Effect of Glycemic Control Using Different Antidiabetic Drugs on Cognitive Functions in Experimentally-Induced Type 2DM: A Comparative Study

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ABSTRACT
Background: Neurocognitive impairment is recognized as the fourth microvascular complications of type 2 diabetes mellitus (T2DM). **Objectives:** To compare the effect of glycemic control on cognitive functions in diabetic rats by using different anti-diabetic drugs. **Materials and Methods:** Fifty male albino rats were equally categorized into: Control group, Janumet group, Diabetic non-treated group, Diabetic+Insulin group and Diabetic+Janumet group. At the end of 4 weeks, neurocognitive assessment was done by using Morris water maze (MWM) and Y-maze tests. Blood samples were collected to estimate glycemic state, lipid profile and total antioxidant capacity level (TAC). Rats' brains were extracted. The right half of the brain was utilized for lipid peroxidation marker (MDA), tumor necrosis factor alpha (TNF-α), superoxide dismutase (SOD), and interleukin 1B (IL-B) measurement. The left half was utilized for histopathological study of the hippocampal tissue. **Results**: Diabetic group showed significant increase in FBG, HBA1C, HOMA-IR, total cholesterol, triglyceride, MDA, TNF-alpha and IL-1B, and decrease in serum insulin, HDL, TAC and SOD. Neurocognitive impairment and hippocampal degenerative changes were also obvious in diabetic rats. Diabetic+ Janumet group demonstrated significant improvement in the measured biomarkers and neurobehavioral tests with restoration of the hippocampal tissue structure in comparison with Diabetic+ Insulin group. However, there was an insignificant difference in lipid profile markers between Diabetic+ Janumet group and Diabetic+ Insulin group. Insignificant difference between Control and Janumet groups regarding all measured parameters was detected.
Conclusion: Both insulin and Janumet drugs alleviates the metabolic, neurocognitive impairment and hippocampal changes associated with T2DM. However, the improvement was more pronounced with Janumet treatment than insulin treatment. The synergistic hypoglycemic, anti-oxidant and anti-inflammatory effects of Janumet's components may account for this improvement.
Keywords: Cognitive functions, Diabetes mellitus, Insulin, Janumet, SOD, TNF-alpha.

INTRODUCTION

DM is a persistent metabolic disorder identified by metabolic irregularities and hyperglycemia. Based on data from 2015 compiled by the International Diabetes Federation, the DM global prevalence among adults is estimated to be around 415 million [1]. **Saedi et al.** define cognition as the process through which expertise, thought, feeling, and practice are utilized to recognize and acquire knowledge [2].

Neurocognitive impairment, the fourth microvascular complication associated with diabetes, may become evident even in the nascent phases of the condition and persist as chronic complications [2]. According to the evidence, oxidative stress has been associated with the development of various neurological disorders. The brain is especially exposed to oxidative damage because of its high oxygen consumption, high polyunsaturated fatty acid concentration, and reduced antioxidant levels (SOD and TAC) [3]. Neuroinflammation may lead to the manifestation of atypical brain functions [4]. Potentially increasing neuronal dysfunction, sustained pro-inflammatory factors release (IL-1B and TNF-alpha) stimulates the activation of additional glia cells, primarily microglia, which in turn initiates the release of pro-inflammatory factors [4]. Metformin hydrochloride and sitagliptin phosphate are components of Janumet® film-coated tablets at strengths of 1000 mg and 50 mg respectively for oral administration. Metformin possesses antioxidant properties that have the potential to reduce neuroinflammation and oxidative stress [5].

Sitagliptin is a DPP-4 Inhibitor and specifically targets the incretin axis. Sitagliptin inhibits the enzymatic degradation and subsequent glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) inactivation, which are the principal incretins implicated in the glucose homeostasis regulation [6]. Additionally, anti-inflammatory effects of sitagliptin have been documented via mechanisms dependent on mitogen-activated protein kinase [6]. The sitagliptin potent antioxidant effect may be attributed to its potential hypoglycemic effect, which reduces reactive oxygen species (ROS) production [7]. For the blood glucose levels management in DM patients, insulin has been widely administered. Its neuroprotective effect is attributed to glutamate levels mitigation rather than glucose levels [8].

The present study was conducted to compare the possible effects of Janumet and insulin on neurocognitive impairment in experimentally-induced type 2DM in rats.

MATERIALS AND METHODS
I- Animals and experimental groups

The study utilized 50 adult male albino rats, with each rodent weighing between 160 and 200 g. Rats were housed at a density of 5 per cage in well-

ventilated enclosures set at room temperature, with a 12-hour artificial light/dark cycle. Free access to water and a balanced standard diet were provided.

Ethical considerations:
The protocols established by the National Institutes of Health (NIH) of the United States were followed in regard to the care and laboratory animals' utilization [9]. The study received ethical committee approval (N:10/2020PHYS21) from the Institutional Review Board of Menoufia University, Egypt's Faculty of Medicine.

