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Article

Reprotoxic activities of vildagliptin administration in male Wistar rats

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Vildagliptin is an oral hypoglycemic agent used in the management of diabetes. Some oral antidiabetic drugs have been implicated in reproductive toxicity.The objective of this study was to investigate the effects of daily administration of vildagliptin at different dosages (0.35 mg/kg B.W., 0.70 mg/kg B.W. and 1.40 mg/kg B.W.) to male Wistar rats for 8 weeks. Sperm parameters, serum concentrations of testosterone, follicle stimulating hormone and luteinizing hormone and the histology of the testis of the rats were assessed. Another set of rats were also treated for 8 weeks and allowed to recover and the same parameters were assessed in them. Fertility study was conducted by determining their litter size. The results showed that vildagliptin administration significantly reduced sperm count and motility of the treated rats. It also significantly increased the number of abnormal sperms. Serum level of testosterone was significantly decreased while luteinizing hormone and follicle stimulating hormone levels showed no significant change. The histoarchitecture of the testis of the treated rats appeared visibly normal. The litter size was also significantly reduced. Most of the changes observed were dose dependent. However, these parameters were restored towards normal in the recovery group. Our results suggest that vildagliptin adversely affected sperm parameters, affected litter size and disrupted the pituitary – gonadal axis. These changes were however reversed upon cessation of drug administration.

Keywords: Vildagliptin. Sperm. Testosterone. Testis. Rats.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which there are high blood glucose levels over a prolonged period of time due to deficiency in insulin secretion and/or action (WHO, 2014). It is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Alberti, Zimmet, 1998). Acute complications of untreated diabetes mellitus include diabetic ketoacidosis and hyperglycaemic hyperosmolal state. Serious long term complications include cardiovascular diseases, chronic kidney disease, foot ulcers and damage to the eyes (Kitabchi *et al.*, 2009). Antidiabetic agents consist of any of the several drugs that are used to control the level of glucose in the blood. The type of antidiabetic drug used depends on the nature of the diabetes, age, severity of the illness, as well as other factors (Harrigan *et al.*, 2001). The classes of antidiabetic drugs include insulin, biguanides, thiazolidinediones, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, glucagon-like peptide agonists, dipeptidyl peptidase-4 inhibitors, amylin analogues and sodium-dependent glucose co-transporter-2 (SGLT-2) inhibitors (Moses *et al.*, 2014). Each class of antidiabetic drugs has its own mechanism of action, although some mechanisms are interrelated. If adequate glucose control is not attained using a single oral agent, a combination of agents with different mechanisms of action may have additive therapeutic effects and result in better glycaemic control (Luna, Feingos, 2001).

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Vildagliptin is an oral antidiabetic agent that enhances pancreatic islet cell responsiveness to glucose (Mathieu, Degrande, 2008). It is an oral hypoglycaemic agent of the new dipeptidyl peptidase-4 inhibitor class of drugs (Ahren *et al*., 2004). It acts by inhibiting the inactivation of glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) allowing them to potentiate the secretion of insulin in beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas (Croxtall, Keam, 2008). It has been shown to reduce hyperglycaemia in type 2 diabetes mellitus.

Diabetes mellitus can affect both the male and female reproductive systems (Amaral *et al*., 2008). In the long term, diabetes mellitus can affect the blood vessels and the nerves of the male genitals. The damage to nerves or blood vessels of the penis makes it difficult to achieve or maintain erection. Oxidative stress induced by hyperglycaemia have been reported to damage the male reproductive system (Zhao *et al*., 2004). In streptozocin-induced diabetic rats, the reproductive organs' weights, sperm content and motility as well as serum testosterone were adversely affected (Hassan *et al*., 1993). Diabetes melltus in men has a direct effect on fertility by significantly altering their sperm ribonucleic acid (RNA). Patients with diabetes mellitus have been reported to have a significant decrease in their ability to repair deoxyribonucleic acid (DNA) damage (Blasiak *et al*., 2004). Reduced sperm DNA quality is known to be associated with decreased embryo quality, low embryo implantation rate and higher miscarriage rates (Ozumba *et al*., 2004).

Some oral antidiabetic drugs have also been reported to cause reproductive complications. They include metformin which has been shown to have an inhibitory effect on androgen production (Mansfield *et al*., 2003). It has also been reported to induce degenerative changes in the structure of testes, epididymis and seminal vesicles (Ferreira *et al*., 2015). Pioglitazone was reported to have deleterious effects on the structure of the male reproductive system in experimental animals (Ayuob *et al*., 2015).

This study aimed at investigating the effects of vildagliptin on the male reproductive system by determining its effect on sperm parameters and some reproductive hormone level. It also assessed its effects on the histology of the testis and also on litter size.

