Global Scientific JOURNALS

GSJ: Volume 8, Issue 11, November 2020, Online: ISSN 2320-9186
www.globalscientificjournal.com

ATHEROSCLEROSIS DOWNSIZING EFFECTS OF SILDENAFIL CITRATE ON BLOOD LIPID PROFILES (SERUM AND HEART) IN ALUMINUM CHLORIDE - TREATED MALE WISTAR RATS

BAKARE, O. S.[1]

[1]*Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria*

Corresponding Author:

BAKARE, O. S.[1]

Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

E mail: bakfemsonline@gmail.com. Telephone +2348068902669

Abstract:

The present study evaluates the atherosclerosis downsizing effects of sildenafil citrate on lipid profiles indices of male rats treated with aluminum chloride with a view to evaluating the risks of atherosclerosis which may lead to coronary heart disease and stroke. Since the advent of sildenafil for treatment of erectile dysfunction (ED), its use has enjoyed wide patronage the world over, following reported effectiveness. Indeed, the advent of sildenafil and other PDE5 inhibitors saw to the management of erectile dysfunction, thus leaving the male folks with having to battle with the other common male sexual dysfunction-premature ejaculation (PE). In this study, an attempt was made in assessing the effects of sildenafil on serum and heart lipid profile using male albino Wistar rats induced with aluminium chloride. Twenty (20) healthy and active male rats (Wistar rat) of average weight 140±176 g were randomly divided into 4 groups (n = 5), thus; control, negative control (35 mg/kg $AlCl_3$), 50 mg/kg of Sildenafil, 50 mg/kg of Sildenafil and 100mg/kg $AlCl_3$. The drugs were administered every day orally for two weeks. All animals had access to food and water. Blood was collected from each animal via cardiac puncture and blood lipid profile (serum and heart) were assessed. Standard lipid profiles method was adopted in assessing the lipid profiles. Heart triglycerides, HDL, LDL and cholesterol increased significantly ($p<0.05$) following administration of sildenafil. Serum HDL, LDL and cholesterol decreased significantly ($p<0.05$) following administration of sildenafil. Conclusively, considering the serum cholesterol depleting effects of sildenafil (anti-atherosclerosis), it is

important to tame their abuse, as this could affect processes that depend largely on serum lipids for their proper functioning such as synthesis of steroid hormones.

Key word: Sildenafil citrate, aluminium chloride, lipid profiles, oxidative stress, atherosclerosis

Background: Blood lipid profile refers to quantitative analysis of serum total cholesterol, serum triglycerides, serum VLDL-cholesterol, serum LDL-cholesterol and serum HDL-cholesterol in order to evaluate the risks of atherosclerosis, fatty liver coronary heart diseases, hypertension, etc. (Gupta, 2013). The quantitative analysis of these serum lipids is mainly required to evaluate the risks of atherosclerosis which may lead to coronary heart disease and stroke (Gupta, 2013). In this study, sildenafil citrate plays a central role, as it has link with cardiovascular functions.

Introduction: Metals may have serious effect on the male reproductive system directly when they target specific reproductive organs or indirectly, when they act on the neuroendocrine system (Pizent *et al.*, 2012; Verstraeten *et al.*, 2008). Among them Aluminum (Al) is the most widely distributed metal in the environment (Kumar and Gill, 2009). Aluminum is the most prevalent metal and the third most important abundant element in earth's crust, only oxygen (49.5 %) and silicon (26 %) occur more commonly than aluminum (8 %). In biological systems, aluminium is present only in trace amounts. In no case, Aluminium has been shown to have a definite biological function and it is poorly absorbed and efficiently eliminated when absorption does occur; aluminium is described mainly in bone, liver, testes, kidney, and brain. Aluminium accumulation in tissues and organs results in their dysfunction and toxicity (ATSDR, 1990). Metal such as aluminium have been shown to affect spermatogenesis in rodents and humans, which can lead to low sperm count, abnormal sperm morphology and poor semen quality (Verstraeten *et al.*, 2008). Due to its reactivity, aluminum in nature is found only in combination with other elements such as sulphate, chloride etc. An experiment reported that aluminium induced toxicity in epididymis, vas deferens, seminal vesicle and ventral prostate in mice (Chinoy *et al.*, 2005a). According to another study, it has been shown that aluminium chloride induced reproductive toxicity and exerted a significant adverse effect on the steroidogenesis (Yousef *et al.*, 2005). It has been shown in a study that, *in vitro*, aluminium chloride provoked deterioration in sperm motility and viability and enhancement of free radicals and alterations in enzyme activities on rabbit sperm (Yousef *et al.*, 2007). Alterations in the metabolism of testis and epididymis, leading to a reduction in fertility rate in mice treated with aluminium chloride were also observed in an individual study (Yousef and Salama, 2009). Studies have reported that aluminium block voltage-gated calcium channels, thereby impairing gonadotrophin secretion in the hypophysis with resultant low sperm counts (Llobet *et al.*, 1994). Erectile dysfunction (ED) is the persistent inability to achieve and maintain an erection adequate for satisfactory sexual performance. The probability of erectile dysfunction increases with ageing and the presence of some disease conditions such as diabetes mellitus, hypertension, hypercholesterolemia, ischemic cardiac disease, depression and obesity (NIH, 1993). From an epidemiological evaluation, infertility due to male factor was ranged from 20% to 70% and infertility rates were highest in Africa and Eastern Europe (Agarwal *et al.*, 2015). There are evidences to show that sperm counts have been declining over the last 50 years, with a consequent increase in male infertility (Olayemi, 2010). The treatment of male infertility includes administration of androgens or gonadotropins, aphrodisiacs like sildenafil citrate or surgery and assisted reproductive technology.

Sildenafil Citrate: A white to off-white crystalline powder, is manufactured as blue, film-coated rounded-diamond-shaped tablet (Viagra) with the equivalent amount of sildenafil 25 mg, 50 mg and 100 mg. Sildenafil has molecular

