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Altered Cerebellar Metabolic Parameters in Bromazepam Treated Rats: Implications of Gradual Cessation Protocol

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Abstract

Objectives: Many reports avail on the physiological implications of benzodiazepine and bromazepam use and misuse. The aim of the study was to investigate the effect of gradual cessation protocol of bromazepam administrations on cerebellar metabolisms in female rats.

Methods: Twenty-five female rats weighing 150-160g were randomly divided into five groups of five rats each. Administration of a single daily oral dose of 1.15mg/kg body weight of bromazepam was done for nine days. Cessation was thereafter done three, six and nine days respectively. Cerebellar levels of glycogen, glucose and lactate, blood/cerebellum glucose ratio, cerebellar glucose/glycogen ratio, ataxia index and other parameters were determined.

Results: Bromazepam administration caused significant reduction in cerebellar glycogen. Bromazepam-induced depression in glycogen content was also observed 3days after cessation. However, restoration of the glycogen occurred and peaked 6 days after cessation. Plasma/ Cerebellar glucose ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day cessation groups respectively. Cerebellar glucose/glycogen ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day cessation groups respectively. Bromazepam administration significantly increased ataxia index when compared with control, 3-day, 6-day and 9-day cessation groups respectively. Ataxia index correlated negatively

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with cerebellar glycogen ($r = -0.712$, $P<0.05$) and positively with cerebellar glucose/ glycogen ratio ($r = 0.917$, $P<0.05$) respectively.

Conclusion: The results of the study indicated the adverse but time-dependent reversible effects of bromazepam on cerebellum metabolic parameters in adult female Wistar rats.

Keywords: Cerebellum, Lactate, Glycogen, Bromazepam, Coordination, Ataxia Index

INTRODUCTION

Cerebellum is a part of rhombencephalon and it is situated in the posterior cranial fossa. It averagely weighs 150g in adult male (1). Like other brain areas, its outer part is made of gray mater formed from nerve cell bodies and inner gray mater made of nerve fibers (2). Functionally, cerebellum consists of cerebrocerebellar, spinocerebellum and vestibulocerebellum. Cerebellum plays role in balance and posture. It is concerned with motor coordination, a process which involves motor planning and time-based (1) integration between sensory input and motor output (2). Cerebellar is also a center for motor learning. Increase in cerebellum activities occurs when the position of head in space changes (1). Besides this, exposure to chemicals also affects cerebellar functions. Drugs with documented influences on cerebellum include alcohol, antipsychotic drugs and sedatives including bromazepam.

Bromazepam is a moderate lipid-soluble benzodiazepine with characteristic bromine and pyridine rings at positions 7 and 5 respectively (3). Ratified for public use in 1974, it is available in many countries except United States of America. As a mood-altering medicine, bromazepam is generally employed as anti-anxiety, anticonvulsant and hypnotic drug (4). Like alcohol, bromazepam acts by binding with GABA A receptors thereby enhancing the inhibitory effects of Gamma Amino Butyric Acid in the brain and leading to reduction in CNS activity (5). Common side effects associated with bromazepam include drowsiness, dizziness, attenuated working memory, impaired attention and motor coordination with severity being dose-dependent (6).

Like other drugs, bromazepam is degraded by hepatic cytochrome P-450 system into hydroxybromazepam (6). Studies on the likely effect of overdose or long-term administrations of the drug indicated indistinct speech, depressed respiratory function, cognitive deterioration, impaired attention, coma, decreased libido, reduction in hemoglobin and packed cell volume, decreased sperm count and percentage live sperm cells and elevated percentage of dead spermatozoa and severe abnormal sperm morphology (7), reduced LH, FSH and testosterone secretions (8). Others include psychomotor function and ataxia.

Ataxia is a condition in which there is impaired coordination of voluntary movements (1). It represents deviation in the functionality of neural structures involved in coordination. Physiological functions impaired include motor precision, locomotion, articulation, vision, swallowing among others. These include the vestibular apparatus, cerebral cortex, and cerebellum. While vestibular ataxia is motor incoordination resulting from abnormality in vestibular apparatus, sensory ataxia refers to incoordination that stems from loss of proprioceptive inputs to the brain or derangement of brain areas such as parietal lobe and thalamic nuclei which are involved in proprioception.

