Journal of Pharmacy & Allied Medicine

JPAM, 1(2), 78-85, 2023
https://doi.org/10.58985/jpam.2023.v01i02.10
ISSN (Print): 3005-2726

Research Article

Alpha lipoic acid improved spatial working memory and reduced acetylcholinesterase levels in type-2 diabetic Wistar rats

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Article Information
Received: *01 August 2023*
Revised: *27 August 2023*
Accepted: *30 August 2023*

Academic Editor
Prof. Dr. Christian Celia

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Keywords
Alpha lipoic acid, blood glucose level, spatial working memory, diabetes mellitus, acetylcholinesterase

Abstract

Diabetes mellitus is one of the most common metabolic disorders that is associated with many complications such as memory impairment and dementia. Alpha lipoic acid (ALA) has been shown to have antihyperglycaemic and antioxidant effects. This study aimed to evaluate the effects of ALA on spatial working memory, and acetylcholinesterase level in type-2 diabetic Wistar rats. Thirty rats were divided into six groups of five each (n=5). Diabetes mellitus was induced using a high-fat diet for six weeks followed by a single low dose of streptozotocin (40 mg/kg) intraperitoneally and followed by a high-fat diet for another two weeks. Group I served as normoglycemic control and received 1 ml/kg normal saline, group II, III, IV, V, and VI were diabetic and received 1 ml/kg normal saline, glibenclamide 1 mg/kg, ALA 100 mg/kg, ALA 200 mg/kg and ALA 400 mg/kg respectively. All administrations were done orally for 21 days. Blood glucose level was determined before and after administrations, spatial working memory was determined using spontaneous alternation in the Y-maze, and the serum was used for the determination of acetylcholinesterase level. The results showed that ALA at 200 mg/kg significantly increased percentage alternation on day 21 with values of 69.73 ± 2.67% compared to the untreated group (57.97 ± 2.27%). Serum acetylcholinesterase level was significantly ($p < 0.05$) reduced (78.62 ± 1.66 U/ml) in the rats that received 400 mg/kg ALA compared to the untreated group (88.28 ± 1.82 U/ml). The findings of this study suggest that ALA improved spatial working memory and acetylcholine activity in Wistar rats.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease or disorder that has been associated with complications in the nervous system especially the peripheral nervous system (PNS) and several organs in the body, such as the kidney, eyes, and brain [1]. Among the complications of DM, cognitive dysfunctions are relatively less addressed [2]. DM is characterized by high blood glucose levels as a result of insufficient insulin for the body's needs. Type-1 diabetes (T1DM) is an autoimmune disease that results in beta-cell destruction. It usually presents in childhood, accounts for 5-10% of all diabetes, is associated with the presence of islet-cell antibodies, and patients require lifelong insulin. Type-2 diabetes (T2DM), the most common form of the disease, is influenced by lifestyle factors, such as age, pregnancy, and obesity, but has a strong genetic component. Multiple genes are thought to be involved, each producing a small effect on T2DM

risk [3].

Cognitive impairment has been linked with diabetes mellitus (DM). Hence, prompting new demands for accurate quantification of cognitive dysfunction among persons with diabetes [4, 5]. Researchers suggested that cognitive dysfunction should be listed along with the chronic complications of DM like diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy [6]. Comorbid cognitive impairments in the diabetic population are evident in clinical practice [7–9]. It has not been studied comprehensively in the preclinical setting where the emphasis is significantly given to research on the amelioration of the macro- and microvascular complications of DM [10]. Positive correlations have been found to exist between DM and cognitive impairment. It has been suggested that the central nervous system (CNS) is a crucial target for diabetic complications. these complications include micro-vascular diseases; insulin resistance; activation of polyol pathway; increased formation of advanced glycation end-products (AGEs) and activation of protein kinase C. All these could serve as a mechanism underlining the pathogenesis and progression of cognitive impairments in DM [6,11].