Following an adaptation period of 7 days, the rats were categorized into 5 groups (n=10):
1- Control group: In which rats were administered a saline solution with a concentration of 0.2ml/100 gm. B.W /day via subcutaneous injection, in addition to being given 1 ml of saline orally daily for a duration of 4 weeks,
2- Janumet group: For a duration of 4 weeks, rats were subjected to oral gavage of Janumet (4 ml/kg/day) and subcutaneous injection of saline at a concentration of 0.2 ml/100 gm. B.W/day [10].
3-Diabetic group: In which rats were induced diabetic through the provision of an unrestricted high-fat diet for duration of 2 weeks. This diet composition comprised 25% protein, 58% fat, and 17% carbohydrates (representing a total kilocalories percentage). After a fasting period of 12 hours, a solitary intra-peritoneal streptozotocin injection (35 mg/kg BW) was injected into the rats to mitigate the risk of hypoglycemia, a solution containing 5% glucose was subsequently administered [11]. After 72 hours of STZ administration, an evaluation of the fasting blood glucose (FBG) level was conducted. Diabetes was diagnosed in rats whose blood glucose levels went over 200 mg/dL, and selection was based on this criterion [10].
4-Diabetic +Insulin group: In which DM was induced as in Diabetic group, and insulin was administrated from the first day with injection of 10 IU/kg to rats whose blood glucose levels within the range of 200 to 300 mg/dL. Conversely, rats whose blood glucose levels surpassed 300 mg/dL were administered an injection of 20 IU/kg. A single subcutaneous injection of insulin was administered daily [12]. Concomitantly, 1 ml of saline was taken orally each day for duration of 4 weeks.
5-Diabetic +Janumet group: In which induction of DM was observed as in Diabetic group, after which Janumet was administered via the same route and dose in Janumet group for duration of 4 weeks. Concomitantly, saline was subcutaneously injected at a rate of 0. 2 ml/100gm B.W/day for 4 weeks.

II- Study design
Following the intervention, an evaluation of the rats' spatial memory and learning capabilities was conducted utilizing the MWM and Y-maze tests.

Subsequently, a series of lipid profile indicators, involving total cholesterol, triglyceride, and high-density lipoprotein (HDL) levels, total anti-oxidant capacity (TAC), fasting blood glucose (FBG), serum insulin, glycosylated hemoglobin (HbA1C), and HOMA-IR, were quantified using retro-orbital blood samples. Subsequently, rats were scarified by cervical decapitation and their brains were removed. Then, each brain was partitioned in 2 halves equally. In order to assess super oxide dismutase (SOD), malondialdehye (MDA), and interleukin-1 B (IL-B) and tumor necrosis factor- α (TNF-α) the right half was used. The left hemisphere of each brain was used for histopathological analysis of the hippocampal tissue via H and E staining.

Neurobehavioral Tests
MWM test: As an evaluation of learning and spatial memory, the MWM test was performed after the four-week study period had concluded. Four equal quadrants were determined for a circular pool measuring 60 cm in height and 170 cm in diameter; these quadrants were designated as follows: east, south, north, and west. The escape platform, measuring 10 cm in diameter, was positioned in a fixed location within one of the quadrants and concealed at a depth of 2 cm beneath the water's surface. For four consecutive days, escape latency, or the time required to reach the concealed platform, was monitored. The procedure adopted was done according to **Hu et al.** [1].
Y-maze test: This test was performed to assess learning and spatial memory. It was composed of Plexiglas that had been painted black. Each arm had dimensions of 15 cm in width, 40 cm in length, and 11 cm in height. The arm converged at its longest axis in a 15 cm-diameter equilateral triangular central region. The procedure adopted was done according to **Ahshin-Majd et al.** [13].

Biochemical assays
A) Blood sampling
Blood samples were collected from the retro-orbital venous plexus after 12 hours fasting. A total of 4 mL of blood was collected; of this, 3 mL was transferred to a sterile graduated tube, cooled for 15 minutes, and then centrifuged for an additional 15 minutes at 3000 r.p.m (rotation per minute) [14]. For the purpose of determining the glycemic profile (serum insulin, FBG, and HOMA-IR; Spinreact kit, SPAIN); and lipid profile (total cholesterol, triglycerides, and Spinreact kit, SPAIN), the serum was collected in a dry, sterile tube. TAC level and HDL levels (Human kit, Germany; Bio-diagnostic Company, Egypt) were also measured. In order to detect the HbA1c concentration, the remaining 1 ml of the blood was transferred into an EDTA tube (Teco Diagnostics, USA).
B) Preparation of brain tissue homogenate
The right half brain tissue which was prepared for tissue homogenization was stored at -30°C for 24 hours in Physiology Department, then sent to Central

Lab. at the Faculty of Medicine Menoufia University for estimation of MDA (Bio diagnostic Company, Egypt), The activity of SOD (Bio diagnostic Company, Egypt), TNF-alpha (Sun red histotechnology company, Egypt) and IL-1B (Sun red histotechnology company, Egypt).

Histopathological assessment of the hippocampal tissue

Hematoxylin and eosin staining

Each rat's left hemisphere of the brain was immersed in 10% formaldehyde for a duration of two hours. Following the removal of brain tissues, the hippocampus was isolated and immersed in a fresh formaldehyde solution for a duration of 24 hours. After being dehydrated with ethanol, the sample underwent a comprehensive xylene cleaning and paraffin embedding. Coronal sections with a thickness of 5 μm were acquired through the utilization of a Leica RM 2025 microtome (USA). Subsequently, glass slides were prepared from the sections and stained with H and E [15].