Cerebellar ataxia is motor incoordination that occurs when cerebellum malfunctions (2).

Many studies have identified likely mechanisms of chemical induced ataxia. For instance, diazepam was shown to reduce cyclic guanosine monophosphate (cGMP) in cerebellar slices dose-dependently (9). Administration of diazepam dose just below the toxic level enhanced malondialdehyde levels in cerebellum and brainstem and decreased mitochondrial glutathione reductase level (6). Castro *et al.*, (2009) (3) showed increased lipid peroxidation in the cerebral cortex and cerebellum and high level of carbonyl production in the striatum of rodent brain. Diazepam was shown to reduce serum glucose (10) and its prolonged use reported to result in decreased packed cell volume and hemoglobin concentration in rats (11). Chronic administration of diphenylhydantoin reduced brain metabolisms using [14C] deoxyglucose technique (12) in humans. Using 18-fluorodeoxyglucose, diazepam decreased whole brain metabolic rate without causing alteration in specific region.

In addition to non-invasive glucose uptake procedure, cerebellar lactate, cerebellar glycogen and glucose levels, glucose and glycogen ratio and plasma and cerebellum glucose ratio are indices of cerebellar metabolisms. The aim of the study was to determine the effect of gradual cessation protocol of bromazepam administrations on cerebellar lactate, cerebellar glycogen and glucose levels, glucose and glycogen ratio and plasma and cerebellum glucose ratio in female rats.

MATERIALS AND METHODS

Animal Care and management

Twenty-five female Wistar rats weighing between 150g-160g were used for the study. They were obtained from the Animal house of the Department of Physiology, University of Benin, Nigeria. They were housed in standard cages designed as previously reported (13-15) at room temperature and 12hr light/12hr dark cycle. The animals were acclimatized for 1 week. All rats were fed pelletized grower mash (standard chow) and distilled water *ad libitum.*

Ethical certification

The study (MED/294738) was conducted in line with the guidelines of National Institute of Health (NIH) for the use of laboratory rats. Consent and unwritten approval of the Research and Ethics Committee of the above University and department were received before the study.

Reagents and animal treatments

A sachet of bromazepam (3 mg, manufactured by May and Baker, Nigeria) was purchased from a registered pharmaceutical company in Auchi. A tablet of bromazepam was dissolved in 5 milliliters of distilled water to form a stock solution. An oral therapeutic dose of 1.15 mg/kg body weight was administered singly between 8.00 am and 10.00 am once per day.

Study design

The rats were randomly divided into five groups of five rats per group.

Control group (CTRL): received 0.3ml of distill water once per day.

Bromazepam-treated group (9d BR): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days.

Bromazepam (BR) withdrawal group I (3d BR-wd): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days followed by 3-day cessation.

Bromazepam withdrawal group II (6d BR-wd): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days followed by 6-day cessation.

Bromazepam withdrawal group III (9d BR-wd): received an oral dose of 0.3ml/155g body weight of bromazepam once per day for nine days followed by 9-day cessation.

Evaluation of body weight

Body weight was measured before and in the course of the study and prior to euthanasia using weighing scale. Change in weight was calculated as (Final weight – Initial weight)/ Initial weight X 100%.

Evaluation of fasting blood glucose

Following twelve hours of overnight fasting, blood glucose level was assessed using glucometer (Roche, Germany).

Ataxia index

The beam apparatus was designed according to the method of Tinh *et al.,* (2011) (16) and consisted of 1.1m beam with a flat surface of 30mm width, 40cm above the base level. All rats were allowed to tread the beam during the training session just before the coordination evaluation was done. Ataxia index was scored as the time taken for rats to tread from one end of the beam to the other end. In cases where rats turned towards the underside of the beam, the test was repeated after 20 minutes of rest. Where it became consistent, a value of 120 seconds was awarded. Ataxia index was conducted at the end of the study.

Plasma and tissue preparations

The duration of the study was 18 days. At the end of the experiment, rats were euthanized through cervical dislocation between 8-10am after 12 hours overnight fasting. Blood was collected into lithium heparin bottle for packed cell volume and hemoglobin determinations. Whole cerebellum was excised and preserved in Phosphate Buffer solution for biochemical analysis.

Determinations of packed cell volume and hemoglobin concentration

Packed cell volume

It was based on the application of centrifugal force (at 12,000RPM) to recover blood cells from anticoagulant blood in a tube.

