The Journal of Anatomical Sciences
Email: anatomicaljournal@gmail.com

J. Anat Sci 14(1)

Neurobehavioural and Microscopic Evaluation of the Therapeutic Potential of Trans-cinnamaldehyde on the Cerebral Cortex of Insulin-resistant Wistar Rats

Olorunnado SE[1, 5, a], Enya JI[2, 3], Oghenevurinri AL[4], Akinola OB[5]

[1]Department of Human Anatomy, School of Medicine and Pharmacy, University of Rwanda
[2]Department of Anatomy, Faculty of Basic Medical Sciences, Pamo University of Medical Sciences, Port Harcourt, Rivers State, Nigeria
[3]eNeuro Lab, Pamo University of Medical Sciences, Port Harcourt, Nigeria
[4]Department of Anatomy, Faculty of Basic Medical Sciences, Babcock University, Ogun State, Nigeria
[5]Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria

Corresponding Author: Samson EO

ABSTRACT

Insulin-resistance and type-2 diabetes are associated with altered cognitive function, both conditions have been linked to neurodegenerative disease which is a progressive impairment in the functionality of the brain usually resulting from loss or death of neurons traceable to multiple causes. The aim of this study was to explore the therapeutic potentials of trans-cinnamaldehyde (TCA) on the behavioral and histomorphology of cerebrum of high fat diet (HFD) and streptozotocin (STZ) induced insulin-resistant in Wistar rats. Forty Wistar rats were fed with HFD for 8 weeks and then treated with STZ (30 mg/kg intraperitoneally) to induce insulin resistance. 40 and 60 mg/kg of TCA were orally administered for 4 weeks once daily after the induction of the insulin resistance. Thereafter, open field test (OPT) was used to determine line crossing and rearing frequency, histological examination (haematoxylin and eosin (H&E) and Cresyl fast violet (CFV-to show the Nissl substance in neurons and cell nuclei), and immunohistological assessments to quantify the level of beta-amyloid, Glial fibrillary acidic protein (GFAP) and neuronal nuclear protein (NeuN), were conducted. HFD and STZ induced insulin- resistant caused behavioral alteration, changes in cerebral histoarchitecture, pyknotic pyramidal neurons, sparse Nissl distribution, hypertrophied astrocytes, amyloid plaque formation, however TCA administration to insulin-resistant rats reduced pyknosis, astrogliosis, and neurodegenerative changes in the cerebrum when compared with untreated insulin-resistant rats. The study concluded that TCA protected the cerebrum from insulin-resistance-induced behavioural deficits and neuronal degeneration. The study recommended that trans-cinnamaldehyde be explored as a therapy for insulin-resistance-induced neurodegenerative changes

Keywords: Cerebrum, High-fat Diet, Insulin-resistant, Trans-cinnamaldehyde.

INTRODUCTION

Insulin resistance is a state of impairment in the blood glucose-lowering effect of circulating or injected insulin which is the central feature of type 2 diabetes mellitus (T2DM) and metabolic syndrome [1,2]. There exist basic and clinical evidences that supports the fact that incidence of neurodegenerative diseases is more common among patients with insulin resistance than the general population [3] .Neurodegenerative disease is a progressive impairment in the functionality of the brain usually resulting from loss or death of neurons traceable to multiple causes including insulin resistance, and it disproportionally affects the elderly and worsens with age[4]. The causative factor for neurodegeneration is attributable to specific or general neuronal impairment[4,5]. The resulting neurodegenerative changes lead to a compromise in some specific neuronal functions such as hearing, vision, memory or general brain function. Most neurodegenerative disorders are commonly associated with some age-related symptoms such as obesity, insulin resistance and diabetes[6,7]. Neurodegenerative diseases are generally characterized by cellular accumulation of misfolded proteins, ROS production due to mitochondrial dysfunction, and disruption of the autophagy machinery in neuronal cells[7].Several findings reveal that hyperglycemic patients have a higher prevalence of global cognitive impairment and greater cognitive decline[8,9] when compared to normoglycemic population. Insulin resistance and diabetes have therefore been implicated as a risk factor for dementia[10].