MATERIAL AND METHODS

Animal care and management

A total of 80 male and 80 female Wistar strain rats weighing between 160 - 180 g (8 – 10 weeks old) were used for the study. The rats were obtained from the Animal House section of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife. They were housed in plastic cages and allowed to acclimatize for two weeks before the commencement of the study. They were kept under normal environmental conditions with the natural light/dark cycle. Food and water were provided *ad libitum*. The standard rodent pellets used as their food were obtained from Ladokun Feeds Limited, Ibadan, Nigeria. The methodology of this study and use of animals were reviewed and approved by the Health Research and Ethics Committee, College of Health Sciences where this research was carried out. All the rats were cared for in accordance with the 'Guide for Care and Use of Laboratory Animals' (ILAR, 1996).

Drug preparation

The vildagliptin tablets used for this study were produced by Novartis Pharmaceutical Company, Basel, Switzerland. Three different doses of vildagliptin dissolved in distilled water (0.35 mg/kg, 0.70 mg/kg and 1.4 mg/kg body weight) were administered to the rats orally using the oral cannula. These doses were arrived at by extrapolating from currently administered human doses.

Experimental protocol

The male rats were divided into four categories (A, B, C and D). Each category was subdivided into four groups, making a total of 16 groups and each group had 5 male rats. Category A contained groups 1, 2, 3 and 4. Category B contained groups 5, 6, 7 and 8. Category C contained groups 9, 10, 11 and 12 while Category D contained groups 13, 14, 15 and 16. The female rats were divided into two categories (D and E). Each category was further divided into 4 groups with each group containing 10 animals.

The following procedures were carried out in the rats in category A. Group 1 served as the control and

each of the five rats received 1.4 mL/kg of distilled water daily for 8 weeks. Each rat in Group 2 received 0.35 mg/kg of vildagliptin daily for 8 weeks. Rats in Group 3 received 0.70 mg/kg of vildagliptin daily for 8 weeks. Each rat in Group 4 received 1.4 mg/kg of vildagliptin daily for 8 weeks. Twenty-four hours after the last administration, the rats were sacrificed by exanguination under urethane anesthesia (25%; 0.6 mL/100 g). Rats in category B (Groups 5, 6, 7 and 8) were administered the same doses of vildagliptin as those in category A. However, they were allowed to recover for another 8 weeks after which they were also sacrificed under urethane anesthesia.

Fertility study was carried "OUTT" using the male rats in categories C and D. In category C, Group 9 rats served as the control and each rat received 1.4 mL/kg of distilled water daily for 8 weeks. Each rat in Group 10 received 0.35 mg/kg of vildagliptin daily for 8 weeks. Rats in Group 11 received 0.70 mg/kg of vildagliptin daily for 8 weeks while each rat in Group 12 received 1.4 mg/kg of vildagliptin daily for 8 weeks. Thereafter, they were allowed to cohabit with untreated female rats (ratio of 1 male to 2 female rats). This same procedure was carried out in rats in Category D but they were allowed to recover for another 8 weeks after cessation of drug administration. They were then cohabited with untreated female rats at the same ratio. Successful mating was confirmed by the presence of copulatory (vaginal) plug and the size of the litters produced in each group was noted and recorded.

Anesthetic protocol and autopsy

The body weights of the rats were taken at the beginning of the experiment and the time of autopsy (Hanson digital weighing balance, San Francisco, USA). All the male rats in categories A and C were sacrificed at the specific times stated above. Blood was collected via cardiac puncture into plain bottles and serum was obtained. The testes and epididymis were removed, trimmed of adhering fat and weighed (Camry weighing balance, Guangdong, China). The right epididymis is used immediately for sperm parameter analysis, while the right testis was fixed in Bouin-Hollande's solution for further histological studies.

Hormonal assay

Serum levels of testosterone, follicle stimulating hormone and luteinizing hormone were measured with the Enzyme Linked Immunosorbent Assay (ELISA) technique using the ELISA kit (Roche diagnostics, USA) and following the manufacturer's instruction. It was read using the Utrao microplate reader (Shanghai, China) at 450 nm wavelength.

Analysis of sperm characteristics

Semen was obtained from the right cauda epididymis and used to determine the progressive sperm motility, viability, count and morphology as earlier described [W.H.O. manual, 1987, Raji *et al*., 2005].

Histological studies

The right testis which was earlier fixed in Bouin-Hollande's solution was further processed routinely for paraffin embedding. Five microns microsections were obtained using the rotary microtome and processed for Haematoxylin and Eosin Stain (H&E) according to the method described by Wilson (2013).

Fertility studies

The litter size determination was done for the rats in category B as earlier described. The number of the pups given birth to was noted and recorded for each group as the litter size.