Hemoglobin

Hemoglobin concentration was measured using the popular Sahli's method.

Determination of cerebellar catalase

Catalase activity was determined according to the method Sinha (1971) (17).

Principle

The method was based on the fact that dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate. The Chromic acetate produced was measured colorimetrically at 570nm.

Cerebellar metabolic parameters

Cerebellar glycogen assay

Principle

Degradation into protein and free saccharide occurred when the tissue was placed in boiling solution of potassium hydroxide. In contrast, glycogen was stable in the alkali solution. After potassium hydroxide treatment, glycogen was precipitated with 96% ethanol, washed, diluted and hydrolyzed in H_2SO_4. Glycogen hydrolysis produced free glucose, which was determined by enzymatic reaction in the presence of glucose oxidase. Glucose was converted by glucose oxidase to gluconic acid. The by-product of this reaction was H_2O_2. The hydrogen peroxide formed reacted under catalysis of peroxidase with phenol and 4-aminophenazone to form red-violet product suitable for photometric determination.

Cerebellar glucose assay

Principle

Glucose oxidase (GOD) catalyzed the oxidation of glucose to gluconate. The hydrogen peroxide (H_2O_2) produced was detected by a chromogenic oxygen acceptor, phenol, 4-Aminophenazone (4-AP) in the presence of peroxidase using standard laboratory procedure.

Cerebellar lactate assay

It was done using Enzyme Linked Immunosorbent Assay.

Statistical analysis

Statistical analysis was done using One-way ANOVA at five rats per group. Pairwise comparison was done using Least Square Difference (LSD).

RESULTS

Effect of Bromazepam and its gradual cessation on cerebellar metabolic parameters

Administration of bromazepam for nine days (Table 1) significantly reduced cerebellar glycogen when compared with control group. There was also a significant reduction in cerebellar glycogen three days after cessation when compared with control group. When compared with bromazepam treated rats, bromazepam cessation for three days, six days and nine days significantly increased cerebellar glycogen respectively. When compared with 6-day cessation group, bromazepam cessation for three and nine days respectively decreased cerebellar glycogen.

Bromazepam treatment for nine days significantly decreased cerebellar glucose when

compared with 3 days, 6 days and nine days bromazepam cessation respectively.

Cerebellar lactate significantly reduced 6 days and 9 days after bromazepam cessation when compared with 3 days-bromazepam withdrawal group respectively.

Plasma/ Cerebellar glucose ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day bromazepam cessation groups respectively.

Cerebellar glucose/glycogen ratio was significantly higher in bromazepam treated rats when compared with control, 3-day, 6-day and 9-day bromazepam cessation groups respectively.

Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively. * represents significant difference (P< 0.05) from control. abcd represent significant difference from 9d BR, 3d BR-wd, 6d BR-wd and 9d BR-wd respectively.

Effect of Bromazepam and its gradual withdrawal on Ataxia Index

Bromazepam administration for nine days significantly increased ataxia index when compared with control, 3-day, 6-day and 9-day cessation groups respectively (Figure 1).

Table 1. Effect of Bromazepam and its gradual withdrawal on cerebellar metabolic parameters

Parameters	**Groups**				
	CTRL	**9d BR**	**3d BR-wd**	**6d BR-wd**	**9d BR-wd**
Cerebellar Glycogen (PPM)	33.700 ± 2.435	5.300 ± 0.095*bcd	26.900 ± 2.593*	37.800 ± 1.961bd	29.400 ± 1.804
Cerebellar Glucose (mg/dl)	3.940 ± 0.114	2.686 ± 0.40bcd	4.660 ± 0.680	5.050 ± 0.621*	5.230 ± 0.604*
Cerebellar Lactate (mmol/L)	0.336 ± 0.004	0.310 ± 0.0251	0.350 ± 0.016	0.300 ± 0.000[b]	0.288 ± 0.022[b]
Plasma/Cerebellar Glucose Ratio	18.147 ± 1.094	38.969 ± 6.478*bcd	21.649 ± 2.689	14.490 ± 1.515	13.550 ± 1.645
Cerebellar Glucose/Glycogen Ratio	0.121 ± 0.012	0.512 ± 0.084*bcd	0.170 ± 0.010	0.133 ± 0.012	0.179 ± 0.215