There were recommendations on the use of complementary therapies such as antioxidant supplementation in patients with metabolic abnormalities to boost their nutritional status and immune system [12]. Existing evidence has proven the beneficial effects of several antioxidant supplements including Curcumin [13,14], pentoxifylline [15], and lycopene [16,17] on reducing oxidative stress and inflammation which are secondary to persistent hyperglycemia. Oxidative stress and inflammation appear to be major drivers in the pathogenic mechanisms underlying the cognitive impairments in DM. Antioxidants and anti-inflammatory supplements like alpha lipoic acid could be potential therapeutic tools for preventing and treating cognitive impairments.

2. Materials and methods

2.1 Animals

A total of thirty (30) male Wistar rats weighing 200–250 grams were used for the study. The animals were housed in plastic cages under standard laboratory conditions with free access to food and water. Animals were allowed for two weeks for acclimatization to the laboratory environment before the commencement of the experiments. The animals were handled by principles guiding the use and handling of experimental animals per the Universal declaration of animals rights proclaimed in Paris on 15 October, 1978.

2.2 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).

2.3 Induction of diabetes and experimental design

The rats were fasted for about 13 hours before the commencement of the experiment but were allowed water *ad libitum* throughout the experiment. The normal groups were fed with standard rat feed only while the high-fat diet fed groups were as described by Magalhães *et al.*, [18] with little modification. The high-fat diet (HFD: 35% commercial feed, 25% groundnut, 25% fat, and 15% groundnut oil) was used for the induction of obesity and DM. rats were fed for six (6) weeks followed by a single dose of streptozotocin (STZ) 40 mg/kg intraperitoneally (IP) followed by high-fat diet for another 2 weeks. Rats fasted for 12 hours and fasting blood glucose levels were measured to confirm the establishment of diabetes [18]. Rats with fasting blood glucose levels of 16 mmol/L were considered diabetic and selected for the study [18]. The fasting blood glucose level estimation was done between 0700 – 0900 hours on day 0 (pre-treatment) and day 21 (post-treatment). The rats were divided into six (6) groups of five (5) rats each (n = 5). All drug administrations were done orally for 21 days as follows; Group I served as normal control and received 1 ml/kg 0.9% normal saline; Group II, III, IV, V, and VI were all diabetic and received 1 ml/kg 0.9% normal saline, 1 mg/kg glibenclamide, 100 mg/kg ALA, 200 mg/kg ALA, 400 mg/kg ALA respectively.

2.4 Y-maze test (Spontaneous alternation version)

In this version, each mouse is placed in the Y-maze for 6-8 minutes and the number of arms entered as well as the sequence of entries is recorded and a score is calculated to determine the alternation rate. An

alternation is defined as entry into all three arms consecutively. For instance, if an animal makes the following arm entries: A, C, B, C, A, B, C, A, C, A, B, C,A; the animal has made 13 arm entries 8 of which are correct alternations. 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 x100). A high alternation rate is indicative of sustained spatial working memory as the animals must remember which arm was entered last to not re-enter it [19].

2.5 Determination of fasting blood glucose level

The blood samples were obtained from the rat tail vein on days 0 (pre-treatment) and 21 (post-treatment). A digital glucometer was used to measure the blood glucose levels using the glucose oxidase principle [46] using the digital glucometer (Accu-Check Advantage, Roche Diagnostic, Germany), and results were expressed in mmol/L [47].

2.6 Termination of experiment and sample collection

On day 21 (3 weeks after the treatment period), all rats were subjected to light anesthesia by exposing them to chloroform soaked in cotton wool placed in an anesthetic box. Blood samples of about 5 ml were collected from the heart of each rat from all groups by cardiac puncture. The samples were collected in Eppendorf tubes and allowed to clot. Thereafter, the serum was separated by centrifugation at 3000 rpm for 10 minutes. The serum was used for acetylcholinesterase assay using an acetylcholinesterase enzyme (AChE) ELISA kit (Cat-bio-14126, Shanghai Coon Koon Biotech Co., Ltd, Shanghai, China) according to the manufacturer's instruction.

2.7 Statistical analysis

Statistical Package for the Social Sciences version 22 (SPSS 22) was used to analyze the data. Data obtained were presented as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) was employed to compare the level of significance between experimental groups. Tukey's *post hoc* was conducted to determine the degree of difference between groups. Values of $p < 0.05$ were considered significant.