weight of 666.7 with a solubility of 3.5 mg/ml in water. It is relatively lipophilic (Log $D_{7.4}$ = 2.7) with a weakly basic centre in the piperazine tertiary amine (pKa=6.5) chemically named as 1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo [4, 3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate (Pfizer Labs DoPI, 2006). Sildenafil citrate (Viagra®) is a vasoactive agent, which became available worldwide in 1998 for the treatment of male erectile dysfunction. The drug acts by inhibiting the phosphodiesterase type 5 (PDE5) enzymes that specifically degrade the cyclic guanosine monophosphate (cGMP), responsible for the nitric oxide induced smooth muscle relaxation and vasodilatation (Turko *et al.,* 1999). PDE5 is found in particularly high concentration in the *corpus cavernosum*, the erectile tissue of the penis; it is also found in the retina and vascular endothelium. Increased cGMP concentration results in vasodilation and an increased inflow of blood in penis tissue which facilitates the generation and maintenance of an erection (Salonia *et al.,* 2003; Jackson *et al.,* 2005). Recently, it has been found that the vasodilatory effects of sildenafil also help to reduce symptoms of pulmonary arterial hypertension and it is thus also applied for this medical indication (Wang *et al.,* 2014; Doganci *et al.,* 2015). During sexual stimulation, mechanism of penile erection is resulted through the release of nitric oxide in the *corpus cavernosum*. Nitric oxide then activates guanylatecyclase, which results in increased level of cGMP. This produces smooth muscle relaxation in the *corpus cavernosum*, which allows inflow of blood. Phosphodiesterase type 5 (PDE-5) is responsible for the degradation of cGMP in the *corpus cavernosum*. Sildenafil is a selective inhibitor of PDE5, thus increasing the cGMP level in the *corpus cavernosum*. It has no direct relaxant effect on human *corpus cavernosum* and has no effect in the absence of sexual stimulation at recommended doses (Pfizer Labs DoPI, 2006). Sildenafil is broken down in the liver by hepatic metabolism using cytochrome p450 enzymes, mainly CYP450 3A4 (major route), but also by CYP2C9 (minor route) hepatic isoenzymes. The major product of metabolisation by these enzymes is N-desmethylated sildenafil, which is metabolised further. This metabolite also has an affinity for the PDE receptors, about 40% of that of sildenafil. Thus, the metabolite is responsible for about 20% of sildenafil's action. Sildenafil is excreted as metabolites predominantly in the feces (about 80% of administered oral dose) and to a lesser extent in the urine (around 13% of the administered oral dose). If taken with a high-fat meal, absorption is reduced; the time taken to reach the maximum plasma concentration increases by around one hour, and the maximum concentration itself is decreased by nearly one-third (Deveci *et al.,* 2004).

Lipids have an important role in the functional activity of sperm cells, sperm viability, maturity, capacitation and fertilization (Maqdasy *et al.,* 2013). Lipids defined as biological substances that are generally hydrophobic in nature and in many cases soluble in organic solvents (Smith, 2000). These chemical properties cover a broad range of molecules, such as fatty acids, phospholipids, sterols, sphingolipids, terpenes, and others (Christie, 2003). Lipid classes are fats, oils, waxes, and complex lipids involved in various biological processes such as sterols, phospholipids, glycolipids, lipoproteins and sphingolipids (Vilhemsen *et al.,* 2005). Lipids are first absorbed from the small intestine and emulsified by bile salts which are synthesized from cholesterol in the liver, stored in the gallbladder and secreted following the ingestion of fat. Lipids are insoluble in plasma, thus their transport is mediated by lipoproteins which differ in particle size, com position and density. These are chylomicrons (CYM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). All of them have a hydrophobic core containing TG and cholesteryl ester (CE) and a polar periphery with phospholipids (PL), cholesteryl (C) and apolipoproteins (Mathews *et al.,* 2000). The pharmacokinetics of sildenafil following single dose of intravenous and oral administration has been determined in mouse, rat and dog. After the single dose

of oral sildenafil administration, the low bioavailability was occurred in any species of mouse, rat, rabbit, dog and man. It was due to the pre-systemic hepatic first-pass effect of sildenafil (Shin *et al.,* 2006). Sildenafil and its major metabolite; N-desmethyl metabolite (UK-103, 320) are highly bound to plasma protein but the binding is independent on the concentrations over the range of 0.01-10 μg/ml. The mean proportion of plasma protein binding in rats and man are 95 % and 96-97 % respectively (Pfizer Labs DoPI, 2006). However, according to a population pharmacokinetic study of patients with ED, the apparent volume of distribution (V/F) after oral administration is 3.5 L/kg (Milligan *et al.,* 2002). The report of Pfizer to FDA has shown that the percentage bioavailability values of sildenafil in male rats and man are 15-23 % and 41 % respectively (Nichols *et al.,* 2002). Sildenafil is cleared primarily via the metabolism (Pfizer Labs DoPI, 2006). It is metabolized by CYP2C9 (major route) and CYP3A4 (minor route) and converted mainly to its active metabolites N-desmethylated sildenafil (UK-103, 32), which has a similar property on PDE-5 with the potency of around 50 % of the parent drug. In the case of intravenous administration, it has the same elimination half-life with its parent drug at ~ 0.3 hours in male rat and ~ 2.4 hours in man (Walker *et al.,* 1999). After oral administration, sildenafil and its metabolite are eliminated with the half-life of ~ 0.4 hours in male rat and ~ 4 hours in man (Milligan *et al.,* 2002). Sildenafil is excreted as metabolites predominantly in the feces (approximately 73-88 % of administered oral dose) to a lesser extent in the urine (approximately 6-15 % of administered oral dose) (Muirhead *et al.,* 2002). Studies in rat, mouse and dog show the action of sildenafil is mainly terminated by the metabolism and less than 10 % of the unchanged parent drug is recovered in the feces of these animals (Milligan *et al.,* 2002). However, there is no recovery of radioactivity of sildenafil from the feces of man (Walker *et al.,* 1999). The clearance of sildenafil is reduced in the elderly patients (age >65) with severe renal impairment ($CL_{cr} \leq 30$ml/min) or hepatic cirrhosis (Muirhead *et al.,* 2002).

Aluminium Chloride: Aluminum is the trivalent cation that does not undergo redox changes. Extensive experimental evidences demonstrate both, in vitro and in vivo, that high aluminium concentrations cause oxidative stress. Oxidative stress is an imbalance between free radical generation and the antioxidant defense system. Oxidative stress induced by aluminium is one of the major contributing factors to male reproductive disorders. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects (Jomova and Valko, 2011). Despite the low oxygen tensions that characterize the testicular microenvironment, testis remains vulnerable to oxidative stress due to the abundance of highly unsaturated fatty acids and the presence of potential ROS generating systems (Aitken and Roman, 2008). Although aluminum (Al) is a relatively low redox mineral, it can induce oxidative damage through multiple mechanisms. Excessive free radicals generation by aluminium may cause impairments in mitochondrial bioenergetics and may lead to the generation of oxidative stress which might be one of the causes of reproductive disorders (Kumar and Gill, 2009). There are numerous studies that have examined aluminum's potential to induce toxic effects in humans or laboratory animals exposed via inhalation, oral, or dermal exposure. It is widely accepted that nervous system is the most sensitive target of aluminum toxicity and it may induce cognitive deficiency and dementia when it enters the brain. Besides this cardiotoxic, nephrotoxic and hepatotoxic effects have also been provoked by aluminium (Geyikoglu *et al.,* 2012). Aluminium ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals. Alteration in the histology of testis (Buraimoh *et al.,* 2012) deterioration in spermatogenesis and sperm quality; enhancement of

free radicals and alterations in antioxidant enzymes (Yousef *et al.,* 2007; Yousef *et al.,* 2009); interruption in sex hormone secretion (Guo *et al.,* 2005); and biochemical changes in testis and other accessory reproductive organs (Chinoy *et al.,* 2005a; 2005b) are some of the aspects suggested that Aluminium exposure causes adverse impact on male reproduction.

Materials and Methods:

Chemicals and Reagents

Aluminium chloride, Distilled water, EDTA, normal saline, Blood lipid profile kits.