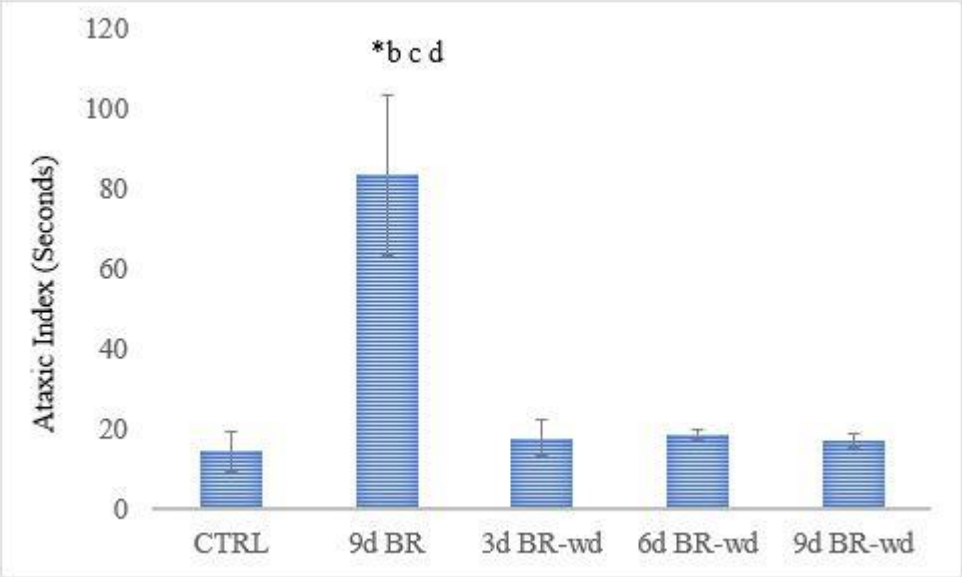

Figure 1. Effect of Bromazepam and its gradual withdrawal on ataxic index. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively. * represents significant difference (P< 0.05) from control. abcd represent significant difference from 9d BR, 3d BR-wd, 6d BR-wd and 9d BR-wd respectively.

Correlation between ataxia index and cerebellar metabolic parameters

There was a significant strong negative correlation between cerebellar glycogen and ataxia index (Table 2). There was also a significant strong positive correlation between cerebellar glucose/glycogen ratio and ataxia index.

Effect of Bromazepam and its gradual withdrawal on Red Blood Cell

Bromazepam administration for nine days caused a significant decrease in packed cell volume and hemoglobin concentration (Table 3) when compared with control, 3-day, 6-day and 9-day cessation groups.

Table 2. Correlation between ataxia index and cerebellar metabolic parameters

Pearson correlation	**Ataxia index**
Cerebellar glycogen	- 0.712*
Cerebellar glucose/glycogen ratio	0.917*
Cerebellar lactate	0.056
Blood/cerebellar glucose ratio	0.357

Effect of Bromazepam and its gradual withdrawal on %weight change

Percentage weight change increased significantly in 9-day cessation group when compared to control group (Figure 2).

Table 3. Effect of Bromazepam and its gradual withdrawal on Red Blood Cell

Parameters	Groups				
	CTRL	9d BR	3d BR-wd	6d BR-wd	9d BR-wd
Packed cell volume (%)	46.0 ± 0.949	39.5 ± 0.158*	40.5 ± 0.158*	40.5 ± 0.160*	46.7 ± 0.967
Hemoglobin (g/dl)	15.00 ± 0.316	13.22 ± 0.053*	13.50 ± 0.053*	13.49 ± 0.0526*	15.60 ± 0.322

Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively. *represents significant difference (P< 0.05) from control.