Data Analysis

Data collected was presented as mean ± SEM. The control and test groups were compared using t-test, ANOVA and Student-Newman-Keuls post hoc analysis. The level of significance was taken at p-value < 0.05 (Snedecor, Cochran, 1980).

RESULTS

In general, all the animals were observed to have taken the food and water given to them as at when needed. There were no clinical symptoms observed in the experimental rats.

Effects of Vildagliptin on body weight of the rats

Administration of vildagliptin to rats for 8 weeks decreased their body weights when compared with the control. However, there was an increase in the body weight of rats in the recovery group when compared with the control (Figure 1).

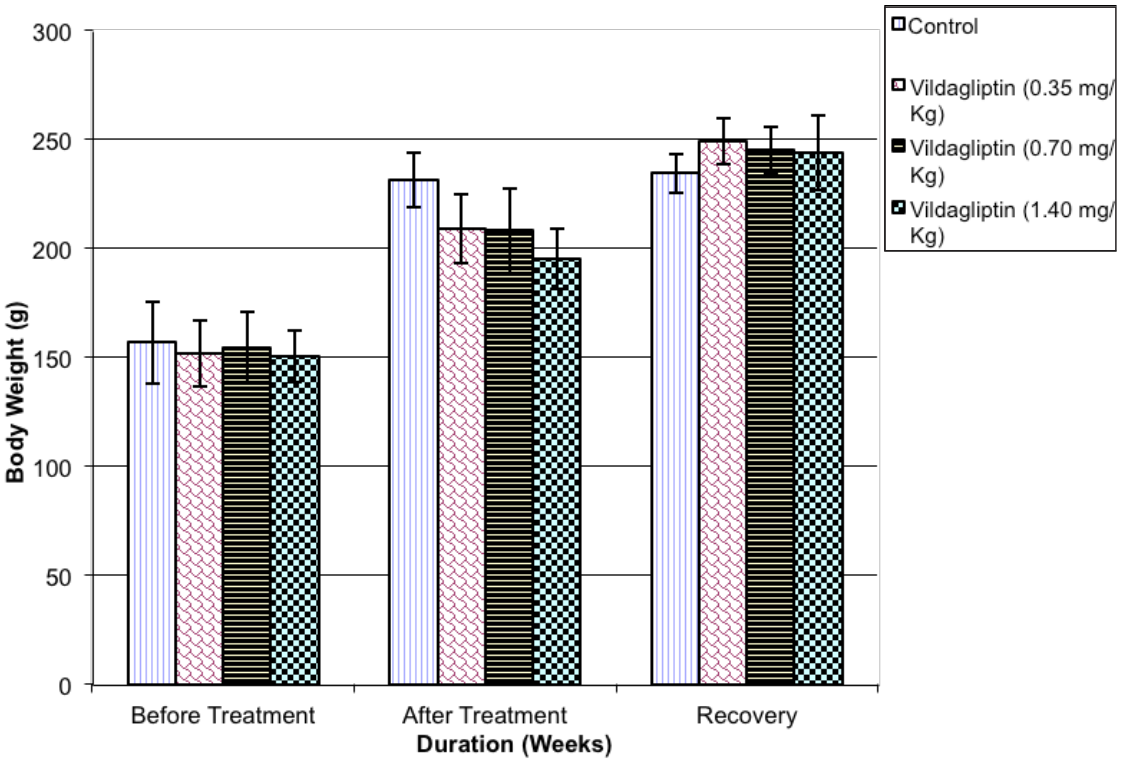

FIGURE 1 – Effects of vildagliptin on body weight of male Wistar rats.
Graph showing the effects of vildagliptin on the percentage weight gain male Wistar rats.
** = significantly different from control ($p < 0.01$).
*** = significantly different from control ($p < 0.001$).

Effect of Vildagliptin on relative reproductive organ weight

There was no significant change in the relative testicular weight of both the treated and recovery rats when compared with the control (Figure 2). A significant decrease ($p < 0.05$) was observed in the relative epididymal weight of rats treated with the highest dose of vildagliptin for 8 weeks when compared with the control. There was a significant decrease ($p < 0.05$) was also observed in the relative epididymal weight of rats in the recovery group when compared with their control. (Figure 3).

