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Original Research Article

Deleterious Effects of Oxidative Stress on Cognitive Decline in Wistar Rats Made Diabetic by Alloxane Monohydrate

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Abstract: ***Background and objective:*** Free radical chain reaction is a condition where there is an imbalance between free radicals and antioxidants in the body. This imbalance can lead to cellular and tissue damage, which is of particular concern in the context of cognitive decline associated with diabetes. The aim of this study was to identify molecular and cellular markers of oxidative stress in diabetic rats. ***Material and methods:*** The study involved 24 Wistar rats, classified into 3 groups, normal (GT), untreated diabetic (DTN) and treated with D-erythrodihydrosphingosine (inhibitor of Sphingosine kinases 1 and 2) (DTT), fed glucose and food to prevent hypoglycaemia, and subjected to the behavioural test including the 8-arm radial maze. RT-PCR was then used to assess the expression of pro-oxidant (8-hydroxydeoxyguanosine: 8-OHGD) and anti-oxidant (glutathione: GSH and superoxide dismutases: SOD) markers of oxidative stress. ***Results:*** The study compared GT with DTN and DTT in a maze task and showed that diabetes affects working memory in diabetic rats. GSH and SOD levels varied according to health status and treatments administered. High levels of GSH and SOD in DTT and DTN suggest high oxidative stress. Low levels in GTs indicate a normal state without significant oxidative stress. The study also found a significant difference between rat groups in the expression of 8-OHGD in the prefrontal cortex. Untreated diabetic rats had higher levels of 8-OHGD, indicating increased oxidative DNA damage due to the oxidative stress associated with diabetes. This shows that diabetes causes increased production of free radicals, leading to cell damage. ***Conclusion:*** This work shows that diabetes induces cognitive decline via oxidative stress.

Keywords: Cognitive impairment, diabetes, rats, Oxidative stress.

INTRODUCTION

Oxidative stress' is a new concept in the biological and medical sciences. In other words, the cell can no longer control an overabundance of harmful oxygen radicals. It is now accepted that oxidative stress, although not a disease in itself, could be a trigger for several diseases or lead to complications as they progress, as is the case with diabetes (Bonnefont-Rousselot *et al.*, 2000). Diabetes mellitus is a variety of metabolic diseases mainly characterised by hyperglycaemia caused by defects in secretion, action or associated anomalies of both (Sharma *et al.*, 2000).

Several recent studies have revealed that chronic diabetes in untreated patients leads to the

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deposition of interneuronal amyloid plaques in the brain, resulting in neuropathological symptoms as in Alzheimer's disease (AD) (Maceyka *et al.*, 2012). Recent evidence from patients with diabetes also suggests that insulin resistance leading to type 2 diabetes results in cognitive dysfunction (Maceyka *et al.*, 2012). Furthermore, evidence suggests that successful management of chronic diabetes does not lead to complete reversal of cognitive deficits (Maceyka *et al.*, 2012; Biessels *et al.*, 2006).

The cognitive dysfunction caused by diabetes is not fully understood. In addition, various mechanisms cause oxidative stress and free radical production in diabetes mellitus. This oxidative stress exacerbates the disease and leads to chronic complications associated with diabetes. As a result, clinical and preclinical studies on rodents have shown that excessive oxidative stress causes neurodegeneration, resulting in impaired glucose metabolism and impaired synaptic transmission mediated by several signalling pathways, in this case bioactive sphingolipids, in particular sphingosine kinases (sphks), which play an important role in regulating nerve cell survival and death (Alvarez *et al.*, 2007). Hence the interest of this study, which aims to determine the impact of oxidative stress markers on cognitive dysfunction in diabetic rats.

Cognitive decline, a complex and multifactorial phenomenon, represents a major challenge for neuroscience and modern medicine. This pathological process is often observed in chronic conditions such as diabetes, which affects millions of people worldwide. Oxidative stress, a condition resulting from an imbalance between the production of free radicals and the body's ability to neutralise them, is increasingly recognised as a key contributory factor in the development of cognitive decline, particularly in diabetic individuals.

In this study, we focus on the deleterious effects of oxidative stress on cognitive decline in diabetic rats.

2. MATERIALS AND METHODS

2.1. Animals and treatments

Our study was carried out in the laboratory of the Marien Ngouabi Faculty of Health Sciences, in the experimental neuropathology unit. Male Wistar rats aged seven (07) to ten (10) weeks from the Faculty of Health Sciences animal house were purchased and used. They were housed in polystyrene cages and maintained under optimal temperature and humidity conditions (21 ± 1◦C and 55 ± 2% humidity) under a 12 h light/dark cycle and free access to food and water. Animals were acclimatised to laboratory conditions for 2 weeks prior to the start of the experiment. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Heath in the United States.

The animals were divided into three (03) groups of 08 rats each and treated as follows:

- Group 1 received distilled water and served as a control group (GT);
- Group 2 consisted of rats made diabetic and given distilled water (DTN);
- Group 3: diabetic rats treated with D-erythro-dihydrosphingosine: SPK1 and SPK2 inhibitor (DEDHS). (DTT)

The products (distilled water and DEDHS) were administered orally for 28 days.

2.2. Diabetes Induction and Blood Glucose Measurement

Diabetes was induced by a single intraperitoneal administration of alloxane monohydrate at a dose of 150 mg/kg body weight. Animals were placed in individual cages with free access to food and 5% glucose solution to avoid hypoglycaemic shock. Three days after administration of alloxan monohydrate, diabetes was confirmed by measuring blood glucose levels using a glucometer (On Call Plus II). A drop of blood obtained through a small incision at the tip of the tail was used to measure blood glucose levels on days 1, 7, 14, 21 and 28. Only rats with blood glucose levels above 180 mg/dl were selected for the experiment.

