Supported By
tetfund
TERTIARY EDUCATION TRUST FUND

AMELIORATIVE ROLE OF PIOGLITAZONE ON HIPPOCAMPAL NEURODEGENERATION IN HIGH-FAT DIET AND STREPTOZOTOCIN-INDUCED TYPE 2 DIABETIC RATS

Olushola O. Oluyomi[1], Olumayowa K. Idowu[1,2†], Oluwatomisin O. Faniyan[2], Olawale I. Oluyomi[3], N'Guessan J-B. Oussou[4], Omosola F. Anifowose[5]

[1]Department of Anatomy, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.
[2]Department of Anatomy, College of Medicine, University of Lagos, Lagos, Nigeria.
[3]Department of Biochemistry, School of Life Science, Federal University of Technology, Minna, Nigeria.
[4]Department of Nature Sciences, Laboratory of Physiology, Pharmacology and Pharmacopeia, Nangui Abrogoua University, Abidjan, Côte d'Ivoire.
[5]Department of Physiology, Faculty of Basic Health and Medical Sciences, Ajayi Crowther University, Oyo, Nigeria.

[†]Corresponding author:
Phone: +2348066955642, email: saintmayorfa@yahoo.com, Orcid ID: https://orcid.org/0000-0002-6605-3266

Received: December 13, 2021 Accepted: March 20, 2022

ABSTRACT Co-administration of high-fat diet (HFD) and Streptozotocin (STZ) has been reported to induce insulin resistance (IR) in Wistar rats. Peroxisome proliferator-activated receptors-gamma (PPARγ) agonist drugs such as pioglitazone (PIO) have been used to effectively treat various metabolic-related diseases. Tis study aimed to determine the role of pioglitazone on hippocampal structure and function in rat model of type 2 diabetes mellitus. Twenty-four male Wistar rats (200 g ± 20) were divided into four groups (control, HFD+STZ, PIO, HFD+STZ+PIO). The animals were fed on high-fat diet for 12 weeks concurrently with streptozotocin injection (30 mg/kg bw i.p) for 5 consecutive days to induce insulin resistance form of type 2 diabetes mellitus. Pioglitazone (20 mg/kg bw, orally) was administered for 2 weeks. After the administration period, neurobehavioural evaluation, oxidative stress markers, indices for insulin resistance and histological changes were assessed. Co-administration of high fat diet and streptozotocin significantly increased blood glucose and insulin levels alongside increased MDA and decreased GSH level in HFD+STZ compared to control. However, pioglitazone significantly reduced blood glucose and insulin levels, decreased MDA and increased GSH levels in HFD+STZ+PIO. Histological assessment also demonstrated severe series of cellular damages in HFD+STZ. On the contrary, pioglitazone potentiates its neuroprotective role via presence of mild neuronal damages in the hippocampus of HFD+STZ+PIO. In conclusion, Pioglitazone has potentiated its ameliorative role on hippocampal degeneration by improving cognitive function in high-fat diet and streptozotocin-induced type 2 diabetes mellitus via its activities on neurobehavioural deficits, oxidative stress and hippocampal cellular architecture.

Keywords: Ameliorative, Diabetes, Hippocampus, Insulin resistance, Neurodegeneration, Pioglitazone.

INTRODUCTION

Neurodegenerative changes within the hippocampus are common features of type 2 diabetes mellitus (T2DM) (Moodley and Chan, 2014). Clinical and preclinical studies have suggested connections between T2DM and cognitive dysfunction, and indeed, complaints of cognitive impairment in T2DM have also been linked with hippocampal insulin resistance (Chiu *et al.*, 2007; Umegaki, 2014). In the hippocampus, insulin participates in the formation of neural circuits, synaptic connections and facilitates neuroplasticity (Grillo *et al.*, 2019). Results from previous studies have provided the evidence that

damage to the hippocampus may result in behavioral, memory, navigation and exploratory deficits (Rubin *et al.*, 2014). Structural and functional deficits in synaptic plasticity, as well as learning and memory impairments have been observed in rodent models of T2DM (Biessels and Reagan, 2015).