3. Results

To determine the effect of ALA on fasting blood glucose level (Table 1), diabetic animals were given different doses of ALA (100 mg/kg, 200 mg/kg, and 400 mg/kg) daily for 21 days, and blood glucose level was checked at the beginning (day 0) and end (day 21). We observed a significant ($p < 0.05$) decrease in the fasting blood glucose level across the three doses after 21 days, especially in the group administered ALA 400 mg/kg (8.60 ± 0.68 mmol/L) compared to the first day of administration (20.44 ± 1.01 mmol/L). Also, the group that received 400 mg/kg ALA showed significant [F (5, 30) = 55.51, $p < 0.0001$)] reduction (Group VI: 8.60 ± 0.68 mmol/L) in fasting blood glucose level compared to the diabetic control group (Group II: 20.06 ± 0.70 mmol/L).

Table 1. Effect of ALA on Fasting Blood Glucose Levels of Type-2 Diabetic Wistar Rats.

Groups	Day 0 (mmol/L)	Day 21 (mmol/L)
Normal + NS (1 ml/kg)	4.88 ± 0.50	4.84 ± 0.25^{b}
DM + NS (1 ml/kg)	19.96 ± 1.20^{a}	20.06 ± 0.70^{a}
DM + Glib (1 mg/kg)	19.76 ± 1.02^{a}	7.02 ± 0.79^{a,b,c}
DM + ALA (100 mg/kg)	20.86 ± 1.40^{a}	10.02 ± 0.71^{a,b,c}
DM + ALA (200 mg/kg)	21.44 ± 1.14^{a}	8.78 ± 0.94^{a,b,c}
DM + ALA (400 mg/kg)	20.44 ± 1.01^{a}	8.60 ± 0.68^{a,b,c}

Values having superscript letters a, b, and c are statistically significant ($p < 0.05$) compared with the normal control group (Normal + NS), diabetic control group (DM + NS), and day 0 respectively. NS: Normal saline; DM: Diabetes mellitus; Glib: Glibenclamide; ALA: alpha lipoic acid.

We also observed a significant ($p < 0.05$) increase in the percentage alternation (Fig. 1) across the three doses of ALA after 21 days of administration with the highest change observed at the dose of 100 mg/kg (67.63 ± 2.61%) compared to day 0 (pre-treatment) of the same group (55.36 ± 1.83%).

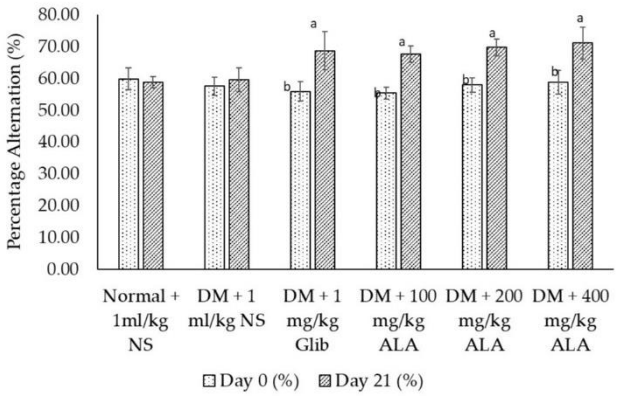

Figure 1. Effect of ALA on Spatial Working Memory using Y-maze test in Type-2 Diabetic Wistar Rats.

(Values with error bars having different superscripts letters a,b are significant ($p < 0.05$); a and b = compared with day 0 (same group), and c = compared with diabetic control (DM + NS). **NS:** Normal saline; **DM:** Diabetes mellitus; **Glib:** Glibenclamide; **ALA:** alpha lipoic acid).

There was no significant [F (5, 30) = 1.84, $p > 0.05$)] increase in percentage alternation between the different groups on the same day (day 21).

To determine the effect of ALA on serum AChE level (Fig. 2), diabetic animals were given different doses of ALA (100 mg/kg, 200 mg/kg, and 400 mg/kg) daily for 21 days and serum AChE level was determined after the administration. We observed a significant [F (5, 30) = 14.82, $p < 0.0001$)] decrease in the serum AChE level of rats administered ALA 400 mg/kg (78.62 ± 1.66 U/ml) compared to the diabetic control group (88.28 ± 1.82 U/ml).