- **Ponderable evolution:** The rats selected were weighed using an electronic balance from the first day before the onset of diabetes (start of the manipulation) and then throughout the days (7, 14, 21 and 28).

2.3. Assessment of cognitive deficit

Behavioural analysis was used to assess cognitive dysfunction using the 8-branch maze test:

- **Radial arm maze**

According to Olton and Samuelson (1976), the concept of the eight (8) branch maze is widely used to assess memory and spatial information management abilities in different species of animals. The maze consists of a centre from which eight branches emerge, arranged like the spokes of a wheel. A food pellet (45 mg) was placed at the end of each branch and was not replaced during the test, allowing a maximum of 8 rewards to be obtained. Firstly, with the diet, the body weight of these animals was reduced to 85% of its initial values, and this weight was maintained throughout the study. In the centre of the platform, the animals were placed facing the same branch for each trial and for each rat (the branches of the maze were numbered from 1 to 8). The animal continued to get lost in the maze until it encountered eight branches (four legs had to reach the entrance edge). The errors observed during the branch visits were recorded in chronological order. The different groups of animals were tested after each week, with an interval of seven (07) days.

2.4. Collection of Behavioural Test Parameters

Each stage of the behavioural tests was recorded using a camera in order to make better use of the various parameters used as variables in each test.

2.4.1. Rat sacrifices and seahorse sampling

Once the treatment had been completed after 28 days, the rats in three (03) groups were euthanised by cervical dislocation and then decapitated. Once the brains had been removed from the skull, the brain structure, in particular the hippocampus, was rapidly harvested and cleaned with phosphate-buffered saline. These samples were then stored in sterile vials and immediately frozen at -80°C for use in molecular analysis.

2.5. Molecular analysis of the sphingosine 1 phosphate (S1P) signaling gene

2.5.1. DNA extraction from rat hippocampal tissue

DNA was extracted using the "ReliaPrepTM gDNA Tissue (Promega)" kit, in accordance with the manufacturer's instructions. The amount of DNA in each sample was assessed using Qubit 3.0 fluorescence technology (Qubit® 3.0 Fluorometer, Life Technology). This assay enabled us to evaluate the amount of DNA in ng/µL.

2.5.2. Amplification by RT-PCR.

Extracted DNA underwent PCR using the Fast-track diagnostics kit.

Mode opératoire:

Step 1: Preparing the Mix

Ingrédients	Volume for one sample
Buffer (Tampon)	12,5 µl
Ppmix (SONDE)	1,5 µl
Enzyme	1 µl
Amorce sens	0,5 µl
Amorce antisens	0,5 µl
ADN	9 µl

Primers for markers of pro-oxidative stress genes, in particular 8-hydroxideoxyguanosine (8-OHGD) and anti-oxidants, glutathione and superoxide dismutases (GSH and SOD), as well as β2-microglobulin (eurofins®, France).

Table 1: Sequence of primers used

Target	Forward Sequence 5'—3'	Reverse Sequence 5'—3'	T° de fusion
8-OHdG	GGGCGGGAGAAGATAAGTCG	GGATGAGCCGAGGTCCAAAA	59.95 °C
SOD	TCCGTCGGCTTCTCGTCTTG	CTTGCCTTCTGCTCGAAGTG	59.90 °C
GSH	GGACAACGAGCGAGTTGGGA	CTCGGAAGTACACCACAGCA	59.85 °C
B2-µGLOBINE	TCGCAACCTCAGGAACAGAC	CAGGAAAGGGGGCTTAGTGG	60 °C

Second step: Mic (thermocycler) programming

Step	Times	Temperature
Initial denaturation	3 min	94°C
Denaturation	30 secondes	94 ° C
Hybridization	8 secondes	94 ° C
Final Extension	34 secondes	60°C
Cycles number	40	

Step 3: Expression of sphingosine 1 phosphate (S1P) signaling gene

We evaluated this expression using Livak's method with the formula:

Rq = 2^-(ΔΔCt). A positive value of relative quantification (Rq) corresponds to overexpression and a negative value to underexpression. S1P expression in each sample was performed in duplicate and the level normalized to β2-microglobulin.

2.6. Statistical analysis

The mean of the data was expressed ± SD. Comparison between two groups was performed using Student's t-tests. Results were examined using ANOVA for multiple comparisons. Figures and graphs were created using GraphPad Prism 5 software. A value of $P < 0.05$ was considered statistically significant.

3-RESULTS

3.1. Body weight of Wistar rats

Rats in different groups were weighed from D 1 to D 28. Group 1 (GT) comprised normal rats and group 2 (DTN) diabetic rats without sphingosine kinase inhibitors 1 and 2. Group 3 (DTT) comprised diabetic rats with sphingosine kinase 1 and 2 inhibitors. Statistical analysis using the Kruskal-Wallis test showed a significant weight reduction from day 7 onwards. (P =0.027, for each group, n =8). These results support the hypothesis that hyperglycemia reduces body weight.

