ASSESSMENT OF THERAPEUTIC, PROLONGED AND OVERDOSE TREATMENT WITH DIAZEPAM ON LIPID PROFILE AND GONADAL STEROID LEVEL OF MATURED MALE ALBINO RATS

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ABSTRACT

The effect of therapeutic, prolonged and overdose treatment with diazepam on lipid profile and gonadal steroid (Testosterone) level of matured male albino rats was evaluated. A total number of 20 male rats (average weight of 160g) were grouped into four of 5 rats each. Group A received a normal therapeutic dose of 0.012 mg /Kg body weight. Group B received an Overdose of 0.036mg/kg body weight. Group C received a prolonged dose of 0.012mg/kg body weight for 28 days. Group D did not receive any treatment and served as the control group. Groups A and B were treated for two weeks while Group C was treated for four weeks. Blood samples were collected from Groups A and B after two weeks and from groups C and D at the end of week 4. Analysis for cholesterol (CHOL), triglycerides (TRYG), Low-density lipoproteins (LDL-C), high-density lipoproteins (HDL-C) and Testosterone hormone were evaluated. Results obtained showed a significant increase ($p<0.05$) in plasma CHOL level in groups that received the Prolonged dose (1.12 ± 0.13mg/dl) and overdose (1.10 ± 0.14mg/dl) when compared to the control (0.93 ± 0.10mg/dl). There was a significant reduction ($p>0.05$) in TRYG for the groups that were administered with normal and prolonged normal doses. The LDL-C levels for the groups administered with overdose (0.68 ± 0.13mg/dl) and prolonged dose (0.68 ± 0.13) was significantly increased when compared with the control (0.48 ± 0.05mg/dl). However, there was a significant decrease in the value of HDL-C for the overdose and prolonged dose groups when compared with the control. The results obtained for testosterone showed that there was a significant decrease ($p<0.05$) in the groups that received overdose (0.95 ± 0.10mg/dl) and prolonged (0.83± 0.05mg/dl) doses of diazepam respectively when compared to the control group (1.45 ± 0.06mg/dl). This study has provided evidence of the adverse effects of overdose and prolonged intake of diazepam on lipid profile and testosterone which is reproductive hormone in matured male albino rats.

Keywords: Lipid profile, testosterone, diazepam, therapeutic dose, prolonged dose, overdose.

INTRODUCTION

Diazepam also known as benzodiazepines is a long-acting, medium-potency benzodiazepine that is used as an anticonvulsant and for anxiolysis, sedation, and myorelaxation. It was first marketed as Valium by Hoffmann-La Roche, It possesses anxiolytic, anticonvulsant, sedative, muscle relaxant, depressant and amnestic properties (Mandrioli *et al.*, 2008). Benzodiazepines are used for their anxiolytic, sedative hypnotic, muscle relaxant, and anticonvulsant properties in the treatment of a variety of neuropsychiatric disorders (Bateson 2002; Tan *et al.*, 2011) including anxiety and depression, which are often related to disturbances in the activity of hypothalamic-pituitary-adrenal (HPA) axis (Arborelius *et al.*, 1999). Diazepam is commonly used to treat a range of conditions including anxiety, alcohol

withdrawal syndrome, benzodiazepine withdrawal syndrome, muscle spasms seizures, trouble sleeping, and restless legs syndrome (Calcaterra *et al*., 2014). It may also be used to cause memory loss during certain medical procedures and it can be taken by mouth, inserted into the rectum, injected into a muscle, or injected into a vein. It may also be used in some surgical procedures to induce amnesia and reduce anxiety (Cascade *et al.*, 2008). It is a core medicine in the World Health Organization's Essential Drugs List, the minimum medical needs for a basic health-care system (WHO Model List, 2005). The severity of diazepam-induced adverse effects forces physicians to exercise caution and pay attention to side effects when prescribing this drug. (Crestani *et al*., 2001). Williams *et al.*, (1984) found that benzodiazepines act as Calcium channels antagonists as they can produce a complete inhibition of voltage-dependent Ca^{2+} uptake. Another study suggested that the $Na+/Ca^{2+}$ exchange carrier in mitochondria may be a common receptor for diazepam and calcium channel blockers (Matlib *et al*., 1983). The endocrine control of Leydig cell steroidogenic activity by luteinizing hormone (LH) or follicle-releasing hormone (FSH) has been exerted through their respective receptors coupled to the Ca^{2+}mediated signalling pathway (Tomic *et al*., 1995).Calcium ion is implicated in diverse cellular functions in both germ cells and somatic cells in the testis, particularly, mediating the responses to endocrine hormones and local regulators in genital tracts (Berridge *et al*., 2003). A common belief is that the Ca^{2+} influx and efflux should be tightly regulated to maintain the intracellular Ca^{2+} homeostasis, and an alteration in the Ca^{2+} transport across the cell membrane could result in a drastic impact on spermatogenesis and steroidogenesis. (Yamaguchi 2005).