The cerebrum constitutes the largest part of the brain with functions such as learning, memory, sensory perception which are critical for survival, it is divided into four distinct lobes: temporal, parietal, occipital and frontal[12].

Over some decades, there has been a geometrical increase in efforts concerning finding treatment for neurodegenerative diseases. To this end, phytochemical derivatives have been used to combat various pathological conditions[13].

Cinnamon is one of the most commonly used flavouring agents in the beverage and food industry globally. It is also well recognized for its medicinal properties and one of its major essential oil is trans-cinnamaldehyde (TCA)[14].

TCA is a clear yellow liquid derived from cinnamon, it has been reported to possesses antimicrobial activity, antioxidant, cholesterol-lowering, antineoplastic, antibacterial and antifungal properties [13,14]. TCA has been reported to inhibit the production of nitric oxide(NO) and (interleukin-1β) IL-1β and expression of (inducible nitric oxide synthase) iNOS and (cyclooxygenase-2) COX-2 by suppressing activation of (tumor necrosis factor-α) NF-κB in LPS-stimulated microglia as a model of activated microglia[15] . However, there are few or no studies that examine the therapeutic potentials of TCA on the cerebrum of insulin-resistance rats. The aim of this study was to explore the therapeutic potentials of trans-cinnamaldehyde (TCA) on neurobehavioral performance and histomorphology of cerebral cortex of high fat diet (HFD) and streptozotocin (STZ) induced insulin-resistant in Wistar rats.

MATERIAL AND METHODS

Ethical Approval: The approval for this research was given by the University of Ilorin Ethical Review Committee (UERC) with approval number UERC/ASN/2018/1157. The research was conducted in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals[16].

Animal Acquisition and Handling: Forty (40) adult Wistar rats weighing 160 ± 10 g were purchased from Department of Anatomy, Ladoke Akintola University, Ogbomosho. The rats were housed in the Animal House of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria. The rats were allowed to acclimatize for fourteen days, the rats were fed daily with rat pellets from Ogo-Oluwa feed and flour mill limited, Sango, Ilorin. The rats had access to water *ad libitum*. Standard guidelines for animal handling as approved by UERC were followed.

Composition of High-fat Diet (HFD): The composition of HFD used in this experiment are listed in table 2 bellow

Table 1: showing High-fat Diet composition

Components	Composition (kg)
Maize	5.5
Wheat offal	0.5
Groundnut cake	5.5
Soya meal	12.5
PKC	5
Bone meal	0.5
Fish meal	0.5
Methionine	0.025
Lysine	0.025
Industrial salt	0.0625
Broiler premix	0.0625

Animal grouping and Induction of Insulin-resistance: To induce insulin-resistance, rats were fed with high-fat diet as previously described[17] for eight weeks, and administered single dose 30 mg/kg STZ (Sigma-Aldrich Inc., St. Louis, MO, USA Lot #MKCD4749) intraperitoneally[18] at the end of the eighth week. Blood sample was withdrawn from the tail vein of the rats, the Wistar rats were fasted overnight and fasting blood glucose level was checked using a digital glucometer (Accu-Check, Roche, Belgium). Wistar rats with fasting blood glucose concentrations not less than 200 mg/mol were included in the study.

Trans-cinnamaldehyde treatment: Following the induction of insulin resistance, 40 and 60 mg/kg of TCA (Sigma-Aldrich Inc., St. Louis, MO, USA Lot #MKCD4749) was administered daily for four weeks orally.