Peroxisome proliferator-activated receptors-gamma (PPARγ) agonist drugs such as pioglitazone (PIO)

have been used to effectively treat various metabolic-related diseases (Gu *et al.*, 2013). PIO is a drug of the thiazolidinedione (TZD) class with hypoglycemic properties that agonise the activities of PPARγ receptors. TZD have been found to possess the ability to increase insulin sensitivity, and in turn reduces the blood glucose levels by modulating the transcription of the genes involved in the control of glucose metabolism in the tissue (Paddock *et al.*, 2007; Kim *et al.*, 2019).

Administration of high-fat diet (HFD) concurrently with Streptozotocin (STZ) has been reported to induce insulin resistance (IR) in Wistar rats (Akinola *et al.*, 2018). Therefore, this study is aimed at investigating the role of PIO on hippocampal structure of HFD and STZ-induced type 2 diabetic rats, and the potentials of repurposing the anti-diabetic drug for the treatment of neurodegenerative diseases.

MATERIALS AND METHODS

Ethical approval

Ethical clearance (with Approval number: UERC/ASN/2017/741) was obtained from the Ethical Review Committee of the University of Ilorin, Ilorin, Nigeria. All experimental procedures including care and handling of the animals were carried out in conformity with international standards according to the National Academy of Sciences' Guide for the Care and Use of Laboratory Animals.

Chemicals

STZ and PIO were procured from Sigma-Aldrich Company (USA) and Micro Labs Ltd. (India) respectively. HFD was compounded according to the method of Akinola *et al.* (2018) at God's glory feed-mill in Ilorin, Nigeria. Other materials and reagents were of analytical grade and were purchased locally.

Study Design

Twenty-four adult male Wistar rats with a body weight range of 180 – 220g were sourced from University of Ilorin animal holding. The rats were housed in plastic cages, maintained under standardized conditions (12hours/12hours light/dark cycle) and received adequate care in the animal holding facility of the Faculty of Basic Medical Sciences, University of Ilorin. They were acclimatized for 14 days during which they were fed with standard pelletized feed and water ad libitum, before the commencement of the study. Then the rats were randomly divided into four groups (n=6); control, HFD+STZ, PIO, HFD+STZ+PIO. The control group was fed with standard pelletized feed and distilled water, HFD+STZ received HFD for 12 weeks concurrently with STZ for 5 days consecutively, PIO received PIO only for 2 weeks and HFD+STZ+PIO received HFD for 12 weeks concurrently with STZ for 5 days consecutively and PIO for 2 weeks. Blood glucose levels were evaluated weekly from tail vein blood using a handheld glucometer (Akinola *et al.*, 2018). PIO dissolved in distilled water was administered at a dosage of 20 mg/kg body weight to the group of rats taking the treatment via intra-gastric gavage daily for 2 weeks (Nankar and Doble, 2017). The animals were subsequently fasted for 8 hours (overnight) and then euthanized using intraperitoneal injection of ketamine (Wang *et al.*, 2000). Dissection of the thoracic cavity was performed to access the heart for blood collection. The hippocampi were isolated, homogenized and cryo-preserved for biochemical analysis. Intracardial whole body perfused was performed on all the subjects for histological study using 0.9% normal saline followed by 4% paraformaldehyde.

Behavioral studies

Y-maze neurobehavioral test was conducted to evaluate the spatial memory of the rats across all the groups. After the administration, an initial 3 days training was conducted prior to the test which was recorded for analysis. The Y-maze apparatus comprises three arms labeled A, B and C, which are separated at 120° symmetrically. Each rat was placed at the end of arm A, and allowed to freely explore the three arms of the apparatus for 8 minutes. The percentage alternation of arm visits (where alternation patters such as ABC, BCA or CAB were considered as correct alternation sequences) was calculated as an index for spatial memory (Abdulmajeed *et al.*, 2016).

$$\% \text{ Alternation} = \frac{\text{Number of right decisions}}{\text{Total number of arm entries} - 2}$$

Oxidative stress marker

The hippocampal tissues were carefully removed from the medial temporal lobe of the brain, homogenized in 30% of sucrose and kept frozen for biochemical analysis. Malondialdehyde (MDA) estimation was carried out as described by Pomierny-Chamioło *et al.* (2013) and Glutathione (GSH) estimation was carried out as described by Saing *et al.* (2016).

