



International Journal of Pharmacology

ISSN 1811-7775



Research Article

Pioglitazone and Metformin on Improving Cognitive Dysfunction Caused by Diabetes Mellitus: A Comparative Study

¹Ahmad Alhowail, ¹Abdullah Alomran, ¹Naif Alqefari, ¹Maha Aldubayan and ²Sattam Alenezi

¹Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah 52571, Saudi Arabia

²Department of Pharmacology and Toxicology, Unaizah College of Pharmacy, Qassim University, Unaizah 51911, Saudi Arabia

Abstract

Background and Objective: Diabetes mellitus is a common metabolic disease that impairs cognitive function. For diabetes management, different classes of drugs, including pioglitazone (PIO) and metformin (MET), are also reported to improve the cognitive dysfunction caused by diabetes. This study aims to compare the neuroprotective effect of PIO versus MET on diabetes mellitus (DM) induced cognitive dysfunction in rat models of diabetes. **Materials and Methods:** Six groups of sixty male albino rats ($n = 10$) were randomly created: Control, diabetes, MET, PIO, diabetes+MET and diabetes+PIO. Nicotinamide (120 mg kg^{-1}) and streptozotocin (55 mg kg^{-1}) intraperitoneal injections induced diabetes. The MET and PIO treatments lasted for 14 days. The survival rate, body weight, behavioral tasks (Y-maze, novel object recognition (NOR), elevated plus maze (EPM)) and glucose levels were measured after the completion of the treatments. **Results:** The findings elucidated that diabetes elicited a decrease in the survival rate, body weight and cognitive function, while concurrently provoking an increase in glucose levels. The groups that received PIO and MET exhibited enhancements in survival rate, cognitive function and glucose levels among the diabetic rats, although no significant changes were observed in body weight. **Conclusion:** This study revealed that PIO and MET improved cognitive dysfunction in diabetic rats, however, MET showed better effectiveness than PIO.

Key words: Rats, diabetes mellitus, pioglitazone, metformin, cognitive dysfunction

Citation: Alhowail, A., A. Alomran, N. Alqefari, M. Aldubayan and S. Alenezi, 2023. Pioglitazone and metformin on improving cognitive dysfunction caused by diabetes mellitus: A comparative study. *Int. J. Pharmacol.*, 19: 505-513.

Corresponding Author: Ahmad Alhowail, Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah 52571, Saudi Arabia

Copyright: © 2023 Ahmad Alhowail *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition that is characterized by insulin deficiency, insulin resistance and chronic hyperglycemia due to the inability of tissues to react to insulin and properly metabolize glucose¹. Diabetes mellitus (DM) is a metabolic condition that is characterized by insulin deficiency, insulin resistance and chronic hyperglycemia. Insulin is critical in regulating physiological functions, such as cellular energy sources and metabolism². Insulin is also involved in the preservation of physiological processes. Diabetes mellitus causes disruptions in the metabolism of carbohydrates, lipids and proteins, which leads to long-term complications affecting a variety of vital organs³. The number of people living with diabetes around the world is expected to reach 387 million by the year 2035, as stated in the Diabetes Atlas published in 2015 by the International Diabetes Federation. The DM's progressive effects include problems that impact the morphology and operation of other physiologic systems and organs, subsequent in dementia, cerebrovascular damage, nephropathy, vision loss and erectile impairment^{4,5}. Beyond the disease's physical manifestations, these effects include problems that impact the morphology and operation of other bodily systems and organs. The downstream signaling pathways in both the central and peripheral nervous systems are strongly influenced by insulin signaling, which plays a critical role in memory encoding^{6,7}. This has the effect of making insulin more effective at regulating cognitive processes.

Metformin, also known as MET, is a pharmacological agent utilized for the management of diabetes mellitus. It is classified within the biguanide drug class, which is specifically indicated for its anti-diabetic properties. The primary mechanism of action involves the inhibition of hepatic gluconeogenesis and the enhancement of insulin receptor sensitivity, resulting in a reduction in glucose levels⁸. For decades, metformin has become an effective therapy in DM treatment. It is the commonly used oral antihyperglycemic agent recommended for most newly diagnosed DM patients as first-line therapy⁹. Metformin is also reported to have other beneficial activities and usage, such as obesity management, metabolic syndrome, anti-cancer activity and polycystic ovary syndrome¹⁰. Several lines of evidence have shown the capability of metformin to improve cognitive dysfunction caused by diabetes and neurodegenerative diseases^{11,12}.