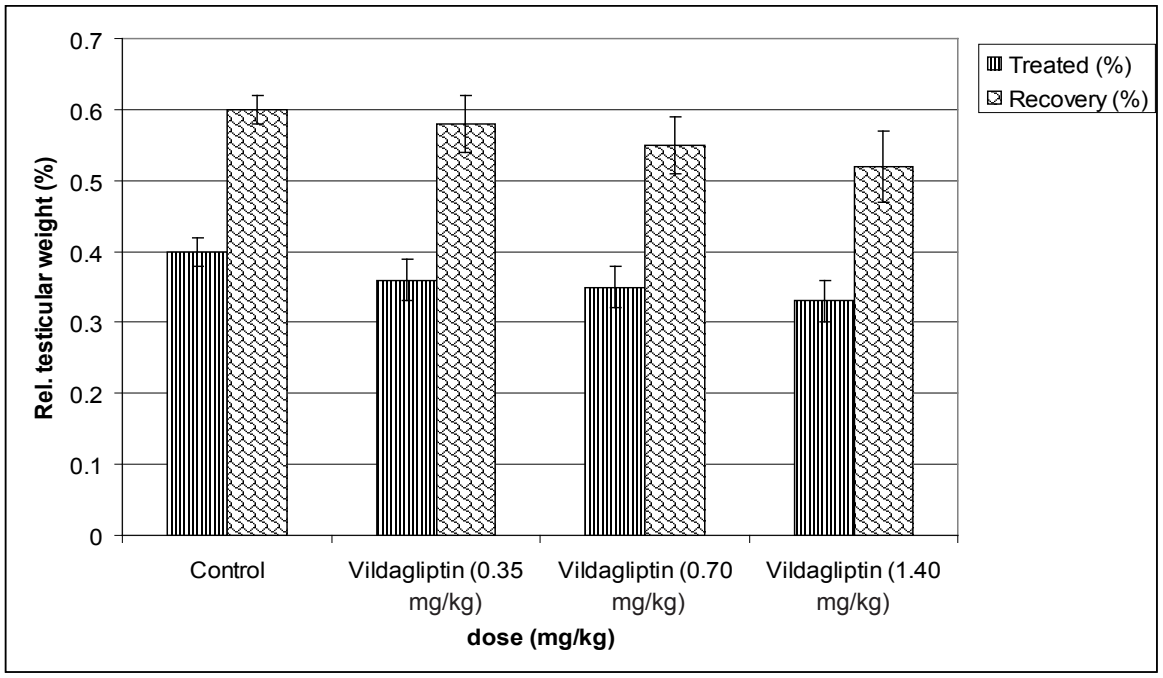

FIGURE 2 – Effects of vildagliptin on relative testicular weight of male Wistar rats.

Graph showing the effects of vildagliptin on the relative testicular weights of male Wistar rats.

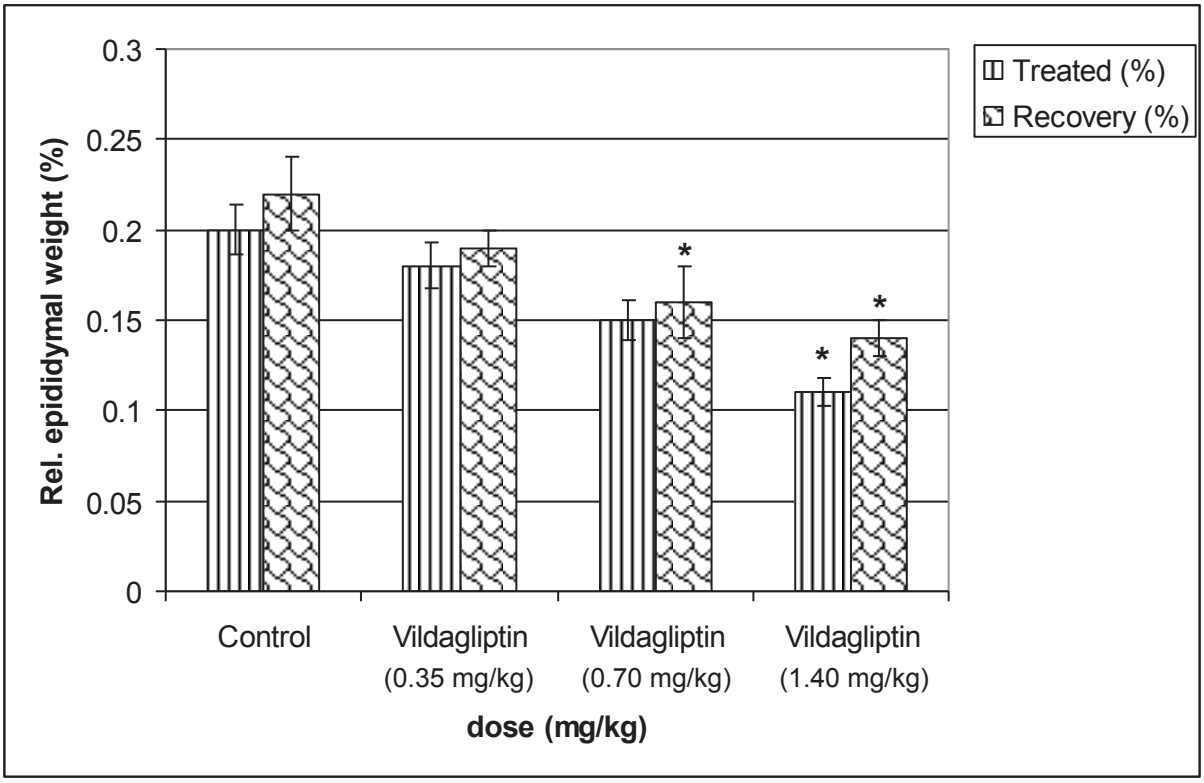

FIGURE 3 – Effects of vildagliptin on relative epididymal weights of male Wistar rats.
Graph showing the effects of vildagliptin on the relative epididymal weights of male Wistar rats.
* = significantly different from control ($p < 0.05$).