It's worth noting that the sudden discontinuation of benzodiazepines can be potentially dangerous or life-threatening for individuals using regularly for extended periods of time, sometimes resulting in seizures or death (Mandrioli *et al.*, 2008). It is highly recommended to taper one's dose by gradually lowering the amount taken each day for a prolonged period of time instead of stopping abruptly.

Infertility is one of the most serious problems faced by some people around the world and the male counterpart contributes half of the infertility cases (Miyamoto *et al*., 2012). Testosterone is the most important androgen (male sex hormone) in men and it is needed for normal reproductive and sexual function. Testosterone is important for the physical changes that happen during male puberty, such as the development of the penis and testes, and for the features typical of adult men such as facial and body hair. Testosterone also acts on cells in the testes to make sperm (Andrology 2015). Testosterone deficit in men may exhibit symptoms such as decreased libido, erectile quality and low or zero sperms in semen (Jose-miller *et al*., 2007). The diagnostic testing can be done from the history of drugs taken, physical examination and of course, semen analysis (Stahl *et al*., 2012). According to Schulte *et al*. (2010) sperm characteristics assessment has been increasingly important in reproductive studies.

Diazepam can alter the lipid profile of an individual. However, cardiovascular diseases due to elevated lipid profile in the plasma (especially plasma LDL-C) may occur after the intravenous administration of diazepam particularly if other anti-seizure agents or central depressants have been administered previously or if diazepam is taken for a long period or an overdose is administered (Hardman and Limbird, 1996). The aim of this study Investigate the effect of various doses and treatment period of diazepam on lipid profile and gonadal steroids of male albino rats

MATERIALS AND METHODS

Preparation of Diazepam stock

The stock solution of diazepam was prepared by dissolving 5 mg of the drug in 100 ml distilled water. The dose to be administered was calculated based on the average body weight of the rats per group.

Experimental design

A total number of 20 male rats (average weight of 160g) were purchased from Physiology animal house in Abuja campus, University of Port Harcourt. They were then grouped into four of 5 rats each
Group A; Normal dose: 0.012 mg /Kg body weight
Group B; Overdose: 0.036mg/kg body weight
Group C; Prolonged dose: 0.012mg/kg body weight ...treated for 21 days.
Group D; Control group: Received no treatment.
This study was carried out for four weeks. At the end of which blood samples were collected.

Method of blood collection

The procedure used was described by Yakubu *et al*. (2005). Each of the adult rats was anaesthetized in chloroform vapour in desiccators and dissected using surgical forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into plain sample tubes and were allowed to stand for 120mins at room temperature to clot, after which they were centrifuged at 3000rpm for 10mins using a bench top centrifuge Uniscope Laboratory Centrifuge (Model 802, Surgifriend Medicals and Essex, England), to obtain the serum. The sera obtained from the samples were carefully removed using Pasteur pipettes, into respective labelled plastic specimen bottles and stored frozen in a bio-freezer until ready for analysis.

Determination of Lipid Profile

Total Cholesterol, triglycerides, LDL and HDL were analysed by kinetic methods kits from Randox, (United Kingdom) using a double-beam spectrophotometer.

Procedure for Testosterone Assay

A hormonal assay kit for Testosterone was supplied by Diagnostic Automation Inc., Calabasa, CA, USA. All other reagents used were of analytical grade and were prepared in a volumetric flask using glass-distilled water. Each of the microplate wells was first formatted i.e. the desired number of wells required for the investigation was collected. The micropipette was then set to the desired volume by turning the knob. 50ul of standards, specimen and control was then added to the appropriate well of the antibody pre-coated micro-titre plate. This was then followed by addition of 100ul of the enzyme conjugate reagent (Biotin) into each well with thorough mixing for 30 seconds. The mixture was then incubated at room temperature for 37^0C for 60 minutes. The incubation mixture was then removed by flicking each plate content into a sink. A washing buffer was then used to remove and flick the micro-titre wells. This was done several times. After the final washing, the plate was then inverted and blot dried by striking plate onto absorbent paper to remove all residual water droplets. Each well was then incubated again at room temperatures for another 20 minutes and this was

followed by addition of 100ul of stop solution to stop the reaction process. Each well was gently mixed by rocking plates for 30 seconds to ensure complete discoloration from blue to yellow. The optical densities at 450nm of the mixture in each well were then read using a micro-titre well reader within 30 minutes. The procedure described in the hormone assay kits was used according to the principle highlighted by (Uotila *et al.*, 1981).