Pioglitazone, also known as PIO, is a pharmacological compound classified as a thiazolidinedione derivative. It functions as an agonist for the Peroxisome Proliferator-Activated Receptor (PPAR)¹³. The PIO has received

approval for the treatment of diabetes mellitus (DM) that is associated with insulin resistance¹⁴. Activation of PPAR agonists such as PIO improves insulin sensitivity, glucose uptake and lipid metabolism¹⁵. In addition, PIO has been effective in treating neurodegenerative diseases, making it a candidate drug for Alzheimer's and Parkinson's diseases¹⁶. Previously published study has also shown that PIO has a beneficial effect on activating the cholinergic system, which improves cognitive dysfunction in rats with experimental dementia¹⁷.

The PIO and MET treatments were aimed at the test to see which one was more effective at reversing the damage that diabetes causes to cognitive function.

MATERIALS AND METHODS

Study area: The study took place between 10th to 30th July, 2023 at the College of Pharmacy, Qassim University in the Kingdom of Saudi Arabia.

Drugs: Pioglitazone hydrochloride (Glados®) and metformin hydrochloride (Metfor®) were procured from Tabuk Pharma Company (Tabuk, Saudi Arabia). Cayman Chemical (Ann Arbor, MI, USA) supplied the streptozotocin and nicotinamide.

Animals: The animal house at Qassim University's College of Pharmacy provided 60 albino male rats weighing between 250 and 350 g for this study. The rodents spent their days in cages with a light-dark cycle of 12 hrs and a temperature of 25.2 °C. The availability of food and water was never limited for them.

Experimental design: The rodents were separated into six groups of ten rats each, with one group serving as the control and receiving no treatment. The MET group members drank beverages with a concentration of MET equal to 3 mg mL⁻¹. The participants in the PIO group drank water containing a concentration of PIO equal to 2 mg mL⁻¹. A single dose of nicotinamide (120 mg kg⁻¹) and streptozotocin (60 mg kg⁻¹) was given to a diabetic group, but nothing else was given to them. A concentration of MET that was 3 mg mL⁻¹ was given to a group of diabetics who also took MET. A diabetic plus PIO group had a concentration of PIO in their beverages equal to 3 mg mL⁻¹, which was then consumed by them. Following the completion of the daily observation of the mortality rate of the rats, the rats' body weights were taken every three days and they were subjected to behavioral examinations.

Y-maze: The Y maze is utilized to evaluate an animal's ability to remember locations within the maze that it has previously explored as well as their capacity to find new locations. The working memory and hippocampus-dependent task performance of rats were evaluated using the Y maze Test. The wooden Y-shaped maze had three arms that were 120° apart and had dimensions of 50, 10 and 18 cm. For better visualization, the arms have been painted a dark brown color. The gadget was placed on the surface. To guarantee that the light was dispersed equally, the light was supplied from above. The animals were given 15 min of free exploration of two arms during the training period. During the trial session, which lasted for a total of 5 min, the animals were given the opportunity to investigate the entire maze, including the recently added arm. There was a 3 hrs break between the two sessions. To track the amount of time and entries in each arm of the exam, the sessions were videotaped. If half of the animal's body penetrated, it was considered to have entered an arm¹⁸. Data has been statistically analyzed using one-way ANOVA followed by Tukey's Test and $p < 0.05$ was counted as statistically significant.

NOR: Memory capacity was evaluated with the help of a NOR test that involved recognizing unfamiliar objects. A wooden box with an open top that was 40×40×40 cm was part of the testing equipment. Two white cups served as the familiarization items and a black box that was the same size as the cups served as the new item. During the training, the rodents were permitted to discover the two cups on their own for fifteen minutes before returning to their respective cages. During the second session, which took place three hours later and lasted for five minutes, one of the teacups was switched out for the new object and the amount of time that was spent examining it was timed with a stopwatch and a video camera¹⁹. The data has been subjected to rigorous statistical analysis utilizing the One-way Analysis of Variance (ANOVA) method, followed by the application of the Tukey's Test and $p < 0.05$ was counted as statistically significant.

EPM: The EPM measures cognitive, memory and anxiety-related abilities. In this investigation, the open arm of the wooden maze was 50 by 10 cm, while the closed arm was 50 by 10 by 30 cm. Side walls of the closed arm were 30 cm tall. A central platform of 10 cm² was located between the arms. The maze stood 50 cm above the ground. Training sessions consisted of ten minutes of the rat being kept at the end of the open arm in front of the central platform before being released to wander the maze. After 3 hrs, the rodent was

put in the same position as during the training session and a video camera was used to record the transfer latency time, which is the amount of time that it took the rodent to locate from the open arm into the closed arm, as well as the total amount of time that the rat spent in the closed arm²⁰. The data subsequently underwent statistical analysis using one-way ANOVA followed by Tukey's Test and $p < 0.05$ was counted as statistically significant.

