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Effect of Alpha Lipoic Acid on Cognitive Function in Normoglycaemic Mice

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ABSTRACT

Neuronal cells depend on glucose as their main source of energy and persistent hyperglycaemia has been shown to affect it. Researchers have investigated the influence of hyperglycaemia on memory in diabetic animals. Both the pathogenesis of memory impairment as well as the progression of the disease was influenced by hyperglycaemia. This research evaluated the effect of alpha lipoic acid on memory in normoglycemic mice. Twenty (20) Swiss albino mice were used for the study and divided into four groups of five (n = 5) each. Group I received normal saline (10 ml/kg) while groups II, III and V received glibenclamide (2 mg/kg), alpha lipoic acid (200 mg/kg) and alpha lipoic acid (400 mg/kg). Working memory was determined using spontaneous alternation in the Y maze and social memory was determined using a novel object recognition task. Results showed that alpha lipoic acid at the two doses (200 mg/kg and 400 mg/kg) after administration for fourteen (14) days did not improve spatial working memory in the Y maze test and discriminative index in novel object recognition task when compared to day 0 (pre-treatment). Also, recognition memory showed no significant ($p > 0.05$) change compared to the control group. Other doses of alpha lipoic acid do not significantly improve spatial working memory and recognition memory in the Y maze test and novel object recognition task. Alpha lipoic acid improved spatial working memory and discriminative index and showed no effect on recognition memory in normoglycaemic mice.

Keywords: Alpha lipoic acid, memory, hyperglycaemia, Y maze, novel object recognition task, dementia.

INTRODUCTION

Alpha lipoic acid (ALA) is a powerful antioxidant and a metal ion chelator for metals that are contained naturally in the body (Memudu and Adanike, 2022). It was reported to be helpful in animal models of a variety of peripheral and central nervous system conditions, including autoimmune encephalomyelitis, cerebral ischemia-reperfusion, diabetic neuropathy, and multiple sclerosis (Fu et al., 2012). In clinical practice, it is commonly used to treat pain-related disorders like diabetic neuropathy (Salehi et al., 2019). There is increasing evidence that dietary changes can successfully stop or slow the age-related deterioration in learning, memory and other neurological disorders (Garkuwa et al., 2021; Fogacci et al., 2020; Jan et al., 2015). Since oxidative stress is a major factor in the pathophysiology of cognitive decline, there is growing interest in the potential of antioxidant supplements to protect against neurodegenerative disorders (Nambirajan et al., 2018; Sies, 2015).

Cognitive function serves a critical role in everyday behaviour and social behaviour. Exposure to toxic substances in development or adulthood impacts their substrates and resulting cognitive functions (Saedi et al., 2016). Memory plays a crucial role in our experiences and is closely linked

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to the limbic system. It involves storing information over time to guide future actions (Deary et al., 2009; Murman, 2015; Reagan et al., 2021; Zhao and Alkon, 2001). Dementia is the most severe kind of cognitive decline in neural function, it ranges from moderate cognitive impairment (MCI), which is the primary form of cognitive decline with age (Kim, 2019). Cognitive decline, which includes difficulties with learning and delayed amnesia, results in substantial degrees of functional reliance and decreased quality of life (Aborisade et al., 2022; Saedi et al., 2016). An increasing number of elderly people are experiencing age-related cognitive deterioration as the global population's age distribution continually increases (Maciejczyk et al., 2019). A complex interaction of hormones that regulate glucose synthesis and utilization keeps fasting and postprandial normal blood glucose levels in this constrained range (Nambirajan et al., 2018; Russell et al., 2016).