The rats were randomly assigned into five(5) groups; Control, Insulin resistance control, TCA only, Insulin resistance + TCA (60mg/kg) and Insulin resistance + TCA (40mg/kg). The table below shows the groupings and treatments

Table 2: Experimental animal groupings and treatment

Groups	Administration	Duration
A-Control	Standard rat feed	Standard rat feed
B-Insulin-resistant	HFD+STZ	8 weeks HFD, STZ
C-TCA	60 mg/kg TCA	4 weeks TCA
D-Insulin-resistant +High TCA	HFD+STZ+ 60 mg/kg TCA	8 weeks HFD+ STZ, 4 weeks TCA
E- Insulin-resistant +Low TCA	HFD+STZ+ 40 mg/kg TCA	8 weeks HFD, 4 weeks TCA

Neurobehavioral Test (Open Field Test): The open field apparatus was made of plywood and measured 72 x 72 x 36 cm high. The floor and walls were painted a neutral white color and the floor was divided into 16 squares, each 18x18 cm, by lines drawn under the clear Plexiglas floor with a blue marker. Line Crossing and rearing frequency were measured to know when the Wistar rats crosses one of the grid lines which separate the squares in the open field with all four feet and when the rats stand on hind legs respectively[19].

Histopathological Studies: After the open field test, the rats were anaesthetized with intramuscular injection of ketamine 30 mg kg−1 and perfused transcardially with sterile Phosphate Buffered Saline (PBS), following 10% formol-saline, and their cerebral cortices were then excised and then fixed in 10% formalin for histological examination using haematoxylin and eosin (H&E), cresyl fast violet (CFV) and Immunohistochemical staining techniques. Immunohistochemistry was used to quantify the level of beta-amyloid, Glia Fibrillary Acidic Protein (GFAP) for astrocyte distribution and Neuronal nuclei (NeuN) for nuclei protein expression using the beta-amyloid, GFAP and NeuN kits (Santa Cruz, Germany) according to the manufacturer's instructions and modified method[20]. Stained sections were viewed under a light binocular microscope (Olympus, NJ, USA) attached to an Amscope Digital Camera (MD500, CA, USA).

Data Analysis: Data obtained from open field test were analyzed using GraphPad Prism® software (Version 8.1) using one way analysis of variance (ANOVA) with Tukey's multiple comparisons test. Significance was set at $p<0.05$.

RESULTS

OFT Assessment: The open field test (OFT) task revealed a decrease ($p<0.05$) in line crossing among the insulin resistant group compared to the control. Treatment with TCA 60 mg/kg increases the line crossing frequency comparable to control (Figure **1 i**). TCA treatment increases rearing frequency in insulin resistant rats at both doses. However, the untreated insulin resistant rats shows reduced rearing frequency (Figure **1 ii B**).