Blood glucose test: On the final day of the trial, a clean, sterile needle was used to puncture the tail vein of each rodent to obtain the purest blood possible. All of the rat groups had their blood sugar levels measured using a wristband-mounted Accu-Chek blood glucose meter. The machinery was operated per the manufacturer's guidelines.

Statistical analysis: The data were analyzed using unidirectional ANOVA and expressed as a mean standard error using the GraphPad Prism 10.0.0.153 software (GraphPad, Boston, Massachusetts, USA). The data were subjected to the Dunnett's Multiple Comparison Test to evaluate the endpoints against the control and $p < 0.05$ was counted as statistically significant.

RESULTS

MET and PIO treatment improves diabetic survival rate: Rat mortality was reduced by 40% in the diabetic rat model whereas the control, MET and PIO groups were not altered. However, diabetic rats treated with MET and PIO improved the survival rate causing 10% death in MET treated and 20% in PIO treated (Fig. 1).

Diabetics body weight did not improve with MET or PIO treatment: Comparing the diabetic rat groups to the control group, the diabetic rat groups had lower body weights. The control, MET and PIO groups, conversely, had increased body weight. However, in diabetic rats, MET and PIO treatments did not enhance body weight loss (Fig. 2).

PIO and MET improve memory in Y-maze: Diabetic rats showed notable reduction in the number of entries and time spent in the novel arm. However, rats that were given PIO and MET were improved the memory function, whereas MET was a better results improving the number of entries by increasing significant (Fig. 3a). Rodents in the MET group spent more time in the new arm, while the other groups did not vary statistically (Fig. 3b).