Equipment and Laboratory Apparatus

Rats cage, feed pan, syringe, hand gloves, bowls, masking tape, permanent marker, test tubes and test tubes rack, EDTA bottles, cotton wool, tissue paper, foil paper, capillary tubes, beaker (1000ml), spatula, measuring cylinder, conical flask, weighing balance, test tubes, dissecting kit, cuvette, haematocrit reader, intubator, spectrophotometer, micropipette, diposable tips, waterbath/microwave.

Sildenafil Citrate

Sildenafil was provided as sildenafil citrate which is a white to off-white crystalline powder of a molecular weight of 666.7. It is present in the drug market as the patent preparation Viagra® that is an oral therapy for erectile dysfunction, formulated as blue, film-coated rounded-diamond- shaped tablets equivalent to 25 mg, 50 mg and 100 mg of sildenafil for oral administration. The rats were given 50 mg of sildenafil once daily for 2 weeks.

Experimental Animals

Twenty (20) healthy and active male rats (Wistar rat) of average weight 140±30g were obtained from the Institute of Medical Research and Teaching (IMRAT) at the University Teaching Hospital Ibadan, Oyo state, Nigeria and were allowed to acclimatize to experimental condition for two weeks. They were housed and grouped in a temperature and humidity controlled environment under a 12 hours light/dark cycle, with their normal rat pellet ration (Top Feeds, Nigeria) and portable water available ad libitum. All procedures were performed in strict accordance with protocols approved by University of Newcastle Animal Care and Ethics Committee, the New South Wales Animal Research Act and Regulations, and the Australian code of practice for care and use of animals for scientific purposes.

Experimental Procedure

The study was performed in two steps. During the first step, made for induction of testicular damage and which lasted 1week, a total of 20 animals were evenly grouped according to their body weight into 4 groups. Group I received an oral administration of distilled water at the dose of 2 ml/kg body weight. Group 2 to 4 received an oral administration of aluminium chloride dissolved in distilled water at the dose of 100 mg/kg body weight according to the procedure of Khattab. The aluminium chloride serves as the toxicants. The second stage of the study which was the treatment period with plant extract lasted 2weeks. During the treatment phase, the different groups of rats were treated as follows: Group 1: Positive control (P-CTR): The animals in this group were not induced with the toxicants

($AlCl_3$). Group 2: Negative control (N-CTR): The animals in this group were induced with the toxicants ($AlCl_3$) at the dose of 35 mg/kg for 2 weeks orally. Group 3: The animals in this group were induced with 50 mg/kg of Sildenafil for 2 weeks through oral pathway. Group 4: They were induced with the 50 mg/kg of Sildenafil and 35 mg/kg $AlCl_3$ for 2 weeks orally.

Animal Sacrifice and Tissue Harvesting

At the end of the 24th day period of stable administration, animals were subjected to fasting over-night and were sacrificed in the next morning. Blood samples were gotten directly from the eyes of the animals using capillary tubes and then poured into an EDTA bottle and taken to the laboratory for the lipid profiles analysis.

Blood Lipid Profiles Analysis

Determination of Lipid Profile

Total triglycerides Assay

Serum total triglycerides concentration was measured by the Tietze (1990) method, as described in the manual of the Randox Total triglycerides kit (Randox Laboratories Limited, United Kingdom). The total number of triglyceride is calculated thus:

$$\text{Triglycerides}\,(mg/dl) = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} * Concentration\ of\ Standard$$

Total Cholesterol Assay

Serum total cholesterol level was measured by the Trinder (1969) method, as described in the manual of the Randox Total cholesterol kit (Randox Laboratories Limited, United Kingdom).

The total cholesterol concentration is obtained as thus:

$$Total\ Cholesterol\ (mg/dl) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} * Concentration\ of\ Standard$$

HDL-Cholesterol Assay

Serum HDL-cholesterol concentration was measured by the NIHCDCS (1992) method, as described in the manual of the Randox HDL-cholesterol kit (Randox Laboratories Limited, United Kingdom).

$$HDL - Cholesterol\ (mg/dl) = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} * Concentration\ of\ Standard$$

Low Density Lipoprotein (LDL).

The concentration of low density lipoprotein is obtained following formula:

$$LDL\ (mg/dl) = \frac{\text{Total cholesterol triglyceride}}{\text{High Density lipoprotein}}$$

Statistical Analysis

Data obtained for lipid parameters were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21 to generate the mean and standard error (mean±SE). Mean generated were separated and compared by Duncan's New Multiple Range Test (DNMRT).

RESULTS

Lipid profiles of heart

Heart Triglycerides

The results of the lipid profiles (Heart total cholesterol, heart triglycerides, heart LDL-cholesterol and heart HDL-cholesterol) in heart are presented in Figure 1-4 accordingly. The results for lipid profiles of heart is also represented in Table 1.

Figure 1 showed the triglycerides contents in heart. Control group recorded the highest content of triglycerides (575.89) followed by 50 mg/kg of sildenafil (479.34), while 35 mg/kg of aluminium chloride ($AlCl_3$) had the least content of triglycerides (208.11). There is significant different ($p<0.05$) between the treatments groups compared to the control.

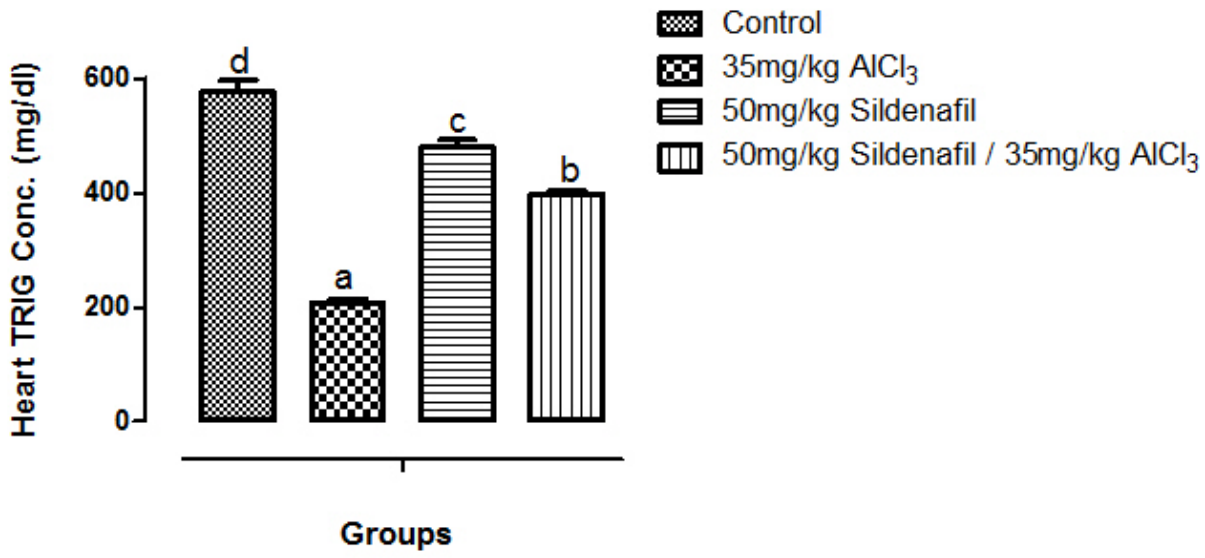

Figure 1: Mean Concentration of Heart Triglycerides

Note: Bars with the same alphabets are not significantly different from each other at ($P>0.05$) using Duncan's New Multiple Range Test (DNMRT)

Heart Low Density Lipoprotein (LDL)

Figure 2 showed the LDL contents in heart. The mean values of LDL across the groups shows that 50 mg/kg sildenafil / 35 mg/kg $AlCl_3$ group recorded the highest content of LDL (8.07) followed by 50 mg/kg of sildenafil (7.84), while 35 mg/kg of aluminium chloride ($AlCl_3$) showed the least content of LDL (7.26). There is no significant different ($p>0.05$) between the treatments groups when compared to the control group except 50 mg/kg sildenafil / 35mg/kg $AlCl_3$ that showed significant different ($p<0.05$) compared to the control.

