

Effect of combined administration of SGLT2 inhibitor (dapagliflozin) and cobalamin on kidney-brain axis in type 2 diabetic rats

M. A. Abdallah^{1,2}, Toka Saber Eata¹, Mohamed H. Hassan¹, Ahmad Mohamad Gaafar¹, Dina M. Allam³, and Eman I. Elgizawy^{1,2}

1Department of Medical Physiology, Faculty of Medicine, Menoufia University, Egypt

2Department of Medical Physiology, Faculty of Medicine, Menoufia National University, Egypt

3 Department of Pathology, Faculty of Medicine, Menoufia University, Egypt

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Abstract

The hallmark of type II diabetes mellitus (T2DM) is selective microvascular damage, particularly to the kidney and brain, where the connection between them is intricate and bidirectional. This investigation sought to examine cobalamin and dapagliflozin's effects on the kidney-brain axis in diabetic rats. Five groups of 50 adult male albino rats were randomly and equally assigned to normal control (NC), diabetic non-treated (DN), diabetic cobalamin-treated (D+Cbl), diabetic dapagliflozin-treated (D+Dapa), and diabetic combined cobalamin & dapagliflozin-treated (D+Cbl+Dapa). T2DM was caused by a high-fat diet and a small dosage of streptozotocin. Following the experiment, a neurocognitive assessment was done. Blood pressure was measured. Urine samples were obtained over a 24-hour period to measure albumin and creatinine levels. Blood samples were collected to estimate glycemic state, lipid profile, renal function tests, IL-6, and TAC. HOMA-IR, LDL-C, UACR, and GFR by cystatin C were computed. Sections of the brain and kidney tissues were examined histopathologically. Diabetic rats revealed a significant increase in FBG, HOMA-IR, HbA1c, total cholesterol, triglyceride, LDL-C, blood pressure, and IL-6, and a reduction in serum insulin, HDL-C, and TAC. Also, diabetic rats exhibited neurocognitive and renal dysfunction, accompanied by morphological alterations in the brain and kidneys. Individual therapy with cobalamin or dapagliflozin significantly improved some of these parameters. The neuro-renal protective effect of cobalamin and dapagliflozin when administered together was greater than the effect of either drug alone, indicating a synergistic effect between them. Therefore, cobalamin plus dapagliflozin can be used as a combination treatment for T2DM microvascular insult.

Introduction

Persistent hyperglycemia and disturbances in protein, lipid, and carbohydrate metabolism brought on by insufficient insulin action, production, or both are hallmarks of diabetes mellitus (DM). The International Diabetes Federation (IDF) estimates that 366 million people worldwide suffered from diabetes in 2011; by 2030, that number is expected to increase to 552 million [1].

Diabetic nephropathy (DN), which is thought to be one of diabetes's most dreaded long-term microvascular consequences, is a major contributor of end-stage renal disease (ESRD). It usually starts with glomerular hyperfiltration and albuminuria, followed by a steady deterioration in renal functioning as prolonged exposure to hyperglycemia damages and disrupts the renal microvasculature and cellular architecture [2].

Additionally, it is becoming more well acknowledged that diabetes end-organ damage can occur in the brain, with cognitive impairment highlighted as one of the many repercussions of DM. The intricate pathophysiology of memory and learning problems in DM has not been fully understood. Several causes have been implicated, including metabolic abnormalities, chronic inflammation, free radical generation, and vascular issues [3].

Diabetic nephropathy and cognitive impairment (kidney-brain axis) may be linked by a variety of factors, including metabolic, vascular, and sociodemographic factors. Higher albuminuria, decreased GFR, and systemic inflammatory indicators are all associated with kidney-brain axis dysfunction in DN patients [4].

A novel class of antidiabetic medication called dapagliflozin works by limiting the kidney's ability to reabsorb glucose. It is an extremely potent and

reversible inhibitor of sodium glucose cotransporter-2 (SGLT-2i). Apart from its anti-diabetic benefits, it has reno-neuroprotective effects by decreasing blood pressure and triglycerides, improving insulin resistance, and inhibiting renal oxidative stress and the inflammatory response [2]. Furthermore, prior studies on dapagliflozin found that it had a neuroprotective effect on DM, as evidenced by enhancements in hippocampal synaptic plasticity and avoidance of cognitive deterioration [5].

Hematopoiesis, neurological and cognitive function, and appropriate lipid metabolism all depend on cobalamin, a water-soluble vitamin commonly referred to as vitamin B12 [6]. Because of its potent anti-inflammatory and antioxidant properties, it can alter inflammatory responses by regulating the transcription factor NFkB. Because of these characteristics, cobalamin is useful in the treatment of a variety of clinical conditions associated with oxidative stress, inflammation, and apoptotic cellular damage [7].

The current investigation studied the effects of dapagliflozin and cobalamin on the kidney-brain axis in type II diabetic rats, as well as the underlying mechanisms.

MATERIALS AND METHODS

With permission from Menoufia University's ethics committee, the current investigation was carried out at the Medical Physiology Department of the Faculty of Medicine at Menoufia University in Egypt (N:3/2022PHYS41). Every procedure was conducted in accordance with the National Research Council's (NRC) 2002 international guide for the care and management of laboratory animals.

Animals and experimental groups

50 adult male albino rats of the local breed, weighing between 150 and 200 grams, were

employed in this investigation. Five rats per cage were kept in a completely ventilated wire mesh cage of its size (80x40x30 cm), with unrestricted access to tap water, a natural day/night cycle, and normal room temperature. For acclimation, they were fed a normal rat pellet diet (NPD) that contained 12% of calories as fat [8] and tap water for one week prior to the experiment. After that, the rats were split into five equal experimental groups, each consisting of ten individuals, at random:

I: Normal control (NC) group:

Normal rats with fasting blood glucose levels below 110 mg/dl, as assessed by rat tail samples using a glucometer (ACCU-CHEK), were selected for this group and were kept with unrestricted access to tap water and standard NPD until the experiment was over [8].

II: Diabetic Non-treated (DN) group:

T2DM was induced by feeding rats a high-fat diet (HFD) for four weeks before administering a single dosage of streptozotocin (STZ) (35 mg/kg of rats' body weight) in 1 milliliter of 0.1M citrate buffer that was injected intraperitoneally [9]. 72 hours following STZ injection, one-touch glucose strips and a glucometer (ACCU-CHEK) were used to measure fasting blood glucose level by tail prick. Rats with fasting blood glucose levels of 250 mg/dl or higher were classified as diabetics [9], and they were permitted to continue eating a high-fat diet till the experiment concluded (4 weeks).

III: Diabetic cobalamin-treated (D+Cbl) group:

After being diabetic as group II, diabetic rats were given cobalamin (1 mg/kg/day) injected intraperitoneally for four weeks [10].

IV: Diabetic dapagliflozin-treated (D+Dapa) group:

After being diabetic as group II, diabetic rats were given dapagliflozin (1 mg/kg/day) diluted in distilled water and delivered orally by gavage every day for four weeks [11].

V: Diabetic combined cobalamin & dapagliflozin-treated (D+Cbl+Dapa) group:

After being diabetic as group II, diabetic rats were given both cobalamin (1 mg/kg/day) injected intraperitoneally and dapagliflozin (1 mg/kg/day) diluted in distilled water and delivered orally by gavage every day for four weeks.

All parameters of the first two groups were measured before the start of the last three groups to confirm the developing renal impairment in the DN group.

Neurobehavioral Tests

Morris water maze (MWM) test: to evaluate rats' spatial memory and learning, the MWM test was performed. A circular pool, measuring 150 cm in diameter and 62.5 cm in depth, was divided into four quadrants: starting positions—north (N), south (S), east (E), and west (W)—evenly distributed around the pool's circumference. A concealed escape platform was placed in the center, submerged one centimeter beneath the water surface. Over four successive days, rats were trained to localize the platform placed beneath the water's surface. There were four trials per session; four distinct starting positions were used in a predetermined order, and then the escape latency, which is the time needed to reach the concealed platform, was monitored. Rats were placed on the platform if they were unable to locate it within 60 sec. Finally, rats were given a memory probe trial two hours following the last training trial, during which the concealed

platform was taken out from the pool, and the probe trial's escape latency was also noted [12].