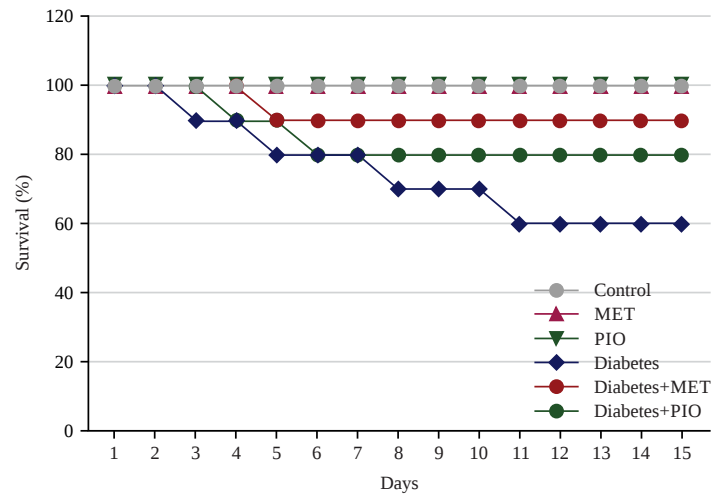


Fig. 1: Effect of diabetes, MET and PIO treatments on the survival rate of rats

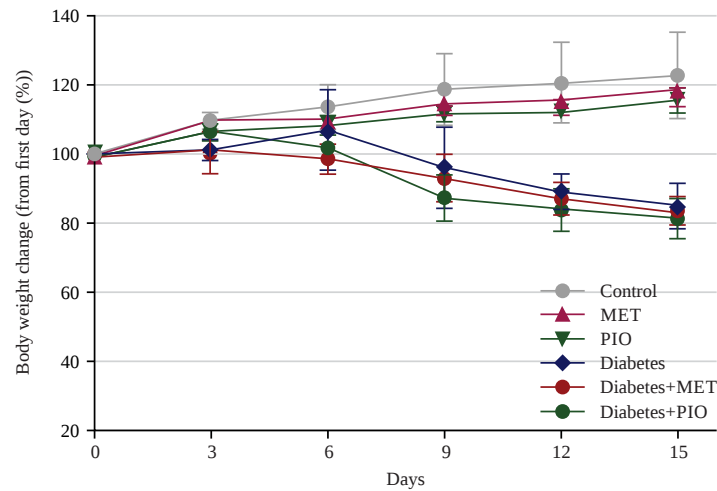


Fig. 2: Effect of diabetes, MET and PIO therapies on rat body weight alterations

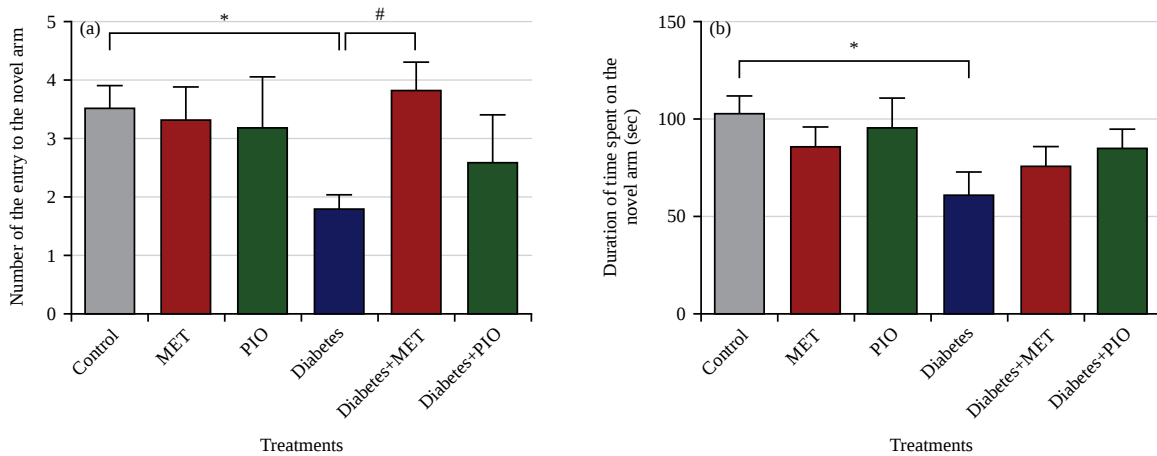


Fig. 3(a-b): Influence that diabetes, (a) MET and (b) PIO have on the Y maze Test

Data are summarized using the mean and standard error and a one-way ANOVA is performed as an analysis step before Dunnett's Test is performed. A probability level of 0.05 was considered to have statistical significance, * $p < 0.05$: Control group and # $p < 0.05$: Diabetes group rats

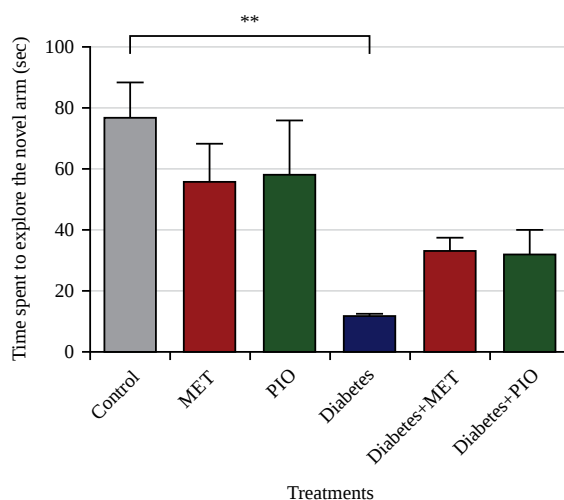


Fig. 4: Impacts of diabetes, MET and PIO on the novel object recognition (NOR) Test

Data were analyzed utilizing a one-way ANOVA tailed with Dunnett's analysis and $**p < 0.05$ as was counted statistically significant

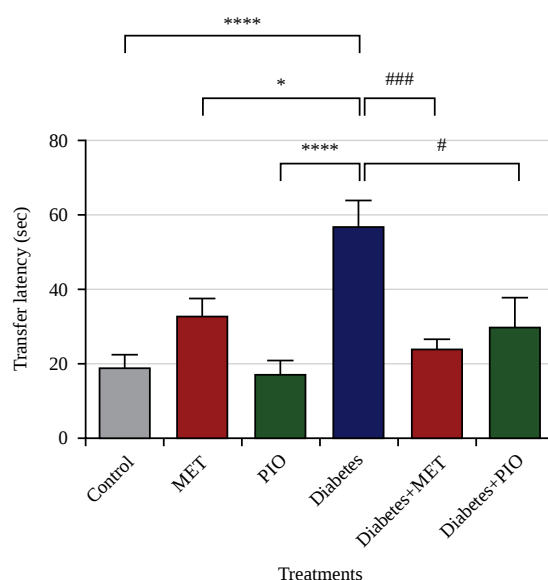


Fig. 5: Impacts of diabetes, MET and PIO treatments on rats on transfer latency time

If the p-value was less than 0.05, then it was counted statistically significant, $*p < 0.05$, $****p < 0.0001$: Control group and $*p < 0.05$ and $***p < 0.001$: Diabetes group rats

PIO and MET improve memory in NOR test performance:

Diabetes reduces the time amounts spent exploring the new object in rats. The MET and PIO treatments showed a slight improvement in behavioral performance during the NOR test, but not significantly related with control groups (Fig. 4).