Statistical Analysis

The arithmetic mean and standard mean error (MEAN±SEM) was calculated for each value. The results were subjected to statistical analysis using student t- test method. Statistical significance was read at $p<0.05$.Values with the same superscript were considered not statistically significant when compared to their respective controls.

RESULTS

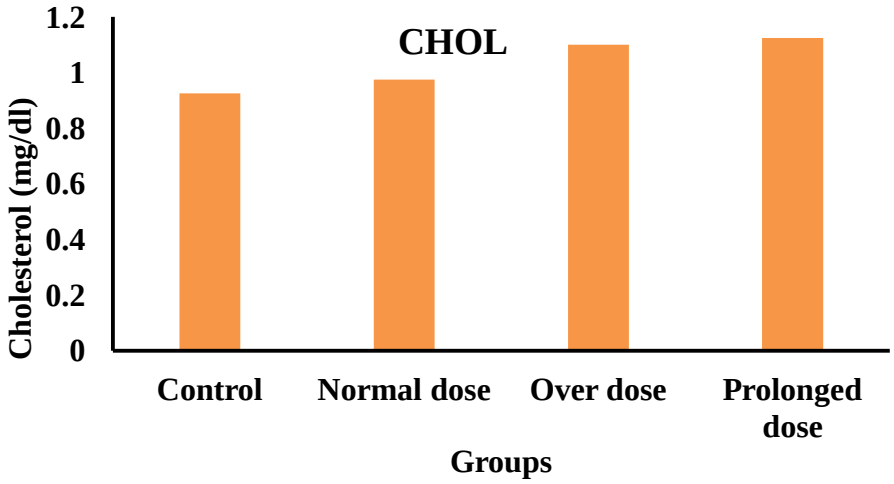

Figure 1: Evaluation of various doses of diazepam on cholesterol levels of matured male albino rats

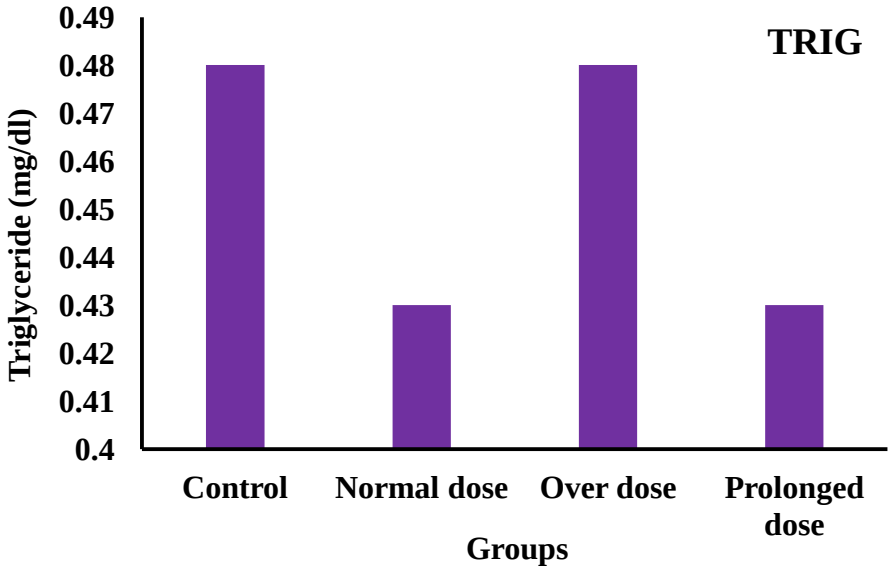

Figure 2: Evaluation of various doses of diazepam on triglyceride levels of matured male albino rats

