Article

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DPP-4 Inhibitor Improved Social Behavior in Acute Kidney Injury in a Murine Model: Possible Role in Mitochondrial Functions

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Background: Kidney disease is usually complicated by multiple organ dysfunctions, such as cognitive impairment and neuropathy. Dipeptidyl peptidase-4 (DPP-4) inhibitors decrease the degradation of glucagon-like peptide-1 (GLP-1), improving glycemic control. This study investigated the effect of acute kidney injury (AKI) on social interaction and investigated the underlying role of inflammation, altered energetics, and the possible mode of action of a DPP-4 inhibitor on the brain in AKI.

Methods: Forty rats constituted the animal model and were distributed into four groups (control, untreated, and treated AKI groups). We evaluated sociability; social novelty preferences in a three-chamber social apparatus; platelet counts; hippocampal mitochondrial enzyme complex (I–V) content by calorimetric methods; serum urea, blood urea nitrogen (BUN), and creatine phosphokinase levels by enzyme-linked immunoassay (ELISA); hippocampal adenosine triphosphate (ATP) content measured by ELISA; hippocampal glial fibrillary acidic protein (GFAP) expression; activity-regulated cytoskeleton-associated protein (Arc); Toll-like receptor 4 (TLR4) expression; and nuclear factor kappa B (NF-κB) expression by real-time polymerase chain reaction (RT–PCR).

Results: The sociability and social novelty indices, all hippocampal mitochondrial complexes (I to V), and platelet, ATP content, were significantly (p value $\leq$ 0.05) lower in the AKI group than in the control group. Serum creatinine, BUN, and creatine phosphokinase (CPK) levels, the relative expression of hippocampal GFAP, Arc, TLR4, and NF-κB were significantly (p value $\leq$ 0.05) increased in the AKI control group compared to those in the control group. Sections from the hippocampal cornu ammonis (CA) 1 (CA1) and 3 (CA3) regions and CA3 regions showed degeneration of pyramidal cells with microglial cell in filtration and the appearance of congested blood capillaries. Vildagliptin exerted a protective effect on uremic encephalopathy induced by AKI, as revealed by social behavior and biochemical measurements in the serum and hippocampus and confirmed by histological examination of the CA1 and CA3 hippocampal areas. The statistical significance was stated in parallel with intergroup variability.

Conclusions: The present study showed the protective effect of vildagliptin on uremic encephalopathy induced by AKI, as revealed by social behavior and biochemical measurements in the serum and hippocampus and confirmed by histological examination of the CA1 and CA3 hippocampal areas. This improvement was attributed to improved mitochondrial function, which positively affects the energetics of the brain, attenuates the inflammatory response and alters the expression of synaptic proteins.

Keywords: hippocampus; AKI; DPP-4 inhibitor; social behavior; synaptic plasticity

Introduction

The most common cause of remote organ dysfunction in critically ill patients is acute kidney injury (AKI), which is characterized by a sudden loss of renal function and the retention of nitrogenous wastes such as urea and creatinine [1]. According to Malek *et al.* [2], extrarenal adverse effects and the involvement of other distant organs, rather than renal failure, are the main causes of the high mortality rate linked with kidney diseases in acute and chronic conditions. Doi and Rabb [3] reported that acute and chronic kidney injuries may impact the central nervous system (CNS). Parts of the brain known as the social brain play a role in social cognition. The hippocampus is one of those areas. The hippocampus is a crucial CNS region for memory consolidation and learning. Following AKI, soluble inflammatory protein synthesis has been linked to hippocampal cellular inflammation [4].

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Recent studies have indicated that injury to one organ may impact other organs. In some cases, peripheral injury during surgery induces an inflammatory response in the hippocampus and affects behavior [5]. AKI additionally triggers cell death within the hippocampus. The retention of nitrogenous end products (uremic toxins), an imbalance in osmolality, and inflammation are among the known potential factors that contribute to brain involvement after AKI. The endothelial vascular injury, neurotoxicity, and cognitive decline associated with the decreased renal clearance of nitrogenous waste products are caused by a variety of uremic retention products, including urea, creatinine, guanidine, and homocysteine [6]. Reactive oxygen species (ROS) are generated when plasma osmolality and serum sodium levels are elevated in the brain, which triggers endothelial damage, disruption of the blood–brain barrier (BBB), and disruption of brain transporters [7].

Following renal ischemia–reperfusion, the expression of the nuclear factor kappa B (NF-κB) pathway and the antiapoptotic gene B-cell lymphoma 2 (Bcl-2) decreases in the brain, indicating that brain tissue is undergoing apoptosis as a consequence of AKI [8].

Following AKI, the hippocampus exhibited evidence of ROS, nitric oxide (NO), and inflammatory mediators. Elevated levels of these substances in the brain after AKI are reportedly associated with neuronal cytotoxicity and apoptosis [8]. Cognitive impairment appears to indicate abnormalities in the structure or function of the hippocampus. According to Barzilay *et al*. [9], albuminuria impairs glomerular endothelial function and increases the risk of diminished cognition. Unfortunately, only a few studies in this field of research can explain exactly how AKI and cognition are related. Dipeptidyl peptidase-4 (DPP-4) inhibition produced renoprotective effects in an experimental model of renal injury induced by vildagliptin (VG), which was mediated by antiapoptotic, anti-inflammatory and antioxidative effects [10]. The administration of VG also significantly reduced abnormal biochemical marker levels and histopathological changes [11].

These findings indicate that by preventing organ crosstalk, therapies that drastically reduce the impact of damage to one organ system may enhance the outcome of another system. Therefore, the present study investigated whether peripheral organ-targeting therapies ameliorate brain injury by reflecting extracranial renal injury through crosstalk between these two organs or by predicting brain injury.

Materials and Methods

Hashemite University's Institutional Review Board granted clearance for the study's protocols, with lot number 5/7/2020/2021 received on 8/3/2021. This study utilized a randomized controlled animal experimental design, with baseline characterization conducted between the control and model groups. Before the trial, 40 mature male albino rats weighing 120–125 g were housed for 15 days in Hashemite University's animal house in Zarqa, Jordan, to allow for acclimatization and to rule out any infections. The rats were kept in plastic cages with three rats per cage and well-ventilated covers under standard circumstances of relative humidity (45%) and 12-hour cycles of light and dark. The rats were fed a daily laboratory rat diet made available *ad libitum* and had free access to water.

We made all efforts to ameliorate harm to animals by housing them in three per cages to avoid isolation stress and maintain all infection control measures. The duration of the procedures was short, and the lowest dose of chemicals that were proven by previous studies was used to exert the desired action. We also considered euthanasia at the end of the study.

Animal Grouping

The rats were divided into two major groups at random:

A-The negative control group (group I): A total of ten rats.

B-Acute kidney injury (AKI) groups: Thirty rats were given intramuscular (5 mL/kg) injections of 50% glycerol (W252506, Sigma Aldrich Co Pvt Ltd, St. Louis, MO, USA) [12]. At least half of the dose was injected into an alternate muscle in the hind limb. The rats were not provided access to water for 24 hours before glycerol injection. Each conscious rat was given an injection and then housed in cages with free access to food and water. Rats with AKI were randomly divided; we standardized the duration of animal manipulation and handling to lessen randomization bias, and the numbers assigned to animals were generated using an online random sequence generator (https://www.randomizer.org/). The rats were divided into the following three groups (n = 10 rats/group):

The untreated AKI control (group II) was injected with glycerol (CAS Number: 56-81-5, Sigma-Aldrich, Germany) at a dose of 5 mL/kg 50% glycerol [12] without further treatment; however, 3 ml of distilled water was administered orally (vehicle).

