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Effect of Short Term Administration of Diazepam and Bromazepam on Lipid Profile of Albino Wistar Rats (*Rattus rattus*)

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Abstract: Diazepam and bromazepam though are restricted drugs are commonly used especially in this part of the world; it is a known fact that some medications has negative effect on lipid profile. The aim of this study is to investigate the effect of short term administration these drugs on albino *wistar* rats. This study investigated the *in-vivo* effect of short term administration of diazepam (DZ) and bromazepam (BZ) at 0.0046 mg/100 g bwt (1), 0.0036 mg/100 g bwt (2), 0.0026 mg/100 g bwt (3) and 0.0016 mg/100 g bwt (4) concentrations on serum lipid profile of *albino wistar* rats. The parameters that were investigated are: total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL). Data obtained were subjected to statistical calculation using SPSS version 18. Mean values (M) ± SD were calculated and One-Way ANOVA test was performed, significance level was calculated at 95 % confidence level ($P < 0.05$). Serum HDL, serum LDL, serum total cholesterol, and serum triglyceride were reduced by both drugs throughout the duration of study in a dose-dependent manner. The reduction was significant ($P < 0.05$) for total cholesterol and triglyceride. Comparing the reducing/increasing effect between the two drugs at similar dosage showed no significant difference. Short term administration of diazepam and bromazepam at these low dosages does not affect negatively total cholesterol, triglyceride, HDL and LDL concentrations.
Keywords: Diazepam, Bromazepam, lipid profile.

INTRODUCTION

Benzodiazepines are minor tranquilizers that act as central nervous system (CNS) depressant through the facilitation of gamma amino butyric acid (GABA), an inhibitory neurotransmitter binding at various GABA receptors throughout the CNS [1].

Benzodiazepines are effective for the following therapeutic actions in short term usage; anxiolytic (anxiety and panic disorder), myorelaxant (muscle spasms, spastic disorders), anticonvulsant (some forms of epilepsy, fits due to drug poisoning), hypnotic (insomnia) and amnesia (sedation for minor operations) [2].

An altered lipid profile is among the traditional biomarkers used in the forecast of increased cardiovascular risk [3]. Lipid profile could be adversely affected by enzyme inducing anticonvulsants, protease inhibitors, diuretics etc. [4], it is therefore important that all patients consuming benzodiazepine including those with high risk of cardiovascular disease need to be protected and some of these individuals are not aware of their health status and only recognized their poor health state when the condition worsen. A lot of persons in this part of the world are ignorant as to their health status and only come into awareness when their poor health condition are fully manifested, hence taking a medication without adequate information on its effect especially on the lipid profile is one of the reasons behind lipid disasters. Result from this study will enlighten users of the drug as to its effect on lipid profile.

MATERIALS AND METHODS

Animals

One hundred and eight *Albino Wistar* rats weighing between 100 g – 110 g of both sex were obtained from Animal house of the University of Port-Harcourt Choba Rivers State Nigeria, acclimatization was done for one week prior to experimentation. Clearance was obtained from the ethics committee of university of PortHarcourt for use of animals for experiment. Water and standard rat chow were provided *ad libitum* and the animals kept under room temperature. At the end of each week three rats were

sacrificed from each groups, the animals were divided into nine groups as follows;

Group 1 (DZ1) consisting of twelve rats administred orally 0.0046 mg/100 g body weight (bwt) of diazepam

Group 2 (DZ2) consisting of twelve rats administred orally 0.0036 mg/100 g bwt of diazepam

Group 3 (DZ3) consisting of twelve rats administred orally 0.0026 mg/100 g bwt of diazepam

Group 4 (DZ4) consisting of twelve rats administred orally 0.0016 mg/100 g bwt of diazepam

Group 5 (BZ1) consisting of twelve rats administred orally 0.0046 mg/100 g bwt of bromazepam

Group 6 (BZ2) consisting of twelve rats administred orally 0.0036 mg/100 g bwt of bromazepam

Group 7 (BZ3) consisting of twelve rats administred orally 0.0026 mg/100 g bwt of bromazepam

Group 8 (BZ4) consisting of twelve rats administred orally 0.0016 mg/100 g bwt of bromazepam

Group 9 (control group) consisting of twelve rats.