Assessment of insulin resistance

Blood samples were collected into heparinized tubes by cardiac puncture at the right ventricle and centrifuged at 3000rpm for 5 minutes at 4ºC. 0.5 ml of the plasma was taken for fasting glucose and insulin analysis. Fasting plasma glucose (FPG) was measured using glucose oxidase method as modified by Ambade *et al.* (2017). While the fasting plasma insulin (FPI) concentrations was measured using rat insulin ELISA kit (Mercodia, Sweden), following the procedure by Akinola *et al.* (2018). Indices for IR was evaluated using homeostatic model assessment of insulin resistance (HOMA-IR); a method for assessing IR from fasting plasma glucose (mmol/L) and fasting plasma insulin (μIU) (Matthews *et al.*, 1985).

$$\text{HOMA-IR} = \frac{(\text{FPI} \times \text{FPG})}{22.5}$$

Histological studies

Brain tissues were excised and a coronal gross dissection was made at the level of hippocampus. The hippocampal tissues were rinsed in 0.25 M sucrose and post-fixed in 10% phosphate-buffer formalin, then processed and stained using Cresyl violet (CV) dye as described by Pilati *et al.* (2008). All the slides were examined under a light microscope (magnification, ×400).

Statistical analysis

Data were reported as mean ± SEM. Statistical analyses and graphs were drawn using Graph Pad Prism 8 software (Graph pad software Inc. USA). Multiple comparisons were done using a one-way ANOVA with Tukey test. Significance was set at P less than 0.05 ($P < 0.05$).

RESULTS AND DISCUSSION

High-fat diet and Streptozotocin induced type 2 diabetes mellitus in Wistar rats

Intraperitoneal injection of multiple low-dose of STZ concurrently with chronic intake of HFD in this study showed severe metabolic outcomes including elevation of blood glucose level typical of IR. This correlates with report by Akinola *et al.* (2018) that multiple injection of low-dose of STZ with either HFD or high-fructose diet induces metabolic alterations related to IR. Induced IR may be due to DNA methylation, inflammatory changes and oxidative damage, including distorted transmembrane insulin receptor, insulin receptor substrate 1, and the downstream signaling molecules, such as Phosphatidylinositol 3 Kinase (PI3K), Protein Kinase-B (PKB or Akt) and glycogen synthase kinase 3-beta (Hotamisligil *et al.*, 1996; Llorens-Martin *et al.*, 2014).

Pioglitazone ameliorates spatial memory deficit in type 2 diabetic rats

Pioglitazone treatment improves spatial memory (Fig. 1A) by significantly increasing ($p<0.05$) the number of alternations and percentage alternations of insulin resistant rats when tested using Y-maze apparatus. Deficits in spatial memory output as observed in the percentage alternation assessment suggests that incidence of memory cognitive impairment in T2DM maybe as a result of IR leading to impaired synaptic plasticity needed for memory formation and its consolidation (Albani *et al.*, 2014). The improved memory and cognitive function brought about by PIO agrees with the report of Denner *et al.*, (2012) who discussed that the convergence of ERK MAPK and PPARγ signaling pathways in Tg2576 mice enhanced cognitive improvement with rosiglitazone; another drug of the TZD class.