The AKI + saline (group III) group received 1.5 mL of normal saline (CAS number 6251615000846, Pharmacological Solutions Industry, Saudi Arabia) by subcutaneous injection at the time of glycerol injection (5 mL/kg of 50% glycerol) and then every 12 hours for 72 hours [13].

The AKI + vildagliptin (group IV) Treatment with daily oral vildagliptin 10 mg/kg (CAS number 62517002725, Galvus, Novartis Pharma Stein AG, Stein, Switzerland) [11] started 1 hour after an injection of glycerol (5 mL/kg of 50% glycerol) and continued with the same dose every 24 hours for 72 hours.

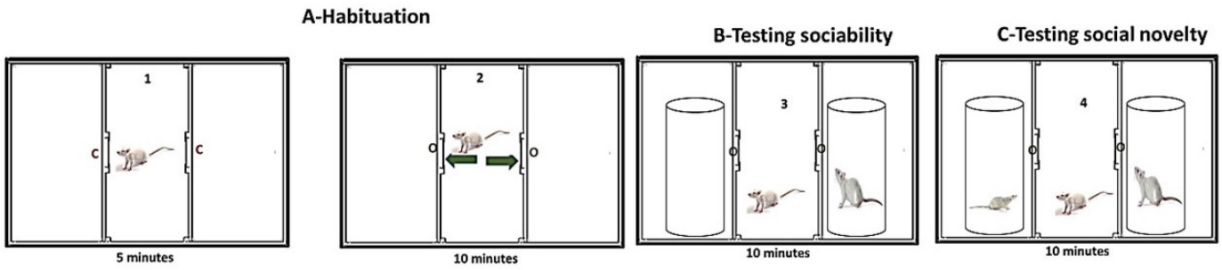

Fig. 1. Testing sociability and social novelty preferences via a three-chamber social apparatus. (A) Habituation: the experimental rats were permitted access to the center chamber only for a five-minute habituation period. The doors were then opened, and the experimental animal was allowed free movement for two intervals of 10 minutes. (B) Testig Sociability: novel animal 1 was first positioned in one of the lateral chambers, and an object was positioned in the opposite chamber. (C) Testing Social Novelty: novel rat 1 was left in its wire cage and novel rat 2 was placed in the wire cage across from it. The sociability index (SI) = time spent exploring the novel object 1 – time spent exploring the novel object/time spent exploring the novel rat 1 + time spent exploring the novel object. The social novelty index (SNI) was calculated as the time spent exploring the novel rat 2 – the time spent exploring the known rat/the time spent exploring the novel rat 2 + the time spent exploring the known rat. C: closed doors; O: opened doors.

Testing Sociability and Social Novelty Preferences

The test set consisted of a social apparatus with three chambers (CAS number: 46502, University of California, CA, USA), one central chamber, and two lateral or side chambers (dimensions: 120 × 58.5 × 40 cm) (Fig. 1). Testing occurred twice: at the beginning of the experiment (following the habituation period) and before administering glycerol. The apparatus's side chamber doors were closed, and the experimental animals (animal under assessment) were placed in the central (middle) chamber. In the corner of the lateral chambers, same-sex stimulus animals (novel rats) were placed in a 15 cm by 15 cm cylindrical stimulus cage with bars that allowed initial nose-to-nose contact but prohibited further contact (Fig. 1).

Habituation: The experimental rats were permitted access to the center chamber only for a five-minute habituation period. The doors were then opened, and the experimental animal was allowed free movement for two intervals of 10 minutes. The entire equipment was disassembled between tests, and every surface was cleaned with a 70% ethanol solution (CAS number: B09N9 WQHF7, Pharma, Egypt).

Assessment of sociability: Novel animal 1 was first positioned in one of the lateral chambers, and an object was positioned in the opposite chamber. The object was an empty cage, the same as the one used to contain the novel rat (the stimulus animal that previously interacted with the animal under assessment), while novel rat 1 is a rat that has never previously interacted with the test animals. The experimental animals were removed from the apparatus and placed in a holding cage when the test was completed.

Testing Social Novelty

Novel rat 1 was left in its wire cage (now known as the known rat), and novel rat 2 was placed in the wire cage across from it (previously vacant). Then, social novelty was introduced, and the experimental animals were reintroduced to the central chamber. The experimental animals were then permitted to engage in 10 minutes of interaction with the well-known and brand-new stimulus animals [14]. The social novelty test was not carried out by rats that did not visit all three chambers during the sociability test.

The sociability index (SI) was calculated as follows: time spent exploring the novel rat 1 – time spent exploring the novel object/time spent exploring the novel rat 1 + time spent exploring the novel object.

The social novelty index (SNI) was calculated as the time spent exploring the novel rat 2 – the time spent exploring the known rat/the time spent exploring the novel rat 2 + the time spent exploring the known rat [15].

Animal Euthanasia

Animals were euthanized by receiving an overdose of pentobarbital (100 mg/kg i.p., NDC-051311-050-01, Dechra Veterinary Products, Hadnall, UK). Then, the rats were euthanized by cervical dislocation. The rats were restrained on the flat surface of the laboratory table, after which the thumb and the first finger of the other hand were used against the back of the neck. Then, we quickly pushed forward and down with the hand while pulling backward with the hand holding the base of the tail. Death was confirmed by lack of pulse, breathing, or corneal reflexes; a response to a firm toe pinch; and an inability to hear respiratory sounds and heartbeats via the use of a stethoscope.

After the mice died, we performed decapitation followed by the extraction of brain tissues. The brains were dissected to determine the ATP content in the hippocampus; mitochondrial enzyme complex (I–V) and glial fibrillary acidic protein (GFAP) expression; and activity-regulated cytoskeleton-associated protein (Arc), toll-like receptor 4 (TLR4), and nuclear factor kappa B (NF-κB) expression, among other parameters.

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 1. Primer sequences for the tissue markers measured in the hippocampus.

	Primer sequence
GFAP (NM_017009.2)	Forward: 5′-AGAGTGGTATCGGTCCAAGTT-3′ Reverse: 5′-TCAAGGTCGCAGGTCAAG-3′
Arc (NM_019361.2)	Forward: 5′-CCCTGCAGCCCAAGTTCAAG-3′ Reverse: 5′-GAAGGCTCAGCTGCCTGCTC-3′
TLR4 (NM_019178.2)	Forward: 5′-GGACTCTGCCCTGCCACCATTTA-3′ Reverse: 5′-CTTGTGCCCTGTGAGGTCGTTGA-3′
NF-κB (NM_001276711.1)	Forward: 5′-TACCCTCAGAGGCCAGAAGA-3′ Reverse: 5′-TCCTCTCTGTTTCGGTTGCT-3′
β-actin (housekeeping gene) (NM_031144.3)	Forward: 5′-CTACCTCATGAAGATCCTCACC-3′ Reverse: 5′-AGTTGAAGGTAGTTTCGTGGAT-3′

GFAP, glial fibrillary acidic protein; *Arc*, activity-regulated cytoskeleton-associated protein; *TLR4*, toll-like receptor 4; *NF-κB*, nuclear factor kappa B.