Drugs and chemicals

Bromazepam (Lexotan) tablet and diazepam (valium) used were made in Switzerland by F. Hoffmann-La Roche Ltd, Basel, randox kit from Randox chemical laboratory and all other reagents used were of analytical grade.

Collection of blood

Blood was collected through cardiac puncture and blood sample collected into plain sample bottles. The bottle containing the blood sample was allowed to stand for one hour to allow clothing after which it was centrifuged at 3000 revolution per minute for ten minutes; the supernatant was separated into clean tube and stored in a refrigerator at – 4 ˚C for subsequent analysis. Repeated thawing and refreezing of samples was avoided.

Estimation of lipid profile

Serum triglyceride and total cholesterol was estimated using randox triglyceride and cholesterol kit [5], serum HDL-cholesterol was estimated using randox HDL-cholesterol kit (precipitation method) [6], while LDL-cholesterol was calculated using the Friedwald's formula [7].

Analysis of results

Data obtained from experimental design and set up were subjected to statistical calculation using SPSS version 18. Mean values (M) ± SD were calculated and One-Way ANOVA test was performed. Significance level was calculated at 95 % confidence level ($P < 0.05$).

RESULTS

Table-1 to 4 shows results of the effect of diazepam and bromazepam on lipid profile of experimental animals. Significant reduction in serum total cholesterol, serum triglycerides and serum LDL was observed in the animals that were administered the drugs in a dose dependent manner while serum HDL was also reduced but the reduction was not significant.

Table-1: Effect of diazepam and bromazepam on serum total cholesterol concentration

Drug Concentrations (mg/L)	Serum total cholesterol concentration (mmol/L)			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
CRTL	3.9 ± 0.1	3.93 ± 0.12	3.97 ± 0.06	3.97 ± 0.15
DZ1	3.07 ± 0.12[a]	3.03 ± 0.06[a]	3.11 ± 0.23[a]	3 ± 0.2[a]
DZ2	3.23 ± 0.23[a]	3.47 ± 0.12	3.17 ± 0.12[a]	3.43 ± 0.06[a]
DZ3	3.57 ± 0.29[b,c]	3.63 ± 0.23[b,c]	3.07 ± 0.29[a]	3.57 ± 0.12[b,c]
DZ4	3.63 ± 0.06[b,c]	3.9 ± 0.17[b,c]	3.57 ± 0.12[c]	3.8 ± 0.1[b,c]
BZ1	2.97 ± 0.06[a]	3.03 ± 0.15[a]	3 ± 0.1[a]	3 ± 0.1[a]
BZ2	3.3 ± 0.1[a]	3.33 ± 0.06[a]	3.4 ± 0[a]	3.03 ± 0.25[a]
BZ3	3.53 ± 0.15[c]	3.83 ± 0.06[b,c]	3.87 ± 0.06[b,c]	3.5 ± 0.1[b,c]
BZ4	3.97 ± 0.12[b,c]	3.9 ± 0.2[b,c]	3.97 ± 0.21[b,c]	3.63 ± 0.15[b,c]

The values are represented as mean ± SD of triplicates, significant difference from the control ($P < 0.05$) is represented by the alphabet a while b represent significant difference from DZ1 and c represent significant difference from BZ1.