Statistical analysis

The results were assessed utilizing version 22.0 of the SPSS software tool. The data were presented in mean±SD notation. One-way ANOVA was used in combination with Tukey's Post-Hoc analysis to compare quantitative variables between the groups that were the subject of the inquiry. A statistically significant P value was one that was less than 0.05.

RESULTS

1) Neurobehavioral Tests

Insignificant difference was observed between the Janumt and Control groups regarding the mean of the duration spent in the target quadrant during the MWM test and the percentage of spontaneous alternation in the Y-maze test (**Figure 1**).

Regarding the mean of the duration spent in the target quadrant, it exhibited a significant decrease in Diabetic group compared with the corresponding values in Janumt and Control groups. Conversely, Diabetic +Janumet and Diabetic +Insulin groups exhibited considerably greater mean compared with Diabetic group. However, this mean continued to be significantly reduced compared with Control and Janumet groups. Furthermore, Diabetic +Insulin group had a significantly lower mean value than Diabetic +Janumet group (**Figure 1**).

The mean value for the percentage of spontaneous alternation was significantly lower in Diabetic group in contrast to both the Janumet and Control groups. On the contrary, it was significantly higher in Diabetic +Insulin and Diabetic +Janumet groups compared with Diabetic group. Nevertheless, in contrast to the Control and Janumet groups, this value remained significantly diminished. Furthermore, Diabetic +Insulin group had a significantly lower value than Diabetic+ Janumet group (**Figure 1**).
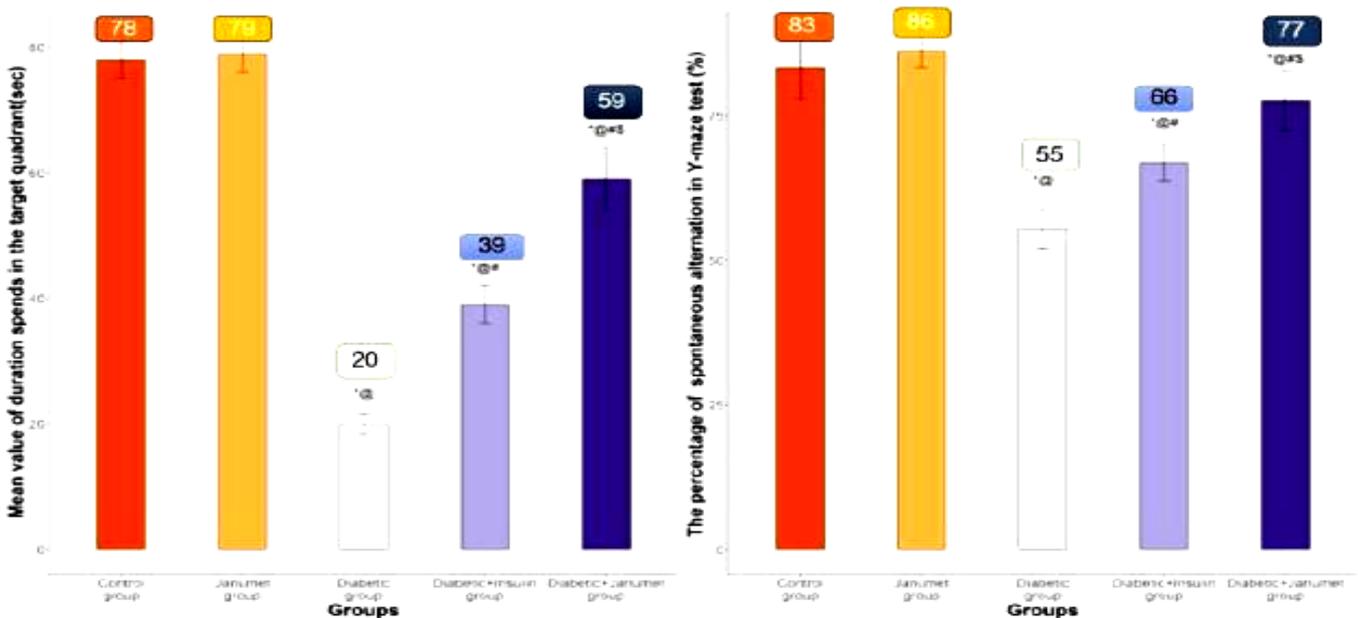
Figure (1): The mean value ±SD of the duration spent in the target quadrant in the MWM test and the percentage of spontaneous alternation in Y-maze test in all studied groups.
*P ≤ 0.05 compared with Control group; @P ≤ 0.05 compared with the Janumet group; #P ≤ 0.05 compared with Diabetic group; and $P ≤ 0.05 compared with Diabetic+ Insulin group. Number of each group=10.

2) Biochemical Tests

There was no statistically significant difference found in the mean values of HBA1C, FBS, serum insulin, and HOMA IR among Janumet and Control groups **(Table 1)**.

However, a statistically significant increase was observed in the mean of FBS and HBA1C in Diabetic group when compared with Janumet and Control groups. Conversely, Diabetic +Janumet and Diabetic +Insulin groups demonstrated the most substantial reductions in the mean of FBS and HBA1C compared with Diabetic group.

On the contrary, Diabetic +Insulin group maintained significantly elevated FBG and HBA1C levels compared with Janumet and Control groups. Furthermore, it was reported that Diabetic +Insulin group had a significantly higher mean value of HBA1C and FBS than Diabetic +Janumet group. On the contrary, there was no significant difference observed in the mean values of HBA1C and FBS between Janumet, Control and Diabetic +Janumet groups **(Table 1)**.