Effects of vildagliptin on sperm parameters

Administration of vildagliptin to rats for 8 weeks significantly decreased ($p < 0.01$) their progressive sperm motility and sperm count while it significantly increased ($p < 0.01$) the number of abnormal sperms (sperm morphology) when compared to the control. This significant change was observed to be dose dependent. There was no significant change in the sperm viability (live/dead ratio) of the treated rats when compared with the control (Table I). However, all these sperm parameters were restored towards the control values in the recovery experiments. (Table II).

TABLE I – Effect of vildagliptin on sperm parameters of Wistar rats (Experimental)

Group	Progressive Sperm Motility (%)	Sperm Viability (live/ dead ratio) %	Sperm count (10^6/mL)	Sperm Cell Morphology (%)
Control	91.25 ± 1.25	95.50 ± 1.20	134.30 ± 4.66	11.76 ± 0.19
Vildagliptin (0.35 mg/kg)	**80.00 ± 3.08	93.25 ± 1.50	128.80 ± 7.45	*13.87 ± 0.78
Vildagliptin (0.70 mg/kg)	**75.00 ± 2.89	93.00 ± 0.41	122.80 ± 6.70	*14.07 ± 0.52
Vildagliptin (1.40 mg/kg)	***70.00 ± 2.78	92.75 ± 0.48	119.31 ± 6.40	*14.41 ± 0.54

* = significantly different from control ($p < 0.05$)
** = significantly different from control ($p < 0.01$)
*** = significantly different from control ($p < 0.001$)

TABLE II – Effect of vildagliptin on sperm parameters of Wistar rats (Recovery)

Group	Progressive Sperm Motility (%)	Sperm Viability (live/ dead ratio) %	Sperm count (10^6/mL)	Sperm Cell Morphology (%)
Control	97.67 ± 1.67	95.35 ± 1.05	136.30 ± 7.27	11.56 ± 0.51
Vildagliptin (0.35 mg/kg)	*88.33 ± 3.41	94.75 ± 0.61	130.70 ± 2.33	12.64 ± 0.32
Vildagliptin (0.70 mg/kg)	*86.67 ± 2.33	94.35 ± 0.75	*126.70 ± 7.62	12.78 ± 0.78
Vildagliptin (1.40 mg/kg)	**81.67 ± 1.68	92.25 ± 1.30	*121.30 ± 7.22	13.53 ± 0.28

* = significantly different from control ($p < 0.05$)
** = significantly different from control ($p < 0.01$)

Effects of vildagliptin on some reproductive hormones

There were no significant changes observed in the serum concentrations of LH and FSH in rats treated daily with vildagliptin for 8 weeks. A similar trend was observed in the recovery rats. There was a decrease in the serum testosterone concentration of the drug-treated rats when compared with the control. There was however a significant increase ($p < 0.01$) in the testosterone concentration of rats in the recovery group when compared with the control (Table III and Table IV).

TABLE III – Effect of vildagliptin on reproductive hormones of Wistar rats (Experimental)

Group	Luteinizing Hormone (ng/mL)	Follicle Stimulating Hormone (ng/mL)	Testosterone (ng/mL)
Control	4.11 ± 0.13	5.68 ± 0.47	10.03 ± 1.28
Vildagliptin (0.35 mg/kg)	3.72 ± 0.14	5.40 ± 0.27	8.77 ± 1.33
Vildagliptin (0.70 mg/kg)	4.07 ± 0.14	6.10 ± 0.73	6.71 ± 1.42
Vildagliptin (1.40 mg/kg)	3.82 ± 0.11	6.36 ± 0.45	5.63 ± 1.29

Results are presented as Mean ± SEM.
Values of the recovery groups are in parentheses

TABLE IV – Effect of vildagliptin on reproductive hormones of Wistar rats (Recovery)

Group	Luteinizing Hormone (ng/mL)	Follicle Stimulating Hormone (ng/mL)	Testosterone (ng/mL)
Control	2.78 ± 0.20	3.68 ± 0.30	11.74 ± 0.36
Vildagliptin (0.35 mg/kg)	2.51 ± 0.23	3.60 ± 0.25	***15.71 ± 0.22
Vildagliptin (0.70 mg/kg)	3.14 ± 0.30	3.87 ± 0.32	***15.22 ± 0.23
Vildagliptin (1.40 mg/kg)	2.44 ± 0.18	3.43 ± 0.41	***14.85 ± 0.49

