Research Article

Effect of Vasodilator (Apresoline) on Reproductive Parameters in Male Wistar Rats

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ABSTRACT

Twelve male Wistar rats (120 - 140 g) were divided into control (distilled water) and vasodilator-treated (0.36 mg/kg) groups (6 per group) for hormonal assay, andrological and histopathological studies. The animals were orally treated on daily basis for 50 days. Plasma testosterone level was assayed using Enzyme-linked Immuno-sorbent Assay (ELISA) and semen analysis was done microscopically. Histology of testes was also done. Data were analysed using descriptive statistics and ANOVA at p=0.05. Treatment of rats with vasodilator (0.36 mg/kg) caused significant ($p<0.05$) increment in testosterone level relative to control. Treatment of rats with vasodilator (0.36 mg/kg) caused significant ($p<0.05$) reductions in progressive sperm motility and sperm count relative to their respective controls. It can therefore be concluded that vasodilator probably have deleterious effect on the reproductive functions in male rats.

Keywords: Vasodilator, Testosterone, Sperm motility, Sperm count, Rats.

INTRODUCTION

Vasodilators are medications that dilate blood vessels. Vasodilators act directly on the smooth muscle of arteries to relax their walls so blood can move more easily through them; they are only used in hypertensive emergencies or when other drugs have failed, and even so are rarely given alone.

Effects of vasodilators on coronary flow and simultaneous release of nitric oxide from guinea pig isolated hearts has been reported [1]. Hemodynamic effects of vasodilators and long-term response in heart failure has been reported [2]. Renal effects of vasodilators in acute heart failure [3] as well as their effects on isolated human uteroplacental arteries [4] have also been reported.

However, due to scanty information from literature on the effect of vasodilator on reproductive parameters in male rats, this study therefore aims at investigating the effect of this antihypertensive agent on these aforementioned parameters in male rats.

MATERIALS AND METHODS

Experimental Animals

Adult male rats weighing between 120 – 140 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water; they were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Afe Babalola University Ethics Committee on guiding principles on care and use of animals.

Drug

Vasodilator (apresoline) tablets (Norvatis Pharma Ltd.) were bought from Danax Pharmacy, Ibadan, Nigeria.

Vasodilator (25 mg) was dissolved in 10 ml of distilled water to give a concentration of 2.5 mg/mL.

The dosage of vasodilator used in this study was in accordance with that recommended by the manufacturer.

Body Weight

Body weight was monitored on weekly basis throughout the duration of the experiment for each rat.

Experimental Design

Twelve male rats (120 – 140 g) were randomly divided into two groups, with each consisting of six animals. The two groups were subjected to the following oral treatments once a day for fifty (50) days:

Group I: received 0.5 mL/100 g of distilled water as control group.

Group II: received 0.36 mg/kg of vasodilator.

Collection of Blood Samples

Twenty four hours (day 51) after the last dosing of the groups, blood samples were collected from all the animals through the medial cantus for the determination of plasma testosterone levels. All the animals were later sacrificed by an overdose of diethyl ether and the testes were removed along with the epididymis for semen analysis.

Hormonal Assay

Plasma samples were assayed for testosterone using the enzyme-linked immunosorbent assay (ELISA) technique using the Fortress kit.

Semen Collection

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

Semen Analysis

Progressive sperm motility: This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27^{O}C) and two drops of warm 2.9% sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using x400 magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish, or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100)[5].

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 [6].

Sperm morphology: This was done by adding two drops of warm Walls and Ewas stain (Eosin/Nigrosin stain can also be used) to the semen on a pre-warmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope using x400 magnification [6]. Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of abnormal spermatozoa was expressed as a percentage of the total number of spermatozoa.

Sperm count: This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5mL formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5mL formol-salline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the Improved Neubauer haemocytometer under the microscope.

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 0.5cm 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 infilterated 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^{O}-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 x40, x100 and x400 magnifications

Statistical Analysis

The mean and standard error of mean (S.E.M.) were calculated for all values. Comparisons between the control and the treated groups were done using one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. Differences were considered statistically significant at $p<0.05$.

RESULTS

Effect on Body weight

Treatment of rats for fifty days with vasodilator (0.36 mg/kg) produced no significant ($p>0.05$) changes in body weight throughout the duration of treatment (Figure 1).