Novel object recognition (NOR) test: to evaluate the rats' capability to identify a new item in a familiar setting. Over the course of three days, each rat underwent three stages of testing: habituation, training, and testing. Rats were put in an open-field apparatus of its size (50 x 50 x 40 cm) and given ten minutes to adjust during the habituation phase. Then, each rat was shown two identical objects for five minutes during the training phase. During the testing phase, which took place a day after the training phase, each rat was shown a new object for five minutes, replacing the old one. The amount of time spent exploring objects was documented. The discrimination index was calculated as [(exploration time of the novel object – exploration time of the familiar object) / total time of exploration × 100%] and the recognition index also was calculated as [exploration time of the novel object / total time of exploration] × 100. Both indices were used to evaluate the cognitive performance of rats [13].

Non-invasive blood pressure measurement

The BIOPAC-MP36 system (BIOPAC System Inc., USA) was utilized to measure blood pressure non-invasively by wrapping a cuff around the tail of adult male rats that were kept in the proper animal restrainers. The body temperature of the tested rats was artificially kept at 37°C by using an animal heating unit (tail heating B), and the small animal tail non-invasive blood pressure system (NIBP 200A) and MP36 software for data simulation were engaged for this study [14].

Urine and blood sampling

A metabolic cage was set up for each rat, and the cage was set over a funnel with wire mesh covering the top. The animal was given

unrestricted access to water while urine was being collected. Samples of urine were centrifuged and kept at -20°C [15].

Overnight fasting blood samples from each rat's retro-orbital venous plexus were obtained and placed in two sterile graduated tubes. One of them contained EDTA to measure HbA1c (Fine Care Company, China), and the other was centrifuged for 15 minutes at 3000 rpm after letting it coagulate at room temperature. Then, the serum was gathered in sterile tubes and stored until it was needed at -80°C.

Biochemical analysis

Fasting blood glucose (mg/dl) (Spectrum Company, Egypt), HbA1c (% of normal) (Fine Care Company, China), TC (mg/dl), HDL-C (mg/dl), TG (mg/dl), serum total protein (g/dl), serum urea (mg/dl), urinary albumin (mg/L), serum & urinary creatinine (mg/dl) (Biomed Diagnostic Company, Egypt), and TAC (μmol/L) (Abcam, UK) were all measured by the enzymatic colorimetric method using test reagent kits.

Serum insulin (μIU/ml) (Diagnostic Automation/Cortez Diagnostic, Inc., USA), serum cystatin C (mg/L) (Glory Science Company, Taiwan,) and IL-6 (pg/ml) (Fine Care Company, China) were all measured using enzyme-linked immunosorbent assay kits.

Calculation of HOMA-IR was done using the following formula: $\text{HOMA-IR} = [\text{fasting serum glucose (mg/dl)} \times \text{fasting serum insulin (μIU/ml)}] / 405$ [16], LDL-C was calculated using the following formula: $\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG} / 5)$ [17]. Concentration of albumin (mg/l) divided by concentration of creatinine (g/l) yielded the UACR (mg/g creatinine) [18]. Also, the formula $\text{GFR} = 100 / \text{serum cystatin C}$ was used to determine GFR [19].

At the end of experimental period, all animals were sacrificed by cervical elongation and dislocation in compliance with the institutional norms for the ethical treatment of laboratory animals. The kidneys and brain were extracted for histological analysis. Animal remains were disposed of in a biosecure and hygienic way.

Histopathological assessment

The kidney and brain tissues were dried and kept in 10% buffered formalin before being treated with xylol. Then, they were individually embedded in paraffin blocks. After that, 5 μ m paraffin-embedded sections were stained with hematoxylin and eosin (H&E) after they had been dewaxed. Finally, a light microscope fitted with 10, 20, 40, and 100 $\times$ lenses was used to examine the sections [20].

Statistical analysis

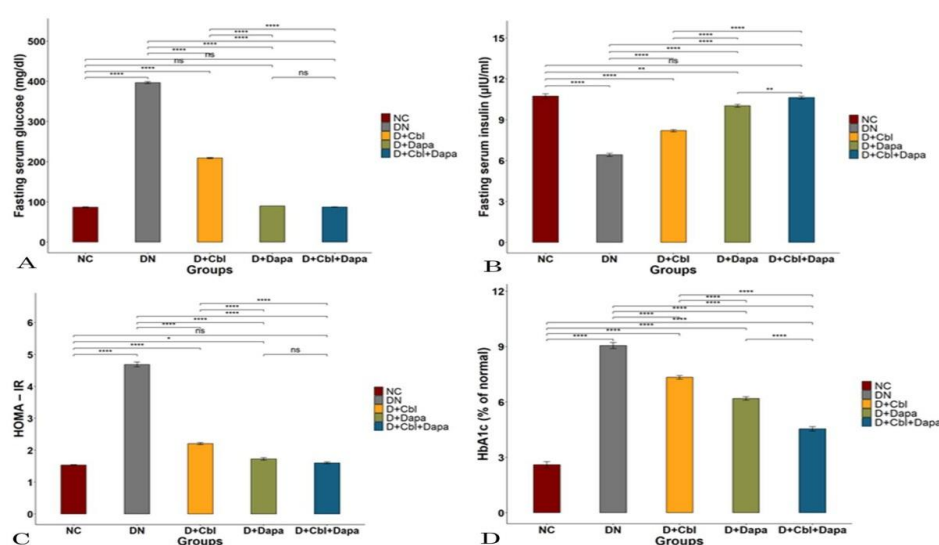
R software, version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria), was used for all statistical analyses. The data's normal distribution was ascertained using the Shapiro-Wilk test. In normal distribution, a one-way analysis of variance (ANOVA) and Tukey Honest Significant Difference (HSD) post hoc test were

used to compare quantitative variables. The mean of the normalized data $\pm$ standard error of the mean (SEM) was used to report the data. $P < 0.05$ was regarded as a statistically significant value.

3.RESULTS

3.1 Glycemic state parameters in all studied groups

The DN group revealed a significant increase ($P < 0.001$) in fasting serum glucose, HbA1c, and HOMA-IR levels with a significant decrease ($P < 0.001$) in serum insulin level compared to the NC group. Although cobalamin and dapagliflozin treatments each alone significantly improved these parameters, the best effect was achieved by their combination, as the D+Cbl+Dapa group exhibited a significant ($P < 0.001$) reduction in fasting serum glucose, HbA1c, and HOMA-IR levels with a significant ($P < 0.001$) elevation in serum insulin level compared to the DN group. The D+Cbl+Dapa group showed that fasting serum glucose and HOMA-IR levels were insignificantly changed ($P > 0.05$), while serum insulin level was significantly increased ($P < 0.001$) and HbA1c level was significantly reduced ($P < 0.001$) when compared to the D+Dapa group.



Figure(1): Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A) fasting serum glucose, (B) fasting serum insulin, (C) HOMA-IR, and (D) HbA1c in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the marks *, **, and **** indicate $P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively.

3.2 Serum lipid profile markers in all studied groups

Figure 2 showed that the DN group exhibited a significant ($P < 0.001$) increase in serum TC, serum TG, and serum LDL-C levels with a significant ($P < 0.001$) decrease in serum HDL-C level compared to the NC group. Although cobalamin and dapagliflozin treatments each alone significantly improved these parameters, the

D+Dapa showed significant improvement compared to the D+Cbl group. Notably, the combination treatment was more effective on the lipid profile than each individual treatment was, as the D+Cbl+Dapa group exhibited a significant ($P < 0.001$) decrease in serum TC, serum TG, and serum LDL-C with a significant ($P < 0.001$) increase in serum HDL-C compared to the D+Cbl and D+Dapa groups.