Pioglitazone ameliorates oxidative stress in type 2 diabetic rats

Evaluation of MDA and GSH were carried out to determine the cellular redox status of the experimental rats. The results showed significant increase ($p < 0.001$) in MDA level (Fig. 1B) and significant

decrease ($p < 0.001$) in GSH level (Fig. 1C) in the HFD+STZ group compared to control. Conversely, HFD+STZ+PIO group showed significant decrease ($p < 0.05$) in MDA and significant increase ($p < 0.05$) in GSH levels compared to HFD+STZ group. The pathophysiology of IR has been traced to multiple factors, one of which is increased oxidative stress (Yaribeygi *et al.,* 2019) similar to increased MDA level in conjunction with decreased GSH level as observed in this study. This might have accounted for the impairment in the bioenergetic system needed for cognitive function by the hippocampal cells leading to decreased performance during the neurobehavioral test. However, reduction in oxidative stress via a lowered MDA end elevated GSH levels were observed in the IR rats after treatment with PIO. This is consistent with Beheshti *et al.* (2019) who described that PPAR-γ agonist improved hippocampal oxidative stress status in lipopolysaccharide-treated rats. These findings revealed that balance in oxidative and antioxidant enzymes activities is vital in cognitive function of the brain especially in the hippocampus saddled with neuroplasticity function.

Pioglitazone improves insulin sensitivity in type 2 diabetic rats

Insulin resistance was evaluated from fasting plasma glucose and fasting insulin levels using HOMA-IR method (Fig. 1D). HFD+STZ groups showed significant ($p < 0.05$) increase in IR compared to control. However, HFD+STZ+PIO group showed statistically significant ($p < 0.05$) decrease in IR compared to HFD+STZ. The high indices of IR demonstrated in the untreated type 2 diabetic rats were indeed reduced in the subjects that received post-induction PIO treatment. This confirms the blood glucose lowering and insulin sensitivity increasing capacity of PIO (Gu *et al.*, 2013; Kim *et al.*, 2019).

Pioglitazone reduces neuronal degeneration in type 2 diabetic rats

Nissl protein demonstration using cresyl violet staining shows that Nissl substance of the control, PIO and HFD+STZ+FEN groups stained positive (pink coloration) to cresyl violet dye with mild traces of neuronal pyknosis and chromatolysis (Fig. 2A, 2C and 2D respectively). However, HFD+STZ group showed areas with severe chromatolysis due to the dissolution of the Nissl body present in the neurons and pyknosis (dark pigmentation) leading to vacuolization of the neurons (Figure 2B). The histoarchitectural derangements indicate onset of neurodegeneration which may be due to redox status imbalance induced by increased blood glucose level (Albani *et al.*, 2014). Interestingly, PIO markedly reduced evidences of hippocampal cellular degeneration suggesting the importance of PPARγ agonist in ameliorating pathological alterations induced by IR (Kobayashi *et al.*, 1992; Avwioro, 2010; Sun *et al.*, 2017).

CONCLUSION

This research reveals that, cognitive impairment associated with IR form of type 2 DM may be linked to increased oxidative stress and hippocampal structural damages. However, the use of PIO, a PPARγ agonist, proved to improve cognition and hippocampal cellular structural integrity despite the detrimental activities of IR.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

ACKNOWLEGEMENT

The authors are grateful to the Department of Anatomy, University of Ilorin, Nigeria, for providing the facility needed towards the success of this research.

REFERENCES

Abdulmajeed WI, Sulieman HB, Zubayr MO, Imam A, Amin A, Biliaminu SA Oyewole LA & Owoyele BV 2016. Honey prevents neurobehavioural deficit and oxidative stress induced by lead acetate exposure in male wistar rats-a preliminary study. *Metab. Brain. Dis.*, 31(1): 37-44.

Akinola O, Biliaminu S, Yawson E & Gabriel M 2018. Comparison of indices of insulin resistance and islet beta-cell dysfunction across rat models of diabetes mellitus induced by modified diets or Streptozotocin. *JKIMSU.*, 7: 1-9.

Albani S, McHail D & Dumas T 2014. Developmental studies of the hippocampus and hippocampal-dependent behaviors: Insights from interdisciplinary studies and tips for new investigators. *Neurosci. Biobehav. Rev.*, 43:183–190.