PIO and MET rescue memory in EPM test: The transfer latency time was significantly lengthened in diabetic rats,

but both the MET and PIO treatments were able to reverse this lengthening (Fig. 5).

PIO and MET reduce levels of blood glucose in diabetes: The levels of glucose in the blood were measured exactly one day after the treatment had been completed. Diabetic rats exhibited a significant elevation in blood glucose levels. However, MET and PIO treatments significantly lower this increase (Fig. 6).

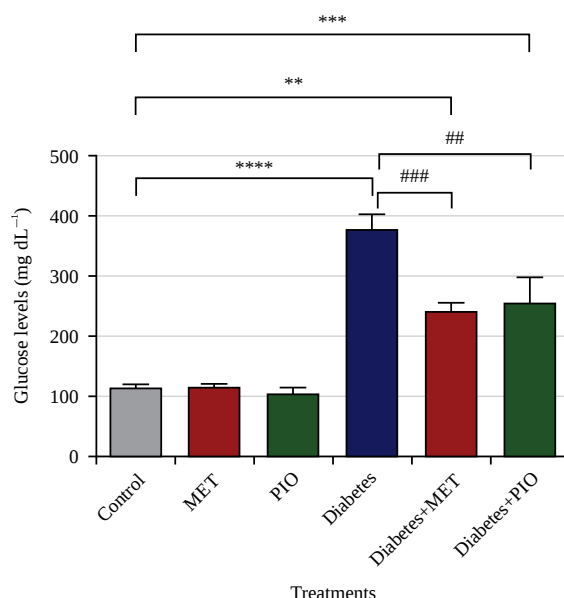


Fig. 6: Effect of diabetes, MET and PIO treatments on rats' blood glucose levels

p<0.01, *p<0.001 and ****p<0.0001: Control group, #p<0.01 and ###p<0.001: Diabetes group rats

DISCUSSION

In the current investigation, the anti-diabetic drugs PIO and MET prevented diabetes-related cognitive impairment in rat models was assessed. By reducing blood glucose levels and improving cognitive impairment, MET and PIO have been shown to improve the quality of life for diabetic patients^{21,22}. The MET has also reduced the risk of occurrence of Alzheimer's disease²³. In addition, the PIO is shown to improve memory impairment caused by diabetes and Alzheimer's and Parkinson's diseases^{24,25}. Therefore, the existing study is aimed to compare the ability and effectiveness of the MET and PIO in improving cognitive impairment caused by diabetes.

The MET exerts its effect by activating the AMPK protein, which, in turn, activation and inhibition of other protein signaling²⁶. For instance, AMPK phosphorylates the Akt and mTOR proteins, which results in the activation of Akt and the inhibition of mTOR. Both of these proteins are well-known for their roles as regulators in cell proliferation and cognitive function^{27,28}. However, PIO, which is a PPAR gamma agonist, has been shown to improve gene transcription and translation of glucose transporters, thereby increasing the amount of glucose absorbed from the bloodstream and lowering blood glucose levels²⁹. Additionally, it helps patients with diabetes who suffer from cognitive decline and slows the advance of Alzheimer's disease³⁰.

The combination of streptozotocin and nicotinamide resulted in a mortality rate of forty percent in rats. On the

other hand, diabetic rats treated with MET had a mortality rate of 10%, while diabetic rats treated with PIO had a mortality rate of 20%. This evidence suggested that the MET and PIO both have a protective effect against diabetes and that they have the potential to improve the complications of diabetes. In addition, neither the MET nor the PIO treatments had any effect on the subjects' body weight in comparison to the controls. However, when related to the controls, the body weight of diabetic rats was significantly lower versus controls and neither the MET nor the PIO treatments were able to reverse this reduction in body weight when compared to the controls. As a result, treatments such as MET and PIO can improve the rate of survival as well as behavioral tasks, but they cannot improve body weight.