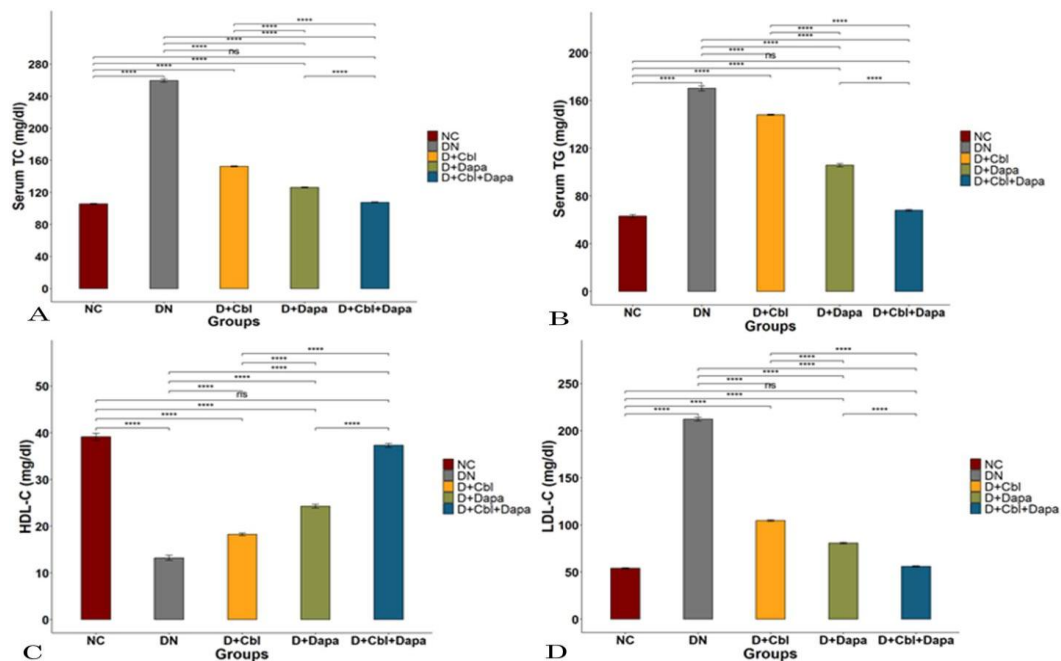


Figure (2): Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A) serum TC, (B) serum TG, (C) serum HDL-C, and (D) serum LDL-C in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the mark **** indicates $P < 0.0001$.

3.3 Renal function parameters in all studied groups

Figures 3 and 4 showed renal function of rats in all experimental groups. The DN group exhibited a significant ($P < 0.05$) elevation in serum urea, serum creatinine, urinary albumin, serum cystatin C, and UACR with a significant ($P < 0.05$) reduction in serum total proteins, GFR by cystatin C, and urinary creatinine when compared to the NC group. Cobalamin and dapagliflozin treatments each alone

significantly improved these parameters when compared to the DN group; however, the D+Dapa showed marked improvement compared to the D+Cbl group. Additionally, the D+Cbl+Dapa group showed a significant ($P < 0.05$) decrease in serum urea, serum creatinine, urinary albumin, serum cystatin C, and UACR with a significant ($P < 0.05$) elevation in serum total proteins, GFR by cystatin C, and urinary creatinine compared to the D+Cbl and D+Dapa groups.

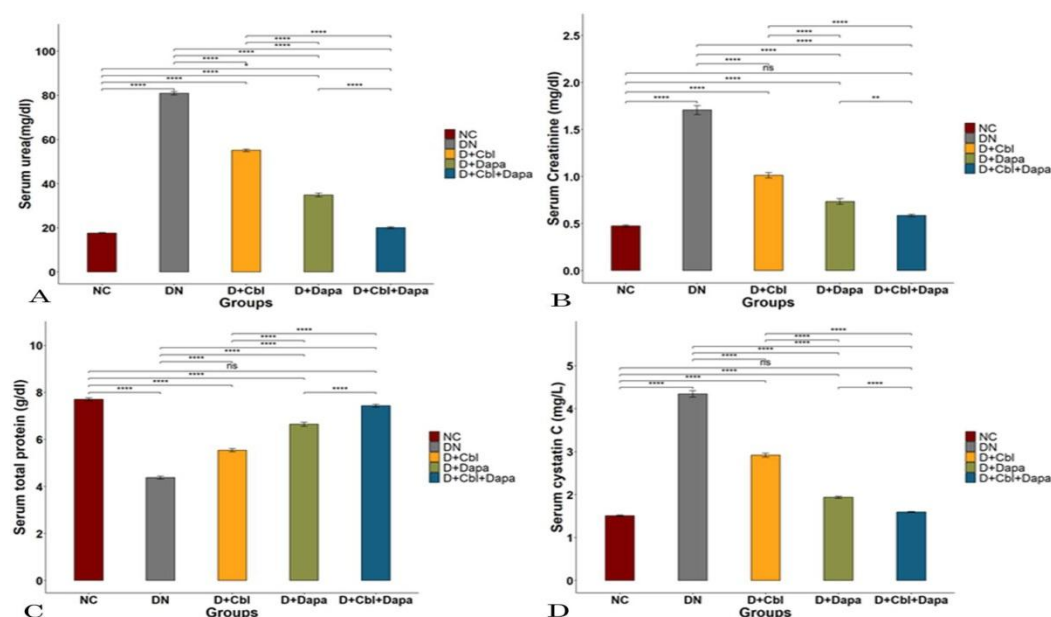


Figure (3): Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A)serum urea, (B) serum creatinine, (C) serum total proteins, and (D) serum cystatin C in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the marks ** and **** indicate $P < 0.01$ and $P < 0.0001$, respectively.

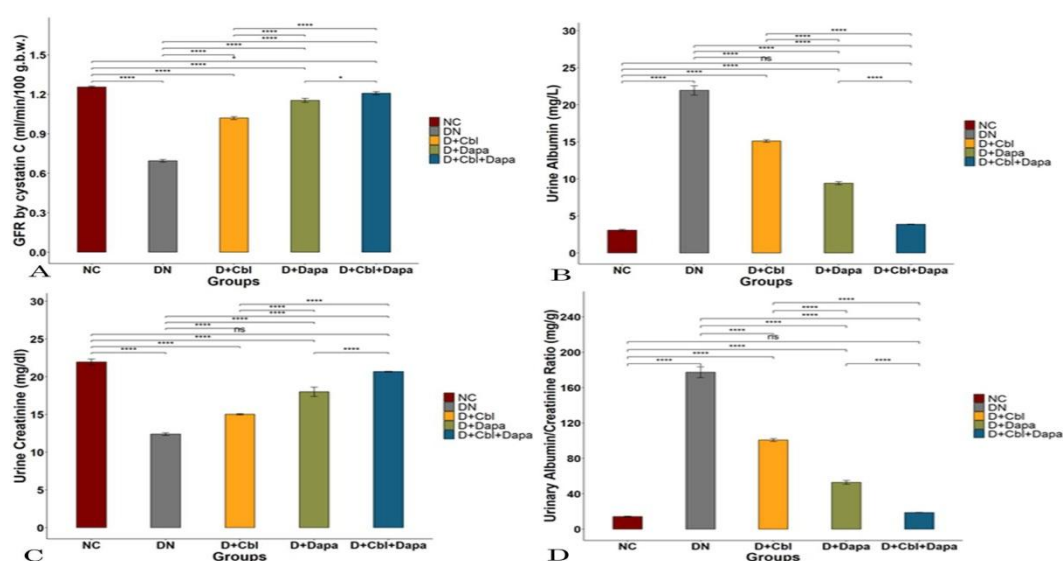


Figure (4):Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A)GFR by cystatin C, (B) urinary albumin, (C) urinary creatinine, and (D) urinary albumin/creatinine ratio in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the marks * and **** indicate $P < 0.05$ and $P < 0.0001$, respectively.