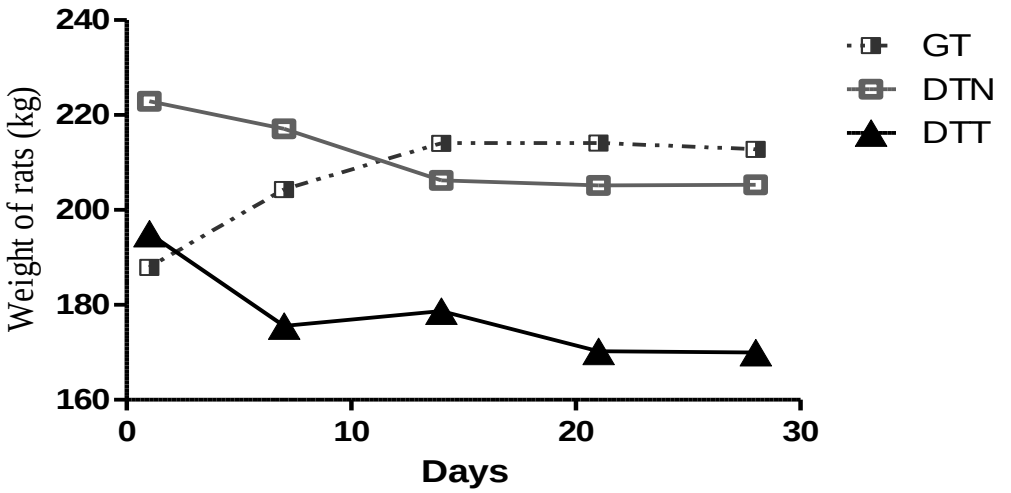


Figure 1: Body weight of the three different groups of Wistar rats

3.2. Glycaemic profile

In rats, alloxan monohydrate at 150 mg/kg was administered to group 2 (DTN) and group 3 (DTT) rats, which were also given an SPK 1 and 2 inhibitor. Rats in the first group (GT) were normal, while rats in the second group (DTN) were diabetic without a sphingosine kinase 1 and 2 inhibitor. The group of diabetic rats with a sphingosine kinase 1 and 2 inhibitor is called DTT. Statistical comparison revealed a significant difference between groups 1 and groups 2 and 3 at D7 (P=0.01, n=8 per group). The results indicate that rats in groups 2 and 3 have diabetes.

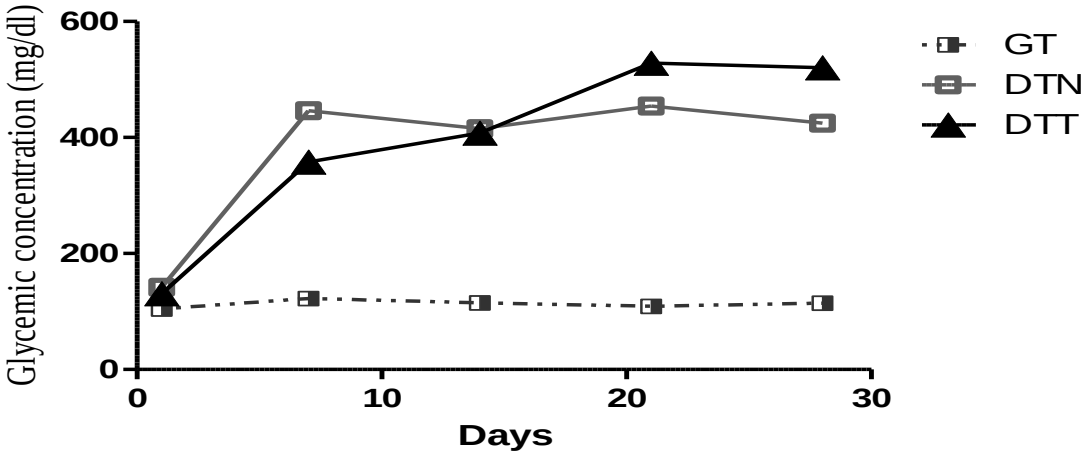


Figure 2: Changes in mean blood glucose levels in three groups of rats

Group 1 (GT) consists of normal rats, group 2 (DTN) of diabetic rats without sphingosine kinase 1 and 2 inhibitors. Group 3 (DTT) was made up of diabetic rats with sphingosine kinase 1 and 2 inhibitors.

3.3. Behavioural analysis

- Radial arm maze

In order to determine whether diabetes had an impact on cognitive abilities, spatial learning was measured in an 8-arm radial maze. The animals were subjected to a working memory test in which, at each session, they had to explore all eight arms of the radial maze. Returning to a previously visited arm was considered an error. In addition to the errors made, the number of correct visits to the arms in the first eight choices was also recorded. There was a significant difference between the group of normal rats and the group of diabetic rats, depending on whether or not they had received a supplemental Sphingosine phosphate kinase 1 and 2 inhibitor (p=0.0029; n=8 per group). These data clearly show that diabetes affects working memory and that the Sphingosine kinase 1 and 2 inhibitor does not improve working memory in diabetic rats.