The effects of diabetes on the brain can include difficulties with learning, memory and cognitive function in both humans and animals (Chen et al., 2021). Diabetes mellitus (DM) is a metabolic disorder and complex disease of the endocrine system that results in high levels of glucose in the bloodstream (Dikalov and Nazarewicz, 2013; Pandey and Dvorakova, 2019). This condition, known as sustained hyperglycaemia, is characterized by a significant amount of circulating glucose in the blood (Katsarou et al., 2017). Most researchers tried to establish a link between hyperglycaemia, and diabetes mellitus with dementia (Akinyemi et al., 2019; Garkuwa et al., 2017). This study examined how ALA affects animals with normal blood sugar levels. By doing so, we hope to gain a better understanding of how ALA and other antioxidants (Garkuwa et al., 2017) impact dementia in animals with diabetes.

MATERIALS AND METHODS

Animals

A total of twenty (20) mice (both sexes) weighing about 18 – 25 grams were used for this research. Before the initiation of the experiment, the rats were acclimatized for 14 days. Standard environmental conditions including temperature, relative humidity and 12-hrs dark/light cycles were maintained. All the animals were fed with vital feed and water *ad libitum* under strict hygienic conditions. The animals were handled by principles guiding the use and handling of experimental animals per the London Declaration of September 1977. Ethical approval was obtained from the Bauchi State University Gadau Committee on Animal Use and Care (BASUG/FBMS/REC/VOL.2/23).

Drugs, Reagents, and Other Materials

All drugs and reagents were obtained commercially and were of analytical grades. The drugs, reagents, equipment, and other materials that were used for the study include alpha lipoic acid purchased from Puritan's Pride Inc. (Ronkonkoma, New York, USA). A digital glucometer was used for blood glucose determination (Accu-Check Advantage, Roche Diagnostic, Germany).

Experimental procedure

Animals were divided into four groups comprising 5 mice in each. Group I served as control and received normal saline (10 ml/kg), group II received glibenclamide (1 mg/kg), group III received ALA (200 mg/kg), and group IV received ALA (400 mg/kg). Administration continued daily for 14 days between 0700 – 0900 hours.

Determination of Blood Glucose Levels

Blood samples for the determination of glucose levels were collected from the tail of the mice on days 0 and 14. This was done using a digital glucometer (Accu check).

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The results were expressed in mg/dl (Schmitt et al., 2011).

Novel object recognition task (NORT)

This study consisted of two phases: a sample phase and a test phase, which were separated by a 24-hour delay. During the sample phase, the mice were presented with two identical objects.. The study involved placing objects in the corners of an arena, with a distance of 15 cm from each adjacent wall. Each mouse was then placed in the center of the arena and given 5 minutes to explore the objects. Before the test phase, all the objects were cleaned with alcohol to remove any olfactory cues.. In the test phase, one of the objects was changed, and each mouse was allowed to explore the objects for 5 minutes. The time spent exploring the two objects was recorded. Time spent exploring the familiar object and the novel object was determined and used for memory tests using the following formulae (Antunes and Biala, 2012; Baxter, 2010; Burke et al., 2010).

Difference (long-term memory) = Tn – Tf (Tn = time spent exploring the novel object, Tf = time spent exploring the familiar object).

$$\text{Discriminative index} = \frac{\text{Tn} - \text{Tf}}{\text{Tn} + \text{Tf}}$$

$$\text{Recognition index} = \frac{\text{Tn}}{\text{Tn} + \text{Tf}} \times 100 \text{ (Antunes and Biala, 2012; Garkuwa et al., 2017)}$$

Spontaneous alternation in Y-maze

During this particular trial, every mouse was individually placed inside the Y-maze for a duration of 6 to 8 minutes. The number of arms entered and the order of entries were recorded, and a score was calculated to determine the rate of alternation. An alternation was counted when the mouse entered all three arms consecutively.. The number of maximum spontaneous alternations is then the total number of arms entered minus two, and the percentage alternation is calculated as ((actual alternations/maximum alternations) x 100) (Hughes, 2004).

Statistical Analysis

Data obtained were expressed as mean ± standard error of the mean (SEM). The data were analyzed using analysis of variance and Tukey's *post hoc* test using the statistical package for social sciences (SPSS) version 22. The value of $p < 0.05$ was taken as significant.