Biochemical Analysis

After social behavior assessment tests, blood samples from the aortas of the dead rats were taken to determine the concentrations of the platelet contents of the mitochondrial enzyme complexes (I–V), the serum urea concentration, the blood urea nitrogen (BUN), and the creatine phosphokinase concentration.

Assessment of Kidney Function

Blood samples were allowed to clot at room temperature for 15–30 minutes to test creatine kinase activity and renal function. The supernatant was then collected by centrifuging the samples at 3000 ×g for 10 minutes at 4 °C. Creatine kinase, blood urea nitrogen, and serum creatinine levels were measured using a QuantiChrom™ Creatinine Assay Kit (Catalog # DICT-500, BioAssay Systems, Hayward, CA, USA), a rat blood urea nitrogen (BUN) enzyme-linked immunoassay (ELISA) Kit (Catalog # MBS2611086, MyBioSource, San Diego, CA, USA) and a Rat CKM ELISA Kit (Catalog #ab187396, Abcam, Cambridge, UK).

Platelet-Mitochondria Preparation

Blood samples were collected from the aorta into heparinized Tyrode's solution, and platelets were isolated as previously described by Bennett *et al*. [16]; then, mitochondria were isolated using a commercially available kit (Catalog #89801, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol.

The following parameters were used to measure mitochondrial complex I enzymatic activity: mitochondrial complex I activity (mitochondrial complex I activity colorimetric assay kit, Catalog # K968-100, BioVision, CA, USA), respiratory complex II activity (succinate dehydrogenase activity colorimetric assay kit, Catalog # K660-100, BioVision, CA, USA), mitochondrial complex III enzymatic activity (mitochondrial complex III activity assay kit, Catalog # K520-100; BioVision, CA, USA), complex IV enzymatic activity (cytochrome oxidase activity colorimetric assay kit, Catalog # K287-100; BioVision, CA, USA) and mitochondrial complex V enzymatic activity (mitochondrial ATP synthase activity assay kit, Catalog # K2112-100, BioVision, CA, USA).

Assessment of Hippocampal ATP Content

Hippocampal tissue was homogenized with a glass homogenizer on ice after being rinsed in ice-cold phosphate-buffered saline (PBS) and weighed to determine the amount of ATP present. After two freeze–thaw cycles, the resultant suspension was subjected to additional damage to the cell membranes. The homogenates were then centrifuged at 1500 ×g for 15 minutes, and the supernatant was utilized to quantitatively measure ATP levels using a Rat Adenosine Triphosphate (ATP) ELISA Kit (Catalog # MBS723034, MyBioSource, San Diego, CA, USA).

Assessment of Mitochondrial Enzyme Complexes

A Mitochondria Isolation Kit for Tissue (Catalog #89801, Thermo Fisher Scientific, Waltham, MA, USA) was used to perform the mitochondrial isolation step on the hippocampal tissue section that was used to evaluate the mitochondrial enzyme complexes (I–V). The same kits described above were subsequently used to measure the levels of the mitochondrial enzyme complexes (I–V) in the purified mitochondria.

Real-Time PCR Analyses

For the mRNA expression of glial fibrillary acidic protein (*GFAP*), activity-regulated cytoskeleton-associated protein (*Arc*), toll-like receptor 4 (*TLR4*), and nuclear factor kappa B (*NF-κB*), real-time PCR analyses were performed.

Total RNA Extraction and Reverse Transcription

The hippocampal tissue utilized for gene expression analysis was subsequently placed in RNA Later Solution (with RNA stabilizing reagent) from Qiagen, Inc., in Va-

lencia, California, at a rate of 10 L per 1 mg of tissue, after which the solution was kept at 80 °C for RNA extraction. Using the RNeasy Mini Kit (Cat. No. 74104, Qiagen, Hilden, Germany), total RNA was isolated from hippocampal homogenates according to the manufacturer's instructions. Using a NanoDrop One/OneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), the absorbance at 260 nm and 280 nm was measured to quantify the concentration and purity of the RNA. The A260/A280 ratio for pure RNA ranged from 1.8 to 2.1. Following the manufacturer's instructions, RNA (1 g) was reverse transcribed using a T100 Thermal Cycler (# 1861096, Bio-Rad, Hercules, CA, USA) and the Maxima First Strand cDNA Synthesis Kit (Catalog number: K2563, Thermo Fisher Scientific, Waltham, MA, USA).

Quantitative Real-Time PCR

Using the Step One Plus Real-Time PCR System (4376357, Life Technologies, Carlsbad, CA, USA) and Maxima SYBR Green/ROX qPCR Master Mix (K0221, Thermo Fisher Scientific, Waltham, MA, USA), real-time PCR was carried out following the manufacturer's instructions. The sequences of the primers used are shown in Table 1.

After correction for B-actin expression, the mRNA expression of each sample was determined. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Histological Examination

After being harvested, the brains were preserved in 10% formaldehyde (CAS number: C3966, Lab Alley, Austin, TX, USA) for two hours. The brains were removed and immersed in fresh formaldehyde for 24 hours, after which they were dehydrated with ethanol (70% for 24 hours, 90% for an hour, and 100% for an hour), cleaned in xylene (CAS number: 106-42-3, Lab Alley, Austin, TX, USA), and then embedded in paraffin. Using a microtome (Leica RM 2025, Leica Microsystems GmbH, Wetzlar, Germany), coronal slices were cut at the level of the hippocampus, mounted on glass slides, and stained using the standard hematoxylin and eosin method. The slides were dried at 65 °C for 1-2 hours and then put into a rack. The rack was dipped in 4 consecutive stain jars containing xylene to remove paraffin, and then the rack was dipped in ethanol to remove xylene. The rack was rinsed with tap water for 5 minutes to remove the ethanol.

To stain the nucleus with hematoxylin (CAS number: 517-28-2, Lab Alley, Austin, TX, USA), the rack was placed in a container filled with hematoxylin for 10 minutes, and the rack was subsequently rinsed with tap water to remove hematoxylin for 10 minutes. The rack was dipped into a jar containing 0.1% HCl (CAS number: 7647-01-0, Lab Alley, Austin, TX, USA) and put into tap water 3~4 times. Following this step, the rack was dipped into a jar containing 0.1% NH4OH 3 times and then into tap water 3~4 times.

To stain the cytoplasm with eosin, the rack was dipped into the following solutions sequentially: eosin, ethanol, acetone, and xylene. Two to three drops of the mountant were dropped on the slide, after which the cover was placed over the slide [17]. At the Histology Department of the College of Medicine at Hashemite University, morphometric studies, photography, and slide examination were all carried out using an Opita light microscope B-383PL and an OPTICKAM B9 digital camera. The number of layers of cornu ammonis 1 region (CA1) and cornu ammonis 3 region (CA3) and the number of apoptotic cells were counted in serial nonoverlapping high-power fields.