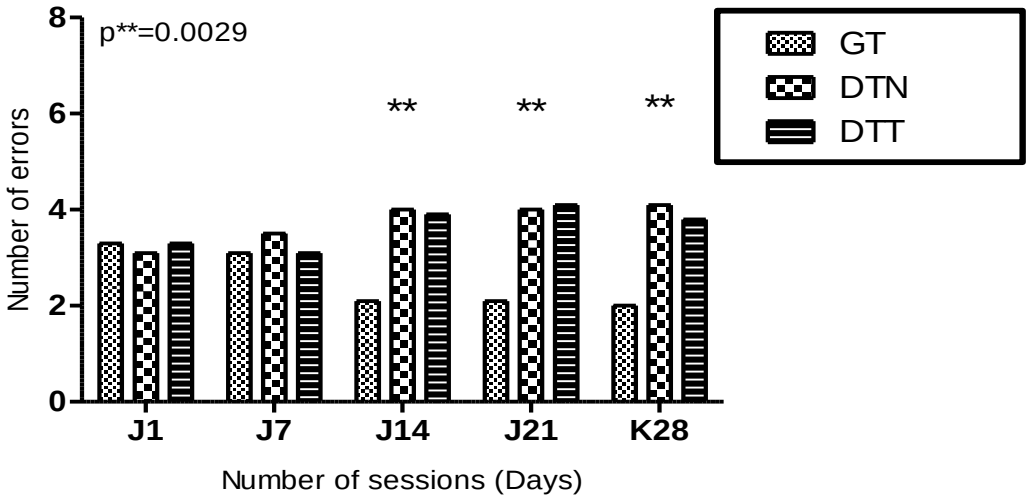


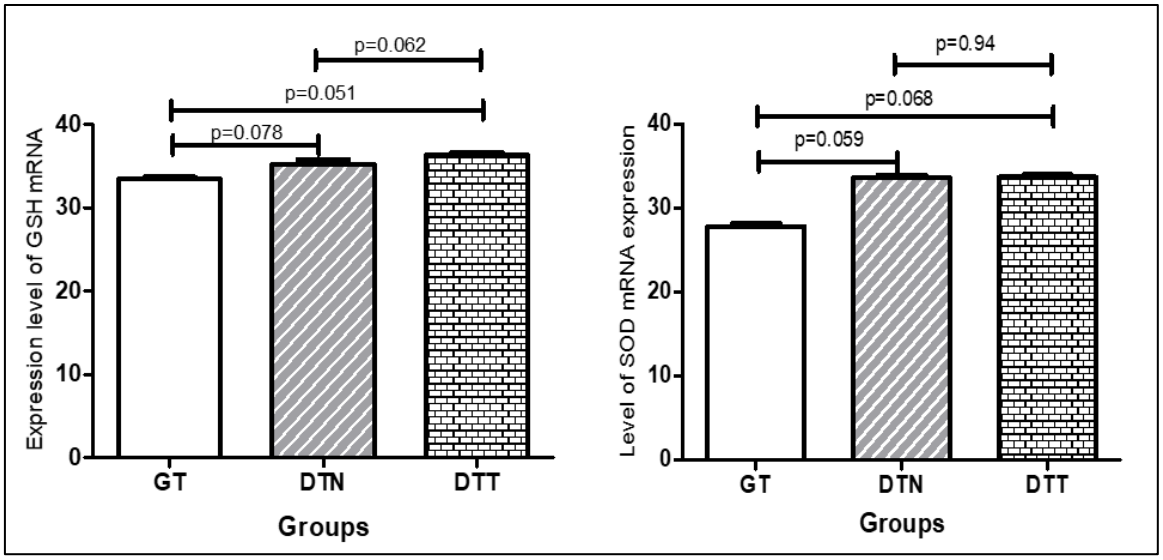
Figure 3: Spatial learning

Group 1 of normal rats (GT) is compared with group 2 (DTN) of diabetic rats without sphingosine kinase inhibitor 1 and 2, and group 3 (DTT) of diabetic rats with sphingosine kinase inhibitor 1 and 2 in a radial arm maze task. The mean number of errors was considered for each session.

3.4. Expression of pro-oxidant and antioxidant stress genes in the prefrontal cortex

Expression of anti-oxidative genes (GSH and SOD) in rats

The activity of two anti-oxidative stress markers (GSH and SOD) was determined by expressing the mRNAs of these proteins. After statistical analysis, our data showed no significant difference between rats in groups 1, 2 and 3 respectively for (p*=0.062 in Figure A) and (p*=0.94; in Figure B). These data suggest that anti-oxidative stress markers (GSH and SOD) do not inhibit or impact diabetes and that inhibition of SPK1 and SPK2 facilitates the expression of hyperglycemia.



A **B**

Figure 4-A: Expression of the GSH gene in rats Figure 4-B: Expression of the SOD gene in rats

¬ Pro-oxidant (8-OHGD) gene expression in the prefrontal cortex of rats

To determine the mRNA expression of the pro-oxidant marker (8-OHGD) in the prefrontal cortex of diabetic rats in order to assess the balance of oxidative stress, we compared the expression levels of three groups. Our data showed a significant difference between rats in group 1 (GT) versus group 2 (DTN) and 3 (DTT) (p***= 0.026; n=8 per group). Untreated diabetic rats had higher levels of 8-OHGD, indicating increased oxidative DNA damage due to the oxidative stress associated with diabetes. This shows that diabetes

results in increased production of free radicals, leading to cell damage. Sphingosine Inhibitor Treated Rats (Lower 8-OHGD Levels). The levels of 8-OHGD in diabetic rats treated with sphingosine inhibitors suggest that these inhibitors have a protective effect against oxidative damage. This may indicate that the inhibitors reduce the production of free radicals or increase the efficiency of antioxidant systems. Control Rats (Low 8-OHGD Levels): The low levels of 8-OHGD in control rats reflect a basal state of normal oxidative stress, without the exacerbated effects of diabetes. This shows that, in the absence of diabetes, there is no excessive production of free radicals, and therefore less oxidative damage to DNA.