A statistically significant reduction was observed in the mean of serum insulin of Diabetic group when comparing with Janumet and Control groups. Conversely, Diabetic +Insulin and Diabetic +Janumet groups demonstrated noticeably elevated mean values of serum insulin compared with Diabetic group. Serum insulin levels in Diabetic +Insulin group were significantly lower than Janumet and Control groups.

Furthermore, it was reported that Diabetic +Insulin group had a significantly lower mean of serum insulin than Diabetic +Janumet group. But between the Diabetic +Janumet, Janumet and Control groups, there was no statistically significant difference in serum insulin levels **(Table 1)**.

Conversely, Diabetic group exhibited a significantly higher mean of HOMA-IR compared with both Janumet and Control groups. Conversely, a statistically significant reduction in the mean of HOMA-IR was detected in Diabetic +Insulin and Diabetic +Janumet groups, as in contrast to Diabetic group. On the other hand, Diabetic +Insulin group demonstrated significantly elevated mean of HOMA-IR when in contrast to both Janumet and Control groups. Furthermore, a significant decrease in the mean of HOMA-IR was detected in Diabetic +Janumet group, as in contrast to the corresponding values in Diabetic +Insulin group.

However, there was no significant difference in the HOMA-IR scores between Diabetic +Janumet, Control, and Janumet groups **(Table 1).**

Table (1): The mean values± SD of FBG, HBA1C, serum insulin and HOMA-IR in all studied groups

Parameters	Control group	Janumet group	Diabetic group	Diabetic+Insulin group	Diabetic+Janumet group	P value
FBS (mg/dl)	110±26.11	115.70±15. 1	460 ±43.518*@	275.5± 36.18*@#	130.60±28.17 #$	<0.001*
HBA1C (%)	2.21±0.24	2.29±0.078	9.97±0.230*@	7.23±0.24*@#	2.41±0.194 #$	<0.001*
Serum Insulin (M IU/L)	2.90 ±0.41	3.02±0.533	1.37±0.298*@	1.80 ±0.10 *@#	2.49 ±0.081 #$	<0.001*
HOMA-IR	0. 8 ± 0.29	0.9 ± 0. 224	1.6±0.377*@	1.23 ± 0.20 *@#	0. 89 ± 0.245 #$	<0.001*

*P ≤ 0.05 compared with Control group, @P ≤ 0.05 compared with Janumet group, #P ≤ 0.05 compared with the Diabetic group, and $P ≤ 0. 05 compared with Diabetic +Insulin group. Number of each group=10.

Insignificant change was detected in the comparison between the mean of serum cholesterol and triglyceride in Janumet and Control groups. In contrast, Diabetic group exhibited significantly higher mean values for serum cholesterol and triglyceride than both Janumet and Control groups.

However, it was observed Diabetic +Insulin and Diabetic +Janumet groups had significantly lower mean values for serum cholesterol and triglyceride when in contrast to Diabetic group. Nevertheless, these values remained significantly elevated when in contrast to Janumet and Control groups.

The mean values of triglyceride and serum cholesterol in Diabetic +Janumet group vs the corresponding value Diabetic +Insulin did not reveal a statistically significant difference **(Table 2)**.

The mean of HDL in Janumet group did not differ significantly from the corresponding value in Control group. On the contrary, the mean of HDL significantly decreased in Diabetic group when in contrast to the corresponding values in Janumet and Control groups.

Conversely, the mean of HDL in Diabetic +Insulin and Diabetic +Janumet groups were significantly greater than Diabetic group. Nevertheless, when in contrast to Janumet group and Control group, these values remained significantly diminished.

On the contrary, there was no significant difference observed among the mean of HDL in Diabetic +Insulin and Diabetic +Janumet groups **(Table 2).**

Table (2): The mean values± SD of serum cholesterol, triglyceride and HDL in all studied groups

Parameters	Control group	Janumet group	Diabetic group	Diabetic +Insulin group	Diabetic+Janumet group	P value
Cholesterol (mg/dl)	152.3±11. 05	123.1±15. 24	351.10± 68.29*@	280.50±33.10*@#	235.5±30. 86*@#	<0.001*
Triglyceride (mg/dl)	78.00 ±17. 34	95.00±12.80	294.60 ±27.68*@	219.40±12.34*@#	202.0 ±14. 37*@#	<0.001*
HDL (mg/dl)	57.7±1.56	58.00±2.30	44.9±2.28*@	50.0±1.49*@#	55 ±2.06*@#	<0.001*

*P ≤ 0.05 when compared to Control group; @P ≤ 0.05 when compared to Janumet group; #P ≤0.05 when compared to Diabetic group. Number of each group=10.

A statistically insignificant difference was detected between Janumet, Control groups, in terms of the mean values of SOD and TAC.