3.4 Serum interleukin-6 and TAC in all studied groups

Figure 5 showed that the DN group exhibited a significant ($P < 0.001$) increase in serum IL-6 level with a significant ($P < 0.001$) decrease in serum TAC level compared to the NC group. Treatment with cobalamin, dapagliflozin, or their

combination significantly improved these parameters, with the best effect achieved by their combination when compared to the DN group. However, the D+Dapa group exhibited a significant ($P < 0.001$) decrease in serum IL-6 level with an insignificant ($P > 0.05$) change in serum level of TAC when compared to the D+Cbl group.

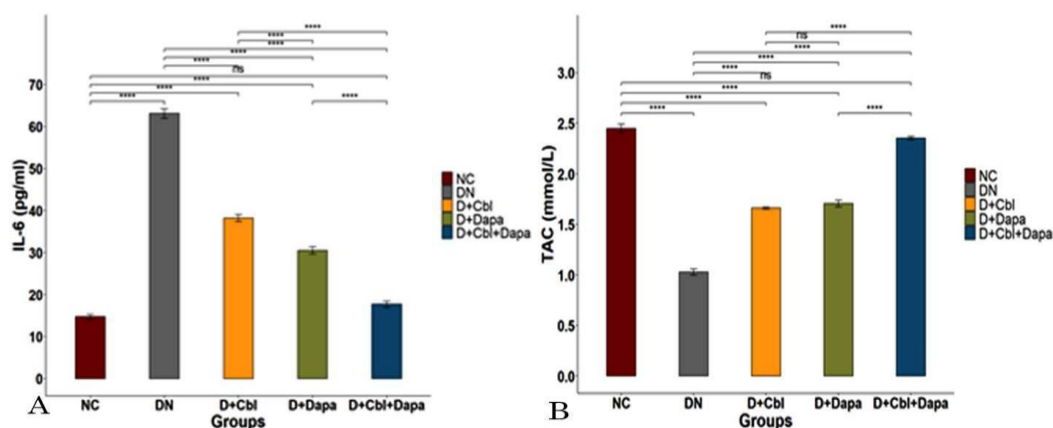


Figure (5): Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A) serum IL-6 and (B) serum TAC in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the mark **** indicates $P < 0.0001$.

3.5 Blood pressure in all studied groups

Figure 6 showed that the DN group exhibited a significant ($P < 0.001$) elevation in SBP, DBP, and MABP when compared to the NC group. In contrast, the D+Dapa and the D+Cbl+Dapa exhibited a significant ($P < 0.001$) reduction in these parameters, while the D+Cbl exhibited an

insignificant change ($P > 0.05$) when compared to the DN group. In the D+Cbl+Dapa group, these parameters were significantly lower ($P < 0.001$) when compared to the D+Cbl group, while they were insignificantly changed ($P > 0.05$) when compared to the D+Dapa group.

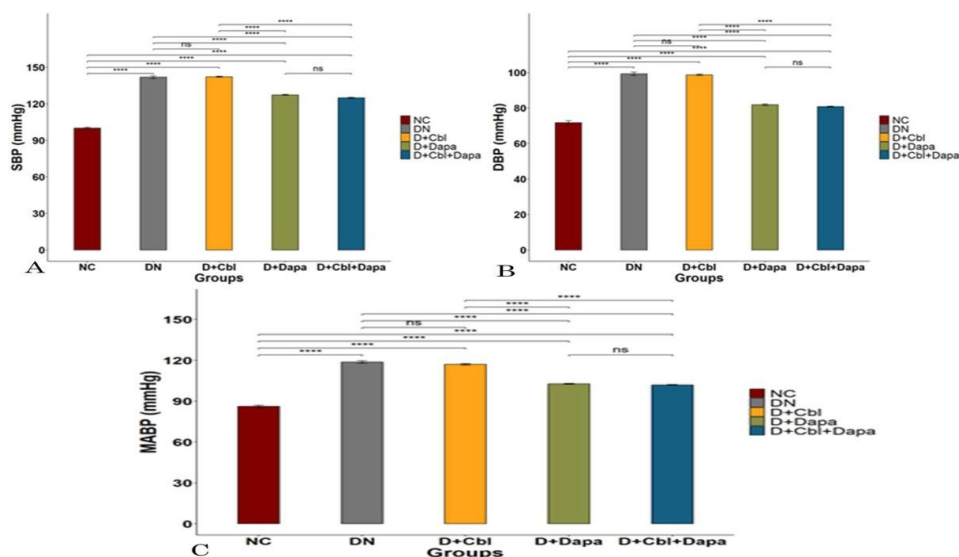


Figure (6): Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A) SBP, (B) DBP, and (C) MABP in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the mark **** indicates $P < 0.0001$.

3.6 Cognitive function parameters in all studied groups

Figure 7 showed cognitive functions of rats in all experimental groups. The DN group exhibited a

significant ($P < 0.001$) elevation in the escape latency of the probe trial of the Morris water maze test with a significant ($P < 0.001$) decrease in the discrimination index and the recognition index of

the novel object recognition test compared to the NC group. Although cobalamin and dapagliflozin treatments each alone significantly improved these parameters, the D+Dapa group showed significant improvement when compared to the D+Cbl group. Moreover, the combination treatment was more effective, as the D+Cbl+Dapa group

exhibited a significant ($P < 0.001$) reduction in the escape latency of the probe trial of the MWM test with a significant ($P < 0.001$) rise in the discrimination index and the recognition index of the NOR test compared to the D+Cbl and D+Dapa groups.

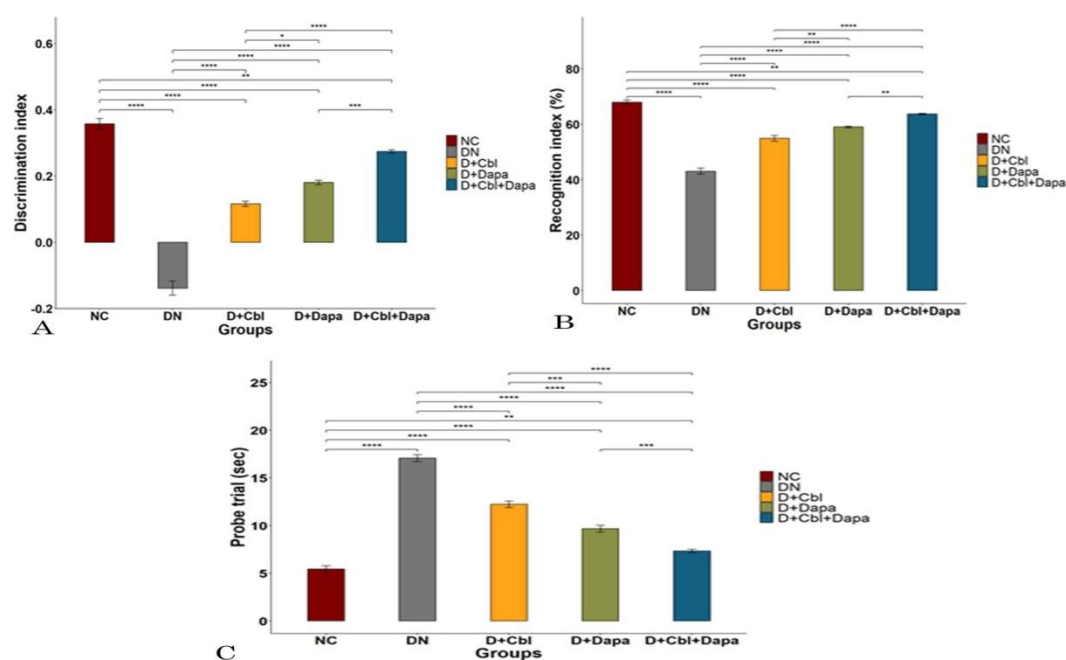


Figure (7): Effect of cobalamin, dapagliflozin, and combined cobalamin and dapagliflozin on (A) discrimination index, (B) recognition index, and (C) probe trial in all studied groups. Number of each group = ten. Results are represented as mean $\pm$ SEM. The significance level was set at $p < 0.05$. "ns" means non-significant, while the marks *, **, ***, and **** indicate $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