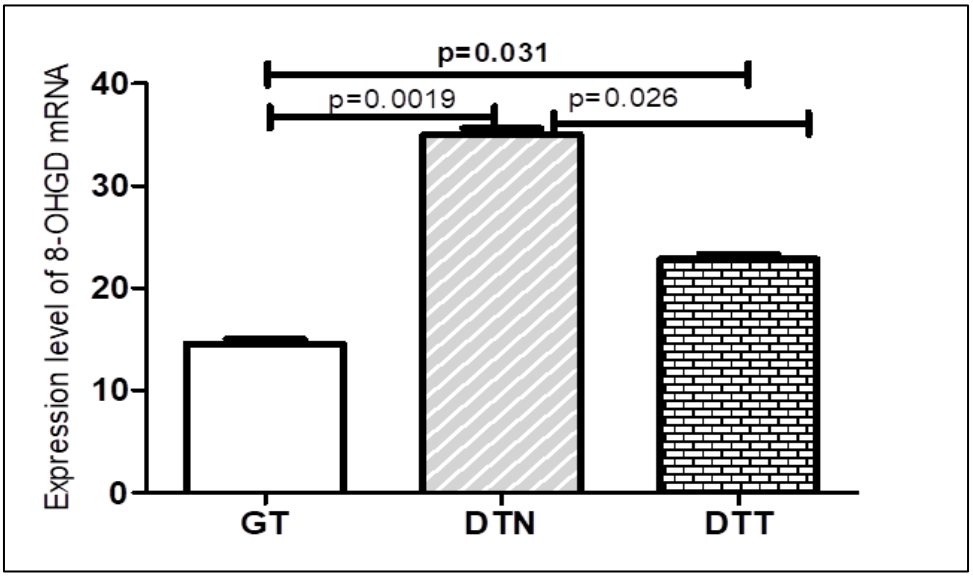


Figure 5: Expression of the pro-oxidative gene (8-OHGD) in rats

4. DISCUSSION

Diabetes induces excessive production of free radicals. If these free radicals are not neutralised by the body's antioxidant mechanisms, they can cause major cellular damage, including alterations at neuronal level. This can lead to cognitive deficits, affecting memory, learning and other essential cognitive functions. (Papachristoforou E *et al.*, 2020).

Hence the interest of this study, which aims to determine the impact of oxidative stress markers on cognitive dysfunction in diabetic rats.

4.1. Relationship between diabetes and cognitive dysfunction

When alloxane monohydrate was administered at 150 mg/kg to rats, we observed a decrease in body weight (Figure 1). This was followed by an increase in blood glucose (Figure 2). In addition, we used the 8-arm radial maze behavioural test to assess memory and learning capacity (Figure 3). These results showed, on the one hand, the induction of diabetes and, on the other, memory dysfunction. (Loubano-Voumbi *et al.*, 2015; Crusio *et al.*, 1999).

Previous studies have shown a link between poorly controlled hyperglycaemia and memory (Bruel *et al.*, 2007; Arendt, 2009). According to Medjdoub (2013), chronic hyperglycaemia is associated with insulin resistance (IR), which also affects parts of the brain. This causes a decrease in autophosphorylation of the insulin receptor and activates the expression of protein kinase C, a protein that dephosphorylates the insulin receptor. According to Craft *et al.*, 2009, insulin receptors (IR) have been identified in the hippocampus and medial temporal cortex, which partly explains the memory problems. Problems with LTP (long-term potentiation) and spatial memory have been observed in mice with reduced IR expression in the hippocampus (Grillo *et al.*, 2015).

In our research, we observed reduced working memory in diabetic rats and problems with exploratory behaviour during spatial learning ($p=0.0029$; $n=8$ per group, Figure 4) due to the number of errors made during open arm entry visits observed in the eight (08) branch radial maze test. It has been shown that type 1 diabetes is often linked to a reduction in reasoning speed and mental flexibility (Brands *et al.*, 2005), and that type 2 diabetes also has an impact on learning and memory (Awad *et al.*, 2004).

According to Allen *et al.*, (2004), cognitive decline over a 7-year follow-up period is more pronounced in patients with diabetes, particularly the elderly. A relationship between diabetes and dementia is common (Duron *et al.*, 2008). The hippocampal synaptic plasticity of elderly insulin-powered diabetic rats is lower than that of young people. Similarly, it appears that the duration of diabetes influences the impact; in insulin-privileged rats, prolonged hyperglycaemia affects hippocampal neurons. The harmful consequences of

hypoglycaemia were also observed. A week later, the diabetic rats were euthanised after receiving high doses of insulin or saline. According to Biessels and Gispen (2005), rats suffering from hypoglycaemia had a greater reduction in neuronal capacity in the hippocampus. Based on this information, it is possible that increased glycaemia has an impact on cerebral metabolism in general, as well as on the molecular mechanisms of memory and learning, which may lead to neurocognitive problems.

4.2. Evaluation of anti-oxidant (GSH and SOD) and pro-oxidant (8-OHGD) markers of hippocampal oxidative stress in diabetic rats associated with cognitive decline

People with diabetes suffer from a constant state of oxidative stress, marked by an overproduction of free radicals through various actions (Matough *et al.,* 2012). They are responsible for the metabolic dysfunctions and degenerative complications of diabetes mellitus, which can affect various organs and functions. According to Sies (1991), when defences are no longer able to retain the reactive oxidising species present, the body suffers cellular damage (protein, lipid and genomic) that disrupts its functioning and accelerates ageing. For example, the brain and erythrocytes are more vulnerable to oxidative stress caused by diabetes because of their high oxygen consumption, high polyunsaturated fatty acid (PUFA) content and low enzymatic antioxidant defence. They are exposed to ROS attacks generated continuously by the high auto-oxidation of glucose, polyunsaturated fatty acids and protein glycation (Kahya *et al.,* 2015; Ayepola *et al.,* 2014; Mahesh and Menon, 2004). According to Defraigne and Pincemail (2008), oxidative stress or oxidative stress are situations where an imbalance occurs in favour of an excess of pro-oxidant molecules, which has harmful consequences for the body and the activity of antioxidant defence systems. Glutathione (GSH) is a tripeptide that acts at different levels in the fight against oxidative stress. It plays a role in the movement of amino acids across the plasma membrane, directly detects the hydroxyl radical and singlet oxygen and contributes to the regeneration of vitamins C and E (Birk *et al.,* 2013; Kurutas, 2015). Superoxide dismutases (SODs) Are metalloenzymes present in mammalian tissues and due to their ability to convert highly reactive superoxide radicals into hydrogen peroxide and molecular oxygen, these enzymes play an essential role in protection against oxidative stress (Valko *et al.,* 2006; Laukkanen, 2016).