Ambade V, Sharma Y & Somani B 2017. Methods for Estimation of Blood Glucose: A Comparative Evaluation. *Med. J. Armed Forces India.*, 54 (2): 131–133.

Avwioro OG 2010. Histochemistry and tissue pathology, principles and techniques. University Press Delta State University, Abraka Nigeria. 2010: 561-568. *University Press Delta State University, Abraka Nigeria ISBN, 978356279,* 561-568.

Beheshti F, Hosseini M, Hashemzehi M, Soukhtanloo M, Khazaei L & Shafei M 2019. The effects of PPAR-γ agonist pioglitazone on hippocampal cytokines, brain-derived neurotrophic factor, memory impairment, and oxidative stress status in lipopolysaccharide-treated rats. *Iran. J. Basic Med. Sci.*, 22(8): 940.

Biessels G & Reagan L 2015. Hippocampal Insulin Resistance and Cognitive Dysfunction. *Nat. Rev. Neurosci.*, 16 (11): 660-671.

Chiu HK, Tsai EC, Juneja R, Stoever J, Brooks-Worrell B, Goel A & Palmer JP 2007. Equivalent insulin resistance in latent autoimmune diabetes in adults (LADA) and type 2 diabetic patients. *Res. Clin. Pract.*, 77(2): 237-244.

Denner L, Rodriguez-Rivera J, Haidacher S, Jahrling J, Carmical J, Hernandez CM Zhao, Y, Sadygov RG, Starkey JM, Spratt H & Luxon BA 2012. Cognitive Enhancement with Rosiglitazone Links the Hippocampal PPARγ and ERK MAPK Signaling Pathways. *J. Neurosci.*, 32(47): 16725–16735.

Grillo C, Woodruff J, Macht V & Reagan L 2019. Insulin resistance and hippocampal dysfunction: Disentangling peripheral and brain causes from consequences. *Exp. Neurol.*, 318: 71-77.

Gu MX, Liu XC & Jiang L 2013. Effect of peroxisome proliferator-activated receptor-gamma on proliferation of airway smooth muscle cells in mice with asthma. *Chin. J Contemp. Ped.*, 15(7): 583–587.

Hotamisligil G, Peraldi P, Budavari A, Ellis R, White M & Spiegelman B 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha and obesity-induced insulin resistance. *Science*, 271(5249): 665-668.

Kim SH, Hong SH, Park YJ, Sung JH, Suh W, Lee KW, Jung K, Lim C, Kim JH, Kim H & Park KS 2019. MD001, a novel peroxisome Proliferator-activated receptor α/γ agonist, improves glucose and lipid metabolism. *Sci. Rep.*, 9(1): 1-12.

Kobayashi M, Iwanishi M, Egawa K & Shigeta Y 1992. Pioglitazone Increases Insulin Sensitivity by Activating Insulin Receptor Kinase. *Diabetes*, 41(4): 476-483.

Llorens-Martin M, Jurado J, Hernandez F & Avila J 2014. GSK-3beta, a pivotalkinase in Alzheimer disease. *Front. Mol. Neurosci.*, 7: 46.

Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D & Turner R 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28(7): 412-419.

Moodley KK & Chan D 2014. The hippocampus in neurodegenerative disease. *Front. Neurol. Neurosci.*, 34: 95-108.

Nankar R & Doble M 2017. Hybrid Drug Combination: Anti-diabetic Treatment of Type 2 Diabetic Wistar Rats With Combination of Ellagic Acid and Pioglitazone. *Phymed.*, 37: 4-9.

Paddock ML, Wiley SE, Axelrod HL, Cohen AE, Roy M, Abresch EC, Capraro D, Murphy AN, Nechushtai R, Dixon JE & Jennings PA 2007. MitoNEET is a uniquely folded 2Fe 2S outer mitochondrial membrane protein stabilized by pioglitazone. *Proc. Nat. Acad. Sci.*, 104(36): 14342-14347.