Statistical Analysis

The results of the data analysis were analyzed using SPSS 21 (IBM SPSS Statistics 21, IBM Corporation, Armonk, NY, USA) and are presented as the mean ± standard deviation (mean ± SD). The Shapiro–Wilk test was used to determine whether the data were normally distributed. Bonferroni post hoc correction was used to evaluate differences in quantitative variables between groups via analysis of variance (ANOVA). The linear relationships between quantitative variables were evaluated using the Pearson correlation coefficient. The results were deemed to be significant at a p value ≤ 0.05 [18].

Results

The following statistical significance is stated in parallel with intergroup variability:

Sociability and Social Novelty Preferences

As shown in Fig. 2, the sociability and social novelty indices were significantly lower (p value ≤ 0.05) in the AKI group than in the control group. In contrast, the two indices were significantly greater (p value ≤ 0.05) in both treated groups than in the AKI control group. However, these values were still significantly lower (p value ≤ 0.05) than those of the negative control group. Additionally, there was a statistically significant difference (p value ≤ 0.05) in both indices between the vildagliptin-treated group and the AKI + saline group.

A statistically significant positive correlation was found between the sociability index and the hippocampal ATP concentration [r = 0.917, p value ≤ 0.01]. Similarly, there was a significant (p value ≤ 0.05) positive correlation between the Social Novelty Index score and the hippocampal ATP concentration [r = 0.975, p value ≤ 0.01] (Fig. 3).

Kidney Function Tests

As shown in Table 2, there was a noticeable decrease in kidney function, as indicated by statistically significant increases (p value ≤ 0.05) in the serum creatinine, BUN, and creatine phosphokinase (CPK) levels in the AKI group compared to those in the control group. Compared to those in the AKI control group, there were significant (p value ≤ 0.05) improvements in the three metrics in both treated

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 2. Serum markers of kidney function in the studied groups.

	Control	AKI	AKI + saline	AKI + vildagliptin
Serum creatinine (mg/dL)	0.159 ± 0.048	1.695 ± 0.217*	0.622 ± 0.133*#	0.312 ± 0.053#@
Serum BUN (mg/dL)	37.4 ± 5.7	87 ± 0.4*	53.95 ± 4.94*#	37.7 ± 2.83#@
Serum CPK (μ/L)	113.95 ± 4.94	857.88 ± 39.9*	693.46 ± 41.12*#	561.32 ± 20.52*#@

* compared to the control group, p value ≤ 0.05; # compared to the AKI group, p value ≤ 0.05; @ compared to the AKI + saline group, p value ≤ 0.05. AKI, acute kidney injury; BUN, blood urea nitrogen; CPK, creatine phosphokinase.

Table 3. Hippocampal and platelet markers of mitochondrial function and energetics.

	Control	AKI	AKI + saline	AKI + vildagliptin
Hippocampal ATP (NMOL/MG PROTEIN)	132.1 ± 5.32	65.48 ± 1.94*	95.37 ± 5.62*#	118.75 ± 1.73*#@
Hippocampal complex I (U/MG PROTEIN)	64.25 ± 7.42	27.92 ± 2.62*	46.46 ± 3.7*#	62.04 ± 2.29#@
Hippocampal complex II (U/MG PROTEIN)	167.48 ± 8.47	81.84 ± 3.78*	118.4 ± 4.48*#	153.3 ± 1.41*#@
Hippocampal complex III (U/MG PROTEIN)	23.98 ± 1.127	11.49 ± 0.8*	16.41 ± 0.406*#	22.3 ± 1.63*#@
Hippocampal complex IV (U/MG PROTEIN)	99.13 ± 3.12	45.67 ± 1.83*	70.32 ± 4.01*#	90.97 ± 1.29*#@
Hippocampal complex V (U/MG PROTEIN)	39.64 ± 1.81	17.03 ± 1.63*	26.6 ± 1.39*#	34.57 ± 0.92*#@
Platelet complex I (U/MG PROTEIN)	43.6 ± 4.34	20.04 ± 2.53*	30.17 ± 4.47*#	38.07 ± 1.44*#@
Platelet complex II (U/MG PROTEIN)	112.6 ± 2.55	60.54 ± 3.36*	89.39 ± 3.08*#	105.7 ± 2.53*#@
Platelet complex III (U/MG PROTEIN)	15.74 ± 1.1	11.66 ± 1.34*	13.95 ± 1.27*#	15.64 ± 0.323#@
Platelet complex IV (U/MG PROTEIN)	128.72 ± 3.25	77.87 ± 3.35*	110.62 ± 1.86*#	119.89 ± 1.195*#@
Platelet complex V (U/MG PROTEIN)	66.35 ± 1.38	36.06 ± 1.939*	48.04 ± 1.74*#	60.58 ± 1.18*#@

* compared to the control group, p value ≤ 0.05; # compared to the AKI group, p value ≤ 0.05; @ compared to the AKI + saline group, p value ≤ 0.05. AKI, acute kidney injury; ATP, adenosine triphosphate.

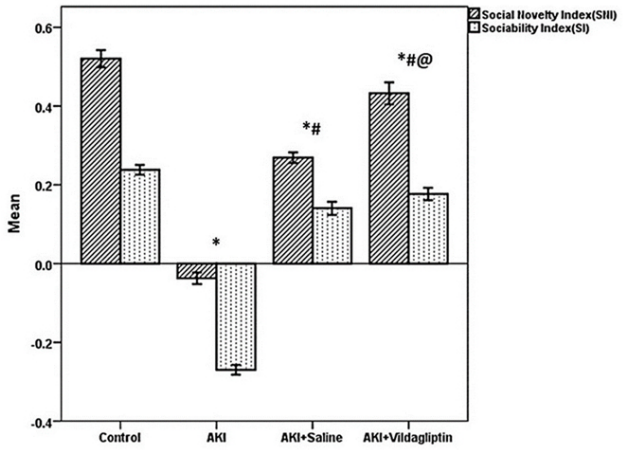

Fig. 2. Sociability and social novelty indices in the studied groups. * compared to the control group, p value ≤ 0.05; # compared to the AKI group, p value ≤ 0.05; @ compared to the AKI + saline group, p value ≤ 0.05. N = 10 rats/group. AKI, acute kidney injury.

groups (saline and vildagliptin). Vildagliptin administration to group IV produced greater improvement than saline administration because there was a statistically significant difference between the two groups (group III) (p value ≤ 0.05). Additionally, the difference in blood creatinine and BUN levels between the vildagliptin-treated and negative control groups was not statistically significant.

Mitochondrial Complexes

Table 3 shows that, compared to those in the control group, all hippocampal mitochondrial complexes (I to V) and platelet counts were significantly lower (p value ≤ 0.05) in the AKI group. Additionally, all the mitochondrial complexes improved following treatment, as evidenced by a statistically significant increase in their values in the AKI + saline and AKI + vildagliptin groups compared to those in the AKI control group (p value ≤ 0.05). In contrast to those in the AKI + saline group, the levels of all ten complexes in the vildagliptin-treated group were significantly greater (p value ≤ 0.05). Except for platelet complex III, all the complexes exhibited a statistically significant difference between the vildagliptin-treated group and the negative control group (p value ≤ 0.05). The significant positive correlation between each type of platelet complex and its corresponding hippocampal complex is shown in Fig. 4 [r = 0.868, 0.973, 0.827, 0.963, and 0.974 for complexes I, II, III, IV, and V, respectively; p value ≤ 0.01], provided evidence that these platelets and hippocampal mitochondrial complexes were correlated.