Table-2: Effect of diazepam and bromazepam on serum triglyceride concentration

Drug Concentrations (mg/L)	Serum triglyceride concentration (mmol/L)			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
CRTL	1.71 ± 0.01	1.73 ± 0.01	1.72 ± 0.01	1.71 ± 0.01
DZ1	1.27 ± 0.06[a]	1.1 ± 0.1[a]	1.3 ± 0.1[a]	1 ± 0.1[a]
DZ2	1.3 ± 0.1[a]	1.4 ± 0.1[a,b]	1.5 ± 0.1	1.3 ± 0.1[a,b]
DZ3	1.53 ± 0.06	1.4 ± 0[a,b]	1.53 ± 0.06	1.37 ± 0.06[a,b]
DZ4	1.6 ± 0.2[b]	1.6 ± 0.1[b,c]	1.63 ± 0.15[b,c]	1.63 ± 0.12[b,c]
BZ1	1.37 ± 0.12[a]	1.3 ± 0[a]	1.27 ± 0.06[a]	1.33 ± 0.06[a,b]
BZ2	1.4 ± 0[a]	1.27 ± 0.06[a]	1.47 ± 0.06	1.5 ± 0.1[b]
BZ3	1.53 ± 0.06	1.53 ± 0.06[b]	1.53 ± 0.06	1.63 ± 0.12[b,c]
BZ4	1.7 ± 0.1[b,c]	1.6 ± 0.2[b,c]	1.63 ± 0.06[b,c]	1.7 ± 0.1[b,c]

The values are represented as mean ± SD of triplicates, significant difference from the control ($P < 0.05$) is represented by the alphabet a while b represent significant difference from DZ1 and c represent significant difference from BZ1.

Table-3: Effect of diazepam and bromazepam on serum HDL concentration

Drug Concentrations (mg/L)	Serum HDL concentration (mmol/L)			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
CRTL	1.13 ± 0.12	1.03 ± 0.06	1.1 ± 0.1[a]	1.03 ± 0.06[a]
DZ1	0.97 ± 0.06	0.8 ± 0.1	0.8 ± 0[a]	0.67 ± 0.06[a]
DZ2	1 ± 0	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
DZ3	1.03 ± 0.06	0.93 ± 0.06	0.77 ± 0.15	0.8 ± 0.1
DZ4	1 ± 0.1	0.83 ± 0.12	0.83 ± 0.12	0.97 ± 0.06[b]
BZ1	0.87 ± 0.06	0.73 ± 0.06[a]	0.7 ± 0.17[a]	0.8 ± 0.1
BZ2	0.97 ± 0.15	0.7 ± 0.1[a]	0.67 ± 0.06[a]	0.8 ± 0.1
BZ3	0.97 ± 0.06	0.9 ± 0	0.87 ± 0.06	0.93 ± 0.06
BZ4	1 ± 0.1	0.9 ± 0.1	0.97 ± 0.06	0.97 ± 0.06[b]

The values are represented as mean ± SD of triplicates, significant difference from the control ($P < 0.05$) is represented by the alphabet a, and b represent significant difference from DZ1.

Table-4: Effect of diazepam and bromazepam on serum LDL concentration

Drug Concentrations (mg/L)	Serum LDL concentration (mmol/L)			
	WEEK 1	WEEK 2	WEEK 3	WEEK 4
CRTL	2.1 ± 0.14	2.2 ± 0.08	2.2 ± 0.17	2.15 ± 0.15
DZ1	1.52 ± 0.10[a]	1.73 ± 0.12	1.74 ± 0.27	1.88 ± 0.21
DZ2	1.64 ± 0.27	1.93 ± 0.14	1.78 ± 0.11	2.14 ± 0.12
DZ3	1.84 ± 0.30	2.06 ± 0.17	1.6 ± 0.30[a]	2.15 ± 0.13
DZ4	1.91 ± 0.15	2.24 ± 0.08	1.99 ± 0.08	2.09 ± 0.15
BZ1	1.48 ± 0.06[a]	1.71 ± 0.2	1.72 ± 0.10	1.59 ± 0.03[a]
BZ2	1.7 ± 0.06	2.06 ± 0.18	2.06 ± 0.08	1.55 ± 0.20[a]
BZ3	1.87 ± 0.18	2.24 ± 0.07	2.23 ± 0.04	1.82 ± 0.17
BZ4	2.13 ± 0.05[b]	2.27 ± 0.01	2.26 ± 0.28	1.89 ± 0.12

The values are represented as mean ± SD of triplicates and significant difference from the control ($P < 0.05$) is represented by the alphabet a.