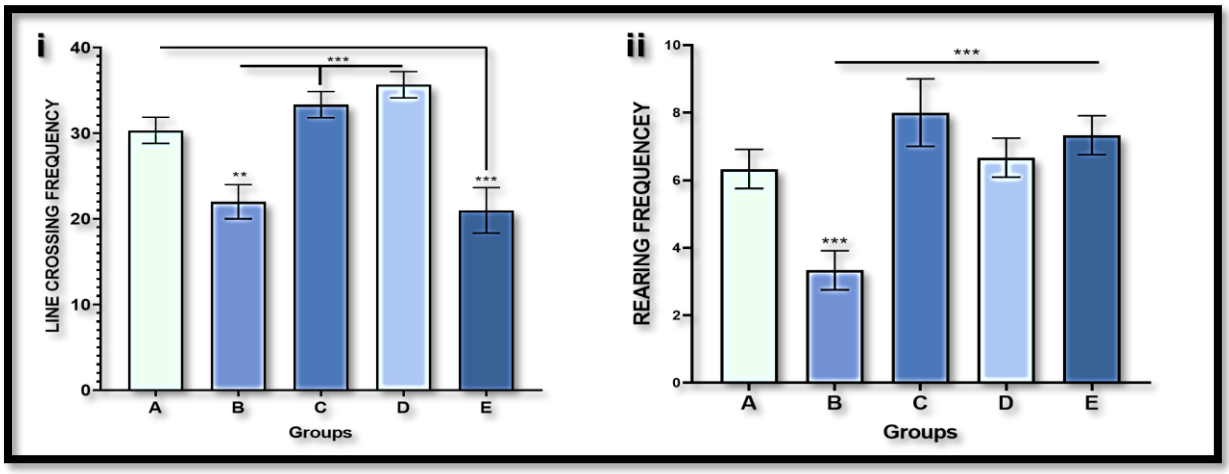

Figure 1 (i-ii): Showing the effects of TCA following the induction of insulin resistance using OFT to demonstrate Line Crossing and Rearing Frequency.

A=control group; B=insulin resistant control group; C=TCA alone treated group; D=insulin resistant treated with TCA 60 mg/kg; E= insulin resistant treated with TCA 40 mg/kg. Data are expressed as mean ± SEM (n=5) and analyzed by One-way ANOVA followed by Tukey's multiple comparisons test.

*All data sets expressed as mean ± SEM. *,** represents p<0.05, p<0.01 and p<0.001.*

Histoarchitectural Observation: H& E stained sections of the cerebral cortex of the control group showed normal cytoarchitecture characterized by large pyramidal neurons. The insulin resistant group showed some degenerative changes characterized by pyknotic pyramidal neurons, reduced pyramidal cell layer and fragmented cells (yellow arrow). However, treatment with TCA showed less degenerative changes and restoration of normal cerebral cortex cytoarchitecutre when compared to the insulin resistant group (Figure 2).

The section of CFV stained cerebral cortex of Wistar rat showed the control and TCA treated groups with intense Nissl stains with very few chromatolytic cells, however the insulin resistant group shows numerous chromatolytic cells with vacuolation (red arrows). Treatment with TCA revealed reduced chromatolysis (Figure 2).

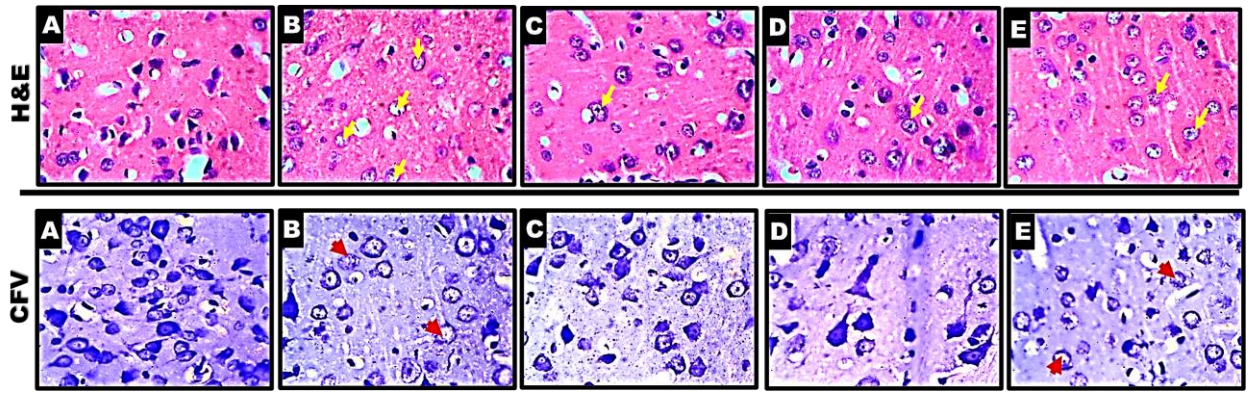

Figure 2: Representative photomicrographs of the cerebrum of Wistar rats using haematoxylin and eosin (H&E) stain and cresyl fast violet (CFV) stain.

A=control group; B=insulin resistant control group; C=TCA alone treated group; D=insulin resistant treated with TCA 60mg/kg; E= insulin resistant treated with TCA 40mg/kg. The yellow arrows show numerous pyknotic nucleus in the untreated insulin resistant group, however treatment with TCA reduced the pyknosis. The red arrows show chromatolytic cells with poor staining intensity.