Hippocampal Tissue Markers

Table 3 reveals a statistically significant decrease (p value ≤ 0.05) in the hippocampal ATP content in AKI group compared to that in the control group. As demonstrated in Fig. 5; a statistically significant increase (p value ≤ 0.05) in the relative expression of hippocampal *GFAP*, *Arc*, *TLR4*, and *NF-κB*. Similarly, compared with those in the AKI con-

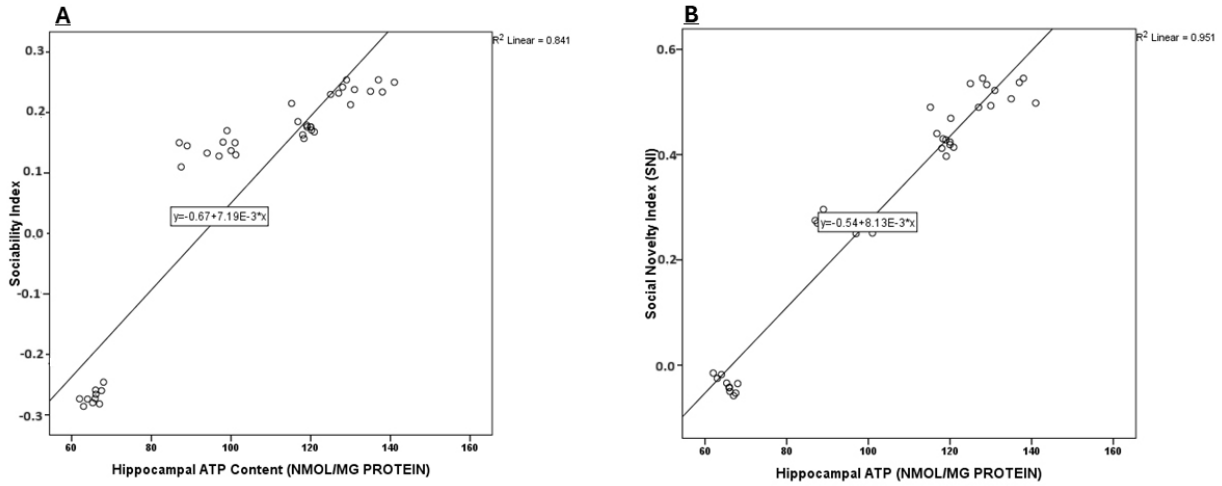

Fig. 3. A significant positive correlation between adenosine triphosphate (ATP) in the hippocampus and both the sociability index (A) and the social novelty index (B).

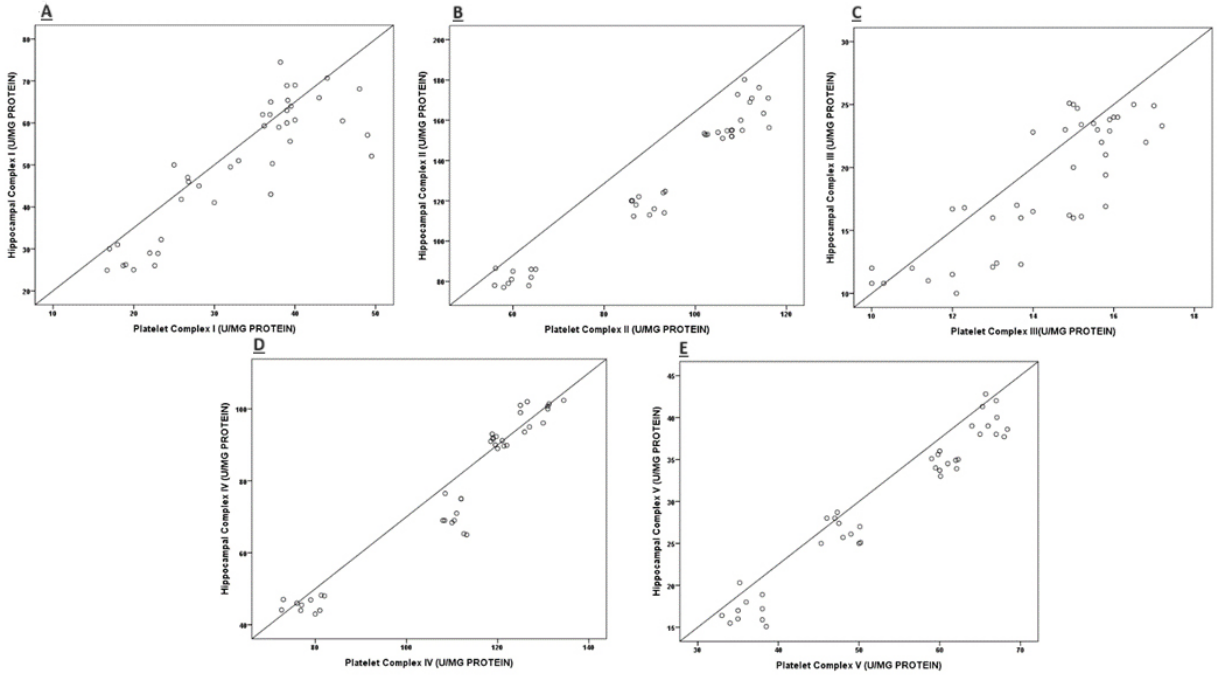

Fig. 4. Significant positive correlations between platelets mitochondrial complexes and the corresponding hippocampal complexes [(A) Complex I; (B) Complex II; (C) Complex III; (D) Complex IV; (E) Complex V].

trol group, the hippocampal *GFAP*, *Arc*, *TLR4*, and *NF-κB* levels in the saline- and vildagliptin-treated groups were significantly lower (p value ≤ 0.05), and the hippocampal ATP level was significantly larger (p value ≤ 0.05) in the saline- and vildagliptin-treated groups. However, the difference in the expression of these hippocampal tissue markers between the two treatment groups and the untreated group was statistically significant (p value ≤ 0.05). The distinction between the AKI + saline and AKI + vildagliptin groups should also be noted.

Histopathology Results

The control section of the CA1 region is formed of small to medium-sized pyramidal cells arranged in 4–6 layers with large, oval vesicular nuclei. AKI sections from the CA1 region showed disarray and degeneration of pyramidal cells with microglial cell infiltration and the appearance of congested blood capillaries. AKI sections treated with saline showed distortion of the cellular architecture of pyramidal cells—an increased number of degenerated pyramidal cells with increased microglial cell infiltration. Treat-

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 4. Morphometric evaluation of the hippocampus in the study groups.

	Control	AKI	AKI + saline	AKI + vildagliptin
Count of layers of CA1	5.33 ± 0.816	5 ± 0.632	4.83 ± 0.753	5.33 ± 0.816
Count of layers of CA3	3.50 ± 0.548	3.17 ± 0.408	3.50 ± 0.548	3.50 ± 0.548
Count of diseased cells in CA1	1.17 ± 1.169	8.33 ± 1.211*	3.17 ± 1.602#	2.67 ± 1.033#
Count of diseased cells in CA3	2 ± 1.265	11.33 ± 1.211*	9.83 ± 0.983*	4 ± 0.894*#

CA, cornu ammonis; AKI, acute kidney injury. * Significant compared to the control group; # Significant compared to the AKI group at p value ≤ 0.05.