The Y-maze, the NOR and the EPM were used to detect the functionality of cognition to identify the cognitive impairment²⁰. This was done by analyzing the short-term memory function³¹. Both the Y-maze and the NOR tests were hippocampal-dependent activities. Memory was shown to be impaired in the diabetic group as demonstrated by a reduction in both the number of entries and the amount of time spent in the Y-novel maze's arm when related to control rats. As a consequence of this finding, the diabetic rats had difficulty differentiating the novel arm from the familiar arms. Although diabetic rats treated with MET or PIO showed improvement in the number of entries and amount of time spent in the novel arm compared with controls, MET and PIO-treated groups did not show any difference in their

performance in Y-maze tasks related to the control group. However, when compared to diabetic rats, rats treated with diabetics plus MET showed a significant improvement. According to these findings, cognitive impairment was probably brought on by diabetes and it was cured by treatments involving MET and PIO. In a similar vein, the findings of the NOR demonstrated that the amount of time spent by diabetic rats investigating a novel object was significantly less than that of control rats. However, treatment with either MET or PIO was able to improve these cognitive impairments. In addition to that, the EPM task was utilized to characterize the rats' cognitive dysfunction. The time it took for the transfer latency to occur was considerably longer in the diabetic rats than in the control rats. The MET and PIO treatments, conversely, were able to reverse this increase in transfer latency time. However, the reduction in time was significantly more significant in the MET treatment ($p < 0.001$) than it was in the PIO treatment ($p < 0.05$). When taken as a whole, these behavioral findings suggest that MET is more effective than PIO treatment in improving cognitive dysfunction.

The regulation of brain function, learning and memory encoding, neuronal survival and synaptic plasticity is greatly influenced by insulin and insulin receptors^{32,33}. Insulin receptors can be found in high numbers throughout the brain, especially in the hippocampus³⁴. In diabetic patients, insulin treatment improves cognitive dysfunction. The activation of insulin receptors activates the insulin receptor substrate and the PI3K/Akt signaling pathway, which traffics the Glucose Transporter 4 (GLUT4) to the cell surface, where it uptakes glucose and lowers hyperglycemia^{18,35}. The present investigation involved the development of DM rat models through the administration of streptozotocin/nicotinamide, which induced hyperglycemia and cognitive dysfunction in the rat subjects. Prolonged high blood sugar may damage the cerebral blood vessels, resulting in a deficiency of blood that reaches the brain, causing ischemia and dysfunction in cognition. It is interesting to note that our data showed that MET and PIO treatments in diabetic rats enhanced memory performance and reduced glucose levels compared with controls, while the MET outcome was more effective than the PIO.

The current study's limitations are that diabetes was induced by streptozotocin and nicotinamide in an experimental rat model. The effects of MET and PIO therapy on mortality, body weight and cognitive function were studied in rats. However, there was no indication of an impact on insulin susceptibility or receptor signaling. Therefore, the effect on insulin receptor sensitivity and signaling as well as metabolic

condition following MET versus PIO is worth mentioning. Although the result indicated that MET versus PIO improves cognitive function and seems to reduce neurotoxicity, the mechanisms of these improvements in cognition function are not fully elucidated. Therefore, further direction and research are necessary to inform the comparison of efficacy in patients with diabetes.

CONCLUSION

This study found that diabetes induces a decline in cognitive function, but MET and PIO have restored this decline in diabetic rats. Behavioral assessments negatively impaired Y-maze, NOR and EPM tasks and were associated with increased glucose levels in diabetic rats, however, MET and PIO therapies have improved all behavioral tasks and reduced glucose levels. Conclusively, MET is more effective than PIO in diabetic patients with cognitive disabilities and manages glucose levels. Therefore, this result could assist practitioners in choosing the most effective anti-diabetic drugs.

SIGNIFICANCE STATEMENT

The current investigation aims to assess and contrast the impact of pioglitazone and metformin on cognitive impairment induced by Type 2 Diabetes Mellitus (T2DM) in an experimental rat model. The T2DM was experimentally induced in male rats through the administration of nicotinamide and streptozotocin. The biochemical assessment involved the measurement of glucose levels, while the cognitive function was evaluated. Rats with diabetes demonstrated a notable elevation in blood glucose levels and experienced cognitive impairment to a significant degree. The findings indicate that the administration of Pioglitazone and metformin reduced blood glucose levels and a notable enhancement in cognitive performance. Ultimately, it is worth noting that metformin exhibits potential superiority over pioglitazone in terms of ameliorating hyperglycemia and hyperglycemia-associated cognitive dysfunction.

REFERENCES

1. Galicia-Garcia, U., A. Benito-Vicente, S. Jebari, A. Larrea-Sebal and H. Siddiqi *et al.*, 2020. Pathophysiology of type 2 diabetes mellitus. *Int. J. Mol. Sci.*, Vol. 21. 10.3390/ijms21176275.
2. Rahman, M.S., K.S. Hossain, S. Das, S. Kundu and E.O. Adegoke *et al.*, 2021. Role of insulin in health and disease: An update. *Int. J. Mol. Sci.*, Vol. 22. 10.3390/ijms22126403.