3.7 Histopathological examination in all studied groups

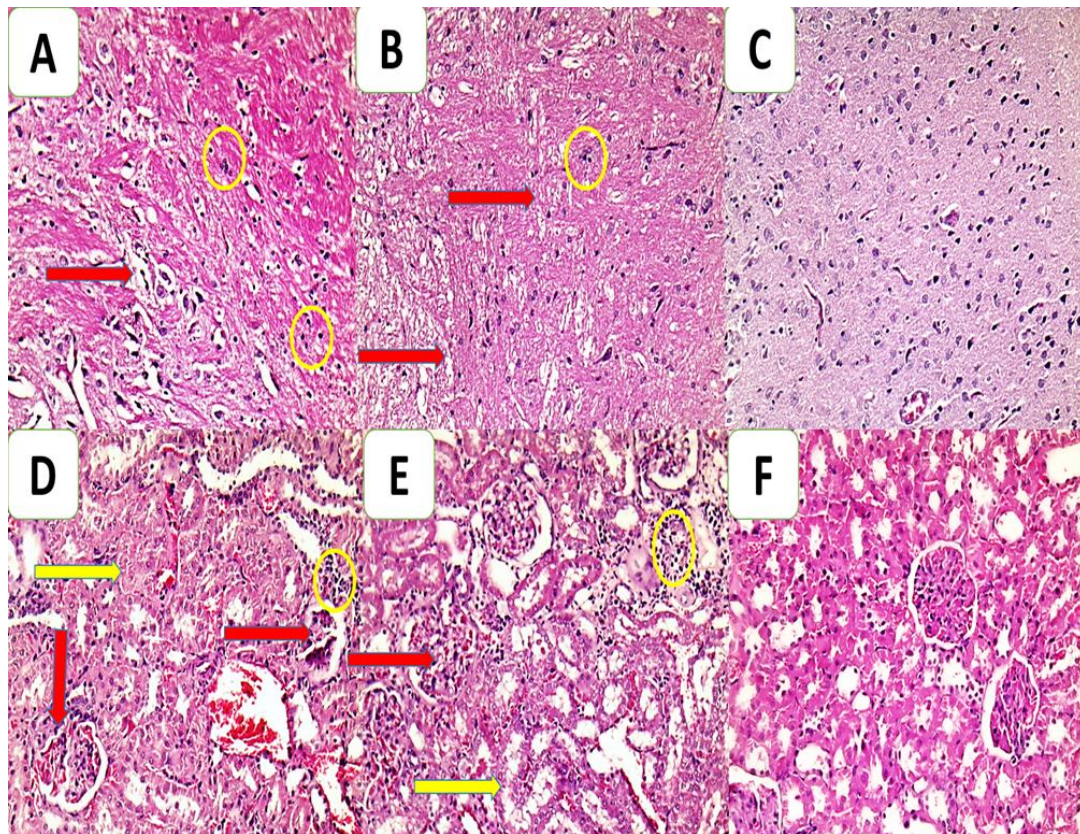
Histopathological examination of renal tissue from the DN group revealed histological alterations in the form of marked glomerular hypertrophy, glomerulosclerosis, marked tubular thickness and fibrosis with severe inflammation, and marked interstitial fibrosis. The D+Cbl group demonstrated marked improvement in histological pictures compared to the DN group. The renal interstitium showed moderate inflammation and fibrosis; however, most renal glomeruli showed moderate glomerular hypertrophy and

glomerulosclerosis. Moreover, the D+Dapa group exhibited significant improvement in histological images; the renal tissues displayed moderate fibrosis of the renal interstitium along with almost normal renal glomeruli and renal tubules. The D+Cbl+Dapa group's renal tubules and glomeruli were almost normal, indicating a remarkable improvement.

Histopathological examination of brain tissue from the DN group revealed histological alterations in the form of marked disorganization of the pyramidal cell layer with degenerative changes and a marked increase in apoptotic

cells. Shrunken pyramidal cells with highly pigmented basophilic cytoplasm and shrunken pyknotic nuclei were observed by the DN group. This was accompanied by severe inflammation, indicated by the presence of infiltrating lymphocytes within the affected regions. Compared to the DN group, the D+Cbl group revealed marked improvement in histological pictures. The pyramidal cell layer showed moderate organization. This was accompanied by moderate inflammation, degenerative changes, and a moderate decrease in apoptotic cells. The

D+Dapa group revealed significant improvement in histological pictures compared to the DN and D+Cbl groups. The pyramidal cell layer appeared quite normal and organized. There was a significant reduction in inflammation, with fewer lymphocytic infiltrations and a significant decrease in degenerative changes and apoptotic cells. The D+Cbl+Dapa group showed significant improvement in histological pictures. The pyramidal cell layer appeared quite normal and organized. Inflammation, degenerative changes, and apoptotic cells disappeared.



Cross sections of brain (A, B, C) and kidney tissue (H&E) (D, E, F) from studied groups:

(A) The DN group showed areas of marked neuronal degeneration, cellular disorganization, gliosis, and loss of structural integrity (red arrow), nuclear degeneration, and inflammation (yellow circle). (B) The D+Cbl group showed an area of moderate neuronal degeneration, loss of structural integrity (red arrow), and moderate nuclear degeneration and inflammation (yellow circle). (C) The D+Cbl+Dapa group showed nearly normal neuronal distribution with no inflammation (H&EX200). (D) The DN group showed an area of marked glomerular hypertrophy/sclerosis (red arrow), tubular thickness, cloudy swelling (yellow arrow), and interstitial inflammation (yellow circle). (E) The D+Cbl group showed an area of moderate glomerular hypertrophy/sclerosis (red arrow), tubular thickness, cloudy swelling (yellow arrow), and moderate interstitial inflammation (yellow circle). (F) The D+Cbl+Dapa group showed normal glomeruli with no inflammation (H&EX200).

DISCUSSION

Diabetes mellitus represents an important microvascular disease concerning the kidney and the brain. It is a chronic, metabolic illness marked by severe abnormalities in glucose metabolism with overproduction of free radicals and glycated proteins, which is responsible for the kidney and brain axis's negative consequences [21].

In the current investigation, the T2DM rat model was efficiently established using HFD combined with a small dosage of STZ injection, as demonstrated by a marked rise in fasting serum glucose, HOMA-IR, and HbA1c, with a marked drop in insulin levels. These results came in line with those of Hfaiedh et al. [21]. Type II diabetes has been shown to be brought on by a low dose of STZ combined with a high-fat diet. It is characterized by decreased insulin production and sensitivity, increased glycogenolysis, gluconeogenesis, and impaired glucose tolerance [22].

It is still necessary to thoroughly clarify the complex pathogenic link and precise regulation mechanisms between renal dysfunction and cognitive impairment. The prevalence of cognitive impairment and deficiencies in one or more important brain functions, such as memory and learning, varies according to the stage of chronic kidney disease (CKD), which is caused primarily by T2DM. Cognitive impairment starts early in chronic kidney disease and coincides with a loss in kidney function [23].