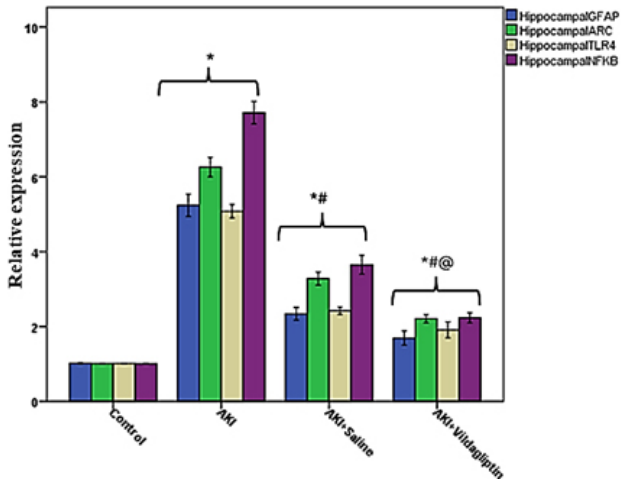

Fig. 5. Hippocampal expression of tissue markers in the studied groups. * compared to the control group, p value ≤ 0.05; # compared to the AKI group, p value ≤ 0.05; @ compared to the AKI + saline group, p value ≤ 0.05. N = 10 rats/group. AKI, acute kidney injury; GFAP, glial fibrillary acidic protein; Arc, activity-regulated cytoskeleton-associated protein; TLR4, toll-like receptor 4; NF-κB, nuclear factor kappa B.

ment of AKI sections with vildagliptin restored the cellular architecture of pyramidal cells with oval vesicular nuclei and the disappearance of microglia (Fig. 6).

The control section of the CA3 region is composed of medium- to large-sized pyramidal cells arranged with large, oval vesicular nuclei and thick, voluminous axon hillocks. The section of the CA3 region in the AKI group showed degeneration and shrinkage of pyramidal cells with pyknotic nuclei and microglial cell infiltration with congested blood capillaries. AKI tissue sections were treated with saline and showed distortion of the cellular architecture of pyramidal cells with increased microglial cell infiltration. Treatment of AKI sections with vildagliptin restored the cellular architecture of pyramidal cells with prominent oval nuclei and thick voluminous axon hillocks, attenuating microglial cell infiltration and capillary congestion (Fig. 7).

Morphometric examination of the hippocampus in the studied groups revealed no significant difference in the number of layers of CA1 and CA3 between the two groups. The number of diseased apoptotic cells was significantly greater (p value ≤ 0.05) in the AKI group than in the control group. Treatment with saline or vildagliptin significantly reduced (p value ≤ 0.05) the number of apoptotic CA1 cells compared to that in the AKI group. The number of apoptotic cells in CA3 was significantly greater (p value ≤ 0.05) in the AKI group than in the control group. Treatment with vildagliptin only significantly reduced the number of apoptotic cells (p value ≤ 0.05) compared to that in the AKI group (Table 4).

Discussion

AKI is characterized by reduced glomerular filtration rate (GFR), and urine output, which leads to fluid and electrolyte imbalances and waste accumulation [19]. The findings of the present study showed a declined kidney function with the onset of AKI, as indicated by the increase serum creatinine, BUN, and CPK levels. The results of the present study agreed with those of Gyurászová *et al.* [20], who reported that two animal models of AKI had significantly elevated serum creatinine and BUN levels. It is still unclear how many organs can malfunction with AKI. A probable explanation for these aberrations could be the accumulation of uremic toxins characteristic of AKI that develop in systemic organs such as the brain and circulation, in addition to oxidative stress and ROS [21].

AKI pathogenesis has been linked to several processes, including endothelial damage, leukocyte infiltration, cytokine release, adhesion molecule production, and apoptosis induction. According to Yap and Lee [22], these mechanisms encourage the extravasation of leukocytes, including neutrophils, lymphocytes, and macrophages, into the kidney. This cellular extravasation triggers the production of cytokines and ROS that triggers systemic inflammatory responses [23].

These inflammatory cytokines contribute to endothelial and epithelial damage, which causes capillary leakage. Additionally, there is further evidence that disruption of the BBB is linked to systemic inflammation. Increased brain vascular permeability, microvascular protein leakage, and disruption of the BBB have all been reported in studies using animal models of AKI [20]. Proinflammatory cytokines might reach the brain through permeable capillaries and disrupt the BBB. The hippocampus is a region that is highly susceptible to oxidative stress and inflammation [24].

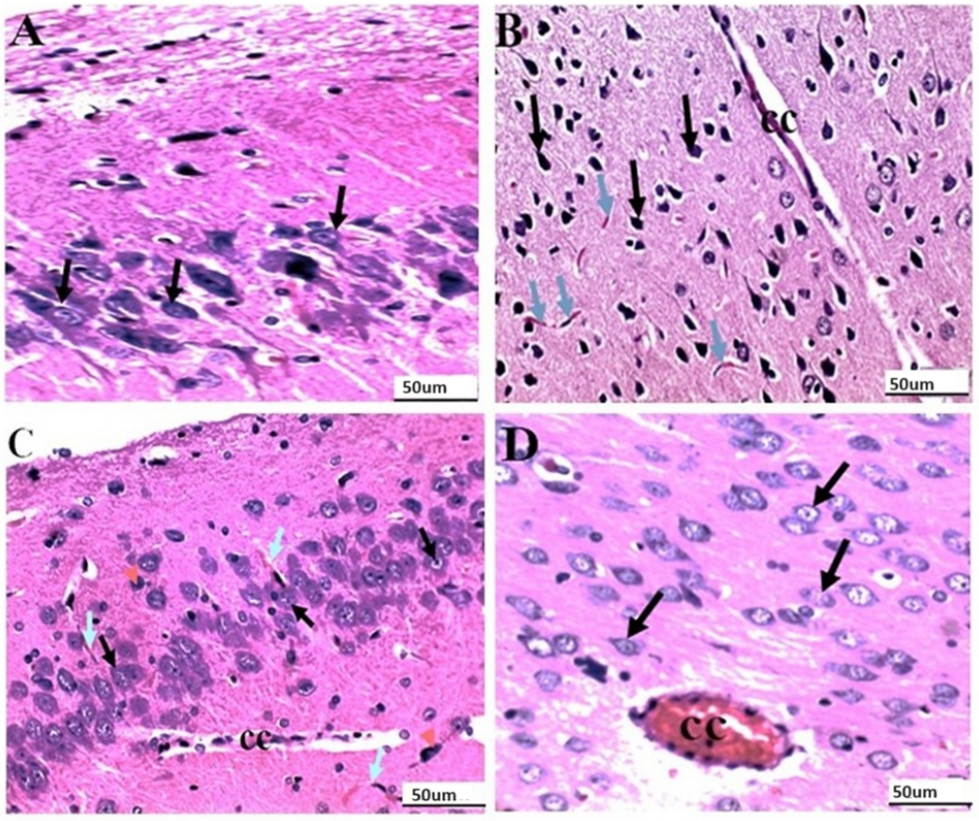

Fig. 6. Photomicrographs of the cornus ammonis 1 (CA1) region. (A) Control section showing small- to medium-sized pyramidal cells (black arrow) arranged in 4–6 layers with large, oval vesicular nuclei. (B) Acute kidney injury (AKI) section showing disarray and degeneration of pyramidal cells (black arrows) with microglial cell infiltration (blue arrows). Notably, congested intraventricular blood capillaries (CCs) were observed. (C) A section from the AKI group was treated with saline, and the images show distortion of the cellular architecture of the pyramidal cells (black arrows). Decreased number of degenerated pyramidal cells (red arrow heads) with decreased number of microglia (blue arrows). A congested blood capillary (cc) was observed. (D) A section of the AKI group treated with vildagliptin showed restoration of the cellular architecture of pyramidal cells (black arrows) with oval vesicular nuclei. The presence of congested blood capillaries (CCs) and the disappearance of microglia were observed (hematoxylin and eosin (H&E) staining × 400).