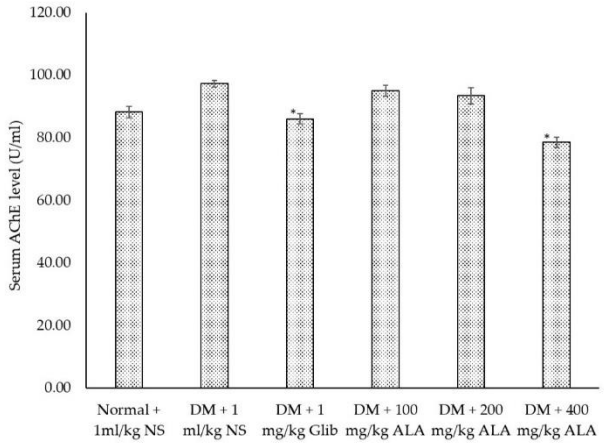

Figure 2. Effect of ALA on Serum AChE Level in Type-2 Diabetic Wistar Rats.

(Values with error bars having * are significant ($p < 0.05$) compared to group II. **NS:** Normal saline; **DM:** Diabetes mellitus; **Glib:** Glibenclamide; **ALA:** alpha lipoic acid; **AChE:** Acetylcholinesterase.)

4. Discussion

Several scientific researchers have over the years associated diabetes mellitus with peripheral nerve damage, Alzheimer's disease, and cognitive impairment. DM also alters the intermediary metabolism of lipids and proteins adversely [20-22]. The use of chemicals such as alloxan and streptozotocin has been used for many decades to induce diabetes mellitus in experimental animals. Other methods used to induce diabetes mellitus include dietary formulations such as a high-fat diet to induce insulin resistance and diabetes mellitus [23-25]. It was postulated that hyperglycemia, oxidative stress, dyslipidemia, and inflammation are the major mediators for the pathogenesis and progression of memory impairment in diabetes mellitus [26, 27]. Alpha lipoic acid (ALA) has been used for some years now as an antioxidant with antihyperglycemic and anti-inflammatory effects. It is also used to treat many ailments because of its wide spectrum of pharmacological activities [28, 29]. Scientific research spanning more than a decade has confirmed the diverse pharmacological effects of ALA and established its ability to act as a chemo-preventive agent as well as a potential therapeutic agent against several chronic diseases. This study investigated the influence of type-2 diabetes on fasting blood glucose levels, cognitive function, and some biomarkers of oxidative stress and the possible modulatory role of ALA.

In this study, we observed that all three doses of alpha lipoic acid significantly ($p < 0.05$) decreased the fasting blood glucose level when compared to the control group. The decrease in the fasting blood glucose level observed in the present study suggests that daily administration of alpha lipoic acid improved the hyperglycaemic condition induced by a high-fat diet/streptozotocin in experimental animals. Indicating that alpha lipoic acid has a strong antihyperglycemic effect. Also, the treatment group and normoglycemic control group showed no significant ($p > 0.05$) difference, hence suggesting that alpha lipoic acid might have led to the recovery of insulin resistance and aided in the transport of glucose into tissues. The findings of the present study agreed with the Ansar *et al.*, [30], in which alpha lipoic acid improved insulin resistance and diabetic condition in type-2 diabetic patient. Also, Elbadawy *et al.* [31] reported an ameliorative effect on peripheral neuropathy, glycated hemoglobin, and lipid profiles in diabetic patient.

A Y-maze test was carried out to determine the spatial memory ability of rats. The Y-maze task was carried out to assess the animals' spontaneous tendency to alternate among the 3 arms of the maze, often linked to working memory. Results obtained in Fig. 1 clearly showed that hyperglycemia and diabetes mellitus might be linked to cognitive decline and memory impairment. We observed a non-significant change in the working memory of the experimental rats in the diabetic control group between day 0 and day 21. A recent discovery of insulin receptors in the brain areas associated with learning and memory, especially the hippocampus has improved the understanding of the possible involvement of insulin in memory encoding [32, 33]. The non-significant change observed in this