Immunohistochemical Observations: In the cerebral cortical sections of control and TCA-treated rats, the expression of astrocytes appeared normal. The insulin resistant groups were marked by increased reactive astrocyte having enlarged size with distinct morphology (black arrows), the TCA alone group shows astrocyte distribution similar to the control, treatment with high and low doses of TCA shows restoration of astrocyte morphology comparable to the control group, (Figs. **3 i**). Normal amyloid deposition was observed in the control group. Insulin resistant untreated group showed multiple amyloid beta (Aβ) plaque deposition (black arrows), TCA alone treated group with amyloid deposition comparable to the control, treatment with TCA (Figure **3ii**) reduces the amyloid burden in the cerebral cortex of Wistar rats.

Increased NeuN immunoreactivity was observed in control with normal cellular architectural layout(red arrows); insulin resistant group showed reduced NeuN immunoreactivity, TCA treatment restores NeuN immunoreactivity (Figure **3 iii**).

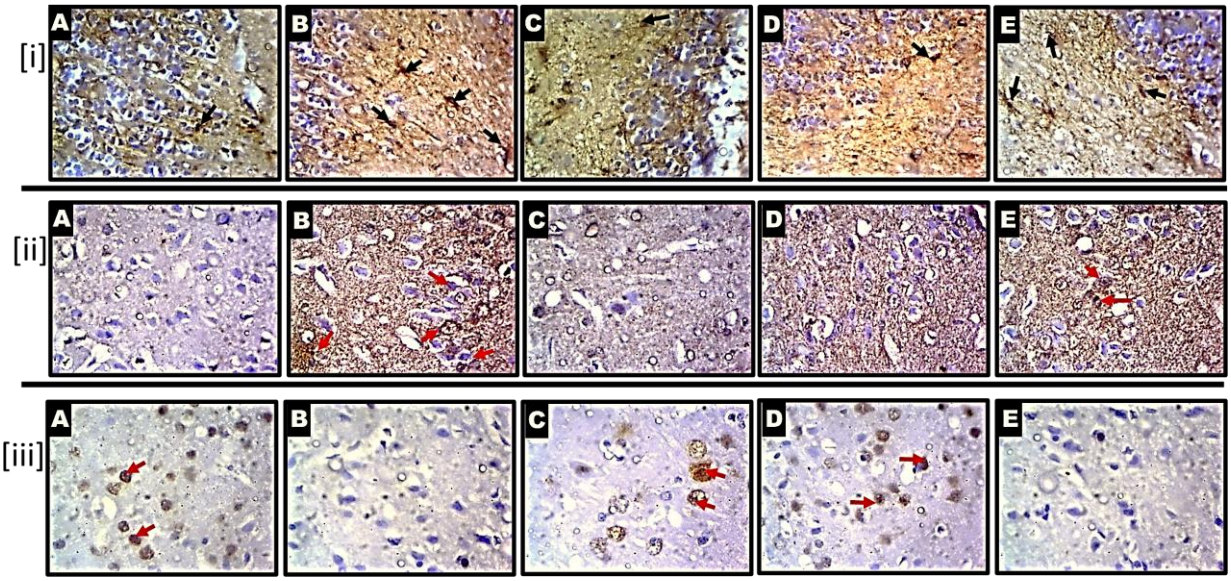

Figure 3: Representative photomicrographs of the cerebrum of insulin resistant Wistar rats showing immunohistochemical demonstration of:

(i) astrocytes; black arrows showing increase astrocyte distribution and morphology among the insulin resistant untreated group, however treatment with TCA reduces the astrocyte expression (ii)amyloid; red arrows show multiple amyloid plaques among the insulin resistant group and (iii) NeuN; the control and TCA treated groups show normal nuclear contents (red arrows). A=control group; B=insulin resistant control group; C=TCA alone treated group; D=insulin resistant treated with TCA 60mg/kg; E= insulin resistant treated with TCA 40mg/kg