Results are presented as Mean ± SEM.
*** = significantly different from control ($p < 0.001$)

Effects of vildagliptin on average litter size

There was a dose dependent significant decrease ($p < 0.01$) observed in the litter size (average number of pups delivered) produced by female rats mated with male rats treated with vildagliptin daily for 8 weeks when compared with the control. A significant increase ($p < 0.05$) in the average number of pups delivered by female rats mated with male rats in the recovery group was observed when compared with the treated group (Figure 4).

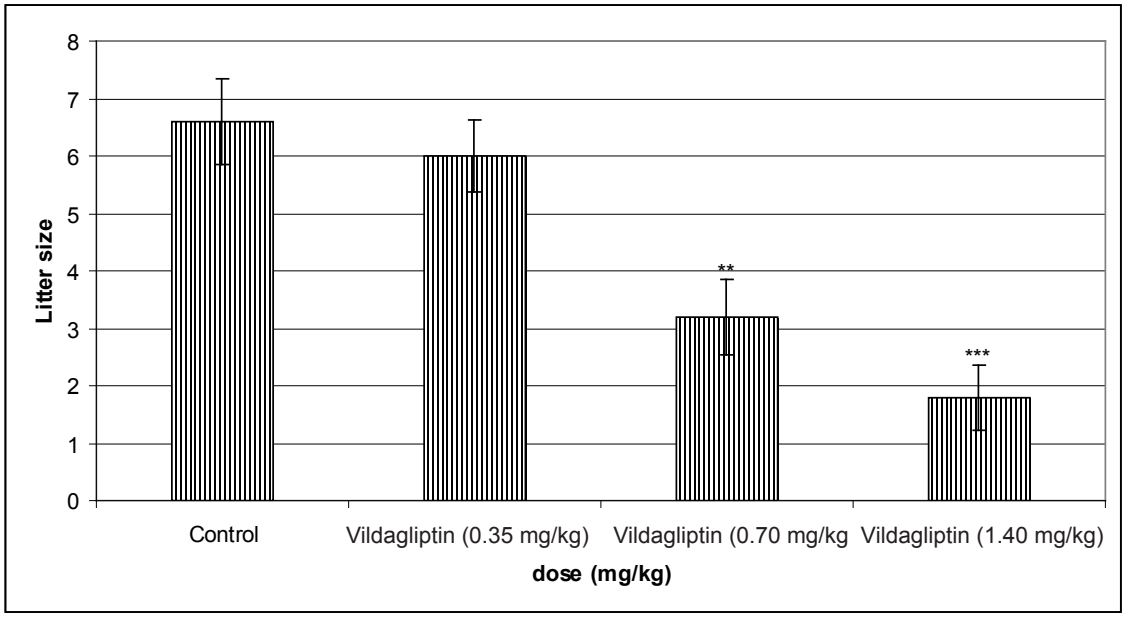

FIGURE 4 – Effects of vildagliptin on average litter size.
** = significantly different from control ($p < 0.01$).
*** = significantly different from control ($p < 0.001$).

Effects of vildagliptin on the histology of the testis of Wistar rats

The seminiferous tubules of rats administered vildagliptin daily for 8 weeks showed normal histoarchitecture even when compared with the control. There were organized layers of spermatogenic cells at different stages of maturation. A similar result was obtained from the testis of rats in the recovery group when compared with control (Figure 5 and 6).

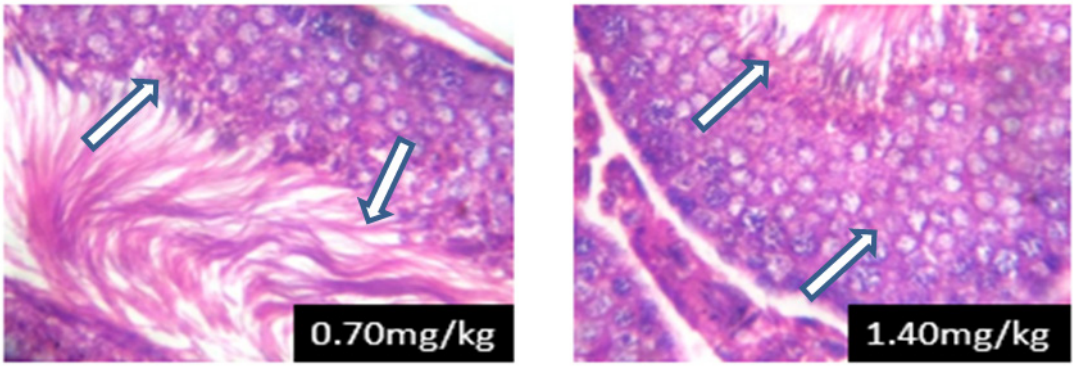

FIGURE 5 – Photomicrograph of the histology of the seminiferous tubules of control and treated rats. H & E X 400 magnification.