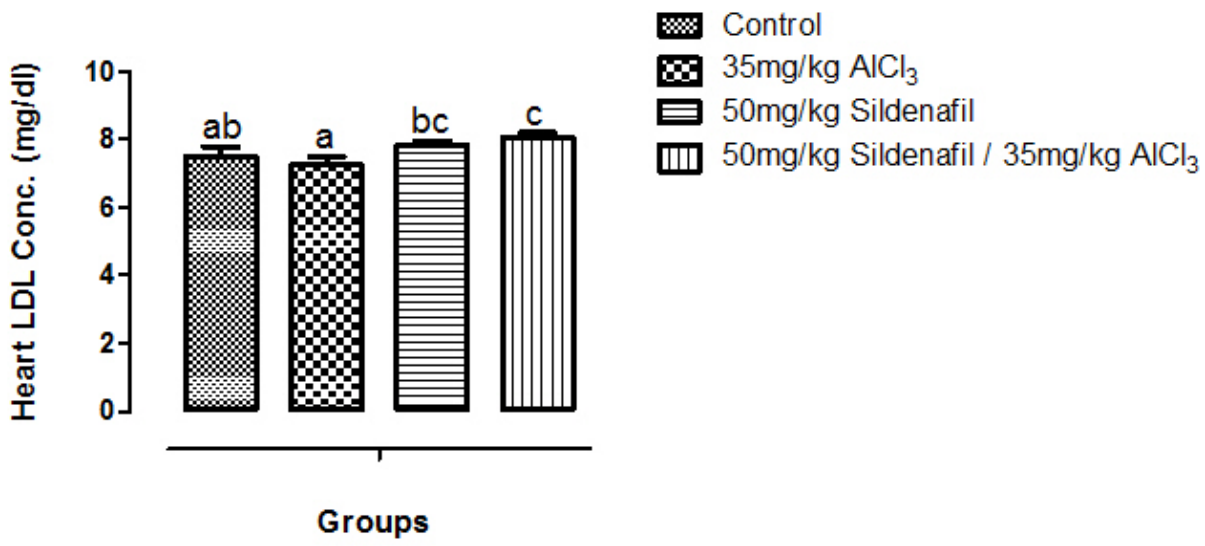

Figure 2: Mean Concentration of Heart Low Density Lipoprotein (LDL)

Note: Bars with the same alphabets are not significantly different from each other at **(**P>0.05) using Duncan's New Multiple Range Test (DNMRT)

Heart High Density Lipoprotein (HDL)

Figure 3 showed the HDL contents in heart. The mean values of HDL across the groups shows that control group recorded the highest content of HDL (0.06) followed by 50 mg/kg of sildenafil (0.04), while 35 mg/kg of aluminium chloride ($AlCl_3$) showed the least content of HDL (0.02). There is no significant different (p>0.05) between the treatments groups when compared to the control group.

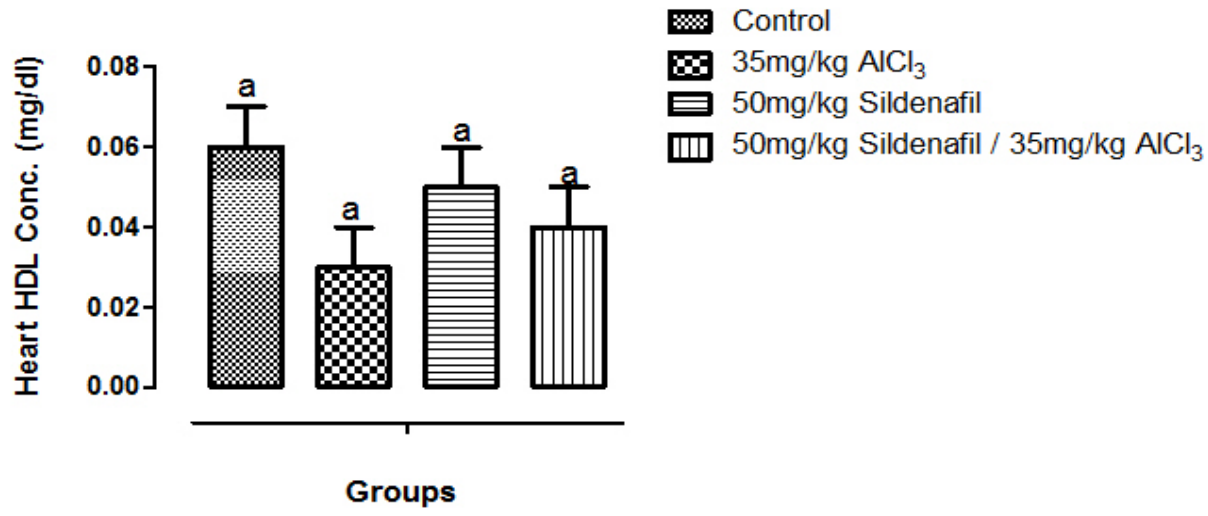

Figure 3: Mean Concentration of Heart High Density Lipoprotein (HDL)

Note: Bars with the same alphabets are not significantly different from each other at (P>0.05) using Duncan's New Multiple Range Test (DNMRT)

Heart Cholesterol

Figure 4 showed the cholesterols contents in heart. The mean values of cholesterols across the groups shows that control group recorded the highest content of cholesterols (382.72) followed by 50 mg/kg sildenafil group (333.06), while 35 mg/kg of aluminium chloride ($AlCl_3$) showed the least content of cholesterols (308.97). There is no significant different (p>0.05) between the treatments groups when compared to the control group.