Effect on Plasma Testosterone Level

Treatment of rats for fifty with vasodilator (0.36 mg/kg) produced significant ($p<0.05$) increment in testosterone level relative to control (Figure 2).

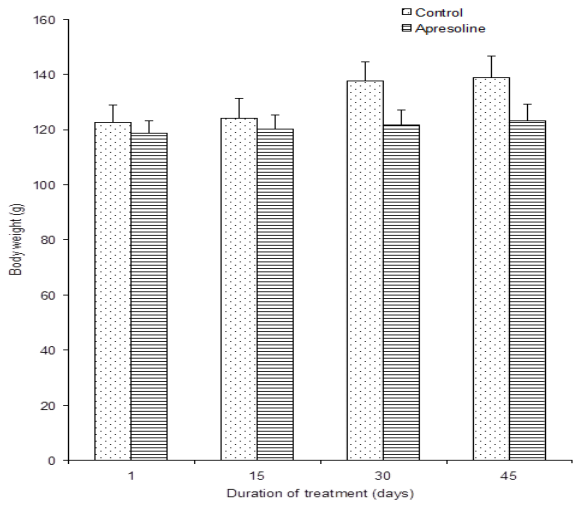

Figure 1: Body weight changes in control rats and rats treated with vasodilator for 50 days (n=6, *p<0.5)

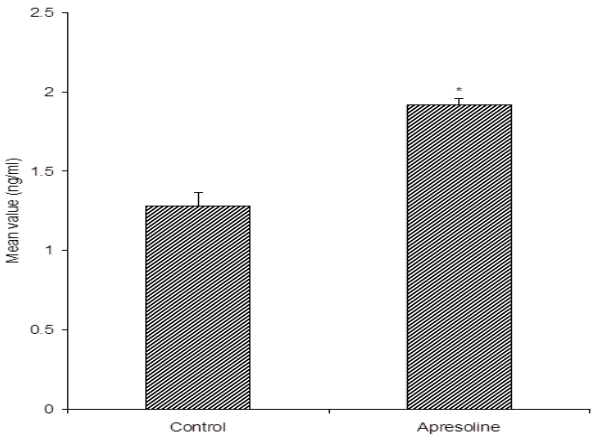

Figure 2: Effect of treatment of rats for 50 days with vasodilator on plasma testosterone level (n=6, *p<0.5)

Effect on Sperm Characteristics

The effect of 50 days treatment of rats with vasodilator (0.36 mg/kg) on sperm characteristics are shown in Figures 3 and 4.

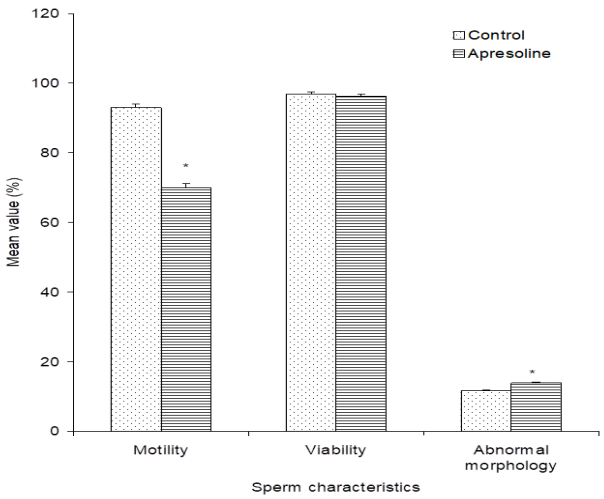

Figure 3: Spermogram showing the effect of 50 days treatment of rats with vasodilator on sperm characteristics (n=6, *p <0.05)

Treatment of rats with vasodilator (0.36 mg/kg) caused significant (p<0.05) reductions in progressive sperm motility, but produced no significant changes (p>0.05) in the percentage of viable sperms relative to their respective controls. Treatment of rats with vasodilator (0.36 mg/kg) caused significant (p<0.05) increase in the percentage of abnormal sperms, as well as induced significant (p<0.05) reduction in sperm count relative to their respective controls.