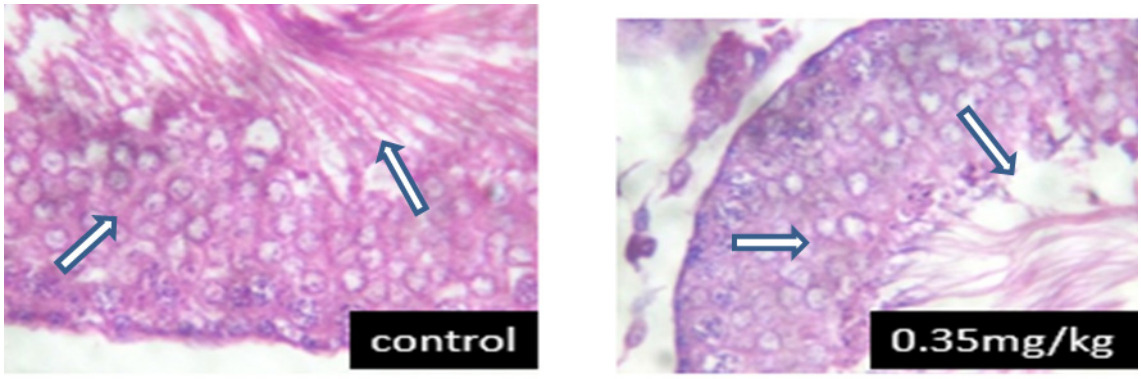

FIGURE 6 – Photomicrograph of the histology of the seminiferous tubules of rats after 8 weeks recovery period. H & E X 400 magnification. The seminiferous tubules appear normal showing spermatogenic cells at different stages of spermatogenesis.

DISCUSSION

This study investigated the effects of graded doses of vildagliptin on sperm parameters, pituitary and gonadal hormones, litter size and the histology of the testis. Three doses were administered orally. The lowest dose, 0.35 mg/kg was a sub-therapeutic dose; the medium dose; 0.75 mg/kg represents the average daily recommended dose while the highest dose; 1.40 mg/kg (not exceeding 100 mg/day) is the maximum daily recommended dose for the treatment of diabetes mellitus (Ahren, Foley, 2008).

The weight loss observed in the treated rats in this study implied that vildagliptin had detrimental effect on body weight. The higher the dose of vildagliptin, the lower the percentage weight gained. A similar finding was reported in a previous study which showed that drug-naïve patients administered vildagliptin exhibited significantly lower chylomicron lipid than placebo-treated patients. This suggests that vildagliptin may reduce percentage weight gain by inhibiting intestinal lipid absorption thereby preventing lipid deposition (Ahren, Foley, 2008). Dicker (2011) reported that vildagliptin increased the markers of fatty acid mobilization and oxidation. This weight loss was reversed in the recovery group which may imply that stoppage of the drug caused weight gain in the treated rats. Some studies have shown that chronic administration of vildagliptin delays gastric emptying but stoppage of the drug increased gastric emptying and lipid absorption thereby leading to weight gain (Croxtall, Keam, 2008). In another study, stoppage of vildagliptin administration caused an increase in appetite thereby leading to weight gain (Bergeson, 2015). There was no significant difference observed in the relative testicular weight of the treated rats. The absence of a significant decrease in relative testicular weight may suggest that the blood testis barrier (Wong, Cheng, 2005) restricted the permeability of vildagliptin into the testicular tissue. A significant decrease was however observed in the relative epididymal weights of rats administered 1.4 mg/kg of vildagliptin (highest dose). This significant decrease was also noticed in the recovery group. It has

been found that the testis to produces some lumicrine factors which help to maintain the weight of the epididymis after an exposure to a low or moderate dose of xenobiotics, including drugs (Lan *et al*., 1998). However, at high doses, this protective mechanism may be overwhelmed. This may partly explain why the high dose of vildagliptin (1.4 mg/kg) produced a significant decrease in the relative epididymal weight. It has also been reported that the blood-epididymis barrier is easily overwhelmed at high doses of xenobiotics when compared with the blood-testis barrier (Franca *et al*., 2012). This might also explain why the high dose of vildagliptin produced significant changes in relative epididymal weight.