RESULTS

To determine the effect of alpha lipoic acid on blood glucose levels, we used a digital glucometer to check the blood glucose level on day 0 (before commencement of ALA), day seven and day fourteen (after the last administration). We observed that ALA does not significantly ($p>0.05$) change the blood glucose level across both doses (Table 1).

Table 1: Effect of alpha lipoic acid on fasting blood glucose level in mice

Groups	FBG (mg/dL) DAY 0	FBG (mg/dL) DAY 7	FBG (mg/dL) DAY 14
Control 10 ml/kg	108.00 ± 5.74	111.00 ± 14.54	104.50 ± 3.12
Glib. 1 mg/kg	114.25 ± 8.11	113.00 ± 8.21	107.00 ± 2.38
ALA 200 mg/kg	83.50 ± 2.53	123.00 ± 4.69	117.00 ± 9.65
ALA 400 mg/kg	100.75 ± 12.92	121.75 ± 11.16	112.25 ± 8.64

ALA: alpha lipoic acid; Glib: glibenclamide; FBG: fasting blood glucose level

We also used the Y maze (spontaneous alternation) before and after ALA administration to evaluate the influence of ALA on spatial working memory. We observed no significant ($p > 0.05$) change in all the doses administered as seen in Figure 1.

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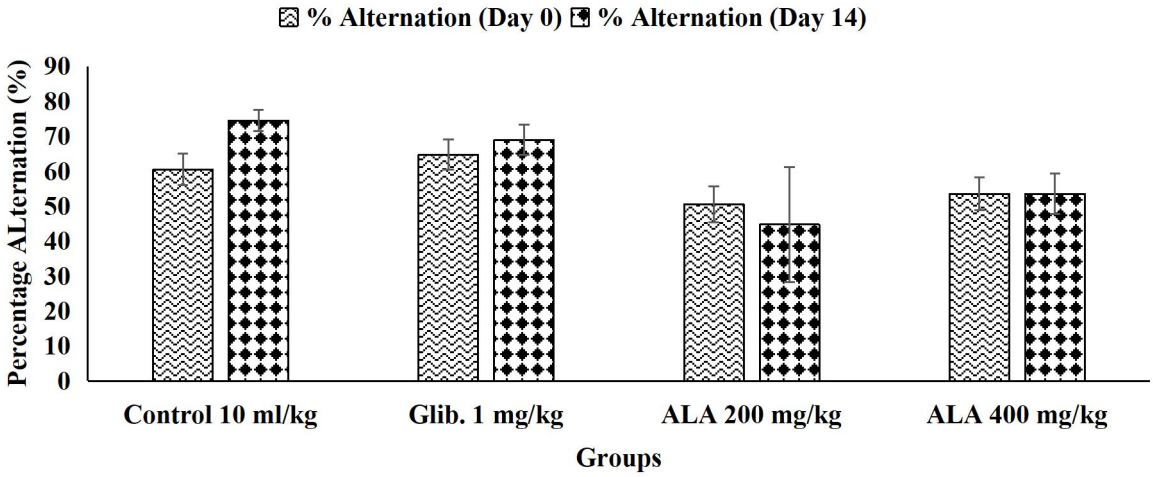

Figure 1: Effect of Alpha Lipoic Acid on Spatial Working Memory in Mice. ALA: alpha lipoic acid; Glib: glibenclamide

To evaluate the effect of alpha lipoic acid on social memory, we used novel object recognition tasks before and after ALA administration. We found no significant ($p > 0.05$) improvement in long-term memory, discriminative and recognitive indices in all the doses of ALA (Table 2).

