inadequate/paucity of information on the effect of the drug on hormonal profile and semen qualities; hence the current study which investigated the effect of oral administration of Ramipril on hormonal profile and semen quality of male rats.

METHODS

Drug and chemicals

ACE Inhibitor (Ramipril) tablets (5 mg per tablet) (May & Baker, UK), formalin and hematoxylin stain.

Drug reconstitution

Ramipril (5 mg) was dissolved in 5 ml of distilled water to give a concentration of 1 mg/mL. The dosage of ramipril used in this study was based on manufacturer's recommendation (5 mg per 70 kg).

Experimental animals

Adult male wistar rats ranging from 120-150 g purchased from the Department of Pharmacy Animal Center, Olabisi Onabanjo University, Sagamu Campus, Ogun State were used for this study.

All the animals were housed and maintained in the animal house of Department of Physiology, Babcock University,

Ilisan, Ogun state, Nigeria. The animals were fed and treated in accordance with the in-house guidelines for animal care.[21] Animals were kept for 14 days in other to acclimatize prior to the investigation. During this time, they were given rodent pellet and water ad libitum and were used with strict adherence to the Ethics Guidelines and Research Policy of Ahmadu Bello University, Zaria. Ethical Approval (ABUCAUC/2021/084) was obtained from the ABU Committee on Animal Use and Care.

Experiment design

Twenty (20) male rats (120-150 g) were randomly divided into 4 groups of 5 rats per group and then treated orally for 28 days as follows:

Group A: Distilled water (control group)
Group B: 5 mg/kg of Ramipril
Group C: 10 mg/kg of Ramipril
Group D: 20 mg/kg of Ramipril

At the end of the experimental period (day 29th), 2 ml of blood samples were collected via ocular puncture from five (5) rats in each group and placed into clean test tubes for the determination of hormonal profiles. All the animals were later sacrificed by cervical dislocation and the testes removed along with the epididymis for semen analysis.

Hormonal assay

The blood samples collected were centrifuged at 2500 rpm for 5 min at 10-25^{o}C to obtain the plasma for analyzing testosterone and follicle stimulating hormone (FSH) using enzyme-linked immunosorbent assay (ELISA) technique according to.[22]

Semen analysis

The testes were removed along with the epididymides. The caudal epididymides were then separated from the testes, blotted with filter papers and lacerated to collect the semen.

Evaluation of sperm motility: Semen samples from the different treatment groups were dropped on a glass slide and viewed under the microscope. A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 200 spermatozoa for each rat. The percentage of sperm motility was analyzed for progressive motile sperm (PMS), non-progressive motile sperm (NPMS) and non-motile sperm (NMS) distinguished by the movement of the sperm cells.[23]

Estimation of mean sperm count: This was carried out according to the method of.[24] The epididymal content was obtained by macerating with fine scissors known weights of the caput and caudal epididymides in a glass petri dish containing warmed buffered physiological saline in the ratio of 1:10 w/v. After vigorous pipetting, the suspension was separated from tissue fragments by filtering it through an 80 μm stainless mesh. A tissue-free aliquot was loaded into the Neubauer haemocytometer. Five different counts were done for each sample, and the mean were taken as the mean count for each male rat.

Sperm count = $\frac{\text{Total number of sperm cells in the cytometer}}{\text{Mean value}}$

Where; mean value = Five.

Sperm morphology: A fraction of each of the sperm suspension was examined by placing the solution (10:1) for 30 mins on a glass slide. The slide was examined for percentage abnormalities in every 200 spermatozoa observed on each slide and five air dried smear was prepared on glass slide for each sample according to.[24]

Sperm viability (Life/dead ratio): This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope using x400 magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated.[25]

Testicular histology: After removing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hours, 95 % alcohol for 2 hours, 100 % alcohol for 2 hours, 100 % alcohol for 2 hours and finally 100 % alcohol for 2 hours. The tissues were then cleared to remove the alcohol, the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten Paraffin wax for 2 hours in an oven at 57^{o}C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5 μm). The satisfactory ribbons were picked up from a water bath (50-55^{o}C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before

immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x 400 magnification.