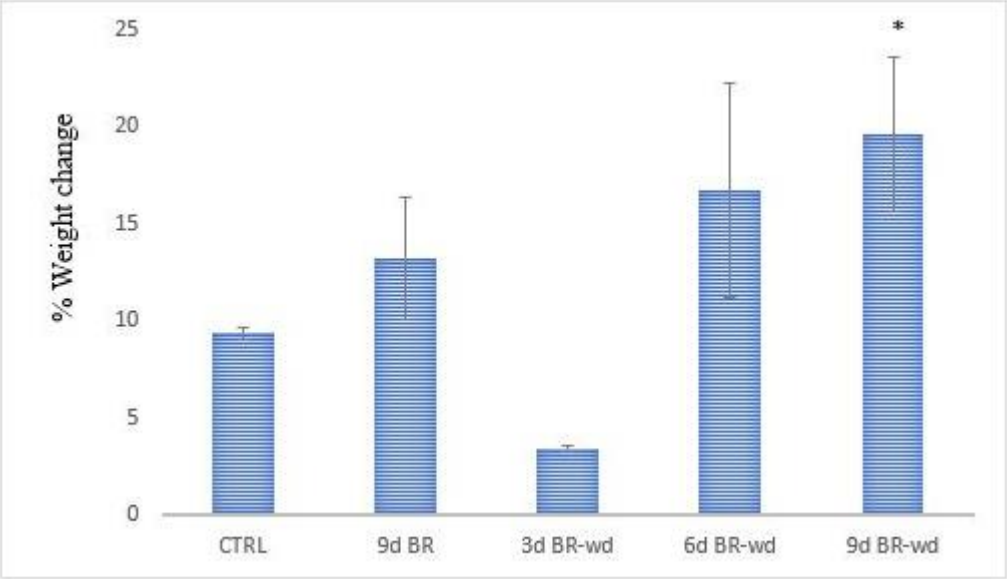

Figure 2. Effect of Bromazepam and its gradual withdrawal on % weight change. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively.* represents significant difference (P< 0.05) from control.

Effect of Bromazepam and its gradual withdrawal on cerebellar catalase

Cerebellar catalase was unaffected by bromazepam or cessation pattern (Figure 3).

Effect of Bromazepam and its gradual withdrawal on fasting blood glucose

When compared with control group, fasting blood glucose significantly increased in rats administered bromazepam for nine days and in 3-days cessation group (Figure 4).

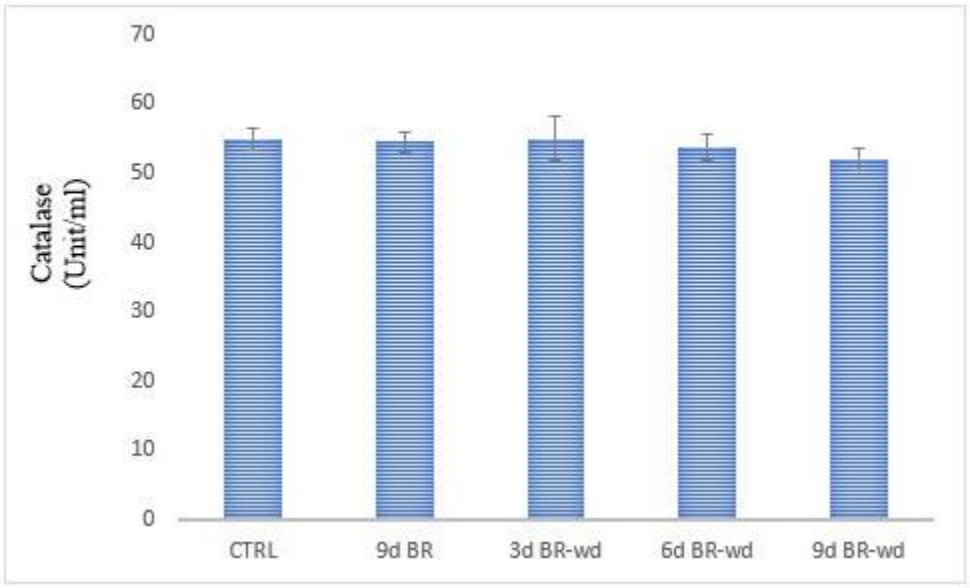

Figure 3. Effect of Bromazepam and its gradual withdrawal on cerebellar catalase. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively.* represents significant difference (P< 0.05) from control.