On the contrary, the mean values of TAC and SOD were significantly lower in Diabetic group when in contrast to the corresponding values in Janumet and Control groups. However, the mean values of SOD and TAC in Diabetic+ Insulin and Diabetic+ Janumet groups were significantly higher than those in Diabetic group. Nevertheless, these values remained considerably diminished compared with both Janumet and Control groups. Additionally, it was observed that Diabetic+ Janumet group had significantly higher mean values of SOD and TAC in contrast to Diabetic+ Insulin group (**Table 3**). There was non-significant difference observed in the mean of MDA between Janumet group and the corresponding value in Control group. In contrast, the mean of MDA in Diabetic group was significantly higher than in Janumet and Control groups. Nevertheless, Diabetic+ Insulin and Diabetic+ Janumet groups exhibited significantly lower mean of MDA values compared with Diabetic group. However,

these values remained significantly higher values compared with Janumet and Control groups. Furthermore, the mean of MDA in Diabetic+ Janumet group was decreased significantly compared with the corresponding value in Diabetic+ Insulin group (**Table 3**).

The Janumet group did not show a significant difference in mean values of TNF-alpha and IL-1B in contrast to Control group.

On the contrary, the mean values of TNF-alpha and IL-1B were significantly elevated in Diabetic group in contrast to both Janumet and Control groups. However, when in contrast to Diabetic group, the mean values for TNF-alpha and IL-1B were significantly lower in Diabetic+ Insulin and Diabetic+ Janumet groups. Nevertheless, these values remained significantly elevated when in contrast to Janumet and Control groups. Also. A statistically significant decrease in the mean values of TNF-alpha and IL-1B was detected in Diabetic+ Janumet group as opposed to Diabetic+ Insulin group (**Table 3**).

Table (3): The mean values± SD of SOD, TAC, MDA, IL-1B and TNF-alpha in all studied groups

Parameters	Control group	Janumet group	Diabetic group	Diabetic+ Insulin group	Diabetic + Janumet group	P value
SOD (mg/dl)	23.5±2.36	24. 7±2.66	9.9±1. 66 *@	14.8±1.31 *@#	17.9±1.66*@ #$	<0.001*
TAC (mM/L)	0.28±0.04	0.27±0.05	0.06±0.02 *@	0.13±0.016 *@#	0.20±0.04 *@#$	<0.001*
MDA (nmol/gram tissue)	40.82±3.13	37.06±5. 83	65.66±3.63*@	57.6±2.06 *@#	50.33±3.19*@ #$	<0.001*
IL-1B (p g/l)	309.3±55. 95	313.9±57.9	565.2±62.20*@	469.33±25.03*@#	394±31.07*@#$	<0.001*
TNF-alpha (ng/l)	25.63±2.93	27.77±2.33	44.2±2.65*@	37.2±1.75*@#	32.3±2.67*@#$	<0.001*

*P ≤ 0. 05 vs Control group, @P ≤ 0. 05 vs Janumet group, #P ≤ 0. 05 vs Diabetic group, and $P ≤ 0. 05 vs Diabetic+ Insulin group. Number of each group=10.

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3) Histopathological results