Statistical analysis

The results obtained were expressed as mean ± standard error of mean (SEM), statistical analysis of the data was done using One Way Analysis of Variance (ANOVA), followed by Dunnett's post hoc test, differences were considered statistically significant at $p< 0.05$.

RESULTS

Effect of Ramipril on Hormonal profile

Ramipril administration produced a significant ($p< 0.05$) dose dependent decrease in testosterone level of the treated rats at all the tested doses used compared to distilled water group. However, the levels of follicle stimulating hormone in the treated rats increased non-significantly at 5 mg/kg and 10 mg/kg and decreased at 20 mg/kg relative to control group (Table 1).

Table 1: Effect of 28 days oral administration of ramipril on testosterone and Follicle Stimulating Hormone

Treatment groups	Testosterone	Follicle Stimulating Hormone
Distilled water	4.37 ± 1.65	0.95 ± 0.0041
5 mg/kg	1.40 ± 0.27*	0.98 ± 0.0071
10 mg/kg	0.17 ± 0.16*	0.98 ± 0.0071
20 mg/kg	0.93 ± 0.01*	0.95 ± 0.0075

Values are expressed as mean ± standard error of mean (SEM); *($p<0.05$) considered significant compared to the control group; n=5

Effect of ramipril on semen quality

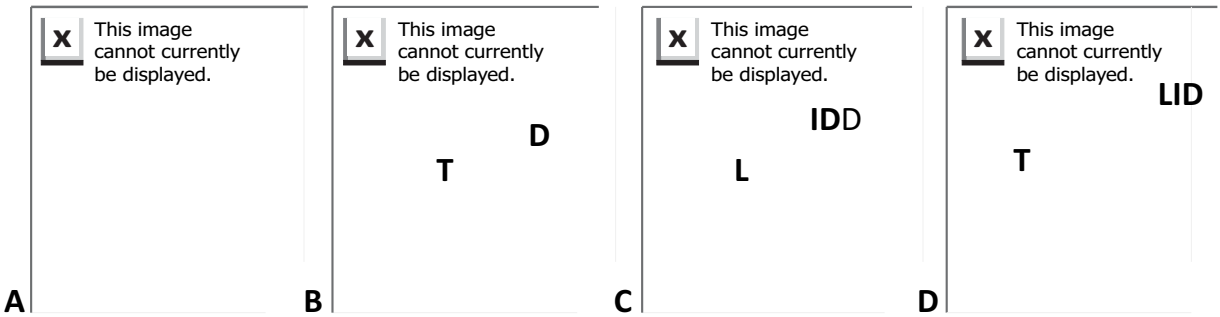
Treatment with ramipril at all doses induced a significant ($p<0.05$) reduction in sperm motility of the treated rats compared to the control group. There was also a non-significant dose dependent decrease in sperm count and an insignificant increase in the percentage of morphologically abnormal sperm cells of the treated rats. The sperm vitality of the treated rats increased at 10 mg/kg and 20 mg/kg dose of ramipril (Table 2).

Table 2: Effect of 28 days oral administration of ramipril on sperm parameters

Treatment groups	Sperm count	Sperm morphology	Sperm motility	Sperm viability
Distilled water	14.40 ± 5.60	4.00 ± 0.0	62.50 ± 32.5	85.00 ± 0.01
5 mg/kg	14.07 ± 1.62	3.75 ± 0.25	31.67 ± 4.41*	85.00 ± 0.01
10 mg/kg	11.95 ± 2.34	3.00 ± 0.71	31.25 ± 6.58*	90.30 ± 3.20
20 mg/kg	10.35 ± 2.82	3.00 ± 1.00	30.25 ± 5.25*	90.50 ± 3.80

Values are expressed as mean ± standard error of mean (SEM); *($p<0.05$) considered significant compared to the control group; n=5

Effect of Ramipril on Testicular Histology



A) Photomicrograph showing normal testicular tissue (x400), B) Photomicrograph showing distortion of the spermatogonia cells and thickening of sertoli cells and leydig cells (x400), C) Photomicrograph showing irregular distribution of spermatogonia cells and loss of Sertoli cells as well as the leydig cells (x400), D) Photomicrograph showing loss and irregular distribution of spermatogonia cells, thickening of seminiferous lumen and interstials lining of leydig cells and loss of sertoli cells (x400).