group might be a result of the increase in the insulin level and its possible role in memory encoding and or consolidation. However, the group that received different doses of ALA showed significant increase in spatial working memory after administration compared to day 0 (pre-treatment). This improvement might be associated with the reduction in the level of AChE level as seen in the present study. Increased phosphorylated *tau* proteins have been shown to increase the expression and activity of AChE in the synaptic cleft and hence, decrease the activity of acetylcholine (a neurotransmitter implicated in memory formation) [43]. It might also be due to the lipid lowering effect of ALA in hyperglycaemic rats [49]. Dyslipidaemia is believed to be among the major players in the pathogenesis and progression of memory impairment in diabetes. Also, it could be due to the blood glucose-lowering effect of ALA and decreased shunting of glucose into the hexosamine pathway. Glucose shunting into the hexosamine pathway and activation of the polyol pathway has been linked to the onset and progression of cognitive decline among the diabetes population through neuronal injury [34]. These pathways might promote additional vascular ROS production that activates proinflammatory pathways associated with key molecular events in atherogenesis [34–36]. The results obtained in the high-dose of ALA treated group showed a significant ($p< 0.05$) increase in percentage spontaneous alternation in the Y maze test when compared to the diabetic control group on day 21. This is an indication that ALA at a high dose may improve spatial working memory. The group that received standard antidiabetic drugs did not show any significant increase in spatial working memory compared to the diabetic control group on day 21. Increased ROS generation, polyol pathway activation, advanced glycation end products, and glucose shunting into the hexosamine pathway cause neurotoxicity, cognitive impairment, neuronal death, and end-organ damage due to hyperglycaemia [37–39]. This finding was similar to the Villasana *et al.* that showed ALA prevents radiation-induced impairments in spatial memory retention in the water maze probe trials following reversal learning [40]. Also, Memudu and Adanike reported that Alpha lipoic acid reverses scopolamine-induced spatial memory loss and pyramidal cell neurodegeneration in the prefrontal cortex of Wistar rats [41]. Attenuation of spatial working memory impairment of ALA also reported [48].

One of the major neurotransmitters found in the hippocampus that help in memory formation is acetylcholine. Acetylcholine like other neurotransmitters binds to both presynaptic to regulate its secretion and postsynaptic receptors and activate it. The activity of which is also controlled by the effect of acetylcholinesterase (AChE) [42-44]. AChE is a key enzyme in the cholinergic nervous system which is commonly associated with β-amyloid plaques and neurofibrillary tangles (NFT). Increasing evidence suggests that both β-amyloid protein (Aβ) and abnormally hyperphosphorylated tau (P-tau) can influence AChE expression [43]. In this study, we observed a significant decrease in the amount of acetylcholinesterase in rats administered ALA 400 mg/kg (figure 2) compared to the diabetic control group. This is a clear indication that at this dose, the activity of AChE was significantly reduced hence increasing the effect of Ach at the postsynaptic membrane Ach receptors. This is an important indication of a possible reason for the improved memory observed in the present study. This reduction might be accompanied by a decrease in tau protein phosphorylation and plaque formation. AChE inhibitors (AChEI) are thought to be important enhancers of cholinergic transmission. They act by inhibiting the effect of AChEs. Aβ peptides influence AChE levels, thus Aβ might be responsible for increased AChE around plaques [44]. This finding conforms to that of Arivazhagan *et al.* who reported that lipoic acid is effective in the restoration of the activity of acetylcholinesterase in aged rats [45].

5. Conclusions

Results obtained in the present study demonstrated that Type-2 diabetes mellitus causes memory impairment, which significantly improved after 21 days of administration of ALA. Spatial working memory was improved by ALA's antihyperglycemic and acetylcholinesterase reducing effects.

Authors' contributions

UAG conceptualized the design of the study. MIAS, AA, and MUK supervised the study and prepared the first draft of the manuscript. UAG, MIAS, and AA helped in the data collection and analysis. UAG and

MUK did the interpretation of the results. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to express their gratitude to the Head of the Department of Physiology who permitted the study. Also, the Head and Staff of the Natural Product Research Laboratory, Department of Biochemistry; the Dean of Faculty of Basic Medical Sciences all of Bauchi State University Gadau.

Funding

The author did not receive any financial aid.

Availability of data and materials

All relevant data are within the paper and its supporting information files. Additional data will be made available on request according to the journal policy.