The dentate gyrus and the hippocampus proper were visible in sections of the control group's hippocampal tissue stained with H and E. The four regions of cornu ammonis (CA1, CA2, CA3, and CA4) make up the hippocampus proper. Areas CA1 and CA3 are composed of the deep polymorphic layer, the middle pyramidal layer, and the surface molecular layer. The three layers that make up dentate gyrus are the molecular layer, the middle granular cell layer, and the polymorphic layer (**Figure 2(A-C)**). The Janumet group's sections displayed similar picture to the control group (**Figure 2(D)**). Conversely, compared to the Control group, major structural alterations were seen in the regions from the Diabetic group. Pyramidal cells in the CA1 and CA3 regions are smaller, and the layer of pyramidal cells appears thinner, with dilated, congested blood vessels. Perineural space around deteriorated pyramidal cells is also visible in CA3 regions. Dentate gyrus regions exhibit reduced granule cell size and a noticeable tissue loss (**Figure 2(E-H)**). Compared to the Diabetic group, sections from the Diabetic+ Insulin group showed fewer degenerative alterations. The pyramidal cell structure in the CA1 and CA3 regions appears to have improved, with fewer shrunken pyramidal cells and an increase in the thickness of the pyramidal cell layer (**Figure 2(I-K)**). Interestingly, sections from Diabetic+ Janumet group showed a picture nearly similar to Control group (**Figure 2(L-N)**).
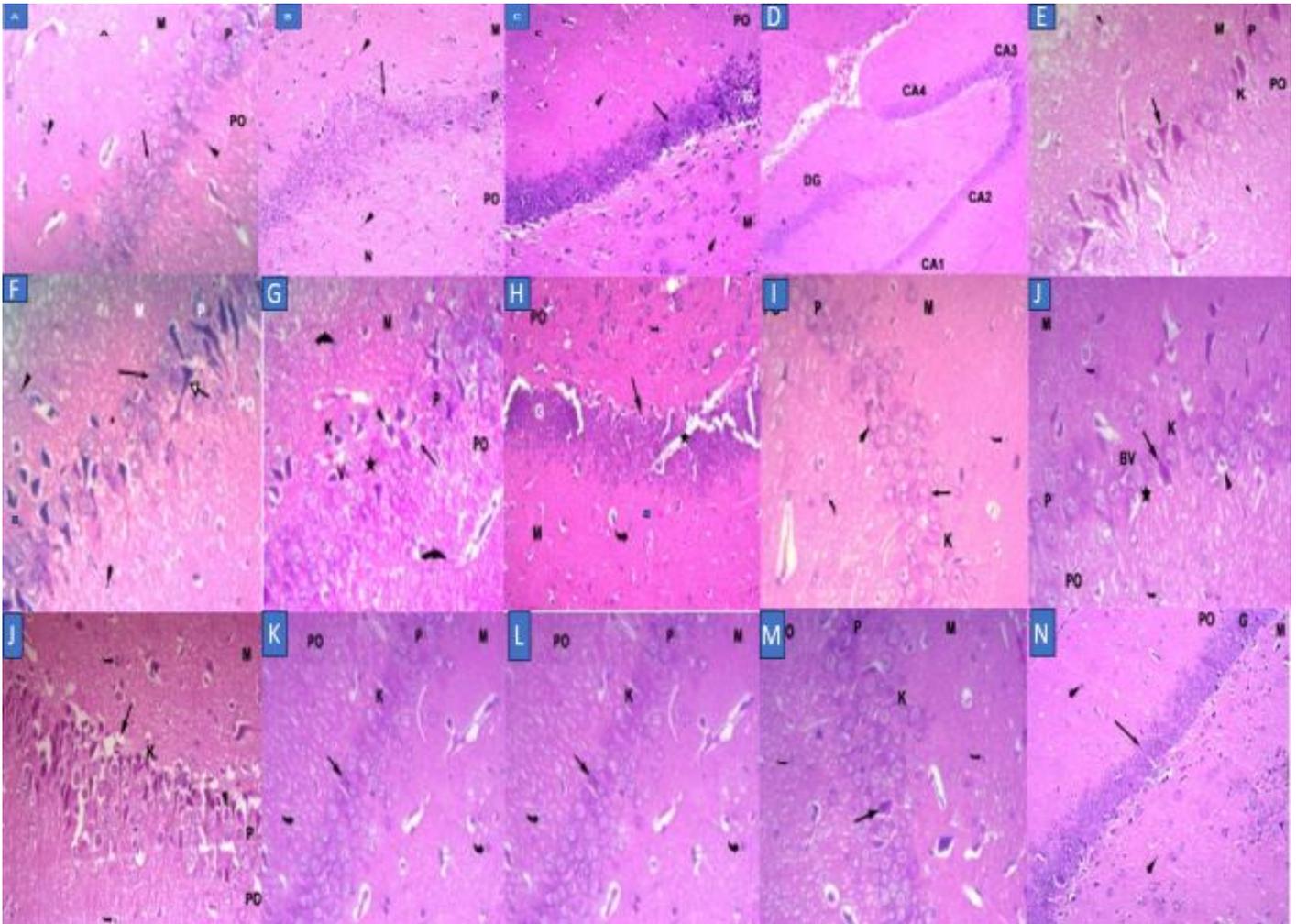
Figure (2): A Photomicrograph of H and E sections of the hippocampus of the different groups.
A) The control group CA1 areas are composed of three layers: the deep polymorphic layer (PO), the middle pyramidal layer (P), and the surface molecular layer (M). Three to four rows of densely packed, uniformly distributed pyramidal neurons with sparse neuropil between make up the pyramidal layer. Every cell has a single, spherical, massive, vesicular nucleus with two to three conspicuous nucleoli, and basophilic cytoplasm (↑). **B)** Control group displaying the CA3 regions, which are composed of the deep polymorphic layer (PO), middle pyramidal layer (P), and superficial molecular layer (M). The pyramidal layer's image is identical to that of CA1. **C)** The control group displayed the dentate gyrus (DG), which is composed of three layers: the polymorphic layer (PO), the intermediate granular cell layer (G), and the molecular layer (M). The DG is visible as a black structure. There is minimal interstitial tissue between many granular cell neurons that make up the granular layer. **D)** Janumet group showing a picture similar to the Control group. **E)** A diabetic group containing some shrunken pyramidal cells with dark eosinophilic cytoplasm and pyknotic nuclei, encircled by pericellular space (K) in CA1, as well as a shrunken pyramidal cell with darkly pigmented basophilic cytoplasm and pyknotic nuclei (arrow). **F)** Diabetic group showing CA1 areas that is similar to the image **(E)** and shows obvious thin pyramidal cell layer with dilated congested blood vessel. **G)** Diabetic group revealed shrunken pyramidal cells with deeply stained basophilic cytoplasm, shrunken pyknotic nuclei (K), perineural space surrounding degenerated pyramidal cells (arrowhead), some pyramidal cells with degenerated karyolitic nuclei (arrow) and marked decrease in the thickness of pyramidal cell layer (star) in CA3. **H)** Diabetic group demonstrating shrunken granule cells with basophilic cytoplasm and shrunken pyknotic nuclei (arrow) and marked loss of tissue are clear (star) in DG. **I)** Diabetic+ Insulin group showing CA1 areas: an apparent improvement in the structure of pyramidal cell but still seen some shrunken pyramidal cells with shrunken deeply stained pyknotic nuclei (hollow arrow), perineural space surrounding degenerated pyramidal cells (arrow), some shrunken pyramidal cells with dark eosinophilic cytoplasm and pyknotic nuclei and surrounded by pericellular space (K). **J)** Diabetic+ Insulin showing CA1 areas that is similar to the image **(I)** and shows dilated congested blood vessel (BV). **K)** Diabetic+ Insulin showing CA3 areas: an apparent improvement but still seen few shrunken pyramidal cells with deeply stained pyknotic nuclei (K), perineural space surrounding degenerated pyramidal cells (arrowhead) and some pyramidal cells with degenerated karyolitic nuclei (arrow). **L)** Diabetic+ Janumet group showing CA1 areas: very few shrunken pyramidal cells with shrunken deeply stained pyknotic nuclei (hollow arrow) and perineural space surrounding degenerated pyramidal cells. Pyramidal cells with karyolitic pale-stained nuclei (arrow). **M)** Diabetic+ Janumet group showing CA3 areas: an increase of the thickness of pyramidal cell layer but still seen very few shrunken pyramidal cells with shrunken deeply stained pyknotic nuclei (K), perineural space surrounding degenerated pyramidal cells (arrowhead) and some pyramidal cells with degenerated karyolitic nuclei (arrow). **N)** Diabetic+ Janumet group showing DG: the granular layer restored its normal structure, which formed of multiple granular cell neurons with few interstitial tissues in-between.