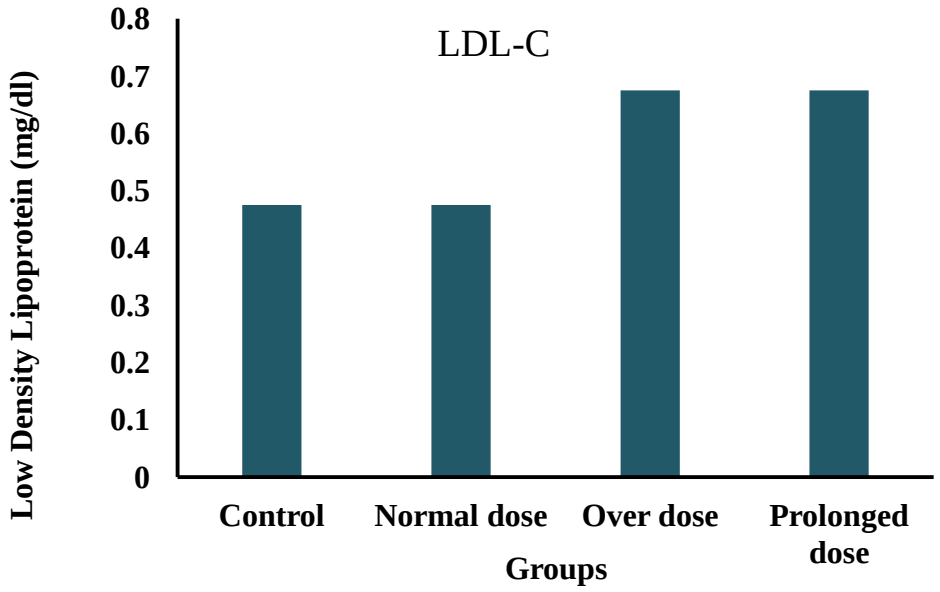

Figure 3: Evaluation of various doses of diazepam on LDL-C levels of matured male albino rats

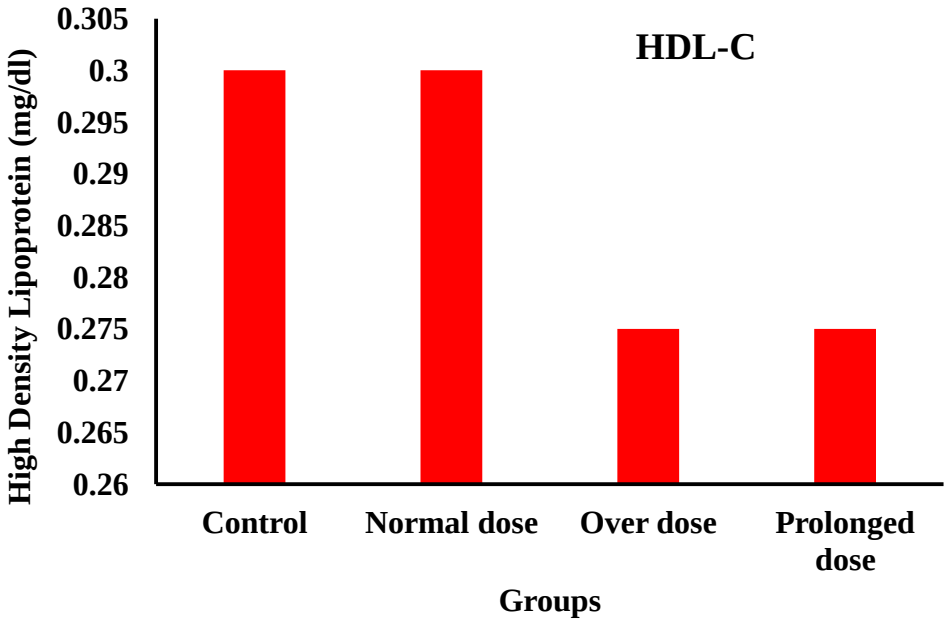

Figure 4: Evaluation of various doses of diazepam on HDL-C levels of matured male albino rats