In this study, a dose dependent reduction was observed in the epididymal sperm count of the treated rats. Some anti-diabetic drugs such as metformin have also been reported to significantly reduce sperm counts in animal studies (Bertoldo *et al*., 2014). In another study, glibenclamide (a very common antidiabetic drug) was also reported to decrease the sperm count of treated rats (Hakim *et al*., 2008). The amount of sperm produced is directly proportional to the rate of spermatogenesis within the testes. However, testosterone plays a very vital role in the process of spermatogenesis. Testosterone is produced by the Interstitial cells of Leydig within the testis (Boron, Oulapaep, 2009). The decrease observed in the sperm count of the treated rats could be linked to the decrease in their serum testosterone levels as recorded in this study. Metformin which is also an antidiabetic drug has been reported to reduce serum testosterone levels in experimental animals (Bertoldo *et al*., 2014). Other studies have shown that a significant reduction in serum testosterone levels also led to a reduction in sperm count (Kumar *et al*., 2010; Bebb, 2011; Crosnoe-Shipley *et al*., 2015). Also, the protective effect of the blood-testis barrier applies only to the seminiferous tubules rendering the testicular interstitium more susceptible to the effect of xenobiotics. This makes the Interstitial cells of Leydig easily exposed to the negative effect of xenobiotics thereby causing reduced testosterone synthesis in the Leydig cells (Setchell, 2008). This may partly explain why vildagliptin caused a reduction in serum testosterone levels. There was an increase in LH and FSH levels in the treated rats. The rise in the levels of serum gonadotropins; FSH and LH recorded in this study, may be due to the decrease recorded in serum testosterone level. This is because a decrease in testosterone level will act via negative feedback to increase LH and FSH levels and vice versa (Boron, Oulapaep, 2009). This was observed in the recovery study. The sperm count of the recovery rats showed a dose dependent decrease, which implies that the effect of the drug on sperm count is deleterious and the chance of recovery worsens as the dose increases. This was also reported in previous studies which showed that the deleterious effects of drugs on sperm counts become more difficult to reverse at higher doses of the drugs (Ahmed, Kurkar, 2014). However, there was an increase in the sperm count of the recovery rats when compared with that of the treated rats. The percentage sperm viability of the treated rats did not show any significant deleterious effect from the data obtained. However there was a significant dose dependent reduction observed in the progressive sperm motility and percentage of abnormal sperms of the drug-treated rats. Agrawal and Vanha-Perttula (1986) reported that dipeptidyl peptidase-IV (DPP-4), an enzyme that regulates their forward motility is high in the cauda epididymis where sperms acquire their motility (Kao *et al*., 2008). Vildagliptin has been reported to inhibit production of this enzyme (Prato, 2007). This may explain why vildagliptin, being a dipeptidyl peptidase-IV (DPP-4) inhibitor, may produce deleterious effects within the cauda epididymis thereby reducing sperm motility. However, this adverse effect on sperm motility was reversed in the recovery experiment. In addition, there was a significant increase in the percentage of abnormal sperms in the drug-treated rats and this was observed to be dose dependent. A previous study demonstrated that the transfer of DPP-4 present in the semen via some vesicles regulates the normal morphological development of sperm cells (Arienti *et al*., 1997). This may, therefore, partly explain why vildagliptin, an inhibitor of DPP-4 may induce abnormal morphological changes in the sperm cells. Similarly, metformin which is also an antidiabetic drug is known to cause increased abnormal sperm morphology in experimental animals (Bertoldo *et al*., 2014). However, the adverse effect of vildagliptin on sperm morphology was reversed in the recovery experiment. Furthermore, a significant reduction was observed in the litter size when the drug-treated male rats were mated with untreated female rats. This was also observed to be dose dependent. This could be due to significant decrease in the progressive sperm motility, sperm count, testosterone level with a significant increase in the percentage of abnormal sperms of the treated rats recorded in this study. Findings from a previous study (Arienti *et al*., 1997) showed that chemical substances that have a significant adverse effects on sperm count, sperm motility and sperm morphology have a great possibility of

reducing litter size. Ahmed and Kurkar (2014) reported a strong association between low serum testosterone levels and reduced litter size in animal studies.

This study showed that vildagliptin administration for 8 weeks did not cause any observable degenerative changes on the histoarchitecture of the testis especially within the seminiferous tubules. This may be due to the presence of the protective blood-testis barrier which restricted the permeability of vildagliptin to cause any observable deleterious effects in the histology of the seminiferous tubules. However, previous studies have shown that the mere absence of any observable degenerative effect on relative testicular mass does not entirely exclude testicular damage as the effect may be molecular or better observed using other staining techniques such as immunohistochemical stains (Ekhoye *et al*., 2013).

In conclusion, this study showed that long term administration of vildagliptin may cause significant adverse effects on the male reproductive system which appear to be dose dependent. However these adverse effects appeared to be reversed towards control values in the recovery experiments upon cessation of vildagliptin administration.

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