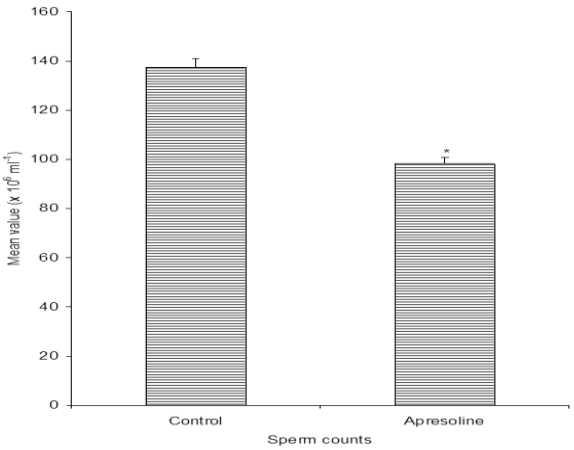

Figure 4: Spermogram showing the effect of 50 days treatment of rats with vasodilator on sperm count (n=6, *p<0.05).

Histopathological Effect

Plates 1 and 2 respectively show the transverse sections through the testes of control rat and rat treated with vasodilator (0.36 mg/kg) for fifty days.

Treatment of rats with vasodilator (0.36 mg/kg) caused no visible lesion in the testes of rat, which is similar to what was observed in the control rats.

Plate 1: Effect of 50 days treatment of rat with distilled water (control) on rat testis (×400).

Photomicrograph showing normal viable germinal epithelium (GE) with no visible lesion seen.

Plate 2: Effect of 50 days treatment of rat with vasodilator (0.36 mg/kg) on rat testis (x400)

Photomicrograph showing normal viable germinal epithelium (GE) with no visible lesion seen.

DISCUSSION

This study has shown that the treatment of rats with vasodilator caused no significant changes in body weight of rats. This suggests that the vasodilator was not toxic to the animals [7]. This could also be due to the absence of androgenic property in this drug, since it has been reported that androgens possess anabolic activities [8]. It could also be due to absence of anorectic and lipolytic

properties in this drug [9]. Similar result was reported by [10] in *Vernonia amygdalina* extract treated rats.

The drug induced significant increase in testosterone level which was not expected. The plausible explanation for this observation could be as a result of direct damage to the testes by vasodilator, since it has been reported that any direct damage to the testis is likely to impair gonadal response to FSH and LH [11]. Contrary report was given by [7] in rats treated with aspirin. This increase in testosterone levels could also indicate that vasodilator stimulates the mechanism involved in the process of hormone synthesis in the Leydig cells.

Vasodilator caused significant decrease in sperm motility. This suggest that the drug was able to permeate the blood-testis barrier with a resultant alteration in the micro-environment of the seminiferous tubules, since it has been reported that the decrease the in sperm motility caused by chemical agents was due to their ability to permeate the blood-testis barrier [12] and thus creating a different micro-environment in the inner part of the wall of the seminiferous tubules from the outer part [13]. Similar report was given by [14] in rats treated with *sarcotemma acidum* extract.

There was an insignificant decrease in sperm viability as well as a significant increase in the percentage of morphologically abnormal sperm cells induced after treatment of rats with vasodilator. This could be due to the ability of the drug to either interfere with the spermatogenic processes in the seminiferous tubules and epididymal functions which may result in alteration of spermatogenesis [15, 16]. Similar result was reported by [17] in isolated tetracyclic steroid treated rats.

Sperm count is considered to be an important parameter with which to access the effect of chemicals on spermatogenesis [18]. Spermatogenesis is influenced by the hypothalamic adenohypophysial-Leydig cell system relating gonadotropin releasing hormone, luteinizing hormone and androgen. This implies that the decrease in sperm count caused by vasodilator in the treated rats might not be as a result of plasma level of testosterone, because this hormone has been reported to be important in the initiation and maintenance of spermatogenesis [19]. Similar report was given by [20] in *Terminalia chebula* extract treated rats.

Photomicrographs revealed that rats treated with vasodilator presented with normal germinal epithelium with no visible lesions. Similar results were reported by [21] in rats treated with *Hibiscus macranthus and Basella alba* extracts. This suggests that the vasodilator has no toxic effect on the exocrine function of the testes at histological level [7].

CONCLUSION

In conclusion, this study has shown that the vasodilator (apresoline) has spermatotoxic or antispermatogenic effect in male rats. However, the effect of this drug on human reproductive functions is unknown. Nevertheless, considering these findings in animal models, it is recommended that moderation should be exercised in the consumption of this drug by those taking it for therapeutic purposes.

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