Pilati N, Barker M, Panteleimonitis S, Donga R & Hamann M 2008. A Rapid Method Combining Golgi and Nissl Staining to Study Neuronal Morphology and Cytoarchitecture. *J. Histochem. Cytochem.*, 56(6): 539–550.

Pomierny-Chamioło L, Moniczewski A, Wydra K, Suder A & Filip M 2013. Oxidative Stress Biomarkers in Some Rat Brain Structures and Peripheral Organs Underwent Cocaine. *Neurotox. Res.*, 23(1): 92–102.

Rubin R, Watson P, Duff M & Cohen N 2014. The role of the hippocampus in flexible cognition and social behavior. *Front. Hum. Neurosci.*, 8: 742.

Saing T, Lagman M, Castrillon J, Gutierrez E, Guilford F & Venketaramana V 2016. Analysis of glutathione levels in the brain tissue samples from HIV-1-positive individuals and subject with Alzheimer's disease and its implication in the pathophysiology of the disease process. *BBA. Clin.*, 6: 38–44.

Sun L, Yuan Q, Xu T, Yao L, Feng J, Ma J, Wang L, Lu C & Wang D 2017. Pioglitazone Improves Mitochondrial Function in the Remnant Kidney and Protects against Renal Fibrosis in 5/6 Nephrectomized Rats. *Front. Pharmacol.*, 8: 545.

Umegaki H 2014. Type 2 diabetes as a risk factor for cognitive impairment: current insights. *Clin. Interv. Aging.*, 9: 1011–1019.

Wang Y, Huang C, Cao Y & Han J-S 2000. Repeated administration of low dose ketamine for the treatment of monoarthritic pain in the rat. *Life Sci.*, 67(3): 261 – 267.

Yaribeygi H, Sathyapalan T, Atkin S & Sahebkar A 2020. Molecular Mechanisms Linking Oxidative Stress and Diabetes Mellitus. *Oxid. Med. Cell. Longev.*, 2020.