DISCUSSION

The mechanisms by which insulin resistance and diabetes alter brain function are not clearly understood, there is no accurate method to diagnose and effectively treat insulin-resistance induced cognitive dysfunction[21]. Findings from this study reveals that HFD/STZ reduced line crossing and grooming frequency, while groups treated with TCA shows increased line crossing and grooming frequency. Oxidative stress is well established for its impairing effects on motor and locomotory activities, mostly through activating the hypothalamic–pituitary–adrenal (HPA) axis, which promotes glucocorticoid excess in rodent, leading to free radical overproduction[22] and inhibiting neurogenesis, reducing brain volume and cause dendritic atrophy in cerebral cortex and hippocampus pyramidal neurons and as well plays a key part in the progression of aging and age-related neurodegenerative illnesses including Alzheimer's disease[22, 23,24,25]

Some studies suggest that HFD/STZ administration causes a long-term reduction in glucose and glycogen metabolism in the cerebral cortex, hippocampus, and other parts of the brain, impairing learning, memory, and motor abilities[22,26] . Thus, in the HFD/STZ model, decreased brain oxidative metabolism is the primary cause of degenerative alterations. In this research, a significant reduction in line crossing and

rearing frequency following HFD/STZ administration which was significantly ameliorated by the administration of TCA is in accordance with reports on impairment in motor functions in diabetic rats [24,27.]

The degenerative changes observed in the cerebral cortex of insulin-resistant rats in this study may be the result of redox imbalance. The protein synthesis mechanism of the neuronal cells may be affected by neuronal fragmentation or pyknosis and disruption in Nissl profile, which may then have an impact on vital cellular processes and neurological functions[28].

Rough endoplasmic reticulum, or Nissl bodies, is found in the cell bodies of neurons. As there is a redox interaction between the trio of mitochondria, endoplasmic reticulum, and peroxisomes in their involvement in reactive oxygen species (ROS) production, endoplasmic reticulum has also been linked to the production of ROS[29]. The administration of TCA therefore, is vital in restoring the integrity of neuronal somatic rough endoplasmic reticulum, to maintain their capacity to produce protein for optimal neuronal functions.

Findings in this study documented astrocytic morphology and distribution using GFAP immunostaining. HFD/STZ induced-insulin resistance changed the morphology of astrocytes, resulting in diffused distribution of reactive astroglia and the presence of reactive astrocytes cells within the cerebral cortex, an indication of astrogliosis. The intrinsic brain defense system and control of brain homeostasis are both significantly influenced by astrocytes[30]. Thus, astrocytes are important for neuronal survival. Interestingly, the blood-brain barrier (BBB) which delivers neurotrophic factors to neurons is made up of astrocytes as a critical component. It helps to improve cognition, learning, and memory by influencing neuronal immune response, possible excitotoxicity, and synaptic plasticity. Astrocytic dysfunction can be a major contributor to neurodegeneration. The glial scar left behind by reactive astrocytes may prevent axon regeneration and exacerbate neurological dysfunctions linked to psychiatric disorders[31]. Additionally, TCA treated rats after HFD/STZ administration, showed moderate deposition of aggregated Aβ protein in parallel with increased NeuN expression in the cerebral cortex. It was reported that Aβ accumulation induces neuronal loss through stimulation of apoptotic pathways, hence the observed decrease in NeuN immunoreactivity among the insulin resistant group[32] .However, a medicinal plant extract such as TCA that could ameliorate these adverse neurological effects following STZ administration to induce insulin resistant, should be considered in the management of such clinical conditions.

In conclusion, the results of this study shows that TCA administration improves neurobehavioural activities in Wistar rat model of insulin resistance and reduces neurodegenerative changes in the cerebral cortex.