In our study, to assess the expression of anti-oxidative stress genes (GSH and SOD) in the hippocampus of rats, we expressed the mRNAs of these proteins. Indeed, the activity of two antioxidant stress markers (GSH and SOD) was determined for (p*=0.06 in Figure 4- A; n=8 per group,) and (p*=0.94 in Figure 4- B; n=8 per group,) our data after statistical analysis did not reveal any significant difference between rats in groups 1, 2 and 3. These data suggest that antioxidant stress markers (GSH and SOD) do not inhibit or have no effect on diabetes and that inhibition of SPK1 and SPK2 facilitates the expression of hyperglycemia.

However, we had compared mRNA expression levels of Pro-oxidant markers (8-OHGD) in the hippocampus of diabetic rats to assess the balance of oxidative stress (Pro-oxidant. and Anti-oxidant). 8-Hydroxideoxyguanosine (8-OHdG) is an essential biomarker for measuring oxidative stress and DNA damage. Formed when reactive oxygen species (ROS) oxidise guanine in DNA, 8-OHdG reflects the extent of oxidative damage, making it essential for understanding various health problems. Our results revealed significant differences between rats in group 1 (GT) and group 2 (DTN) and group 3 (DTT) (p***= 0.026; n=8 per group, Figure 5). According to these data, we can suggest that the pro-oxidant stress marker (8-OHGD) affects hyperglycemia and therefore the expression of diabetes, while the inhibitor of SPK1 and SPK2 inhibits hyperglycemia.

According to the results of this study, there was an imbalance in redox status in favour of pro-oxidants, leading to oxidative stress in the brain at the level of the hippocampus. This can be explained by the significant expression (p***= 0.026; n=8 per group, Figure 5) of 8-OHDG mRNA in diabetic animals, as well as the non-expression of GSH and SOD mRNAs respectively (p*=0.06 in Figure 4- A; n=8 per group) and (p*=0. 94 in Figure 4- B; n=8 per group) due to their free radical neutralising effect. In this situation, other authors have shown the same effects with experimental diabetes, where there is an impairment of the antioxidant system, an increase in lipid peroxidation and a decrease in erythrocyte GSH (Vural *et al.,* 2001; Mahesh and Menon, 2004; Ozkol *et al.,* 2013). In parallel with our results, several studies have reported a disruption of antioxidant systems in diabetes. The reduced activity of antioxidant enzymes could be due to glycation of their active site, as has been observed for erythrocyte SOD. In vitro glycation studies have also revealed changes in the enzymatic activities of catalase, GSH reductase and GSH-Px, suggesting that these enzymes are modified during diabetes. Furthermore, according to Da Silva Haeser *et al.,* (2007) and Ceretta *et al.,* (2012), chronic hyperglycaemia can have serious consequences for the brain structures involved in cognitive and behavioural functions. The pathological change affecting key elements of neuronal cell structure is attributed to free radical attacks, which are known to cause cellular damage at the brain level. According to the results of our study, increased oxidative stress in the hippocampus led to cognitive impairment in diabetic rats. This led to impaired working memory and disturbances in exploratory behaviour (p=0.0029; Figure 3; n=8 per group,) by the number of errors made during open arm entry visits observed during the eight (08) branch radial maze test. In agreement with our results, Several rodent studies have shown that diabetic animals exhibit

behavioural deficits when subjected to behavioural tests such as the Open Field Test (OFT), Elevated Plus Maze (EPM) and forced swim test (FST) (Haider *et al.,* 2013). Furthermore, persistent diabetes leads to oxidative stress in the prefrontal cortex and hippocampus, resulting in functional dysregulation and altered synaptic neuroplasticity caused by ROS (Tian *et al.,* 2016; Castillo-Gómez *et al.,* 2015; De Morais *et al.,* 2014). According to Nitta *et al.,* (2002) and Rosso *et al.,* (2017), these brain regions involved in depressive pathophysiology face cellular degradation that can lead to neuronal atrophy, driven by decreased expression of neuronal growth factors such as BDNF, which plays an essential role in neuronal survival and function. However, during the experimental phase of diabetes, cognitive disturbances and depressive disorders were observed due to a deficit in hippocampal neurogenesis and altered synaptic plasticity, probably as a result of hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, which leads to an increase in circulating glucocorticoids such as corticosterone (Magariños *et al.,* 2000; Stranahan *et al.,* 2008). Furthermore, fluoxetine treatment has been shown to reverse depressive behaviour and promote neurogenesis in hippocampal regions in mice exposed long-term to the stress hormone corticosterone (David *et al.,* 2009). According to Moretti and colleagues (2012), during chronic unpredictable stress in an animal model of depression, fluoxetine demonstrated a significant decrease in MDA levels in the cortex and hippocampus, as well as an increase in CAT activity in depressed individuals. According to Li *et al.,* (2003), the cytoprotective properties of fluoxetine and its limiting effect on the excessive production of ions (ca2+) are responsible for the protective effect of this substance against oxidative stress. On the other hand, other research has shown that fluoxetine blocks cytochrome P450, which plays a role in ROS synthesis (Nieuwstraten *et al.,* 2006; Gałecki *et al.,* 2009). Cognitive decline: a potential consequence. Because of its high oxygen consumption, the brain is particularly vulnerable to oxidative stress. The damage caused by free radicals can impair cognitive functions, leading to Memory problems: Difficulty remembering new information, loss of memories.