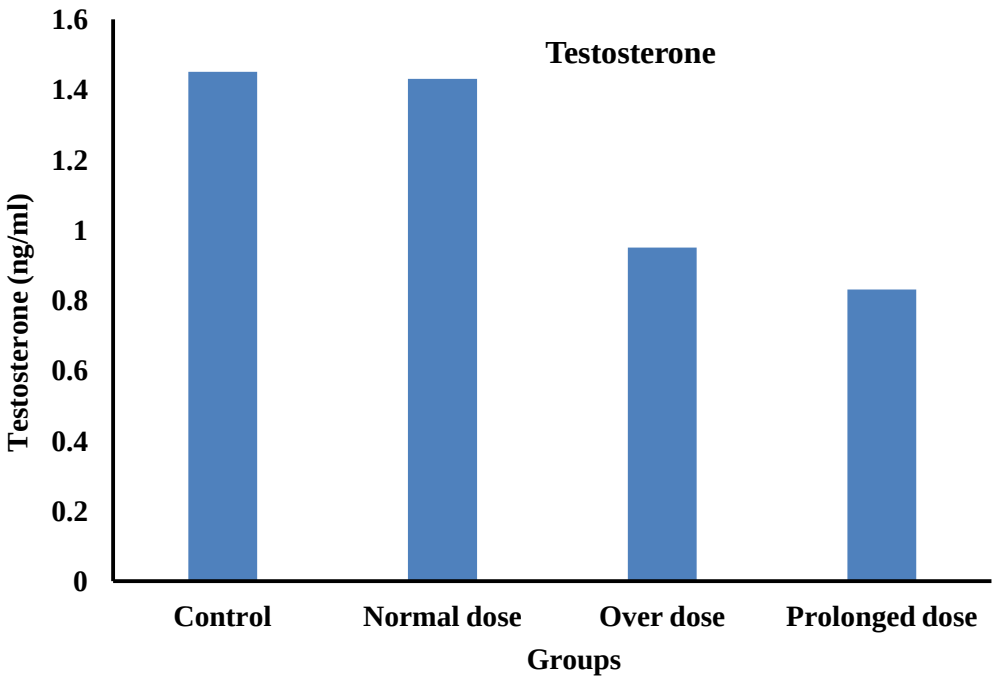

Figure 3: Evaluation of various doses of diazepam on Testosterone levels of matured male albino rats

DISCUSSION AND CONCLUSION

Diazepam, which is benzodiazepine medication has been used to relieve anxiety when taken in small doses but can induce sedation when taken in sufficient doses and is currently being used in Nigeria. The results obtained from this study revealed that after two and four weeks of diazepam dose administration, there was a significant increase ($p<0.05$) in plasma cholesterol level in group that received the Prolonged dose when compared to the control, normal therapeutic dose and Overdose groups.), this increase in total cholesterol may be as result of the diazepam cause disturbance in the liver metabolism, this results are in agreement with the report that there is an increase in cholesterol synthesis with long term diazepam treatment (Jezeque *et al*., 2008), these findings also point to the side effect of the drug. In Triglyceride, there was no significant increase ($p>0.05$) in the groups that received the normal therapeutic, overdose and prolonged doses in triglyceride and high-density lipoprotein cholesterol respectively when compared to the control group. HDL-C is also called the "good" cholesterol and no significant increase indicate that diazepam plays no role in lowering the risk of heart disease. Individuals with higher levels HDL cholesterol have a fewer problem with cardiovascular disease while those with low HDL-C have an increased risk (Barter *et al*; 2007).There was no significant increase ($p>0.05$) of LDL-C in the group that received the normal therapeutic dose when compared to the control group. However, there was a significant increase ($p<0.05$) when the control group (0.48 ± 0.05mg/dl) was compared to the groups that received the overdose (0.68 ± 0.13mg/dl) and prolonged dose (0.68 ± 0.13mg/dl). LDL-C is known as the "bad" cholesterol and its increase in overdose and prolonged doses indicate that if diazepam is not taken in the normal dose it can increase the risk of cardiovascular diseases. The results obtained for testosterone showed that there was a significant decrease ($p<0.05$) in the groups that received overdose (0.95 ± 0.10mg/dl) and prolonged (0.83 ± 0.05mg/dl) doses of diazepam respectively when compared to the control group (1.45 ± 0.06mg/dl) and the group that received the normal therapeutic dose (1.43 ± 0.17mg/dl).According to Shahrak *et al.*, (2008), a decrease in the level of testosterone

will eventually be reflected on the peripheral sex organ functions including epididymis and testis and infertility can be caused by androgen deficiency or low testosterone level.

In conclusion, the result of this study on the effect of diazepam on lipid profile and testosterone hormone shows that there was no significant increase in plasma triglyceride and high-density Lipoprotein cholesterol (HDL-C) which is known as the good cholesterol but a significant increase was observed in total cholesterol and Low-density Lipoprotein cholesterol. This increase in LDL-C implies that it is the major cause of the increase in the total serum cholesterol. Therefore diazepam should be taken with caution in order to reduce the risk of hypercholesterolemia which is a risk factor for cardiovascular diseases that are associated with increased plasma total cholesterol and low-density Lipoprotein. Furthermore, this present review provides evidence of adverse effects of overdose and prolonged intake of diazepam on testosterone which is a reproductive hormone. The implication being that overdose or prolong intake of diazepam may lead to infertility in male albino rats.

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