Funding: Nil

Conflict of Interest: The authors declare that they have no conflict of interest

REFERENCES

1. Feldman, B. & Eva, L.Insulin resistance in the nervous system.*Trends in Endocrinology and Metabolism*, 2012; 23(3), 133-141.
2. Czech, M. P. Insulin action and resistance in obesity and type 2 diabetes. *Natural Medicine*, 2017; 23(7), 804-814.
3. Teixeira, M..M. Passos, V.M.A. & Barreto, S. M. Association between diabetes and cognitive function at baseline in the Brazilian Longitudinal Study of Adult Health (ELSA- Brasil) .*Scientific Reports*, 2020; 10, 1596. https://doi.org/10.1038/s41598-020-58332-9
4. Despa, F., DeCarli, C. Amylin: What might be its role in Alzheimer's disease and how could this affect therapy? *Expert Review of Proteomics*, 2013; 10(5), 403- 405.
5. McKusick, V.A. Mendelian Inheritance in Man and its online version. *American journal of human genetics*, 2007; 80(4), 588-604.
6. Reddy, S., Pugazhenthi. L. Qin, . H. Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease, *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*, 2017; 1863(5), 1037-1045.
7. Madhusudhanan, J., Suresh, G., Devanathan, V. Neurodegeneration in type 2 diabetes Alzheimer's as a case study. *Brain and Behavior*, 2020; 10(5).
8. Elham, S. Mohammad, R. Firoozeh, F. Mohammad, A. Diabetes mellitus and cognitive impairments. *World Journal of Diabetes*, 2016; 7(17), 412-422.
9. Lindsay, A., Krish, C., Justin, Y., James, W. Diabetes and Cognitive Impairment. *Current Diabetes Reports*, 2016; 16(9), 87.
10. Kannappan S, Jayaraman T, Rajasekar P, Ravichandran M. Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed Rat.Singapore Med J 2006; 47:(10) 858-863.
11. Bekhedda H, Menadi N, Demmouche A, Ghani A, Mai H, Histological study of the effects of aluminum chloride exposure on the brain of wistar rats female, Journal of Drug Delivery and Therapeutics. 2020; 10(3-s): 37-42
12. Saeideh, M., Shokoufeh, H., Fazlullah, K. & Mojtaba, Z. Cinnamon,a promising prospect towards Alzheimer's disease. *Pharmacological Research*, 2018; 130, 241-258.
13. Yeon-Ji J, Ji-Yeon C, Yun-Joong K, Sang-Gi M, Geun-Pyo H. Physical and antimicrobial properties of trans-cinnamaldehyde nanoemulsions in water melon juice. Food Science and Technology 2015; 60: 444-451.
14. Kollanoor JA, Darre MJ, Hoagland TA, Schreiber DT, Donoghue AM, Donoghue DJ. Antibacterial effect of Trans-cinnamaldehyde on Salmonella enteritidis and Campylobacter jejuni in chicken drinking water. J Applied Poultry Res 2008;17(4):490-497.
15. Chen YF, Wang YW, Huang WS, Lee MM, Wood WG, Leung YM, Tsai HY. Trans-Cinnamaldehyde, An Essential Oil in Cinnamon Powder, Ameliorates Cerebral Ischemia-Induced Brain Injury via Inhibition of Neuroinflammation Through Attenuation of iNOS, COX-2 Expression and NFκ-B Signaling Pathway. Neuromolecular Med. 2016 Sep;18(3):322-33.
16. National Research Council. Guide for

the Care and Use of Laboratory Animals, The National Academies Press, 2011. Eight Edition. Washington, DC.