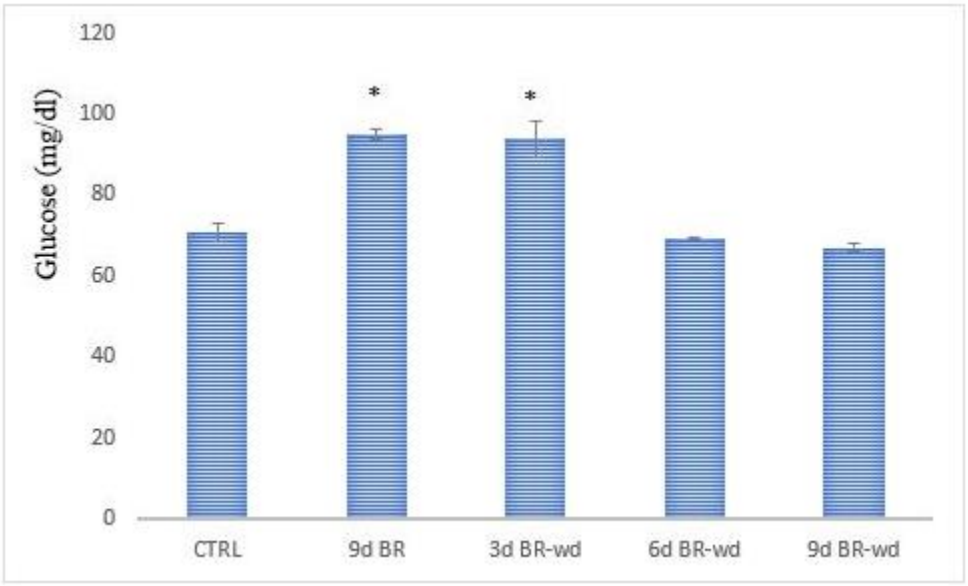

Figure 4. Effect of Bromazepam and its gradual withdrawal on fasting blood glucose. Data are expressed as mean ± SEM (n=5). Alphabets d, BR and wd stand for days, Bromazepam and Drug cessation respectively.* represents significant difference (P< 0.05) from control.

DISCUSSION AND CONCLUSION

The study investigated the effect of bromazepam administration and gradual cessation protocol on cerebellar metabolisms in female Wistar rats. Like other body cells, glucose is an important substrate for Adenosine Triphosphate (ATP) production (18). Using non-invasive 18-fluorodeoxyglucose, works have shown diazepam depressed brain metabolisms (19-22). However, few data exist on the effect of diazepam on cerebellar metabolisms. It was observed from the study that despite the fact that bromazepam administration for nine days did not significantly affect cerebellar glucose levels, an increase was noticed following 6 days and 9 days cessation groups when compared with control group.

In addition to evaluation of glucose utilization using positron emission tomography (PET) and other non-invasive techniques, blood/cerebellum glucose ratio used in this study tends to relate plasma glucose level to cerebellar glucose level. A high blood/tissue glucose ratio reflects reduced tissue glucose uptake. In diabetes mellitus, Cushing syndrome, acromegaly and stress (23), a high blood glucose alongside reduced glucose uptake is not impossible. The result of blood/cerebellum glucose ratio indicated that bromazepam treatment elevated the ratio when compared with healthy control rats implying that bromazepam reduced cerebellar glucose uptake. Fortunately, this trend concurred with findings from human and animal studies using non-invasive method (21,22). The only salience is that this is the first study where the ratio will be used in evaluating cerebellar glucose uptake. The present study showed that the highest blood/cerebellum glucose ratio occurred when rats were treated for nine days with bromazepam. Contrary to previous studies (10), we observed that bromazepam administration for nine days resulted in increased fasting blood glucose level when compared with health control rats. Metabolic demand is known to reduce during sleep with characteristic decrease in brain glucose uptake and utilization and attendant reduction in glucose-production mechanisms evidenced by lowered blood glucose. We have no skepticism about the possibility of fasting blood glucose increasing even despite bromazepam- induced depression of brain activities. This increase in fasting blood glucose might be a result of dawn effect and Somogyll rebound (24), These are well known mechanisms that come into play to mitigate hypoglycemia and they are often associated with release of hyperglycemic hormones. In the present study, we also observed that there was an increase in fasting blood glucose three day cessations.

Another finding of the study was the reduction in cerebellar glycogen level three days of bromazepam withdrawal when compared with healthy rats. Glycogen is a stored form of glucose and an important energy reserve. Depression in its level in the body tissue may result in decline in glycogenolysis and consequent dependence on other energy yielding alternative such as gluconeogenesis. Apart from derivation of glucose from amino acids and fatty acids which

occur virtually in all body tissues, lactate is another non-hexose energy source. A transport protein known as monocarboxylate transporter-1 is known to be responsible for lactate uptake by the brain. As far as the study was concerned bromazepam administration caused no significant decrease in cerebellar lactate level. However, 3-day cessation was characterized by a significant increase in cerebellar lactate level when compared with 6-day and 9- day cessation groups. This implied that even though, bromazepam administration for 9days did not affect cerebellar glycogen level, peak lactate level occurred three days cessation. We also noticed that glycogen restoration following bromazepam challenge peaked 6 days cessation.