-Learning difficulties: Slower learning processes, concentration problems. And behavioural changes: anxiety, depression, irritability.

This work proves that diabetes induces cognitive decline via oxidative stress, and the precise mechanisms by which oxidative stress induces cognitive decline in diabetics are complex and multiple. These include Damage to neurons and synapses: Free radicals damage the cell membranes, proteins and DNA of neurons, altering their function and connections. And brain inflammation: -Stimulation of inflammatory processes in the brain, which can contribute to neurodegeneration. In addition, the cerebral microvasculature is altered: the brain's small blood vessels are damaged by oxidative stress, leading to a reduction in the supply of oxygen and nutrients to the neurons.

5. CONCLUSION

Our research shows that rats with diabetes, whether or not they were treated with a sphingosine kinase 1 and 2 inhibitor, showed reduced body weight and marked deficits in working memory, illustrating the cognitive complications associated with this condition. We also note that diabetes leads to a significant increase in oxidative damage to hippocampal DNA, as evidenced by the elevated levels of 8-OHGD in untreated rats. However, the use of sphingosine inhibitors reduces this damage, suggesting a potential protective effect against oxidative stress. This research highlights the critical role of oxidative stress in diabetes-related complications and proposes sphingosine 1 and 2 inhibitors as a potential intervention to reduce these negative effects. Further studies are needed to confirm these results and explore the underlying mechanisms in order to develop effective treatments for diabetic patients.

REFERENCES

- Alvarez, S. E., Milstien, S., & Spiegel, S. (2007). Autocrine and paracrine roles of sphingosine-1-phosphate. *Trends in Endocrinology & Metabolism*, *18*(8), 300-307.
- Allen, K. V., Frier, B. M., & Strachan, M. W. (2004). The relationship between type 2 diabetes and cognitive dysfunction: longitudinal studies and their methodological limitations. *European journal of pharmacology*, *490*(1-3), 169-175.
- Arendt, T. (2009). Synaptic degeneration in Alzheimer's disease. *Acta neuropathologica*, *118*, 167-179.
- Ayepola, O. R., Brooks, N. L., & Oguntibeju, O. O. (2014). Kolaviron Improved Resistance to Oxidative Stress and Inflammation in the Blood (Erythrocyte, Serum, and Plasma) of Streptozotocin-Induced Diabetic Rats. *The Scientific World Journal*.
- Awad, N., Gagnon, M., & Messier, C. (2004). The relationship between impaired glucose tolerance, type 2 diabetes, and cognitive function. *Journal of clinical and experimental neuropsychology*, *26*(8), 1044-1080.
- Biessels, G. J., & Gispen, W. H. (2005). The impact of diabetes on cognition: what can be learned from rodent models?. *Neurobiology of Aging*, *26*(1), 36-41.
- Biessels, G. J., Staekenborg, S., Brunner, E., Brayne, C., & Scheltens, P. (2006). Risk of dementia in diabetes mellitus: a systematic review. *The Lancet Neurology*, *5*(1), 64-74.
- Birk, J., Meyer, M., Aller, I., Hansen, H. G., Odermatt, A., Dick, T. P., ... & Appenzeller-Herzog, C. (2013). Endoplasmic reticulum: reduced and

oxidized glutathione revisited. *Journal of cell science*, *126*(7), 1604-1617.