Table 2: Effect of Alpha Lipoic Acid on Social Memory in Mice

Groups	Difference (s) Day 0	Difference (s) Day 14	DI Day 0	DI Day 14	RI (%) Day 0	RI (%) Day 14
Control 10 ml/kg	5.02 ± 2.13	3.48 ± 1.10	0.04 ± 0.02	0.03 ± 0.01	51.79 ± 0.83	51.36 ± 0.43
Glib. 1 mg/kg	4.00 ± 1.78	9.00 ± 6.12	0.03 ± 0.01	0.07 ± 0.04	51.60 ± 0.69	53.33 ± 2.11
ALA 200 mg/kg	-0.75 ± 2.17	2.25 ± 1.31	-0.01 ± 0.02	0.02 ± 0.01	49.73 ± 0.82	51.04 ± 0.59
ALA 400 mg/kg	-8.50 ± 8.70	-2.75 ± 0.75	-0.07 ± 0.07	-0.03 ± 0.01	46.61 ± 3.34	48.68 ± 0.37

ALA: alpha lipoic acid; Glib: glibenclamide; DI: discriminative index; RI: recognitive index.

DISCUSSION

Recently, scientific research has focused on investigating the activities of many antioxidants on diabetic animals, dementia and other diseases but has not established whether these supplements have any influence on normal animals. Persistent hyperglycaemia has a negative effect on body cells causing many complications including micro- and macrovascular complications. Results showed that administration of ALA for fourteen days did not have a glycaemic effect on normoglycaemic mice. This is important considering how ALA have been investigated and shown to possess antihyperglycaemic effect. Hence, this indicates that ALA as a supplement may not lower the blood glucose level beyond the acceptable range.

The innate curiosity to explore previously unvisited areas was used as an index of spatial working memory. The result showed that there was no significant change between the ALA groups and the two control groups. This indicates that ALA at both 200 mg/kg and 400 mg/kg does not affect the memory faculty of the animals. Many antioxidants are beneficial to the spatial working memory of diabetic experimental animals treated with ALA (Garkuwa et al., 2017; Imran et al., 2020). The results of this study further corroborate these findings supporting that ALA reverses diabetes-induced working memory impairment without affecting the memory of

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normal animals (Memudu and Adanike, 2022; Villasana et al., 2013; Zhang et al., 2019).

Long-term, discriminative and recognitive memories were assessed. From the results long-term memory of the mice was not improved significantly. Also, both discriminative and recognitive indices were not affected. This further adds to the beneficial role of ALA on memory. Other reports have demonstrated that ALA influences long-term memory (Farr et al., 2012) but not recognition memory (Villasana et al., 2013). Memory impairment and dementia in diabetic animals have been linked to hyperglycemia, inflammation, oxidative stress, and dyslipidemia.Findings from this study further indicated that ALA has no effect on memory in normal animals.

CONCLUSION

In conclusion, ALA did not affect the fasting blood glucose levels and did not affect spatial working memory, long-term memory, discriminative and recognitive indices in mice.

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REFERENCE

Aborisade, O. B., Charles–Davies, M. A., Owolabi, M. O., and Agbedana, E. O. (2022). Obesity and Dyslipidemia as Risk Factors of Vascular Cognitive Impairment in Adult Hypertensive Nigerian. *Journal of Advances in Medicine and Medical Research*, 334–342.

Akinyemi, R. O., Owolabi, M. O., Ihara, M., Damasceno, A., Ogunniyi, A., Dotchin, C., Paddick, S.-M., Ogeng'o, J., Walker, R., and Kalaria, R. N. (2019). Stroke, cerebrovascular diseases and vascular cognitive impairment in Africa. *Brain Research Bulletin*, *145*, 97–108.

Antunes, M., and Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. In *Cognitive Processing* (Vol. 13, Issue 2, pp. 93–110).

Baxter, M. G. (2010). "I've seen it all before" Explaining age-related impairments in object recognition. Theoretical comment on Burke et al. (2010). In *Behavioral Neuroscience* (Vol. 124, Issue 5, pp. 706–709).

Burke, S. N., Wallace, J. L., Nematollahi, S., Uprety, A. R., and Barnes, C. A. (2010). Pattern Separation Deficits May Contribute to Age-Associated Recognition Impairments. *Behavioral neuroscience*, *124*(5), 559–573.