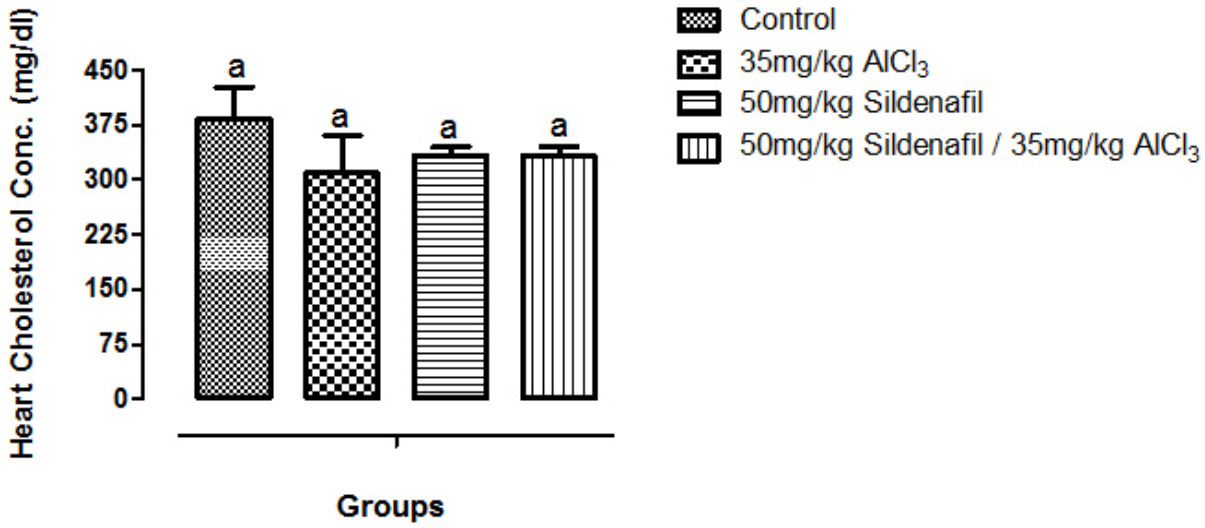

Figure 4: Mean Concentration of Heart Cholesterol

Note: Bars with the same alphabets are not significantly different from each other at **(**P>0.05) using Duncan's New Multiple Range Test (DNMRT)

Lipid Profiles of Serum

Serum Low Density Lipoprotein (LDL)

The lipid profiles of serum (serum total cholesterol, serum triglycerides, serum VLDL-cholesterol, serum LDL-cholesterol and serum HDL-cholesterol) are presented in Figure 5-7 accordingly. The results for lipid profiles in serum is also represented in Table 2.

Figure 5 showed the LDL contents in heart. Control group recorded the highest content of LDL (7.96) followed by 50 mg/kg sildenafil / 35 mg/kg $AlCl_3$ group (7.77), while 50 mg/kg of sildenafil group had the least content of LDL (7.59). There is significant different (p<0.05) between the treatments groups compared to the control.

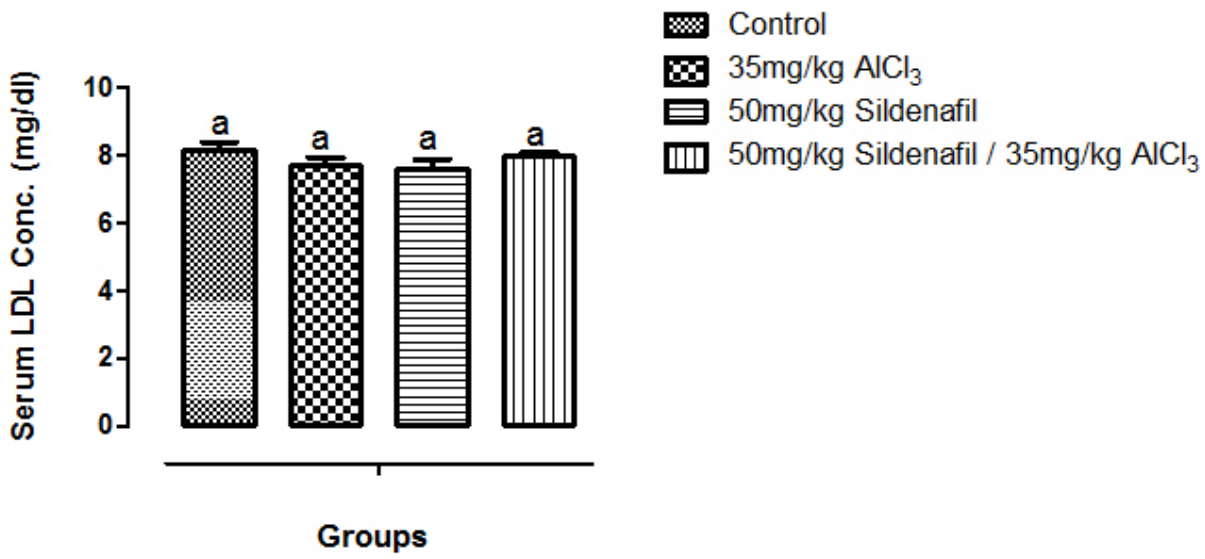

Figure 5: Mean Concentration of Serum Low Density Lipoprotein (LDL)

Note: Bars with the same alphabets are not significantly different from each other at **(**P>0.05) using Duncan's New Multiple Range Test (DNMRT)

Serum High Density Lipoprotein (HDL)

Figure 6 showed the HDL contents in heart. Control group recorded the highest content of HDL (0.30) followed by 35 mg/kg $AlCl_3$ group (0.18), while 50 mg/kg sildenafil / 35 mg/kg $AlCl_3$ group had the least content of HDL (0.10). There is significant different ($p<0.05$) between the treatments groups compared to the control.

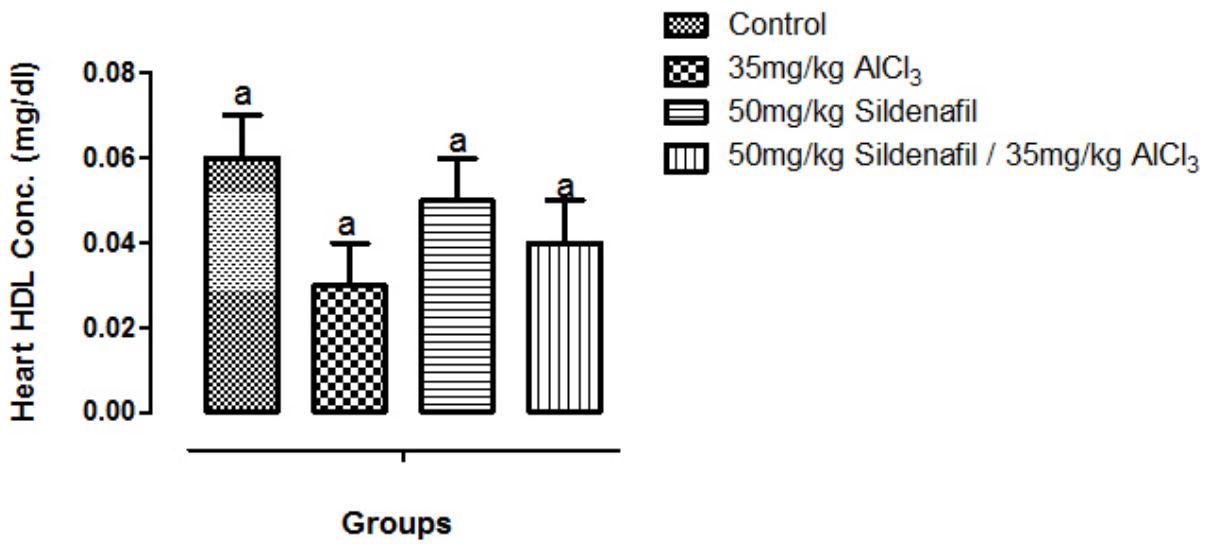

Figure 6: Mean Concentration of Serum High Density Lipoprotein (HDL)

Note: Bars with the same alphabets are not significantly different from each other at **(**P>0.05) using Duncan's New Multiple Range Test (DNMRT)

Serum Cholesterol

Figure 7 showed the cholesterols contents in heart. Control group recorded the highest content of cholesterols (0.30) followed by 35 mg/kg $AlCl_3$ group (0.18), while 50 mg/kg sildenafil / 35 mg/kg $AlCl_3$ group had the least content of cholesterols (0.10). There is significant different ($p<0.05$) between the treatments groups compared to the control.