DISCUSSION

DM type 2 is often initiated by a combination of obesity and insulin resistance. A potential correlation has been identified between type 2 diabetes and cognitive decline [2]. Janumet drug is a combination of metformin and sitagliptin. Metformin exhibits antioxidant characteristics that may ameliorate oxidative stress and neuroinflammation [5]. Through pathways dependent on mitogen-activated protein kinase, the anti-inflammatory effects of sitagliptin have been apparent [6]. The possible hypoglycemic impact of sitagliptin, which lowers the formation of ROS, may be the cause of its strong antioxidant action [7]. The neuroprotective impact of insulin therapy is due to glutamate levels modulation rather than glucose levels [8].

In the current investigation, learning and spatial memory were assessed using Y-maze tests and the MWM. A significant reduction in the proportion of spontaneous alterations in the Y-maze test and the duration spent in the target quadrant during the MWM test indicated that Diabetic group exhibited neurocognitive impairment. These findings were in contrast to those of Control and Janumet groups. These results were consistent with those previously reported [16]. Diabetic patients may experience cognitive impairments as a result of cerebral vasculature complications or metabolic dysfunction [16]. Conversely, the results of our study indicated that Diabetic+ Janumet group exhibited a statistically significant increase in the proportion of spontaneous alternations in the Y-maze test and the duration of time spent in the target quadrant in the MWM test, respectively, compared with Diabetic group. The extent of this enhancement was significantly higher in Diabetic+ Janumet group when compared with Diabetic+ Insulin group.

When comparing Diabetic group to Janumet and Control groups, we observed a significant increase in FBG, HbA1C, and HOMA-IR. These findings were consistent with previous data [17]. This phenomenon may be accounted for the development of insulin resistance syndrome in the rats, which is distinguished by obesity, moderate hyperglycemia, hypertriglyceridemia, and hypercholesterolemia, following 2 weeks of feeding them a high-fat diet (HFD) [18]. HbA1c levels exhibit a direct correlation with blood glucose levels. Due to the fact that its binding to hemoglobin molecules is concentration-dependent, an increase in blood glucose levels results [19]. Diabetic+Janumet group demonstrated significantly reduced values of FBG, HbA1c and HOMA-IR when in contrast to Diabetic+ Insulin group. The aforementioned findings were consistent with the previous ones [18,20]. By increasing glucose uptake in adipose and muscle tissues and facilitating the translocation of the glucose transporter GLUT4 from intracellular to cell surface sites, insulin therapy reduces the impact on blood glucose levels [20].

Metformin has the ability to enhance insulin sensitivity by rapidly increasing insulin receptor activation and signaling, mostly through insulin-receptor substrate-2. Sitagliptin functions as a DPP-4 inhibitor by augmenting GLP-1 levels, consequently inducing insulin secretion while impeding glucagon secretion, which accounts for its potent hypoglycemic effect [18].

Compared with Janumet group and Control group, there was a statistically significant decrease in serum insulin levels observed in Diabetic group. This finding was consistent with previous result [16,18]. In the current study, the decreased serum insulin levels observed in the Diabetic group may have been caused by the STZ administration. Beta cells are destroyed and biological macromolecules are fragmented as a result of the alkylating properties of STZ [21]. Ingestion of HFD for 2 weeks resulted in obesity-induced insulin resistance and hyperinsulinemia, however, destruction of Beta cells by STZ resulted in progression to hypoinsulinemia [22].

Compared with Diabetic group, the Diabetic+ Insulin and Diabetic+ Janumet groups exhibited a significant elevation in serum insulin levels in the current study. This result corroborated the preceding findings [18,23,24]. However, the Diabetic+ Janumet group exhibited a significant elevation in serum insulin levels compared with Diabetic+ Insulin group. Emerging evidence indicates that diabetic patients' insulin secretion increased dramatically following a period of intensive insulin therapy [23]. The significant elevation in serum insulin levels observed with metformin can be attributed to its potential mechanisms of action, which include stimulation of GLP-1 secretion [24]. The augmented insulin level observed in Diabetic+ Janumet group, may be mechanistically explained by the synergistic effects of sitagliptin and metformin in enhancing insulin release in various experimental DM models [25]. Furthermore, our research demonstrated a noteworthy increase in lipid profile indicators (total cholesterol and triglyceride levels) in Diabetic group compared with Janumet and Control groups. Conversely, the mean HDL level was considerably diminished in Diabetic group compared with Control and Janumet groups. These results were consistent with prior research [18,26]. In T2DM rats, dyslipidemia is a prominent characteristic. By activating hepatic lipase, insulin resistance increases the flow of free fatty acids (FFA) from adipocytes and the hepatic esterification of FFA to TG [26].