Diabetic nephropathy that developed in the DN group could be approved by a significant elevation in serum urea, serum creatinine, urinary albumin, serum cystatin C, and UACR, with a marked reduction in serum total proteins, GFR by cystatin

C, and urinary creatinine when compared to the NC group, and that was consistent with previous studies by Yang et al. [2]. Hyperglycemia, dyslipidemia, inflammation, and oxidative stress have all been directly or indirectly connected to the pathophysiology of diabetic nephropathy[9]. Also, it has been documented that systemic hypertension, through its additive glomerular hypertension, exacerbates nephropathy [24]. In diabetes mellitus, oxidative stress not only harms mesangial cells and the glomerular filtration barrier, but it also has an impact on renal hemodynamics and metabolism, resulting in renal interstitial fibrosis and proteinuria [2].

Also, cognitive functions were assessed in our investigation using MWM and NOR tests, and the DN group exhibited significant cognitive impairment when compared to the NC group. So, the DN group showed obvious symptoms of renal dysfunction and cognitive impairment, indicating a disturbed kidney-brain axis.

Patients with renal dysfunction frequently have endothelial dysfunction. Proteinuria leakage and decreased glomerular filtration are signs of poor endothelial function in the kidney. The inflammatory or oxidative response is amplified by uremic toxicity and other metabolic disorders, and this can either directly or indirectly damage the brain vasculature and hasten cognitive loss [23]. An earlier study found that high urea damaged the tight junction proteins and actin cytoskeleton in cultivated endothelial cells, which in turn caused the BBB to collapse [25]. Also, following endothelial dysfunction, HbA1c seeps out of the vessels and contributes to the pathophysiology of renal disease. Remarkably, CKD patients are more

likely to have poorer memory and executive function if their HbA1c levels are higher [23].

The Alzheimer's disease (AD) subtype may be predicted by albuminuria, a sign of aberrant kidney function that is also linked to cognitive impairment. Also, total brain shrinkage was substantially correlated with both elevated UACR and decreased eGFR levels [26]. In addition, a previous study revealed that people with renal dysfunction are more likely to have poor cognitive attention and executive function performance if their serum cystatin C level is higher [27].

Following cobalamin and dapagliflozin treatment, the kidney-brain axis, which was significantly compromised by diabetes, demonstrated a noteworthy functional recovery, as when compared to the DN group, we observed that the D+Cbl and D+Dapa groups exhibited a significant decrease in fasting serum glucose, HOMA-IR, and HbA1c, with a marked elevation in insulin levels. These results aligned with earlier observations [28, 29]. Cobalamin supplementation may improve glycemic control and insulin sensitivity in diabetics [30]. This can be explained by the dominant role of cobalamin in the utilization of carbohydrates and its ability to mitigate oxidative damage [28]. In addition, dapagliflozin inhibited the renal reabsorption of glucose, thereby acting in an insulin-independent manner by preventing glucose toxicity. Also, by downregulating the hepatic glucagon receptors, hepatic glucagon signaling is suppressed. Beta cells may be directly impacted, and other hormones, including glucagon-like peptide-1, may also be impacted [31].

Furthermore, the DN group exhibited dyslipidemia, as evidenced by marked elevations

in TG, TC, and LDL-C levels, as well as a significant drop in HDL-C. Following our findings, other studies have revealed altered lipid profiles in diabetic rats [9, 22]. Diabetic dyslipidemia increases ROS generation, activates inflammatory pathways, causes insulin resistance, and promotes macrovascular and microvascular consequences [32].

Conversely, the D+Cbl and D+Dapa groups exhibited significant modulation in lipid profile markers compared to the DN group. These findings were in harmony with previously documented findings [29, 33, 34]. According to Boachie et al., cobalamin has anti-hyperlipidemic properties, and because it contributes to the conversion of methyl malonyl-CoA (MM-CoA) to succinyl-CoA, its deficiency is linked to an altered lipid profile and metabolic diseases. A deficit in cobalamin impedes this process, causing MM-CoA to accumulate. This inhibits the rate-limiting enzyme of fatty acid oxidation (CPT1—carnitine palmitoyl transferase), resulting in lipogenesis [35]. In like manner, dapagliflozin had a positive effect on the lipid profile because it induces a metabolic switch from glucose utilization to fatty acid oxidation [36].

Another risk factor for cognitive impairment, inflammation, contributes significantly to the cognitive decline linked to renal dysfunction. TNF, transforming growth factor- β , and IL-6 are most commonly linked to the pathophysiology of chronic kidney disease (CKD) and have been shown to play a role in cognitive dysfunction. High levels of IL-6 were also linked to degradation of GABAergic interneurons and poor executive function [26].

Also, it has been shown that oxidative stress has a significant role in neurodegenerative illnesses, such as AD, as it was found that an increase in reactive oxygen species (ROS) generation and a decrease in antioxidant function had a direct impact on synaptic activity and neurotransmission. Excessive oxidative stress results in the formation of A β , a significant pathophysiological feature of cognitive impairment. Oxidative stress also is common in CKD, as the kidney is a metabolic organ that is susceptible to damage from oxidative stress because of the abundance of oxidation reactions in its mitochondria [23].

In the current investigation, the DN group displayed a marked reduction in serum TAC with a marked increase in serum IL-6. Because it creates free radicals through the polyol pathway, non-enzymatic glycation of proteins, and glucose autooxidation, hyperglycemia results in oxidative stress and can cause diabetes complications [37]. Oxidative stress caused by hyperglycemia and dyslipidemia generates ROS, which activates various inflammatory signaling cascades that exacerbate inflammation and promote systemic insulin resistance [32,37].

In contrast, monotherapy with cobalamin and dapagliflozin significantly enhanced serum TAC levels and significantly reduced serum IL-6. Cobalamin has been demonstrated to possess antioxidant and anti-inflammatory characteristics, as it can directly scavenge ROS, specifically reducing superoxide levels. Also, it can indirectly neutralize ROS through modulating glutathione levels. As well as it can modulate the activity of transcription factors, including NF- κ B [6]. Furthermore, dapagliflozin was found to

downregulate several cytokines, such as TNF- α , IL-1 β , and IL-6, by reducing ROS generation [38]. Also, the current study revealed that there was significant elevation of SBP, DBP, and MABP in the DN group when compared to the NC group. These findings were consistent with those previously documented [39]. However, dapagliflozin in diabetic rats significantly reduced SBP, DBP, and MABP. Our findings were in harmony with those of Saleh et al., who found that dapagliflozin provided significant cardiovascular protection and reduced elevated blood pressure in the HFD/STZ rat model. This could be because dyslipidemia, the source of persistent low-grade inflammation on the vascular endothelium that leads to hypertension, was improved, as well as through osmotic diuresis [39].

In contrast, the D+Cbl group showed no improvement in blood pressure results. This finding was also noted by Hajihashemi et al., who found that cobalamin did not affect blood pressure but might promote vasodilatation and increase blood flow in the kidneys because cobalamin is responsible for producing or providing the necessary components for NOS [7].

In line with other research [7, 28], the D+Cbl group demonstrated a notable improvement in renal function tests, which can be attributed to its anti-inflammatory and antioxidant characteristics along with its amelioration of hyperglycemic and lipidemic states. Moreover, dapagliflozin therapy enhanced renal function by inhibiting inflammation, fibrosis, and apoptosis, as well as lowering lipid buildup in the kidneys of HFD-fed rats [11].

Also, cobalamin supplementation reversed the HFD/STZ-induced cognitive impairment in rats.

Our findings were in harmony with another study [34] where they found that supplementation of cobalamin reversed the HFD-induced memory deficit and anxiety behavior patterns in rats. Furthermore, dapagliflozin demonstrated significant neuroprotection in our study, which is consistent with Sa-Nguanmoo, who stated that dapagliflozin may restore cognitive function by improving insulin sensitivity and decreasing brain oxidative stress. This leads to enhanced brain mitochondrial function, as well as decreased brain inflammation and apoptosis, resulting in greater hippocampal synaptic plasticity [40]. Additionally, a prior study found that an SGLT2 inhibitor could boost brain-derived neurotrophic factor, a key modulator of synaptogenesis and synaptic plasticity processes that are critical for memory and learning in the brain [41].