Chen, Q., Cao, T., Li, N. N., Zeng, C., Zhang, S., Wu, X., Zhang, B., and Cai, H. (2021). Repurposing of Anti-Diabetic Agents as a New Opportunity to Alleviate Cognitive Impairment in Neurodegenerative and Neuropsychiatric Disorders. In *Frontiers in Pharmacology* (Vol. 12, Issue May, pp. 1–23).

Deary, I. J., Corley, J., Gow, A. J., Harris, S. E., Houlihan, L. M., Marioni, R. E., Penke, L., Rafnsson, S. B., and Starr, J. M. (2009). Age-associated cognitive decline. In *British Medical Bulletin* (Vol. 92, Issue 1, pp. 135–152).

Dikalov, S. I., and Nazarewicz, R. R. (2013). Angiotensin II-induced production of mitochondrial reactive oxygen species: Potential mechanisms and relevance for cardiovascular disease. In *Antioxidants and Redox Signaling* (Vol. 19, Issue 10, pp. 1085–1094).

Farr, S. A., Price, T. O., Banks, W. A., Ercal, N., and Morley, J. E. (2012). Effect of

DOI: 10.56892/bima.v7i3.489

alpha-lipoic acid on memory, oxidation, and lifespan in SAMP8 mice. *Journal of Alzheimer's Disease*, *32*(2), 447–455.

Fogacci, F., Rizzo, M., Krogager, C., Kennedy, C., Georges, C. M. G., Knežević, T., Liberopoulos, E., Vallée, A., Pérez-martínez, P., Wenstedt, E. F. E., Šatrauskienė, A., Vrablík, M., and Cicero, A. F. G. (2020). Safety evaluation of α-lipoic acid supplementation: A systematic review and meta-analysis of randomized placebo-controlled clinical studies. *Antioxidants*, *9*(10), 1–34.

Fu, Z., R. Gilbert, E., and Liu, D. (2012). Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes. *Current Diabetes Reviews*, *9*(1), 25–53.

Garkuwa, U. A., Ibrahim, B., Balanmalam, A. N., Muhammad, S. R., Muazu, M., Garkuwa, A. H., and Yakubu, A. B. (2021). Curcuma longa supplement increases anxiety-like behaviour and blood glucose levels in Swiss albino mice. *Neuroscience Research Notes*, *4*(1), 1–6.

Garkuwa, U., Alhassan, A., and Tanko, Y. (2017). Effect of Curcumin on Blood Glucose Level and Some Neurobehavioral Responses in Alloxan-induced Diabetic Swiss Albino Mice. *Journal of Advances in Medical and Pharmaceutical Sciences*, *14*(1), 1–7.

Hughes, R. N. (2004). The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience and Biobehavioral Reviews*, *28*(5), 497–505.

Imran, M., Ghorat, F., Ul-haq, I., Ur-rehman, H., Aslam, F., Heydari, M., Shariati, M. A., Okuskhanova, E., Yessimbekov, Z., Thiruvengadam, M., Hashempur, M. H., and Rebezov, M. (2020). Lycopene as a natural antioxidant used to prevent human health disorders. In *Antioxidants* (Vol. 9, Issue 8, pp. 1–27). MDPI.

Jan, A. T., Azam, M., Siddiqui, K., Ali, A., and Choi, I. (2015). *Heavy Metals and Human Health : Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants*. 29592–29630.

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., Jacobsen, L. M., Schatz, D. A., and Lernmark, A. (2017). Type 1 diabetes mellitus. *Nature Reviews Disease Primers*, *3*. https://doi.org/10.1038/nrdp.2017.16

Kim, H. (2019). Cognitive dysfunctions in individuals with diabetes mellitus. *Yeungnam University Journal of Medicine*, *36*(3), 183–191.

Maciejczyk, M., Żebrowska, E., and Chabowski, A. (2019). Insulin resistance and oxidative stress in the brain: What's new? In *International Journal of Molecular Sciences* (Vol. 20, Issue 4).