- Bonnefont-Rousselot, D., Bastard, J. P., Jaudon, M. C., & Delattre, J. (2000). Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes and metabolism*, *26*(3), 163-177.
- Brands, A. M., Biessels, G. J., De Haan, E. H., Kappelle, L. J., & Kessels, R. P. (2005). The effects of type 1 diabetes on cognitive performance: a meta-analysis. *Diabetes care*, *28*(3), 726-735.
- Bruel-Jungerman, E., Rampon, C., & Laroche, S. (2007). Adult hippocampal neurogenesis, synaptic plasticity and memory: facts and hypotheses. *Reviews in the Neurosciences*, *18*(2), 93-114.
- Castillo-Gomez, E., Coviello, S., Perez-Rando, M., Curto, Y., Carceller, H., Salvador, A., & Nacher, J. (2015). Streptozotocin diabetic mice display depressive-like behavior and alterations in the structure, neurotransmission and plasticity of medial prefrontal cortex interneurons. *Brain research bulletin*, *116*, 45-56.
- Ceretta, L. B., Réus, G. Z., Abelaira, H. M., Ribeiro, K. F., Zappellini, G., Felisbino, F. F., ... & Quevedo, J. (2012). Increased oxidative stress and imbalance in antioxidant enzymes in the brains of alloxan-induced diabetic rats. *Journal of Diabetes Research*, *2012*(1), 302682.
- Craft, S. (2009). The role of metabolic disorders in Alzheimer disease and vascular dementia: two roads converged. *Archives of neurology*, *66*(3), 300-305.
- David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., ... & Hen, R. (2009). Neurogenesis-dependent and-independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*, *62*(4), 479-493.
- da Silva Haeser, A., Sitta, A., Barschak, A. G., Deon, M., Barden, A. T., Schmitt, G. O., ... & Vargas, C. R. (2007). Oxidative stress parameters in diabetic rats submitted to forced swimming test: the clonazepam effect. *Brain research*, *1154*, 137-143.
- Defraigne, J. O., & Pincemail, J. (2008). Stress oxydant et antioxydants: mythes et réalités. *Revue médicale de Liège*, *63*.
- de Morais, H., de Souza, C. P., da Silva, L. M., Ferreira, D. M., Werner, M. F., Andreatini, R., ... & Zanoveli, J. M. (2014). Increased oxidative stress in prefrontal cortex and hippocampus is related to depressive-like behavior in streptozotocin-diabetic rats. *Behavioural brain research*, *258*, 52-64.
- Duron, E., & Hanon, O. (2008). Vascular risk factors, cognitve decline, and dementia. *Vascular health and risk management*, *4*(2), 363-381.
- Gałecki, P., Szemraj, J., Bieńkiewicz, M., Zboralski, K., & Gałecka, E. (2009). Oxidative stress parameters after combined fluoxetine and acetylsalicylic acid therapy in depressive patients. *Human Psychopharmacology: Clinical and Experimental*, *24*(4), 277-286.
- Grillo, C. A., Piroli, G. G., Lawrence, R. C., Wrighten, S. A., Green, A. J., Wilson, S. P., ... & Reagan, L. P. (2015). Hippocampal insulin resistance impairs spatial learning and synaptic plasticity. *Diabetes*, *64*(11), 3927-3936.
- Haider, S., Ahmed, S., Tabassum, S., Memon, Z., Ikram, M., & Haleem, D. J. (2013). Streptozotocin-induced insulin deficiency leads to development of behavioral deficits in rats. *Acta Neurologica Belgica*, *113*, 35-41.
- Kahya, M. C., Naziroğlu, M., & Çiğ, B. (2015). Melatonin and selenium reduce plasma cytokine and brain oxidative stress levels in diabetic rats. *Brain injury*, *29*(12), 1490-1496.
- Kurutas, E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition journal*, *15*, 1-22.
- Laukkanen, M. O. (2016). Extracellular superoxide dismutase: growth promoter or tumor suppressor?. *Oxidative medicine and cellular longevity*, *2016*(1), 3612589.
- Li, Y. F., Liu, Y. Q., Huang, W. C., & Luo, Z. P. (2003). Cytoprotective effect is one of common action pathways for antidepressants. *Acta Pharmacologica Sinica*, *24*(10), 996-1000.
- Magariños, A. M., & McEwen, B. S. (2000). Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress. *Proceedings of the National Academy of Sciences*, *97*(20), 11056-11061.
- Mahesh, T., & Menon, V. P. (2004). Quercetin allievates oxidative stress in streptozotocin-induced diabetic rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *18*(2), 123-127.
- Matough, F. A., Budin, S. B., Hamid, Z. A., Alwahaibi, N., & Mohamed, J. (2012). The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos university medical journal*, *12*(1), 5.
- MEDJDOUB, H. (2013). *Contribution à la recherche d'éventuelles activités biologiques de Zygophyllum geslini Coss* (Doctoral dissertation).
- Moretti, M., Colla, A., de Oliveira Balen, G., dos Santos, D. B., Budni, J., de Freitas, A. E., ... & Rodrigues, A. L. S. (2012). Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. *Journal of psychiatric research*, 46(3), 331-340.
- Nieuwstraten, C., Labiris, N. R., & Holbrook, A. (2006). Systematic overview of drug interactions with antidepressant medications. *The Canadian Journal of Psychiatry*, 51(5), 300-316.
- Nitta, A., Murai, R., Suzuki, N., Ito, H., Nomoto, H., Katoh, G., ... & Furukawa, S. (2002). Diabetic

neuropathies in brain are induced by deficiency of BDNF. *Neurotoxicology and teratology*, 24(5), 695-701.

- Ozkol, H., Tuluce, Y., Dilsiz, N., & Koyuncu, I. (2013). Therapeutic potential of some plant extracts used in Turkish traditional medicine on streptozocin-induced type 1 diabetes mellitus in rats. The Journal of membrane biology, 246(1), 47-55.
- Rosso, P., De Nicolò, S., Carito, V., Fiore, M., Iannitelli, A., Moreno, S., & Tirassa, P. (2017). Ocular Nerve Growth Factor Administration Modulates Brain-derived Neurotrophic Factor Signaling in Prefrontal Cortex of Healthy and Diabetic Rats. *CNS Neuroscience & Therapeutics*, 1-11.
- Sharma, A., Kharb, S., Chungh, S. N., Kakkar, R., Singh, G. P. (2000). Evaluation of oxidative stress befor and after control of glycemia and after Vitamin E supplementation in diabetic patients. Metabolism. 49: 160-162.
- Sies, H. (1991). Role of reactive oxygen species in biological processes. Klin Wochenschr 69, 965-8.
- Stranahan, A. M., Arumugam, T. V., Cutler, R. G., Lee, K., Egan, J. M., & Mattson, M.P. (2008). Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nature neuroscience*, 11(3), 309-317.
- Tian, X., Liu, Y., Ren, G., Yin, L., Liang, X., Geng, T., ... & An, R. (2016). Resveratrol limits diabetes-associated cognitive decline in rats by preventing oxidative stress and inflammation and modulating hippocampal structural synaptic plasticity. *Brain Research*, 1650, 1-9.
- Valko, M., Rhodes, C., Moncol, J., Izakovic, M. M., Mazur, M. (2006). Free radicals, metals andantioxidants in oxidative stress-induced cancer. Chemico-biological interactions. 160(1), 1- 40.
- Vural, H., Sabuncu, T., Arslan, S. O., & Aksoy, N. (2001). Melatonin inhibits lipidperoxidation and stimulates the antioxidant status of diabetic rats. Journal of pineal research, 31(3), 193-198.

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