Conversely, Diabetic+ Janumet and Diabetic+ Insulin groups exhibited significant modulation in lipid profile markers compared with diabetic group. These findings were consistent with previously documented findings [18,27,28]. With respect to lipid profile markers, however, Diabetic+ Janumet and Diabetic+ Insulin groups found no significant difference.

In addition to reducing circulating TGs and cholesterol, insulin therapy increased HDL. Insulin is regarded as a highly effective lipoprotein lipase

activator [27]. By inhibiting lipolysis in adipose tissue, metformin decreases the amount of circulating non-esterified fatty acids [18]. Sitagliptin can inhibit the enzyme hormone-sensitive lipase in adipose tissue, which prevents the conversion of TG to FFA and, consequently, the synthesis of cholesterol [28].

In Diabetic group, there was a significant decrease in SOD and TAC and a corresponding increase in MDA level, as observed in the current study. This finding was in contrast to Control and Janumet groups. They were consistent with findings from another research [29]. By stimulating the formation of ROS via a multitude of cellular pathways including non-enzymatic glycation, aldose reductase and oxidative phosphorylation, hyperglycemia damages β-cells and pancreatic tissue [16,29]. A heightened concentration of MDA in individuals diagnosed with diabetes suggests that peroxidative harm may contribute to the progression of diabetic complications. These complications induce cellular damage through the formation of cross-links among membrane components that contain amino groups, resulting in membrane permeability [29].

On the other hand, Diabetic+ Insulin and Diabetic+ Janumet groups exhibited significant improvements in oxidative balance restoration compared with Diabetic group. These findings were consistent with previously documented results [7,22,30]. Diabetic+ Janumet group exhibited a significant increase in SOD and TAC, which was accompanied by a substantial reduction in MDA, in contrast to Diabetic+ Insulin group. Insulin may function as an antioxidant scavenger, as evidenced by the fact that intensive insulin treatment significantly increases the plasma TAC [30]. Metformin exhibits the capacity to diminish ROS by impeding mitochondrial respiration and decreasing advanced glycation end products (AGEs) [5]. Sitagliptin decreases endoplasmic reticulum stress and ROS production, which may account for its potent antioxidant effect [7].

In relation to the inflammatory state, there was a significant increase in IL-1B and TNF-α among Diabetic group compared Janumet and Control groups. These results were consistent with previously documented results. Inflammation is induced by excessive FFA and glucose through the mechanisms of oxidative stress and a reduction in antioxidants [31]. In contrast, IL1-B and TNF-α decreased significantly in the Diabetic+ Insulin and Diabetic+ Janumet groups in contrast to Diabetic group in our study. These findings were consistent with previously reported findings [31-33]. Diabetic+ Janumet group exhibited a significantly reduced levels of IL-1B and TNF-α compared with Diabetic+ Insulin group. By way of non-metabolic pathways, insulin induces anti-inflammatory mediators and inhibits pro-inflammatory cytokines [32]. Metformin has the potential to inhibit pro-inflammatory responses by means of its direct inhibition of NF-kB [31]. Sitagliptin inhibits the NF-kB

signaling cascade and decreases excessive protein accumulation to reduce inflammatory response [33].

The presence of uncontrolled hyperglycemia, dyslipidemia, inflammation, and oxidative stress can now be linked to the decline in learning and spatial memory in Diabetic group, as evidenced by the results of the MWM and Y-maze tests conducted in this research. Hyperglycemia is by far the most significant cause of cognitive impairment [16]. Accordingly, the improvement in the neurocognitive impairment in insulin-treated group and Janumet-treated group was attributed to their strong hypoglycemic effect.

In diabetic group H and E-stained sections; degenerative changes of the hippocampal tissue coincide with the biochemical and neurobehavioral results. There were shrunken pyramidal cells in CA1 and CA3 and shrunken granule cells in DG. These changes induced by diabetes were also present in other study [34], which may attribute to the harmful effects of hyperglycemia and oxidative stress. In contrast, hippocampal tissue in Diabetic+ Insulin group revealed apparent improvement in the structure of pyramidal cell in CA1 and CA3. Similar images showed the effect of insulin on the improvement of hippocampal tissue were previously recorded [35]. Hippocampal tissue in Diabetic+Janumet group revealed apparent improvement in the thickness of layers and shape of cells but still showing very few shrunken pyramidal cells in CA1 and CA3 and also the granular layer of dentate gyrus restored its normal structure. These beneficial effects were consistent with other studies [1,16].

CONCLUSION

Both Janumet and insulin ameliorate the neurocognitive impairment and hippocampal degeneration observed in type 2 DM, according to the current study. It is probable that the hypoglycemic, antioxidant, and anti-inflammatory effects are at play. Furthermore, apart from their function in ameliorating dyslipidemia. In contrast, the improvement in biomarkers, neurocognition, and hippocampal structure was more pronounced in the Diabetic+ Janumet group more than Diabetic+ Insulin group. The synergistic effect of Janumet's components, namely metformin and sitagliptin, in regulating glycemic state in type 2 diabetes may account for this improvement.

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