DISCUSSION

Ramipril, an ACE inhibitor is one of the drugs usually prescribed for the management of hypertension.[15] Several adverse effects including acute liver injury, abnormal kidney function, headache, blurred vision, chest pain, angioedema, low white blood cell count have been associated with long time use of antihypertensive drugs. Studies have shown that repeated doses of drugs may cause temporary or persistent infertility either by affecting pituitary gonadotropins; altering hormonal functions or damaging/triggering changes in sperm parameters such as sperm count, morphology and mortality.[26-27] It is therefore of utmost importance that men be cautious of the drugs taken by them as these can adversely affect fertility. This research work was conducted to evaluate the effect of oral administration of Ramipril on reproductive functions and semen quality of rats.

In this study, there was reduction in testosterone levels of Ramipril treated rats at all doses and an insignificant increase in FSH level at lowest doses used. Testosterone is the male key sex hormone necessary for the initiation and maintenance of spermatogenesis, erections, sex drive as well as the release of sperm.[28] Thus, its low level may lead to decrease production of sperm, reduced sex drive, erectile dysfunction and slow sperm release.

Hypothalamus and pituitary gland are known to control the production of testosterone by the testicles. The hypothalamus releases GnRH (gonadotropin-releasing hormone) that stimulates the pituitary gland to release

follicle stimulating hormone (FSH) which binds to its receptor to initiate spermatogenesis; and luteinizing hormone (LH) that helps in the production and release of testosterone from the gonads.[29-30] Reduction in the testosterone level produced by the oral administration of Ramipril may be due to the adverse effect of Ramipril on the testicles, hypothalamus or pituitary gland.[29,31] The reduction also suggests that ramipril may affect the mechanism involved in the production of the hormone in the leydig cells.

Oral administration of ramipril also produced a decrease in sperm count, sperm motility, sperm viability and sperm morphology of the male rats. Semen analysis involves the assessment of the shape, movement ability and concentration of sperm cells. Poor sperm quality (low sperm count, abnormal sperm shape and low sperm motility) can cause infertility in male.[28] The reduction in the sperm motility of ramipril treated rats may be due to the ability of the drug to penetrate the blood-testes barrier and alter the micro environment of the inner wall of the seminiferous tubules thereby affecting sperm production as reported by[32-33] in rats treated with *Azadirachta Indica* and losartan, respectively. An insignificant increase in the percentage of morphologically abnormal sperm cells coupled with decrease in sperm viability suggests the ability of ramipril to interfere with the process of sperm production in the seminiferous tubules.[33] Ramipril may also disrupt sperm epididymis epithelial cell formation or prevent calcium influx into the sperm as this is needed for sperm production, activation and movement.[34]

Studies have shown that damages to DNA, proteins and lipids of spermatozoa may have resulted from induction of oxidative stress in the testes. This may lead to decrease in sperm count and impairment of sperm function as production of reactive oxygen species (ROS) in the testes has been reported as one of the major reasons for testicular dysfunction and destruction of spermatogenesis which may lead to infertility.[35-36] This suggests that decrease in plasma level of testosterone, sperm count, sperm motility and sperm morphology observed in the study may be due to Ramipril inducing oxidative stress in leydig cells of the testes.

Photomicrograph of the testes revealed some pathological aberrations in the testicular tissue and this suggests the toxic effect of the ramipril on the testes of the treated rats.

CONCLUSION

Based on the findings from this study, it can be concluded that ramipril is capable of adversely affecting the reproductive ability of the treated rats, as testosterone is needed for the production and release of sperm. In addition, ramipril was observed to cause erectile dysfunction and reduced sex drive in the treated rats. This suggests that ramipril should be used with caution especially in expectant fathers.

ACKNOWLEDGEMENT

The authors thank Mr. Dashe Samuel of the Department of Physiology, Babcock University, Nigeria for rendering technical assistance and for allowing access to research facilities. The authors report that there are no competing interests to declare.

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