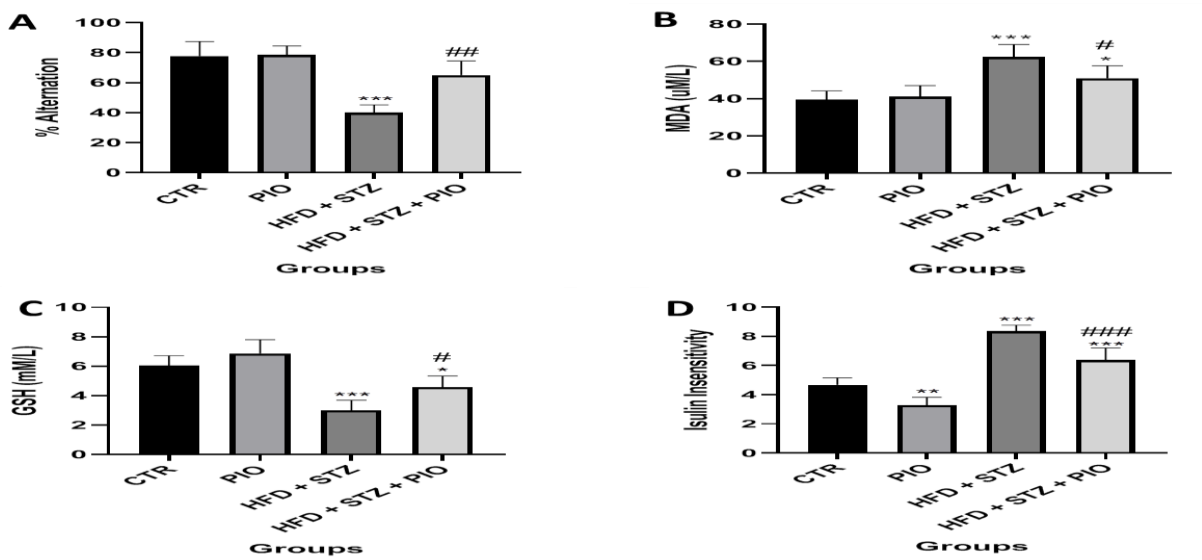

Figure 1: Y-maze test (A), MDA level (B), GSH level (C) and Insulin insensitivity (D) [***: p<0.001, **: p< 0.01, *: p< 0.05 compared to control; ###: p<0.001, ##: p<0.01, #: p<0.05 compared to HFD+STZ; CTR: Control, HFD: High fat diet, STZ: Streptozotocin, PIO: Pioglitazone].

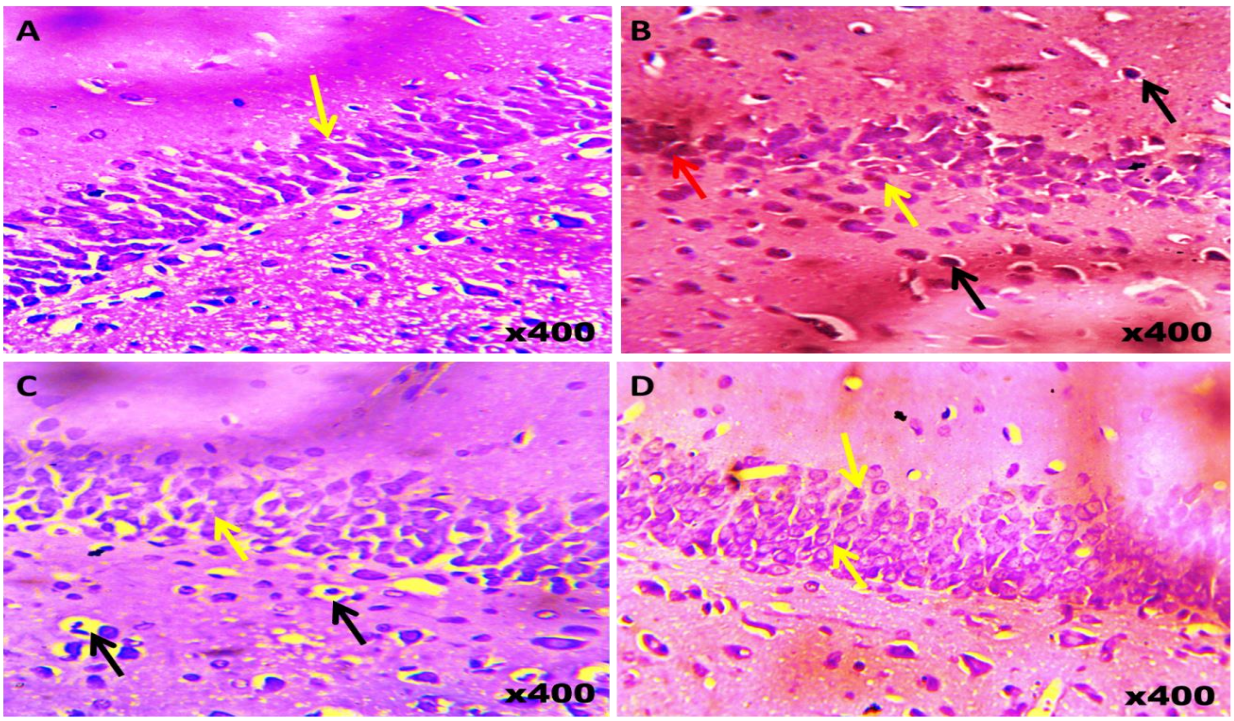

Figure 2: Pioglitazone reduces loss of Nissl bodies in type 2 diabetic rats. (Black arrow: Shrunken nuclei, red arrow: chromatolysis, yellow arrow: neuronal Nissl body, A: Control, B: HFD+STZ, C: PIO, D: HFD+STZ+PIO. Magnification: x 400).