3. Dilworth, L., A. Facey and F. Omoruyi, 2021. Diabetes mellitus and its metabolic complications: The role of adipose tissues. *Int. J. Mol. Sci.*, Vol. 22. 10.3390/ijms22147644.
4. Oh, W., E. Kim, M.R. Castro, P.J. Caraballo, V. Kumar, M.S. Steinbach and G.J. Simon, 2016. Type 2 diabetes mellitus trajectories and associated risks. *Big Data*, 4: 25-30.
5. Cholerton, B., L.D. Baker, T.J. Montine and S. Craft, 2016. Type 2 diabetes, cognition, and dementia in older adults: Toward a precision health approach. *Diabetes Spectrum*, 29: 210-219.
6. Bloemer, J., S. Bhattacharya, R. Amin and V. Suppiramaniam, 2014. Impaired insulin signaling and mechanisms of memory loss. In: *Progress in Molecular Biology and Translational Science*, Tao, Y.X. (Ed.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-12-800101-1, pp: 413-449.
7. Dou, J.T., M. Chen, F. Dufour, D.L. Alkon and W.Q. Zhao, 2005. Insulin receptor signaling in long-term memory consolidation following spatial learning. *Learn. Mem.*, 12: 646-655.
8. Hunter, R.W., C.C. Hughey, L. Lantier, E.I. Sundelin and M. Pegg *et al.*, 2018. Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase. *Nat. Med.*, 24: 1395-1406.
9. Garber, A.J., 1997. Metformin: Mechanisms of antihyperglycemic action, other pharmacodynamic properties, and safety perspectives. *Endocr. Pract.*, 3: 359-370.
10. Marshall, S.M., 2017. 60 years of metformin use: A glance at the past and a look to the future. *Diabetologia*, 60: 1561-1565.
11. Zhang, Q.Q., W.S. Li, Z. Liu, H.L. Zhang, Y.G. Ba and R.X. Zhang, 2020. Metformin therapy and cognitive dysfunction in patients with type 2 diabetes: A meta-analysis and systematic review. *Medicine*, Vol. 99. 10.1097/MD.00000000000019378.
12. Samaras, K., S. Makkar, J.D. Crawford, N.A. Kochan and W. Wen *et al.*, 2020. Metformin use is associated with slowed cognitive decline and reduced incident dementia in older adults with type 2 diabetes: The Sydney memory and ageing study. *Diabetes Care*, 43: 2691-2701.
13. Thangavel, N., M. Al Bratty, S.A. Javed, W. Ahsan and H.A. Alhazmi, 2017. Targeting peroxisome proliferator-activated receptors using thiazolidinediones: Strategy for design of novel antidiabetic drugs. *Int. J. Med. Chem.*, Vol. 2017. 10.1155/2017/1069718.
14. Schernthaner, G., C.J. Currie and G.H. Schernthaner, 2013. Do we still need pioglitazone for the treatment of type 2 diabetes? A risk-benefit critique in 2013. *Diabetes Care*, 36: S155-S161.
15. Leonardini, A., L. Laviola, S. Perrini, A. Natalicchio and F. Giorgino, 2009. Cross-talk between PPAR and insulin signaling and modulation of insulin sensitivity. *PPAR Res.*, Vol. 2009. 10.1155/2009/818945.
16. Durães, F., M. Pinto and E. Sousa, 2018. Old drugs as new treatments for neurodegenerative diseases. *Pharmaceuticals*, Vol. 11. 10.3390/ph11020044.
17. Fernandez-Martos, C.M., R.A.K. Atkinson, M.I. Chuah, A.E. King and J.C. Vickers, 2017. Combination treatment with leptin and pioglitazone in a mouse model of Alzheimer's disease. *Alzheimer's Dementia: Transl. Res. Clin. Interventions*, 3: 92-106.
18. Alharbi, I., H. Alharbi, Y. Almogbel, A. Alalwan and A. Alhowail, 2020. Effect of metformin on doxorubicin-induced memory dysfunction. *Brain Sci.*, Vol. 10. 10.3390/brainsci10030152.
19. Alhowail, A.H., P.D. Pinky, M. Eggert, J. Bloemer and L.N. Woodie *et al.*, 2021. Doxorubicin induces dysregulation of AMPA receptor and impairs hippocampal synaptic plasticity leading to learning and memory deficits. *Heliyon*, Vol. 7. 10.1016/j.heliyon.2021.e07456.
20. Alhowail, A.H., S. Chigurupati, S. Sajid and V. Mani, 2019. Ameliorative effect of metformin on cyclophosphamide-induced memory impairment in mice. *Eur. Rev. Med. Pharmacol. Sci.*, 23: 9660-9666.
21. Seok, H., M. Lee, E. Shin, M.R. Yun and Y.H. Lee *et al.*, 2019. Low-dose pioglitazone can ameliorate learning and memory impairment in a mouse model of dementia by increasing LRP1 expression in the hippocampus. *Sci. Rep.*, Vol. 9. 10.1038/s41598-019-40736-x.
22. Teng, Z., J. Feng, Q. Qi, Y. Dong and Y. Xiao *et al.*, 2021. Long-term use of metformin is associated with reduced risk of cognitive impairment with alleviation of cerebral small vessel disease burden in patients with type 2 diabetes. *Front. Aging Neurosci.*, Vol. 13. 10.3389/fnagi.2021.773797.
23. Ha, J., D.W. Choi, K.J. Kim, S.Y. Cho and H. Kim *et al.*, 2021. Association of metformin use with Alzheimer's disease in patients with newly diagnosed type 2 diabetes: A population-based nested case-control study. *Sci. Rep.*, Vol. 11. 10.1038/s41598-021-03406-5.
24. Saunders, A.M., D.K. Burns and W.K. Gottschalk, 2021. Reassessment of pioglitazone for Alzheimer's disease. *Front. Neurosci.*, Vol. 15. 10.3389/fnins.2021.666958.
25. Yin, Q.Q., J.J. Pei, S. Xu, D.Z. Luo and S.Q. Dong *et al.*, 2013. Pioglitazone improves cognitive function via increasing insulin sensitivity and strengthening antioxidant defense system in fructose-drinking insulin resistance rats. *PLoS ONE*, Vol. 8. 10.1371/journal.pone.0059313.
26. Meng, S., J. Cao, Q. He, L. Xiong and E. Chang *et al.*, 2015. Metformin activates AMP-activated protein kinase by promoting formation of the $\alpha\beta\gamma$ heterotrimeric complex. *J. Biol. Chem.*, 290: 3793-3802.
27. Agarwal, S., C.M. Bell, S.B. Rothbart and R.G. Moran, 2015. AMP-activated protein kinase (AMPK) control of mTORC1 is p53- and TSC2-independent in pemetrexed-treated carcinoma cells. *J. Biol. Chem.*, 290: 27473-27486.