Conflicts of interest

The authors have declared that no competing interests exist.

References

1. Kim, H. Cognitive dysfunctions in individuals with diabetes mellitus. Yeungnam Univ J Med. 2019, 36(3), 183–91.
2. McCrimmon, R.J.; Ryan, CM.; Frier, B.M. Diabetes 2 Diabetes and cognitive dysfunction. Lancet. 2012, 379 (9833), 2291–9. http://dx.doi.org/10.1016/ S0140-6736(12)60360-2
3. Ashcroft, F.M.; Rorsman, P. Europe PMC Funders Group Diabetes mellitus and the β -cell: the Last Ten Years. Cell. 2018, 148(6), 1160–71.
4. de Almeida Faria, A.C.R.; Dall'Agnol, J.F.; Gouveia, A.M.; de Paiva, C.I.; Segalla, V.C.; Baena, C.P. Risk factors for cognitive decline in type 2 diabetes mellitus patients in Brazil: a prospective observational study. Diabetol. Metabol. Synd. 2022, 14(1), 1–13. https://doi.org/10.1186/s13098-022-00872-3
5. Lacy, M.E.; Moran, C.; Gilsanz, P.; Beeri, M.S.; Karter, A.J.; Whitmer, R.A. Comparison of cognitive function in older adults with type 1 diabetes, type 2 diabetes, and no diabetes : results from the Study of Longevity in Diabetes (SOLID). BMJ Open Diabetes Res. Care 2022, 1–11.
6. Vijayakumar, T.; Sirisha, G.;, Begam, F. Mechanism Linking Cognitive Impairment and Diabetes mellitus. Eur. J. Appl. Sci. 2012, 4(1), 1–05.
7. Barrett, E.J.; Liu, Z.; Khamaisi, M.; King, G.L.; Klein, R.; Klein, B.E.K.;, et al. Diabetic microvascular disease: An endocrine society scientific statement. J. Clin. Endocrinol. Metab. 2017, 102(12), 4343–410.
8. Saedi, E.; Gheini, M.R.; Faiz, F.; Arami, M.A. Diabetes mellitus and cognitive impairments. World J. Diabetes 2016, 7(17), 412–22.
9. Bon, R.S.; Ariaratnam, S.; Saher, Z.M.; Mohamad, M. Cognitive Impairment and Its Associated Risk Factors in the Elderly With Type 2 Diabetes Mellitus. Front. Psychiatry. 2021, 12(October), 1–7.
10. Rangel, É.B.; Rodrigues, C.O.; De Sá, J.R. Micro- and macrovascular complications in diabetes mellitus: preclinical and clinical studies. J. Diabet. Res. 2019, 1–6.
11. Pignalosa, F.C.; Desiderio, A.; Mirra, P.; Nigro, C.; Perruolo, G.; Ulianich, L.; et al. Diabetes and cognitive impairment : a role for glucotoxicity and dopaminergic dysfunction. Int. J. Mol. Sci. 2021, 22(22), 12366.
12. Tabrizi, R.; Borhani-Haghighi, A.; Mirhosseini, N.; Lankarani, K.B.; Naghibzadeh-Tahami, A.; Akbari, M.; et al. The effects of alpha-lipoic acid supplementation on fasting glucose and lipid profiles among patients with stroke: a systematic review and meta-analysis of randomized controlled trials. J. Diabet.Metabol. Disord. 2019, 18, 585–95.
13. Xu, C.; Hou, B.; He, P.; Ma, P.; Yang, X.; Yang, X.; et al. Neuroprotective effect of salvianolic acid a against diabetic peripheral neuropathy through modulation of nrf2. Oxid. Med. Cell. Longev. 2020, 2020.
14. Sharifi-rad, M.; Kumar, N.V.A.; Zucca, P.; Varoni, E.M.; Dini, L.; Panzarini, E. et al. Lifestyle, Oxidative stress, and antioxidants : back and forth in the pathophysiology of chronic diseases. Front. Physiol. 2020, 1, 1–21.
15. Chavarría, A.P.; Vázquez, R.R.V.; Cherit, J.G.D.; Bello, H.H.; Suastegui, H.C.; Moreno-Castañeda, L. et al. Antioxidants and pentoxifylline as coadjuvant measures to standard therapy to improve prognosis of patients with pneumonia by COVID-19. Comput. Struct. Biotechnol. J. 2021, 19, 1379–90.
16. Varela, E.L.P.; Gomes, A.R.Q.; da Silva Barbosa dos Santos, A.; de Carvalho, E.P.; Vale, V.V.; Percário, S. Potential benefits of lycopene consumption: rationale for using it as an adjuvant treatment for malaria patients and in several diseases. Nutrients. 2022, 14(24), 5303. https://www.mdpi.com/2072-6643/14/24/5303.
17. Imran, M.; Ghorat, F.; Ul-haq, I.; Ur-rehman, H.; Aslam, F.; Heydari, M. et al. Lycopene as a natural antioxidant used to prevent human health disorders. Antioxidants. 2020, 9, 1–27.
18. De MagalhÃes, D.A.; Kume, W.T.; Correia, F.S.; Queiroz, T.S.; Allebrandt Neto, E.W.; Dos Santos, M.P.; et al. High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: A new proposal. An Acad. Bras. Cienc. 2019, 91(1), 1–14.
19. Hughes, R.N. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci. Biobehav. Rev.