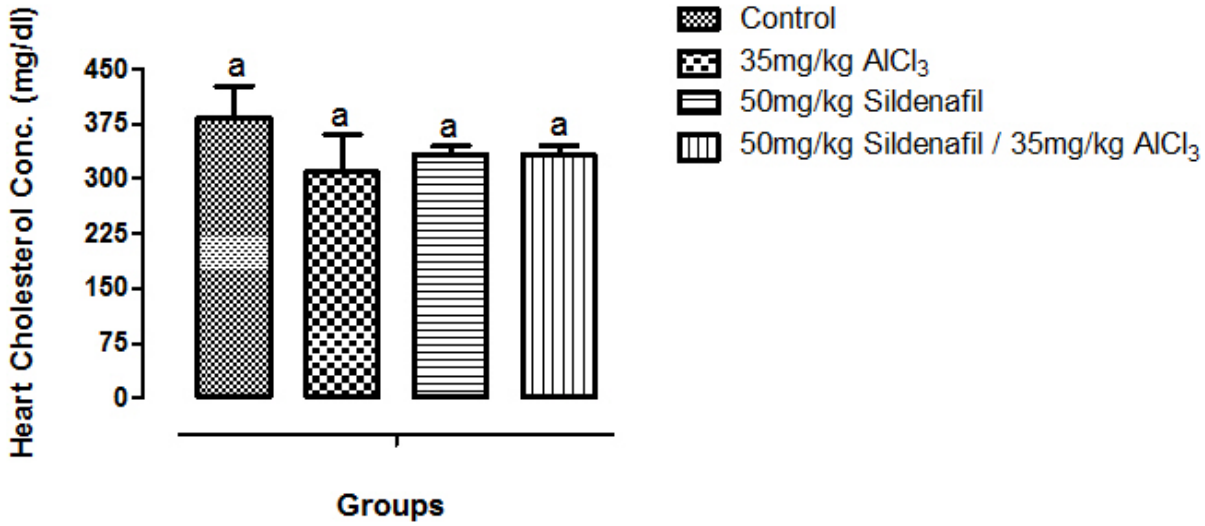

Figure 7: Mean Concentration of Serum Cholesterol

Note: Bars with the same alphabets are not significantly different from each other at **(**P>0.05) using Duncan's New Multiple Range Test (DNMRT)

Table 1: Mean Concentration (Mean±SE) of Lipid Profiles in Heart

Parameters (mg/dl)	Treatments			
	Negative Control	Sildenafil citrate (50 mg/kg)	Sildenafil citrate+$AlCl_3$ (50 mg/kg + 35 mg/kg)	$AlCl_3$ (35 mg/kg)
TRIG.	575.89±2.17^{d}	208.11±0.69^{a}	479.33±0.47^{c}	397.32±0.62^{b}
LDL	7.49±0.29^{ab}	7.26±0.16^{a}	7.84±0.03^{bc}	8.07±0.04^{c}
HDL	0.06±0.03^{a}	0.02±0.01^{a}	0.04±0.01^{a}	0.03±0.00^{a}
Cholesterol	382.72±44.15^{a}	308.97±51.73^{a}	333.06±12.28^{a}	332.63±12.25^{a}

Note: Mean values with the same superscript alphabets in the rows are not significantly different from each other at (P>0.05) using Duncan's New Multiple Range Test (DNMRT).

Table 2: Mean Concentration (Mean±SE) of Lipid Profiles in Serum

Parameters (mg/dl)	Treatments			
	Negative Control	Sildenafil citrate (50 mg/kg)	Sildenafil citrate+$AlCl_3$ (50 mg/kg + 35 mg/kg)	$AlCl_3$ (35 mg/kg)
LDL	7.96±0.24^{a}	7.69±0.25^{a}	7.67±0.30^{a}	7.74±0.04^{a}

HDL	0.30 ± 0.10^{a}	0.18 ± 0.05^{a}	0.12 ± 0.03^{a}	0.11 ± 0.02^{a}
Cholesterol	53.47 ± 8.11^{b}	53.61 ± 0.66^{b}	33.80 ± 3.02^{a}	40.56 ± 4.43^{ab}

Note: Mean values with the same superscript alphabets in the rows are not significantly different from each other at ($P>0.05$) using Duncan's New Multiple Range Test (DNMRT)

Discussion

Since the advent of sildenafil (the first orally administered phosphodiesterase-5 (PDE5) inhibitor) for treatment of erectile dysfunction (ED), its use has enjoyed wide patronage the world over, following reported effectiveness (Pfizer, 2012). Indeed, the advent of sildenafil and other PDE5 inhibitors saw to the management of ED, thus leaving the male folks with having to battle with the other common male sexual dysfunction-premature ejaculation (PE). PDE-5 inhibitors enhance erectile function during sexual stimulation by penetrating into smooth muscle cells and inhibiting PDE-5. This results in decreased degradation of cGMP, which maintains sufficient cellular levels of cGMP in both *corpus cavernosum* and the vessels supplying it. This increases relaxation of the smooth muscle, which dilates the corporeal sinusoids resulting in increased blood flow, allowing an erection to occur. However, it is regrettable that these drugs are currently widely abused the world over. Some undesired effects (predominantly, headache and stomach pain) have been associated with chronic use of sex stimulants like; PDE5 inhibitors, tramadol hydrochloride (an opioid drug used for treatment of PE), herbal therapies, among others (Nna *et al*., 2014).

This study therefore seeks to ascertain effects of sildenafil on serum and heart lipid profile using male albino Wistar rats induced with aluminium chloride. This will provide information on the likelihood to suffer cardiovascular disease based on the heart lipid profiles and serum lipid profiles analysis since alteration in parameters of lipid profile may not be easily discernable, until it becomes life threatening. Aluminum is a trivalent cation that does not undergo redox changes. Oxidative stress induced by Aluminium is one of the major contributing factors to male reproductive disorders. Disruption of metal ion homeostasis may lead to oxidative stress, a state where increased formation of reactive oxygen species (ROS) overwhelms body antioxidant protection and subsequently induces DNA damage, lipid peroxidation, protein modification and other effects (Flora *et al.*, 2008; Jomova, 2011). Lipids have an important role in the functional activity of sperm cells, sperm viability, maturity, capacitation and fertilization (Maqdasy *et al.*, 2013).