17. Akinola, O.B., Sikiru, A.B., Emmanuel, O.Y., Gabriel, M. O. Comparison of indices of insulin resistance and islet beta-cell dysfunction across rat models of diabetes mellitus induced by modified diets or streptozotocin. *Journal of Krishna Institute of Medical Sciences University*, 2018; 7(2), 1-9.
18. Zhang, M., Lv, X.-Y., Li, J., Xu, Z.-G., Chen, L. The Characterization of High-Fat Diet and Multiple Low-Dose Streptozotocin Induced Type 2 Diabetes Rat Model. *Experimental Diabetes Research*, . 2008; https://doi.org/10.1155/2008/704045
19. Brown RE, Corey SC, Moore AK .Differences in measures of exploration and fear in MHC cogenic C57 BL / 6J and B6 – H – 2K – mice behaviour. Genetics, 1999; 26: 263-271.
20. Edelstein, A. D., Mark, A. T., Nenad, A., Henry, P., Ronald, D. V., Nico, S. Advanced methods of microscope control using μManager software. *Journal of Biological Methods*, 20141: e10
21. Shaimaa, N., Amin, S., Mira, F. A histological and functional study on hippocampal formation of normal and diabetic rats, 2013. .doi: 10.12688/f1000research.2-151.v1. eCollection
22. Camm, E. J., Tijsseling, D., Richter, H. G., Adler, A., Hansell, J. A., Derks, J. B., ... & Giussani, D. A. Oxidative stress in the developing brain: effects of postnatal glucocorticoid therapy and antioxidants in the rat. *PLoS One*, 2011; *6*(6), e21142.
23. Jing, Y. H., Chen, K. H., Kuo, P. C., Pao, C. C., & Chen, J. K. Neurodegeneration in streptozotocin-induced diabetic rats is attenuated by treatment with resveratrol. *Neuroendocrinology*, 2013; *98*(2), 116-127.
24. Singh, J. C. H., Kakalij, R. M., Kshirsagar, R. P., Kumar, B. H., Komakula, S. S. B., & Diwan, P. V. Cognitive effects of vanillic acid against streptozotocin-induced neurodegeneration in mice. *Pharmaceutical biology*, 2015; *53*(5), 630-636.
25. Nazir, S., Anwar, F., Saleem, U., Ahmad, B., Raza, Z., Sanawar, M., & Ismail, T. Drotaverine Inhibitor of PDE4: Reverses the Streptozotocin Induced Alzheimer's Disease in Mice. *Neurochemical research*, 2021; *46*(7), 1814-1829.
26. de Oliveira Santos, T., Mazucanti, C. H. Y., Xavier, G. F., & da Silva Torrão, A. Early and late neurodegeneration and memory disruption after intracerebroventricular streptozotocin. *Physiology & behavior*, 2012; *107*(3), 401-413.
27. Sadikan, M. Z., Abdul Nasir, N. A., Agarwal, R., & Mohd Ismail, N. Protective effect of palm oil-derived tocotrienol-rich fraction against retinal neurodegenerative changes in rats with streptozotocin-induced diabetic retinopathy. *Biomolecules*, 2020;*10*(4), 556.
28. Omotoso, G. O., Mutholib, N. Y., Abdulsalam, F. A., & Bature, A. I. Kolaviron protects against cognitive deficits and cortico-hippocampal perturbations associated with maternal

deprivation in rats. *Anatomy & Cell Biology*, (2020). *53*(1), 95.

29. Wanders, R. J., Waterham, H. R., & Ferdinandusse, S. 2016; Metabolic interplay between peroxisomes and other subcellular organelles including mitochondria and the endoplasmic reticulum. *Frontiers in cell and developmental biology*, *3*, 83.
30. Kettenmann, H., & Verkhratsky, A. Neuroglia--living nerve glue. *Fortschritte der Neurologie-Psychiatrie*, 2011;*79*(10), 588-597.
31. Sun, D., & Jakobs, T. C. Structural remodeling of astrocytes in the injured CNS. *The Neuroscientist*, 2012; *18*(6), 567-588.
32. Loo, D. T., Copani, A., Pike, C. J., Whittemore, E. R., Walencewicz, A. J., & Cotman, C. W. Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. *Proceedings of the National Academy of Sciences*, 1993; *90*(17), 7951-7955.