2004, 28(5), 497–505.
20. Akter, K.; Lanza, E.A.; Martin, S.A.; Myronyuk, N.; Rua, M.; Raffa, R.B. Diabetes mellitus and Alzheimer's disease : shared pathology and treatment ? Bri. J. Clin. Pharm. 2010, 71 (3), 365 - 376.
21. Zhang, X.; Alshakhshir, N.; Zhao, L. Glycolytic Metabolism, Brain resilience, and Alzheimer's disease. Front. Neurosci. 2021, 15, 1–19.
22. Ehtewish, H.; Arredouani, A.; El-Agnaf, O. Diagnostic, prognostic, and mechanistic biomarkers of diabetes mellitus-associated cognitive decline. Int. J. Mol. Sci. 2022, 23, 6144.
23. King, A.J.F.F. The use of animal models in diabetes research. Br. J. Pharmacol. 2012, 166(3), 877–94.
24. Patel, D.K.; Kumar, R.; Laloo, D.; Hemalatha, S. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Vol. 2, Asian Pac. J. Trop. Biomed. 2012, 411–20.
25. Ighodaro, O.M.; Akinloye, O.A. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 2018, 54(4), 287–93. https://doi.org/ 10.1016/j.ajme.2017.09.001.
26. Tiwari, B.K.; Pandey, K.B.; Abidi, A.B.; Rizvi, S.I. Markers of oxidative stress during diabetes mellitus. J. Biomarkers. 2013, 2013, 1–8.
27. Maciejczyk, M.; Żebrowska, E.; Chabowski, A. Insulin resistance and oxidative stress in the brain: What's new? Int. J. Mol. Sci. 2019, 20, 874.
28. Duch, D.E. Resin transfer molded high temperature composites. In: International SAMPE Symposium and Exhibition (Proceedings). 1999, 1149–60.
29. Mendoza-núñez, V.M.; García-martínez, B.I.; Rosado-pérez, J.; Santiago-osorio, E.; Pedraza-chaverri, J. The Effect of 600 mg alpha-lipoic acid supplementation on oxidative stress, inflammation, and rage in older adults with type 2 diabetes mellitus. Oxid. Med. Cell Longev. 2019, 2019.
30. Ansar, H.; Mazloom, Z.; Kazemi, F.; Hejazi, N. Effect of alpha-lipoic acid on blood glucose, insulin resistance, and glutathione peroxidase of type 2 diabetic patients. Random. Contr. Trial. 2011, 98(711), 584–8.
31. Elbadawy, A.M.; Elmoniem, R.O.A.; Elsayed, A.M. Alpha lipoic acid and diabetes mellitus : potential effects on peripheral neuropathy and different metabolic parameters. Alexandria J. Med. 2021, 57(1), 113–20. https://doi.org/10.1080/20905068. 2021.1907961
32. Spinelli, M.; Fusco, S.; Grassi, C. Brain Insulin Resistance and Hippocampal Plasticity: Mechanisms and Biomarkers of Cognitive Decline. Front. Neurosci. 2019, 13, 1–13.
33. Reagan, L.P.; Cowan, H.B.; Woodruff, J.L.; Piroli, G.G.; Erichsen, J.M.; Evans, A.N. et al. Hippocampal-specific insulin resistance elicits behavioral despair and hippocampal dendritic atrophy. Neurobiol. Stress. 2021, 15, 100354. https://doi.org/10.1016/j. ynstr.2021.100354
34. Brownlee, M. The pathobiology of diabetic complications: A unifying mechanism. Diabetes. 2005, 1615–25.