28. Alhowail, A., 2021. Potential mechanisms of metformin-induced memory impairment. *Eur. Rev. Med. Pharmacol. Sci.*, 25: 4757-4761.
29. Ahmadian, M., J.M. Suh, N. Hah, C. Liddle, A.R. Atkins, M. Downes and R.M. Evans, 2013. PPAR γ signaling and metabolism: The good, the bad and the future. *Nat. Med.*, 19: 557-566.
30. Michailidis, M., D. Moraitou, D.A. Tata, K. Kalinderi, T. Papamitsou and V. Papaliagkas, 2022. Alzheimer's disease as type 3 diabetes: Common pathophysiological mechanisms between Alzheimer's disease and type 2 diabetes. *Int. J. Mol. Sci.*, Vol. 23. 10.3390/ijms23052687.
31. Rocha, M., D. Wang, V. Avila-Quintero, M.H. Bloch and A. Kaffman, 2021. Deficits in hippocampal-dependent memory across different rodent models of early life stress: Systematic review and meta-analysis. *Transl. Psychiatry*, Vol. 11. 10.1038/s41398-021-01352-4.
32. Mani, V., M. Arfeen, H.A. Mohammed, H.A. Elsis and S. Sajid *et al*, 2022. Sukkari dates seed improves type-2 diabetes mellitus-induced memory impairment by reducing blood glucose levels and enhancing brain cholinergic transmission: *In vivo* and molecular modeling studies. *Saudi Pharm. J.*, 30: 750-763.
33. Alfheaid, H.A., A.A. Alhowail, F. Ahmed, A.K.A. Zaki and A. Alkhaldy, 2023. Effect of various intermittent fasting protocols on hyperglycemia-induced cognitive dysfunction in rats. *Brain Sci.*, Vol. 13. 10.3390/brainsci13020165.
34. Pomytkin, I., J.P. Costa-Nunes, V. Kasatkin, E. Veniaminova and A. Demchenko *et al*, 2018. Insulin receptor in the brain: Mechanisms of activation and the role in the CNS pathology and treatment. *CNS Neurosci. Ther.*, 24: 763-774.
35. McNay, E.C. and J. Pearson-Leary, 2020. GluT4: A central player in hippocampal memory and brain insulin resistance. *Exp. Neurol.*, Vol. 323. 10.1016/j.expneurol.2019.113076.