Long-term cognitive dysfunction in AKI is poorly understood, even though cognitive dysfunction is a well-known side effect of chronic kidney disease (CKD). According to Davey *et al*. [25], cognitive performance deteriorates as kidney function deteriorates. Based on the results of the current study, the AKI group had lower sociability and social novelty indices than did the control group. This finding is consistent with that of another study in which rats exposed to airborne particulate matter (APM) exhibited decreased social play and decreased social behavior in the hippocampus [26], which stimulated systemic oxidative stress and inflammatory cytokine release. According to Malek *et al*. [2], the accumulation of free radicals in the peripheral nervous system coupled with the impairment of BBB integrity produces an accumulation of these free radicals in various parts of the brain, including the hippocampus.

Glial cell causes neuroinflammation when proinflammatory cytokines enter the brain, specifically through the hippocampus [27]. Microglia produce ROS and glutamate in response to inflammatory cytokines producing reactive microgliosis [28]. Activated microglia increase the expression of many receptors and release proinflammatory cytokines. Increased keratinocyte-derived chemokines and granulocyte colony-stimulating factor (G-CSF) have been identified in the hippocampus of rats with ischemic AKI, with increased neuronal pyknosis and microgliosis [29]. GFAP gene activation and protein induction are essential for the development of reactive astrogliosis. These findings are consistent with the results of the present study, which revealed that the relative hippocampal GFAP expression was markedly greater in the AKI group than in the control group.

Another piece of evidence of neuroinflammation in the current study was brain inflammation, as evidenced by the expression of the NF-κB pathway. NF-κB signaling and other signaling molecules induced by neuronal activity and hormones are the key mechanisms that control the expression of the GFAP gene [24]. Therefore, the significant increase in relative hippocampal NF-κB expression observed

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

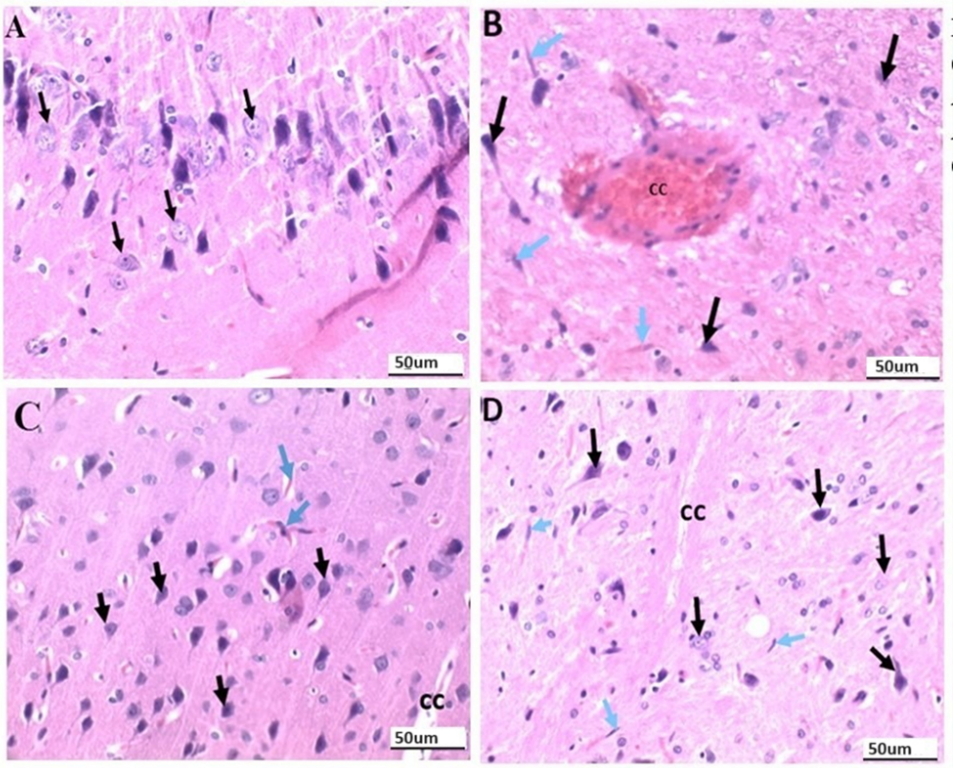

Fig. 7. Photomicrographs of the cornus ammonis 3 (CA3) region. (A) Control section shows large pyramidal cells (black arrows) with large, oval nuclei and a thick voluminous axon hillock. (B) A section of acute kidney injury (AKI) tissue showing degeneration and shrinkage of pyramidal cells in the CA3 region (black arrows) with pyknotic nuclei. Microglial cell infiltration was observed (blue arrows). Congested blood capillary (cc). (C) A section from an AKI mouse treated with saline shows distortion of the cellular architecture of pyramidal cells (black arrow) in the CA3 region. Microglial cell infiltration was observed (blue arrows). The appearance of congested blood capillaries (CCs). (D) A section from an AKI mouse treated with vildagliptin shows restoration of the cellular architecture of pyramidal cells (black arrows) with prominent oval nuclei and a thick voluminous axon hillock. Microglial cell infiltration (blue arrows) decreased in the presence of congested blood capillaries (cc) (hematoxylin and eosin (H&E) staining × 400).

in our study when we compared the AKI group to the control group may also account for the increase in relative hippocampal GFAP expression. NF-κB generally exists in the cytosol, but in some circumstances, which might involve oxidative stress following AKI, it might be activated and transported to the nucleus, where it stimulates the transcription and activation of cytokine genes [2].

The interactions between the brain and kidney may also be influenced by the activation of the innate immune system caused by AKI via TLRs. The TLR family is expressed in glial cells, neurons, and neural progenitor cells, which explains their functions in the regulation of brain physiology [30]. TLRs may trigger apoptosis, neuronal injury, and localized inflammation. In addition, TLR4 might regulate the expression of cytokines during ischemia–reperfusion injury and contribute to neuronal injury [28].

NF-κB is one of the most significant downstream molecules in the TLR signaling pathway since it is essential for triggering protein kinase cascades [31]. The substantial increase in the relative expression of hippocampal NF-κB following the induction of AKI may be explained by the significant increase in the relative hippocampal expression of TLR4 in the AKI group compared to the control group.