Memudu, A. E., and Adanike, R. P. (2022). Alpha lipoic acid reverses scopolamine-induced spatial memory loss and pyramidal cell neurodegeneration in the prefrontal cortex of Wistar rats. *IBRO Neuroscience Reports*, *13*(May), 1–8.

Murman, D. L. (2015). The Impact of Age on Cognition. *Seminars in Hearing*, *36*(3), 111–121.

Nambirajan, G., Karunanidhi, K., Ganesan, A., Rajendran, R., Kandasamy, R., Elangovan, A., and Thilagar, S. (2018). Evaluation of antidiabetic activity of bud and flower of Avaram Senna (Cassia auriculata L.) In high fat diet and streptozotocin induced diabetic rats. *Biomedicine and Pharmacotherapy*, *108*(July), 1495–1506.

Pandey, S., and Dvorakova, M. C. (2019). Future Perspective of Diabetic Animal Models. *Endocrine, Metabolic and Immune Disorders - Drug Targets*, *20*(1), 25–38.

DOI: 10.56892/bima.v7i3.489

Reagan, L. P., Cowan, H. B., Woodruff, J. L., Piroli, G. G., Erichsen, J. M., Evans, A. N., Burzynski, H. E., Maxwell, N. D., Loyo-Rosado, F. Z., Macht, V. A., and Grillo, C. A. (2021). Hippocampal-specific insulin resistance elicits behavioral despair and hippocampal dendritic atrophy. *Neurobiology of Stress*, *15*, 100354.

Russell, W. R., Baka, A., Björck, I., Delzenne, N., Gao, D., Griffiths, H. R., Hadjilucas, E., Juvonen, K., Lahtinen, S., Lansink, M., Loon, L. Van, Mykkänen, H., Östman, E., Riccardi, G., Vinoy, S., and Weickert, M. O. (2016). Impact of Diet Composition on Blood Glucose Regulation. *Critical Reviews in Food Science and Nutrition*, *56*(4), 541–590.

Saedi, E., Gheini, M. R., Faiz, F., and Arami, M. A. (2016). Diabetes mellitus and cognitive impairments. *World Journal of Diabetes*, *7*(17), 412–422.

Salehi, B., Berkay Yılmaz, Y., Antika, G., Boyunegmez Tumer, T., Fawzi Mahomoodally, M., Lobine, D., Akram, M., Riaz, M., Capanoglu, E., Sharopov, F., Martins, N., Cho, W. C., and Sharifi-Rad, J. (2019). Insights on the use of α-lipoic acid for therapeutic purposes. *Biomolecules*, *9*(8), 1–25.

Schmitt, H., Lenzen, S., and Baltrusch, S. (2011). Glucokinase mediates coupling of glycolysis to mitochondrial metabolism but not to beta cell damage at high glucose exposure levels. *Diabetologia*, *54*(7), 1744–1755.

Sies, H. (2015). Redox Biology Oxidative stress : a concept in redox biology and medicine. *Redox Biology*, *4*, 180–183.

Villasana, L. E., Rosenthal, R., Doctrow, S., Pfankuch, T., Garfinkel, M., and Raber, J. (2013). Effects of alpha-lipoic acid on associative and spatial memory of sham-irradiated and 56 Fe-irradiated C57BL/6J male mice. *Pharmacology Biochemistry Behavior*, *103*(3), 487–493.

Zhang, Y., Lv, Y., Si, Y., Zhou, J., Qian, Y., and Bao, H. (2019). α-lipoic acid attenuates spatial learning and memory impairment induced by hepatectomy. *Experimental and Therapeutic Medicine*, 2329–2333.

Zhao, W.-Q., and Alkon, D. L. (2001). Role of insulin and insulin receptor in learning and memory. In *Molecular and Cellular Endocrinology* (Vol. 177). www.elsevier.com/locate/mce