DISCUSSION

Effect of diazepam and bromazepam on lipid profile

Total serum cholesterol was reduced by both diazepam and bromazepam throughout the duration of experiment (table 1), the reduction in total serum cholesterol concentration was significant ($P < 0.05$) at higher doses of 0.0046 mg/100 g bwt and 0.0036 mg/100 g bwt for both diazepam and bromazepam throughout the four weeks experimental period when compared to control. Diazepam had serum cholesterol concentrations of 3.07 ± 0.12* mmol/L at DZ1 and 3.23 ± 0.23* mmol/L at DZ2, and bromazezepam values were 2.97 ± 0.06* mmol/L at BZ1 and 3.3 ± 0.10* mmol/L at BZ2, all at week one. Diazepam although reduced total serum cholesterol concentration more than bromazepam but was not significantly different from bromazepam's reduction, the effect of both drugs at week one remained the same by the end of fourth week.

Triglyceride concentration was decreased significantly in a dose dependent manner by both drugs throughout the duration of experiment as compared to the control ($P < 0.05$) (table 2). At week one DZ1 had a triglyceride concentration of 1.27 ± 0.058* mmol/L, DZ4 1.6 ± 0.02 mmol/L, BZ1 1.37 ± 0.12* mmol/L and BZ4 1.7 ± 0.10 mmol/L while control had 1.71 ± 0.1 mmol/L. Throughout the four weeks this pattern re-occurred, the effect of bromazepam and diazepam compared against each other at similar dosage was not significantly different.

HDL-Cholesterol concentration was reduced not significantly by the varying concentrations of diazepam and bromazepam at week one (table 3). By week two there was significant reduction by bromazepam 0.73 ± 0.05774* mmol/L at BZ1 and 0.7 ± 0.1* mmol/L at BZ2 while diazepam had no significant reduction 0.8 ± 0.1 mmol/L at DZ1 and 0.9 ± 0.1 mmol/L at DZ2 as compared to the control with a cholesterol concentration of 1.03 ± 0.05774 mmol/L. Week 3 showed a significant reduction by both drugs when compared to Control had a HDL-cholesterol concentration of 1.1 ± 0.1 mmol/L, diazepam 0.8 ± 0.0* mmol/L at DZ1 and 0.7 ± 0.1* mmol/L at DZ2, and bromazepam 0.7 ± 0.17321* mmol/L at BZ1 and 0.67 ± 0.05774* mmol/L at BZ2. Bromazepam reducing effect was no longer significant at week four while diazepam continued to elicit a significant reducing effect on HDL-cholesterol. Bromazepam at week four had a HDL-cholesterol concentration of 0.8 ± 0.1 mmol/L at BZ1 and 0.8 ± 0.1 mmol/L at BZ2 while diazepam had a significant reduction of HDL-cholesterol to 0.67 ± 0.05774* mmol/L at DZ1 and 0.7 ± 0.1* mmol/L at DZ2 as compared to the control with a cholesterol concentration of 1.03 ± 0.05774 mmol/L. LDL-cholesterol concentration was reduced by diazepam and bromazepam when compared to the control (table 4), the reduction was not significant at ($p < 0.05$).

High concentration of blood triglycerides, LDL and total cholesterol are linked with increased possibility of atherosclerosis, coronary heart disease etc. [8], consumption of an anxiolytic drug that increases cholesterol, LDL or triglyceride by a potential heart disease patient would be fatal. Result of this experimentation indicates the safety of diazepam and bromazepam, as cholesterol, LDL and triglyceride level were reduced significantly, HDL level was also reduced. This result (significant reduction in triglyceride level) is in support of the work of Garabadu and Krishnamurthy [9] that diazepam ehanced antihyperglycemic and antihypertriglyceridaemic effect of metformin in cooccurring type-2 diabetes mellitus (T2DM) and stress exposed rats.