In this study, the heart triglycerides, HDL and cholesterol increased significantly following administration of sildenafil. Despite the increase, heart triglycerides, HDL and cholesterol was still significantly lower than control values. HDL helps in scavenging cholesterol from the tissue in the presence of Lecithin Cholesterol Acyl Transferase (LCAT) and brings it to the tissue. HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways. HDL is removed by HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. In humans, probably the most relevant pathway is the indirect one, which is mediated by cholesteryl ester transfer protein (CETP). Heart LDL increased significantly following administration of sildenafil and their increase was significantly higher than control group, though the difference was not significant. The results from the heart LDL supports the fact that sildenafil may be atherogenic following prolong use. Atherogenic indices are powerful indicators of the risk of heart disease; the higher the values, the higher the risk of developing cardiovascular disease and vice versa. The higher levels of acetylcoenzyme A may increase the levels of lipid profile (TC, TG and LDL), as observed in heart lipid profiles in this study since it may be utilized for the synthesis of fatty acids and cholesterol. The increase the levels of heart lipid profile (TC, TG and LDL) were in agreement with Manar *et al.* (2013).

The results from the serum HDL, LDL and cholesterols decreased significantly following administration of sildenafil. Cholesterols values recorded for heart in this study were significantly higher following administration of sildenafil. Serum cholesterol is a term that includes the total level of cholesterol that is found in the bloodstream, it includes identifying all types or classes of cholesterol that are found in the system (Mozaffarian *et al.,* 2010). This helpful measurement makes it possible to determine if the balance between the HDL (good cholesterol) and LDL (bad cholesterol) is within acceptable limits. While the presence of HDL is beneficial to maintaining organ health and providing the body with necessary energy, the presence of LDL can lead to blockages that may lead to problems with the heart and lungs (Olaoluwa *et al.,* 2015).

Cholesterol is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. Within the cell membrane, cholesterol also functions in intracellular transport, cell signaling and nerve conduction. It is synthesized in many types of tissues, but principally in the liver and intestinal wall of vertebrates. Cholesterol is essential for the structure and function of invaginated caveolae and clathrin-coated pits, including caveola-dependent and clathrin-dependent endocytosis. The role of cholesterol in

endocytosis of these types can be investigated by using methyl beta cyclodextrin (MβCD) to remove cholesterol from the plasma membrane. Recent studies show that cholesterol is also implicated in cell signaling processes, assisting in the formation of lipid rafts in the plasma membrane, which brings receptor proteins in close proximity with high concentrations of second messenger molecules (Incardona and Eaton, 2000). In multiple layers, cholesterol and phospholipids, both electrical insulators, can facilitate speed of transmission of electrical impulses along nerve tissue. For many neuron fibers, a myelin sheath, rich in cholesterol since it is derived from compacted layers of Schwann cell membrane, provides insulation for more efficient conduction of impulses (Pawlina and Ross, 2006). Cholesterol assays are used to screen for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein disorders (Gressner and Greiling, 1995). Steroid hormones depend largely on serum lipids for their synthesis, although acetyl Coenzyme A also contributes to the pool of substrates (Guyton and Hall, 2010). The concentration of serum total cholesterol determines in part, the concentration of steroid hormones in serum. Furthermore, a reduction in serum cholesterols was observed. Consistent with this study, Abdellatief *et al*. (2014); Ahmed and Kurkar (2014) demonstrated that tramadol reduced serum concentration of total cholesterol. The consequence of this is that steroid hormones concentration will likely be raised in 50 mg/kg sildenafil and lower in 50 mg/kg sildenafil/35 mg/kg aluminium chloride. The significant reduction in serum total cholesterol observed in 50 mg/kg sildenafil treated group may be attributed to the effect of 50 mg/kg sildenafil / 35 mg/kg aluminium chloride since 50 mg/kg sildenafil administered in isolation did not significantly affect total cholesterol.

Conclusion:

In conclusion, considering the serum cholesterol depleting and downsizing effects of sildenafil; it is important to tame their abuse, as this could affect processes that depend largely on serum lipids for their proper functioning such as synthesis of steroid hormones; as this hormone plays a much vital role in fertility and reproduction, viz-a-viz human existence and continuity. Also, the results from the heart triglycerides, HDL and cholesterol suggest that sildenafil may act as a mixed blessing drug; therefore, it must be used carefully and under physician supervision and scrutiny to get its therapeutic benefits and guard against its adverse effects to human existence.

References

(1) Abdellatief, R.B., Elgamal, D.A. and Mohamed, E.E.M. (2014). Effects of chronic tramadol administration on testicular tissue in rats: An experimental study. *Andrologia.* (In Press). 10.1111/and.12316

(2) Agarwal, A., Mulgund, A., Hamada, A. and Chyatte, M.R. (2015). A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology*. 13: 37.

(3) Ahmed, M.A. and Kurkar, A. (2014). Effects of opioid (tramadol) treatment on testicular functions in adult male rats: The role of nitric oxide and oxidative stress. *Clin. Exp. Pharmacol. Physiol.*, 41: 317-323.

(4) Aitken, R.J. and Roman, S.D. (2008). Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity*. 1: 15–24.

(5) ATSDR, Agency for toxic substances and disease registry. (1990). Toxicological profile for aluminum, US department of Health and Human Services. http:// www.atsdr.cdc.gov/toxprofiles/ tp.asp?id=191&tid=34.

(6) Buraimoh, A.A., Ojo, S.A., Hambolu, J.O. and Adebisi, S.S. (2012). Histological study of the effects of aluminium chloride exposure on the testis of Wistar rats, *American International Journal of Contemporary Research.* 2: 114-122.

(7) Chinoy, N.J., Momin, R. and Jhala, D.D. (2005a). Fluoride and aluminium induced toxicity in mice epididymis and its mitigation by vitamin C. *Fluoride*. 38:115–121.

(8) Chinoy, N.J., Sorathia, H.P. and Jhala, D.D. (2005b). Flouride + Aluminum induced toxicity in mice testis with giant cells and its reversal by vitamin C. *Fluoride.* 38:109-114.

(9) Christie, W. W. (2003). Lipid Analysis. 3rd edition. Oily Press, Bridgewater, UK.

(10) Deveci, S., Peşkircioğlu, L., Aygün, C., Tekin, M.I., Dirim, A. and Ozkardeş, H. (2004). "Sublingual sildenafil in the treatment of erectile dysfunction: faster onset of action with less dose. *I*nternational. *Journal of Urology*. 11(11): 989–992.

(11) Doganci, S., Yildirim, V., Yesildal, F., Erol, G., Kadan, M., Ozkan, G., Avcu, F. and Ozgurtas, T. (2015). Comparison of angiogenic and proliferative effects of three commonly used agents for pulmonary artery hypertension (sildenafil, iloprost, bosentan): is angiogenesis always beneficial? *European Rev Med Pharmacol Sci.* 19: 1900-1906.

(12) Flora, M., Mittal, A.M. (2008). Heavy metal induced oxidative stress & its possible reversal by chelation therapy. I*ndian Journal of Medical Research.* 128:501-523.

(13) Geyikoglu, F., Turkez, H., Bakir, T.O. and Cicek, M. (2012). The genotoxic, hepatotoxic, nephrotoxic, haematotoxic and histopathological effects in rats after aluminium chronic intoxication. *Toxicology and Industrial Health.*

(14) Gill, L. S. (1992). Ethnomedical Uses of Plants in Nigeria, University of Benin Press, Benin, Nigeria.