The reduction in renal function was linked with a changed renal histopathological picture, including glomerular hypertrophy, glomerulosclerosis, tubular thickness, and fibrosis, as well as marked inflammation and renal interstitial fibrosis in the DN group. In diabetic rats' kidneys, Jain and Saha et al. noticed thickening of the capillary basement membrane with mesangial hypercellularity. These findings were ascribed to elevated oxidative stress, RAS activation, and the generation of inflammatory cytokines and growth factors. Hyperglycemia and hyperfiltration can also result in classic glomerulosclerosis accompanied by mesangial nodular lesions [42]. Cobalamin ameliorated histopathological alterations of kidney tissue in the D+Cbl group. These findings supported Li et al.'s conclusion that cobalamin reduced kidney damage. [43]. Nevertheless, in the D+Dapa group, dapagliflozin markedly improved

and restored renal histological structure. In addition to its glycemic management, anti-inflammatory, and antioxidant characteristics, dapagliflozin is expected to have direct renoprotective benefits by lowering glomerular capillary pressure and hyperfiltration [2].

Moreover, our histopathological findings regarding brain tissue in the DN group were consistent with those of Kabay et al., who revealed that STZ resulted in areas of neuronal necrosis, damaged blood vessels, and hemorrhagic spots, as well as pyramidal cell degeneration in the diabetic rats. These results may be related to the harmful consequences of oxidative stress and hyperglycemia [44]. In contrast, brain tissue from the D+Cbl group showed considerable improvement in histological pictures. This agreed with Saeedet al., who revealed that cobalamin increases the thickness of the pyramidal cell layer, which restores its normal arrangement and decreases the number of pyknotic and shrunken cells [45]. Nevertheless, dapagliflozin showed good improvement of histological pictures. The pyramidal cell layer appeared quite normal and organized. There was a marked reduction in inflammation, with fewer lymphocytic infiltrations and a marked decrease in degenerative changes and apoptotic cells. Our results were in harmony with another study [46] where they found that dapagliflozin significantly improved capillary congestion, tangles, and plaques while reducing pyknotic cells.

In comparison to the DN, D+Cbl, or D+Dapa groups, diabetic rats treated with cobalamin and dapagliflozin together showed significant improvements in their glycemic status, inflammatory state, oxidative stress, renal

functions, and cognitive functions. The combination of both medications' hypoglycemic, antioxidant, and anti-inflammatory characteristics explains the improvement in every previously evaluated indicator.

limitations

Immunohistochemical staining was not possible due to financial constraints. Including such analysis would have reinforced the study's conclusions and given a more thorough knowledge of the underlying cellular mechanisms.

Conclusion

Both cobalamin and dapagliflozin improve the renal and neurocognitive impairments seen in type 2 diabetes. The hypoglycemic, anti-inflammatory, and antioxidant properties are most likely in play. Dapagliflozin combined with cobalamin significantly improved diabetic rats' glycemic status, renal functions, and cognitive functions. This improvement was greater than that of employing each of them separately.

REFERENCES

1. Alam U, Asghar O, Azmi S, and Malik RA. General aspects of diabetes mellitus. *Handbook of clinical neurology* 126: 211–222, 2014.
2. Yang H, Mei Z, Chen W, Pan Y, Liu L, Zhao R, Ni W, Wang Y, and Fei C. Therapeutic efficacy of dapagliflozin on diabetic kidney disease in rats. *International immunopharmacology* 113: 109272, 2022.
3. Tian X, Liu Y, Ren G, Yin L, Liang X, Geng T, Dang H, and An R. 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, 2016.
4. Ariton DM, Jiménez-Balado J, Maisterra O, Pujadas F, Soler MJ, and Delgado P. Diabetes, albuminuria and the kidney—brain axis. *Journal of clinical medicine* 10: 2364, 2021.
5. El-Safty H, Ismail A, Abdelsalam RM, El-Sahar AE, and Saad MA. Dapagliflozin diminishes memory and cognition impairment in Streptozotocin induced diabetes through its effect on Wnt/ β -Catenin and CREB pathway. *Brain Research Bulletin* 181: 109–120, 2022.
6. van de Lagemaat EE, de Groot LC, and van den Heuvel EG. Vitamin B12 in relation to oxidative stress: a systematic review. *Nutrients* 11: 482, 2019.
7. Hajhashemi S, Hamidizad Z, Rahbari A, Ghanbari F, and Motealeghi ZA. Effects of cobalamin (Vitamin B12) on gentamicin induced nephrotoxicity in rat. *Drug research* 67: 710–718, 2017.
8. Srinivasan K, Viswanad B, Asrat L, Kaul C, and Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological research* 52: 313–320, 2005.
9. Ni Z, Guo L, Liu F, Olatunji OJ, and Yin M. *Allium tuberosum* alleviates diabetic nephropathy by suppressing hyperglycemia-induced oxidative stress and inflammation in high fat diet/streptozotocin treated rats. *Biomedicine & pharmacotherapy* 112: 108678, 2019.
10. Hosseinzadeh H, Moallem S, Moshiri M, Sarnavazi M, and Etemad L. Anti-nociceptive and anti-inflammatory effects of

- cyanocobalamin (vitamin B12) against acute and chronic pain and inflammation in mice. *Arzneimittelforschung* 62: 324–329, 2012.
11. **Jaikumkao K, Pongchaidecha A, Chueakula N, Thongnak L, Wanchai K, Chatsudthipong V, Chattipakorn N, and Lungkaphin A.** Renal outcomes with sodium glucose cotransporter 2 (SGLT2) inhibitor, dapagliflozin, in obese insulin-resistant model. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1864: 2021–2033, 2018.
 12. **Azza A, Ahmed H, El-Samea H, and El-Demerdash E.** The potential effect of caffeine and nicotine co-administration against aluminum-induced Alzheimer's disease in Rats. *J Alzheimers Dis Parkinsonism* 6: 2161–0460.100023, 2016.
 13. **Zou W, Yuan J, Tang Z-J, Wei H-J, Zhu W-W, Zhang P, Gu H-F, Wang C-Y, and Tang X-Q.** Hydrogen sulfide ameliorates cognitive dysfunction in streptozotocin-induced diabetic rats: involving suppression in hippocampal endoplasmic reticulum stress. *Oncotarget* 8: 64203, 2017.
 14. **Das K, Kundu A, Karmakar S, and Ghosh M.** Novel structured diacylglycerol (DAG) rich oleo formulations activate the Nrf2 pathway and impedes NF- κ B translocation to mitigate pre-clinical conditions of hypertension. *Journal of Functional Foods* 64: 103650, 2020.
 15. **Oraby MA, El-Yamany MF, Safar MM, Assaf N, and Ghoneim HA.** Dapagliflozin attenuates early markers of diabetic nephropathy in fructose-streptozotocin-induced diabetes in rats. *Biomedicine & Pharmacotherapy* 109: 910–920, 2019.
 16. **Matthews DR, Hosker JP, Rudenski AS, Naylor B, Treacher DF, and Turner R.** Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *diabetologia* 28: 412–419, 1985.
 17. **Friedewald WT, Levy RI, and Fredrickson DS.** Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry* 18: 499–502, 1972.
 18. **Erman A, Rahamimov R, Mashraki T, Levy-Drummer RS, Winkler J, David I, Hirsh Y, Gafer U, and Chagnac A.** The urine albumin-to-creatinine ratio: assessment of its performance in the renal transplant recipient population. *Clinical Journal of the American Society of Nephrology* 6: 892–897, 2011.
 19. **Perkins BA, Nelson RG, Ostrander BE, Blouch KL, Krolewski AS, Myers BD, and Warram JH.** Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: results of a 4-year follow-up study. *Journal of the American Society of Nephrology* 16: 1404–1412, 2005.
 20. **Slaoui M, and Fiette L.** Histopathology procedures: from tissue sampling to histopathological evaluation. In: *Drug Safety Evaluation: Methods and Protocols* Springer, 2010, p. 69–82.
 21. **Hfaiedh N, Mbarki S, Alimi H, Murat JC, and Elfeki A.** Diabetes-induced damages in rat kidney and brain and protective effects of natural antioxidants. 2013.
 22. **Bin-Jumah MN.** Antidiabetic effect of monollumaquadrangula is mediated via modulation of glucose metabolizing enzymes,