Although an earlier LRC programm prevalence study in 1981 reported elevations of triglycerides, VLDL in diazepam, flurazepam and chlordiazepoxide users (long term users) compared to the entire populations of non-users and matched control non-users, no significant alterations was found in their LDL or total plasma cholesterol [10]. In a study carried out by Mahmoud, Moustafa, Mohamed and Ismail, bromazepam through a mechanism dependent on glutathione concentrations elevated lipid peroxidation [11]. Lipid peroxidation leads to membrane break [12], with subsequent favoring of free radicals production [13], and in hyperlipidaemia lipid peroxidation is high. However elevated level of lipid profile and lipid peroxidation occurred at high dosage consumption of the drugs.

CONCLUSION

Short term consumption of diazepam and bromazepam at the doses stated in this work might not pose a serious concern on blood lipid profile.

REFERENCES

1. Ashton H. The Diagnosis and Management of Benzodiazepine Dependence. Current Opinion in Psychiatry, 2005; 18: 249-255.
2. Ashton CH. Benzodiazepines: How they work and how to withdraw. The Ashton Manual, Aug. 2002 Aug.
3. Upadhyay RK. Emerging risk biomarkers in cardiovascular diseases and disorders. Journal of lipids. 2015;2015.
4. Mantel-Teeuwisse AK, Kloosterman JM, Maitland-van der Zee AH, Klungel OH, Porsius AJ, de Boer A. Drug-induced lipid changes. Drug Safety. 2001 May 1;24(6):443-56.
5. Jiao L, Xiujuan S, Juan W, Song J, Lei X, Guotong X, Lixia L. Comprehensive experiment—clinical biochemistry: Determination of blood glucose and triglycerides in normal and diabetic rats. Biochemistry and Molecular Biology Education. 2015 Jan 2;43(1):47-51.
6. Hirano T, Nohtomi K, Koba S, Muroi A, Ito Y. A simple and precise method for measuring HDL-cholesterol subfractions by a single precipitation followed by homogenous HDL-cholesterol assay. Journal of lipid research. 2008 May 1;49(5):1130-6.
7. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972 Jun 1;18(6):499-502.
8. Larosa JC. Cholesterol and cardiovascular disease: how strong is the evidence?. Clinical cardiology. 1992 Nov 1;15(S3):2-7.
9. Garabadu D, Krishnamurthy S. Diazepam potentiates the antidiabetic, antistress and anxiolytic activities of metformin in type-2 diabetes mellitus with cooccurring stress in experimental animals. BioMed research international. 2014;2014.

10. Hunninghake D, Wallace RB, Reiland S, Barrett-Connor E, Wahl P, Hoover J, Heiss G, Medication and hormones working group. Alterations of plasma lipid and lipoprotein levels associated with benzodiazepine use The LRC program prevalence study. Atherosclerosis. 1981 Oct 1;40(2):159-65.
11. Abdel-Latif MM, El-Naggar MH, Omran MA, Abdel-Nabi IM. Evaluation of the pathophysiological effects of bromazepam on some clinical chemistry parameters of the rat. Egyptian Journal of Biology. 2001;3(2).
12. Santi A, Duarte MM, de Menezes CC, Loro VL. Association of lipids with oxidative stress biomarkers in subclinical hypothyroidism. International journal of endocrinology. 2012;2012.
13. Bouderbala S, Lamri-Senhadji M, Prost J, Lacaille-Dubois MA, Bouchenak M. Changes in antioxidant defense status in hypercholesterolemic rats treated with Ajuga iva. Phytomedicine. 2008 Jun 20;15(6-7):453-61.