(15) Gressner, A.M. and Greiling, H. (1995). Lehrbuch der Klinischen Chernie and Pathobiochemie. 3rd Edition, Schattauer, Sluttgart/New York.

(16) Guo, C.H., Lin, C.Y., Yeh, M.S. and Hsu, G.S.W. (2005). Aluminum-induced suppression of testosterone through nitric oxide production in male mice, *Environment Toxicology Pharmacology*. 19:33-40.

(17) Gupta, P.P. (2013). Textbook of Biochemistry with Biomedical Significance for Medical and Dental Students. Second Edition. CBS Publisher and Distributor. Pp. 166-168.

(18) Incardona, J.P. and Eaton, S. (2000). Cholesterol in signal transduction *Current Opinion in Cell Biol*ogy. 12 (2): 193–203.

(19) Jackson, G., Gillies, H. and Osterloh, I. (2005). Past, present, and future: a 7-year update of Viagra (sildenafil citrate). *International Journal of Clinical Practice.* 59: 680-691.

(20) Jomova, K. and Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*. 283: 65-87.

(21) Jomova, M.V. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology.* 283: 65-87.

(22) Kumar, V. and Gill. K.D. (2009). Aluminium neurotoxicity: Neurobehavioural and oxidative aspects, *Archives of Toxicology*, 83: 965-978.

(23) Llobet, J.M., Colomina, M.T., Sirvent, J.J., Domingo, J.L. and Corbella, J. (1994). Reproductive toxicology of aluminium in male mice. *Fundamamental and Applied Toxicology.* 29: 45-51.

(24) Manar, H.A. and Hebatallah, H.A. (2013). Sildenafil citrate attenuates the deleterious effects of elevated ammonia. *Toxicology Mechanism and Methods.* 23(6): 402–411.

(25) Maqdasy, S., Baptissart, M., Vega, A., Baron, S. and Lobaccaro, J.M. (2013). Cholesterol and male fertility: what about orphans and adopted? *Molecular Cell Endocrinology*. 368: 30-46.

(26) Mathews, K., Holde van, K. E. and Ahem, K. G., (2000). Biochemistry, 3d Ed., Addison, Wesley, Longman.

(27) Milligan, P.A., Marshall, S.F. and Karlsson, M.O. (200). A population pharmacokinetic analysis of sildenafil citrate in patients with erectile dysfunction. *British Journal of Clinical Pharmacology*. 53(1): 45S-52S.

(28) Mozaffarian, D., Micha, R. and Wallace, S. (2010) Effects On Coronary Heart Disease of Increasing Polyunsaturated Fat in Place of Saturated Fat: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *PLoS Med*. **7**: e1000252.

(29) Muirhead, G.J., Rance, D.J., Walker, D.K. and Wastall, P. (2002). Comparative human pharmacokinetics and metabolism of single-dose oral and intravenous sildenafil. *British Journal of Clinical Pharmacology*. 53(1): 13S-20S.

(30) Muirhead, G.J., Wilner, K., Colburn, W., Haug-Pihale, G. and Rouviex, B. (2002). The effects of age and renal and hepatic impairment on the pharmacokinetics of sildenafil. *British Journal of Clinical Pharmacology*. 53(1): 21S-30S.

(31) Nichols, D.J., Muirhead, G.J. and Harness, J.A. (2002). Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. *British Journal of Clinical Pharmacology*. 53(1): 5S-12S.

(32) NIH Consensus Conference. (1993). Impotence, NIH Consensus Development Panel on Impotence. *Journal of American Medical Asso*ciation. 270: 83-90.

(33) Nna, V.U., Ani, E.J., Ofutet, E.O., Ofem, O.E., Iroh, C.E. and Osim, E.E. (2014). Recurrent side effects following chronic recreational use of sexual stimulants among male subjects in Calabar, Cross River State, Nigeria. *Der Pharmacia Lettre*. 6: 56-61.

(34) Olaoluwa, T., Adeyemi, O.O., Olugbenga O.A., Funmilayo, D.O. and Sunday, O.O. (2015). Selected Lipid Profile in the Serum & Tissues of Weaned Male Albino Rats Fed on Processed Atlantic Horse Mackerel (*Trachurus trachurus*). *Advances in Bioscience and Biotechnology*. 6: 286-301.

(35) Olayemi, F.O. (2010). A review on some causes of male infertility. *African Journal of Biotechnology*. 9: 2834-3842.

(36) Pawlina, W. and Ross, M.W. (2006). Histology: a text and atlas: with correlated cell and molecular biology Philadelphia: Lippincott Wiliams & Wilkins. Pp. 230.

(37) Pfizer Labs DoPI, N.Y. (2006). Viagra (sildenafil citrate) tablets. Product insert.

(38) Pizent, A., Tariba, B. and Živković, T. (2012). Reproductive toxicity of metals in men, *Archives of Industrial Hygiene and Toxicology.* 63: 35-46.

(39) Salonia, A., Rigatti, P. and Montorsi, F. (2003). Sildenafil in erectile dysfunction: a critical review. *Curr Med Res Opin.* 19: 241-262.

(40) Shin, H.S., Bae, S.K. and Lee, M.G. (2006). Pharmacokinetics of sildenafil after intravenous and oral administration in rats: hepatic and intestinal first-pass effects. *International Journal of Pharmacology.* 320: 64-70.

(41) Turko, I.V., Ballard, S.A., Francis, S.H. and Corbin, J.D. (1999). Inhibition of cyclic GMP-binding cyclic GMP-specific phosphodiesterase (Type 5) by sildenafil and related compounds. *Mol*

(42) Verstraeten, S.V., Aimo, L. and Oteiza, P.I. (2008). Aluminium and lead: molecular mechanisms of brain toxicity, *Archives of Toxicology.* 82: 789–802.

(43) Vilhemsen, T., Eliasen, H. and Schaefer, T. (2005). Effect of a melt agglomeration process on agglomerates containing solid dispersions. *International Journal of Pharmaceutical Science.* 303: 132-142.

(44) Walker, D.K., Ackland, M.J., James, G.C., Muirhead, G.J., Rance, D.J., Wastall, P. and Wright, P.A. (1999). Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. *Xenobiotica.* 29: 297-310.

(45) Wang, R.C., Jiang, F.M., Zheng, Q.L., Li, C.T., Peng, X.Y., He, C.Y., Luo, J. and Liang, Z.A. (2014). Efficacy and safety of sildenafil treatment in pulmonary arterial hypertension: a systematic review. *Respiration Medicine.* 108: 531- 537.

(46) Yousef, M.I., El-Morsy, A.M. and Hassan, M.S. (2005). Aluminum-induced deterioration in reproductive performance and seminal plasma biochemistry of male rabbits: Protective role of ascorbic acid. *Toxicology.* 215: 97-107.

(47) Yousef, M.I., Kamel, K.I., El-Guendi, M.I. and El-Demerdash, F.M. (2007). An *in vitro* study on reproductive toxicity of aluminium chloride on rabbit sperm: the protective role of some antioxidants. *Toxicology.* 239: 213-223.

(48) Yousef, M.I. and Salama, A.F. (2009). Propolis protection from reproductive toxicity caused by aluminium chloride in male rats, *Food and Chemical Toxicology.* 47: 1168-1175.