- antioxidant defenses, and adiponectin in type 2 diabetic rats. *Oxidative medicine and cellular longevity* 2019: 6290143, 2019.
23. **Xie Z, Tong S, Chu X, Feng T, and Geng M.** Chronic kidney disease and cognitive impairment: the kidney-brain axis. *Kidney Diseases* 8: 275–285, 2022.
 24. **Tomohiro T, Kumai T, Sato T, Takeba Y, Kobayashi S, and Kimura K.** Hypertension aggravates glomerular dysfunction with oxidative stress in a rat model of diabetic nephropathy. *Life Sciences* 80: 1364–1372, 2007.
 25. **Lau WL, Nunes AC, Vasilevko V, Florioli D, Lertpanit L, Savoj J, Bangash M, Yao Z, Shah K, and Naqvi S.** Chronic kidney disease increases cerebral microbleeds in mouse and man. *Translational stroke research* 11: 122–134, 2020.
 26. **Yan Q, Liu M, Xie Y, Lin Y, Fu P, Pu Y, and Wang B.** Kidney-brain axis in the pathogenesis of cognitive impairment. *Neurobiology of disease* 200: 106626, 2024.
 27. **Yaffe K, Kurella-Tamura M, Ackerson L, Hoang TD, Anderson AH, Duckworth M, Go AS, Krousel-Wood M, Kusek JW, and Lash JP.** Higher levels of cystatin C are associated with worse cognitive function in older adults with chronic kidney disease: the chronic renal insufficiency cohort cognitive study. *Journal of the American Geriatrics Society* 62: 1623–1629, 2014.
 28. **Mohamed FE-Z, El-Taweel HM, Alattar RH, Khamis T, Abdel-Aziz A-AF, and El-Dawy K.** The Metabolic Impact of Vitamin B12 in The Context of Metabolic Syndrome. *Journal of Advanced Veterinary Research* 13: 1305–1312, 2023.
 29. **Xue S, Li Y-X, Lu X-X, and Tang W.** Dapagliflozin can alleviate renal fibrosis in rats with streptozotocin-induced type 2 diabetes mellitus. *Experimental and Therapeutic Medicine* 26: 572, 2023.
 30. **Didangelos T, Karlafti E, Kotzakioulafi E, Margariti E, Giannoulaki P, Batanis G, Tesfaye S, and Kantartzis K.** Vitamin B12 supplementation in diabetic neuropathy: a 1-year, randomized, double-blind, placebo-controlled trial. *Nutrients* 13: 395, 2021.
 31. **Millar P, Pathak N, Parthasarathy V, Bjourson AJ, O’Kane M, Pathak V, Moffett RC, Flatt PR, and Gault VA.** Metabolic and neuroprotective effects of dapagliflozin and liraglutide in diabetic mice. *Journal of Endocrinology* 234: 255–267, 2017.
 32. **Al-Trad B, Alkhateeb H, Alsmadi W, and Al-Zoubi M.** Eugenol ameliorates insulin resistance, oxidative stress and inflammation in high fat-diet/streptozotocin-induced diabetic rat. *Life sciences* 216: 183–188, 2019.
 33. **Bhattacharjee S.** Antilipidemic and cardioprotective effects of vitamin B12 and folic acid against arsenic toxicity. 2014.
 34. **Maryoud AM, Elghaba R, Gad GEM, El-desouky S, and Adel M.** Studying the effect of vitamin B12 and folic acid on the levels of brain-derived neurotrophic factor in the blood and the brain of obese male rats. *Bulletin of Egyptian Society for Physiological Sciences* 43: 142–154, 2023.
 35. **Boachie J, Adaikalakoteswari A, Samavat J, and Saravanan P.** Low vitamin B12 and lipid metabolism: evidence from pre-clinical and clinical studies. *Nutrients* 12: 1925, 2020.
 36. **Ahmed WS, Soliman A, Amer AA, El Shahat R, Amin M, Taha R, Awad M, Hamid AA,**

- El-Sayed M, and Eid E.** Effect of dapagliflozin against NAFLD and dyslipidemia in type 2 diabetic albino rats: possible underlying mechanisms. *European Review for Medical & Pharmacological Sciences* 27: 2023.
37. **Samarghandian S, Borji A, and Farkhondeh T.** Attenuation of oxidative stress and inflammation by *Portulaca oleracea* in streptozotocin-induced diabetic rats. *Journal of evidence-based complementary & alternative medicine* 22: 562–566, 2017.
38. **Chi P-J, Lee C-J, Hsieh Y-J, Lu C-W, and Hsu B-G.** Dapagliflozin ameliorates lipopolysaccharide related acute kidney injury in mice with streptozotocin-induced diabetes mellitus. *International Journal of Medical Sciences* 19: 729, 2022.
39. **Saleh S, Hanna G, El-Nabi SH, El-Domiaty H, Shabaan A, and Ewida SF.** Dapagliflozin, a sodium glucose cotransporter 2 inhibitors, protects cardiovascular function in type-2 diabetic murine model. *Journal of Genetics* 99: 46, 2020.
40. **Sa-Nguanmoo P, Tanajak P, Kerdphoo S, Jaiwongkam T, Pratchayasakul W, Chattipakorn N, and Chattipakorn SC.** SGLT2-inhibitor and DPP-4 inhibitor improve brain function via attenuating mitochondrial dysfunction, insulin resistance, inflammation, and apoptosis in HFD-induced obese rats. *Toxicology and applied pharmacology* 333: 43–50, 2017.
41. **Lin B, Koibuchi N, Hasegawa Y, Sueta D, Toyama K, Uekawa K, Ma M, Nakagawa T, Kusaka H, and Kim-Mitsuyama S.** Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovascular diabetology* 13: 148, 2014.
42. **Jain D, and Saha S.** Antioxidant and antihyperglycaemic effects of naringenin arrest the progression of diabetic nephropathy in diabetic rats. *Egyptian Pharmaceutical Journal* 16: 144–151, 2017.
43. **Li F, Bahnson EM, Wilder J, Siletzky R, Hagaman J, Nickeleit V, Hiller S, Ayesha A, Feng L, and Levine JS.** Oral high dose vitamin B12 decreases renal superoxide and post-ischemia/reperfusion injury in mice. *Redox biology* 32: 101504, 2020.
44. **Kabay SC, Ozden H, Guven G, Ustuner MC, Degirmenci I, Olgun EG, and Unal N.** Protective effects of vitamin E on central nervous system in streptozotocin-induced diabetic rats. *Clinical and Investigative Medicine* 32: E314–E321, 2009.
45. **Saeed AA, Harf MAEA, Bdeer SE-A, and Elsherbini HA.** A Study on the possible protective effects of angiotensin II type I receptor blocker (telmisartan) versus vitamin B12 on male albino rat model of Alzheimer. *Zagazig University Medical Journal* 30: 3540–3552, 2024.
46. **Samman WA, Selim SM, El Fayoumi HM, El-Sayed NM, Mehanna ET, and Hazem RM.** Dapagliflozin ameliorates cognitive impairment in aluminum-chloride-induced Alzheimer's disease via modulation of AMPK/mTOR, oxidative stress and glucose metabolism. *Pharmaceuticals* 16: 753, 2023.