The cerebellar glucose/glycogen ratio used in the study represented cerebellar glycogen synthesis level with a high ratio representing deficient glycogen synthesis. Exposure of female rats to bromazepam for nine days caused an increase in cerebellar glucose/glycogen ratio. This result corroborated the outcome of glycogen test in which bromazepam treatment for nine days was found to suppress glycogen content and glycogenolysis. Cerebellar glucose/glycogen ratio was found to reach a zenith level in bromazepam treated group when compared to other groups except healthy control group.

As far as the study was concerned, coordination test was carried out using beam balance method. High ataxia index score occurs when the rats are unable to tread the bar or move towards the underside of the bar after repeated trials and this typically is seen in fear, anxiety and muscle weakness which culminate in poor motor performance. In bromazepam treated rats, high score was obtained in a general consensus. In addition, we observed that ataxia index correlated negatively and positively with cerebellar glycogen and cerebellar glucose/glycogen ratio respectively. This implied that a rise in ataxia index was associated with a decreased cerebellar glycogen and impaired glycogen synthesis respectively. Therefore, in the study, bromazepam-induced ataxia may have been due to reduced cerebellar glycogen and increased cerebellum glucose/glycogen ratio. In addition, ataxia index was lower in all cessation groups when compared to bromazepam treated group.

Moreover, we observed that there was a reduction in weight three days after bromazepam withdrawal. This change in weight might be due to bromazepam cessation effect and readjustment to bromazepam-free life. Weight change recovered fully after nine days of bromazepam withdrawal. We also observe that packed cell volume and hemoglobin concentration were decreased in bromazepam treated rats. Reduction in hematocrit has been previously reported with diazepam use at therapeutic dose (11). Catalase plays in antioxidant defense (25-27). Specifically, it converts hydrogen peroxide into non-toxic water. Catalase analysis was conducted in the study to ascertain if bromazepam use and withdrawal have any influence on cerebellar antioxidant homeostasis. The results showed that bromazepam administration and gradual

withdrawal had no significant effect on cerebellar antioxidant balance.

Bromazepam is a commonly used and abused drug with anti-anxiety, anticonvulsant and hypnotic effects (4, 9). The present study demonstrated not only the possibility of recovery from bromazepam effects but also the recovery pattern. In conclusion, the results of the study indicated the adverse but time-dependent reversible effects of bromazepam withdrawal on cerebellum metabolism in adult female Wistar rats.

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Conflicts of Interest statement

The authors declare no conflict of interest.

Authors' contributions: All authors contributed equally.

REFERENCES

1. Purves D, Augustine GJ, Fitzpatrick D, Hall WC, LaMantia AS, White LE. Neuroscience (4th ed.). New York; W. H. Freeman 2007:197–200.

2. Hodos W. Evolution of Cerebellum. Encyclopedia of Neuroscience (Springer) 2009: 1240–1243.

3. Castro AA, Moretti M, Casagrande TS, Mardnello C, Petronilho F, Steckert AV, Guerrini R, Calo G, Pizzol FD, Queevedo et al. Neuropeptide S produces hyperlocomotion and prevents oxidative stress damage in the mouse brain: a comparative study with amphetamine and diazepam. Pharmacol. Biochem. Behav 2009;21:636-642.

4. Hallett C, Dean BC. Bromazepam. Acute benefit-risk assessment in general practice. Current Medical Research and Opinion 2008;8(10): 683–688.

5. Braestrup C, Squires RF. Pharmacological characterization of benzodiazepine receptors in the brain. European Journal of Pharmacology 1978;48:263–270.

6. Musavi S, Kakkar P. Effect of diazepam treatment and its withdrawal on pro/antioxidative processes in rat brain. Molecular chem biochem 2003;245:51-6.

7. Hadeel MH. The effect of diazepam on some fertility and testosterone levels in healthy adult male rats. Journal of education and science 2011;24:66-79.