35. Zhang, Y.; Lv, Y.; Si, Y.; Zhou, J.; Qian, Y.; Bao, H. α-lipoic acid attenuates spatial learning and memory impairment induced by hepatectomy. Exp. Ther. Med. 2019, 2329–33.
36. Liu, Q.; Wang, Z.; Cao, J.; Dong, Y.; Chen, Y. The Role of Insulin Signaling in Hippocampal-Related Diseases: A Focus on Alzheimer's Disease. Int. J. Mol. Sci. 2022. 23, 14417.
37. Kodl, C.T.; Seaquist, E.R. Cognitive dysfunction and diabetes mellitus. Endocrine Rev. 2008, 29, 494–511.
38. Rajamani, U.; Essop, M.F. Hyperglycemia-mediated activation of the hexosamine biosynthetic pathway results in myocardial apoptosis. Am. J. Physiol-Cell Physiol. 2010, 299(1).
39. Aldosari, D.I.; Malik A, Alhomida AS, Ola MS. Implications of Diabetes-Induced Altered Metabolites on Retinal Neurodegeneration. Front. Neurosci. 2022, 16, 1–10.
40. Villasana, L.E.; Rosenthal, R.; Doctrow, S.; Pfankuch, T.; Garfinkel, M.; Raber, J. Effects of alpha-lipoic acid on associative and spatial memory of sham-irradiated and 56 Fe-irradiated C57BL/6J male mice. Pharmacol. Biochem. Behav. 2013, 103(3), 487–93.
41. Memudu, A.E.; Adanike, R.P. Alpha lipoic acid reverses scopolamine-induced spatial memory loss and pyramidal cell neurodegeneration in the prefrontal cortex of Wistar rats. IBRO Neurosci. Rep. 2022, 13, 1–8. https://doi.org/10.1016/j.ibneur. 2022.05.005.
42. Greenfield, S.A.; Zimmermann, M.; Bond, C.E. Non-hydrolytic functions of acetylcholinesterase The significance of C-terminal peptides. FEBS J. 2008, 275, 604–11.
43. Rajmohan, R.; Reddy, P.H. Amyloid-beta and phosphorylated tau accumulations cause abnormalities at synapses of Alzheimer's disease neurons. J. Alzheimer's Dis. 2017, 57, 975–99.
44. Inestrosa, N.C.; Dinamarca, M.C.; Alvarez, A. Amyloid-cholinesterase interactions: Implications for Alzheimer's disease. FEBS J. 2008, 275, 625–32.
45. Arivazhagan, P.; Ayusawa, D.; Panneerselvam, C. Protective efficacy of α-lipoic acid on acetylcholinesterase activity in aged rat brain regions. In: Rejuvenation Res. 2006, 198–201.
46. Beach, E.F.; Turner, J.J. An enzymatic method for glucose determination in body fluids. Clin. Chem. 1958, 4, 462-465.
47. Rheney, C.C.; Kirk, K.K. Perfomance of three blood

glucose meters. Ann. Pharmacother. 2000, 34(3), 317-321.

48. Zhang, Y.; Lv, Y.; Si, Y.; Zhou, J.; Qian, Y.; Bao, H. α-lipoic acid attenuates spatial learning and memory impairment induced by hepatectomy. Exp. Ther. Med. 2019, 2329–2333. https://doi.org/10.3892/etm.2019.7202.
49. Garkuwa, U.A.; Saleh, M.I.A.; Alhassan, A.; Kawu, M.U. Alpha lipoic acid improved blood glucose level and lipid profile in type-2 diabetic Wistar rats. Gadau J. Pure All. Sci, 2023, 2(2), 106–12.