8. Taher M, Zainab A, Anber NH. Effect of diazepam on the reproductive system in male rats. World Journal of Pharmacy and Pharmaceutical Science 2015;4:60-78.

9. Aerden LAM, Sleinbush H, Markerink-Van Ittersum JL, de Vente J. Neuroch Res 2004;29:1725-9.

10. Khalili M, Mohammad HG, Azam J, Elham Z. Effect of combination therapy with diazepam and glibenclamide on serum lipids and glucose in type 2 diabetic rats. Journal of basic and clinical pathophysiology 2016;4:7-12.

11. Anacletus FC, Onyegema-Okerenta BM. Evaluation of the influence of therapeutic, prolonged and overdose intake of diazepam on hematological indices and liver enzyme markers of male wistar rats. Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences 2017;2:94-102.

12. Melisi JW, Dow-Edwards DL, Kathryn M H, Milhornt TH. Chronic administration of diphenylhydantoin reduces brain metabolisms. Experimental neurology 1988;99:523-530.

13. Ige SF, Adeniyi MJ, Olayinka AT, Kehinde IC. Role of dietary maize formulations in the healing of experimental acetic acid induced ulcerative colitis in male rats. Chin J Physiol 2020;63:156-162.

14. Agoreyo FO and Adeniyi MJ. Pattern of Estrous Cycle and Ovarian Antiperoxidative Activity in Light Deprived Sprague-Dawley Rats Treated with Sodium Selenite. Journal of Medicinal Research and Biological Studies 2018;1:103.

15. Adeniyi MJ, Agoreyo FO, Olorunnisola OL, Olaniyan OT, Seriki SA, Ozolua PO, Odetola AA. Photo-pollution disrupts reproductive homeostasis in female rats: The duration-dependent role of selenium administrations. Chin J Physiol 2020;6:235-43.

16. Tinh N, Holly J, Carlisle AS and Paul HP. Assessment of motor Balance and coordination in mice using the balance beam. J Vis Exp 2011;496:2376.

17. Sinha AK: Calorimetric determination of catalase. Analytical Biochemistry 1972;47 :389-394.

18. Barrett KE, Susan M, Barman SB and Heddwen L. Brooks. Ganong's Review of Medical Physiology (23rd edition). New York: MC graw Hills. 2010;421:391-427.

19. Kelly PAT, Ford I, Mc Culloch J. Effect of diazepam upon local cerebral glucose use on the conscious rats. Neuroscience 1986;19:257-65.

20. Sokoloff S. Relation between physiological function and energy metabolism in the central nervous system. J Neurochem 1977;29:13-26.

21. Pifarre P, Simo M, Gispert JD, Plaza P, Fernandez A and J Pujol. Diazepam and Jacobson's progressive relaxation shows similar attenuating short-term effects on stress-related brain glucose consumption. Eur Psychiatry 2015;30:187-92.

22. Wit H D, Metz J, Wagner N, Cooper M. Effect of diazepam on cerebral metabolism mood in normal volunteers. Neuropsychopharmacology 1991;5:33-41.

23. Adeyemi WJ, Olayaki LA. Diabetes escalates knee osteoarthritis in rats: Evidence of adaptive mechanism, Environmental Toxicology and Pharmacology 2018;6:1-7.

24. Reyhanoglu G, Rehman A. Somogyi Phenomenon. [Updated 2020 Jul 10]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK551525/.

25. Adeniyi MJ, SF, Ige, WJ, Adeyemi, TJ, Oni, PO, Ajayi, SO, Odelola and VT, Adegbola.

Changes in Body Temperature and Intestinal Antioxidant enzymes in Healthy and Colitis Male Rats: Roles of Garcinia kola (Bitter Kola). International Journal of Physiology 2016;4:36-41.

26. Ige SF, Adeniyi MJ, Ademilua OB, Fatola AO, Adeyemi IA.
Allium cepa remediates oxidative stress-mediated hepatic DNA damage in cadmium-exposed rats through enhanced p53 expression and inhibition of bcl2. Int J Biomed Sci 2020;16:11-17.

27. Ige SF, Adeniyi MJ and Iyalla GO. Allium cepa Mitigates Aluminum Chloride-
Induced Hepatotoxicity in Male Wistar Rats. J Biomedical Sci 2017:6:27.