Furthermore, the social and behavioral disorders that occurred in the AKI group may be attributed to the increase in TLR4 expression in the hippocampus. Several studies support this explanation; stimulation of TLR4-expressing monocytes increases the proinflammatory cytokine IL-1B in individuals with autism spectrum disorder, which is manifested by poor social adaptation in conjunction with anxiety [32]. Interestingly, the activation of TLR4 might be correlated with the initiation of multiple forms of oxidative/nitrosamine stress and inflammatory pathways in major depressive disorder involving the NF-κB pathway [33]. Additionally, García-Bueno *et al*. [34] reported an increase in cytokine levels in patients with schizophrenia and bipolar disorders when TLR4 was activated. Furthermore, significant increases in TLR4 mRNA and protein levels have been detected in the peripheral blood of patients with depression [35].

According to the findings of the present study, the relative hippocampal expression of Arc was significantly greater in the AKI group. These findings may be explained by the fact that the Arc/Arg3.1 gene is significantly induced by heat shock and other cellular stress inducers, including ROS, in different cells [36]. Our findings are consistent with those of Rosi *et al*. [37], who demonstrated that after engaging in behavioral exploration, rats with severe neuroinflammation exhibit augmented expression of Arc mRNA and protein. Additionally, they demonstrated that the hippocampal regions with the highest numbers of activated microglia were the only regions with increased expression. The cognitive disorders observed in inflammation-associated diseases may result from this activity-related modification of Arc expression by neuroinflammation [37].

AKI alters the mechanism through which fuel substrates are used, significantly reduces mitochondrial oxidative activity. The process of AKI is significantly influenced by impaired mitochondrial function, which affects energetics, the activation of cell death pathways, and the production of ROS. Increasing amounts of ROS can prompt the opening of the inner membrane anion channel (IMAC) in mitochondria, leading to mitochondrial membrane depolarization [38].

Increased levels of ROS may also result in the opening of the mitochondrial permeability transition pore (MPTP), which subsequently allows metabolites and other solutes to diffuse into the mitochondria and cause swelling, rupture of the outer mitochondrial membrane, release of cytochrome c, and ultimately cell death. In humans and rodents with AKI, swollen mitochondria with ultrastructural disruptions have been reported. Mitochondrial structural abnormalities were observed even before the clinical manifestations of AKI were first detected [39].

The brain needs ATP, which is mostly generated by mitochondria, because of its high degree of energetic support [38]. According to Khacho *et al*. [40], mitochondria are essential for adult neurogenesis and play a role in the maturation of cognition in the brain. The results of the present study revealed that the AKI group had a reduced number of platelets and hippocampal mitochondrial complexes (I to V), in addition to reduced hippocampal ATP, compared with the control group, indicating mitochondrial dysfunction. Glial cell stimulation, which results in mitochondrial damage, and oxidative stress [41], which results in mitochondrial malfunction, could explain the abovementioned findings.

The results of the present study suggested that mitochondrial dysfunction and the accompanying decrease in hippocampal ATP might have additionally contributed to the social and behavioral alterations observed after AKI induction. This was corroborated by the substantial positive correlation between the hippocampal ATP concentration and sociability and social novelty indices. This finding is consistent with several studies that found a strong correlation between mitochondrial dysfunction and several disorders that are linked to cognitive impairment [42].

Our findings revealed significant improvements in renal and hippocampal functions in both the saline- and vildagliptin-treated groups when evaluating the effects of vildagliptin (a DPP-4 inhibitor) and saline as two prospective treatment strategies for AKI. The saline- and vildagliptin-treated groups showed improvements in renal function, as indicated by apparent decreases in the serum creatinine, BUN, and CPK levels. Oliguria and increased serum creatinine levels in AKI patients are accompanied by a decrease in the GFR resulting from hypotension spurred by hypovolemia and decreased cardiac output. Fluid resuscitation is a preventative measure based on increasing the circulation volume and enhancing renal perfusion [43].

A transmembrane glycoprotein with multiple functions, DPP-4, is found in almost every cell [44]. Oral antidiabetic drugs known as DPP-4 inhibitors obstruct the function of the common enzyme DPP-4. The amount of glucagon-like peptide-1 (GLP-1) released by L-cells in the small intestine and circulated in the blood increases when this enzyme is inhibited DPP-4 signaling involves several physiological processes in addition to GLP-1 degradation and glucose control [45].

Kidney function was significantly better in the vildagliptin-treated group than in the saline-treated group in the present study. This was evidenced by decreased serum creatinine, BUN, and CPK levels, with DPP-4 levels appearing highest in the kidneys. In addition to their ability to decrease blood sugar, DPP-4 inhibitors may have renoprotective effects. According to Takagaki *et al*. [46], independent of changes in GLP-1 and glucose levels, DPP-4 inhibitors have many renoprotective effects. Vildagliptin has been proven to have anti-inflammatory, antioxidant, and antiapoptotic effects in numerous animal model studies [47].

Compared to those in the AKI + saline and AKI control groups, the sociability and social novelty indices improved in the vildagliptin-treated group in the present study. Gut-produced DPP-4 penetrates the BBB and is also generated in the brain. It acts on GLP-1 receptors to exert central anti-inflammatory and antiapoptotic effects, thus preventing neuronal damage. Vildagliptin has been shown to enhance memory and cognition by modifying GLP-1, a brain inflammatory biomarker, and oxidant status [48]. Mahmoudi *et al*. [49] reported that functional performance limitations in behavioral tests caused by type 1 diabetes decreased concurrently with decreased hippocampal levels of interleukin 6, NF-κB, TLR4, TNF, and GFAP. A further investigation that supported our findings revealed that administering another DPP-4 inhibitor, omarigliptin, to LPS-injected rats improved their behavior by restoring the oxidant/antioxidant balance, decreasing TLR4 levels, ameliorating neuroinflammation attributed to apoptosis inhibition and restoring GLP-1 levels in cerebral tissues [50].

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Vildagliptin enhance memory and cognition by reducing mitochondrial dysfunction. These findings are consistent with the findings of the present study, which indicated that, compared with those in the AKI group, the hippocampal ATP and hippocampal mitochondrial complex levels (I to V) in the vildagliptin-treated group improved. Vildagliptin restores brain mitochondrial activity and enhance learning and memory functions in rats. These effects might be mediated by the protective effects of GLP-1 against mitochondrial dysfunction because GLP-1 stimulates mitochondrial ATP production and mobilizes intracellular Ca^{2+} [51].

Additionally, GLP-1 enters cells, regulates mitochondrial oxidative phosphorylation, and reduces the production of oxidative stress and ROS [52]. To compare the response of DPP-4 knockdown and wild-type cardiomyocytes to oxidative stress, Lee *et al*. [44] knocked down DPP-4 gene expression in cultured cardiomyocytes. By preserving mitochondrial bioenergetics, lowering intracellular ROS production, and reducing apoptosis-associated protein expression, DPP-4 knockdown mitigated the H2O2-induced decrease in cell viability. Knocking down DPP-4 enhances its ability to prevent oxidative stress by promoting Nrf2 and signaling, which has been linked to protecting mitochondrial function [44].

The hippocampal formation is in the medial aspect of the temporal lobe, lining the lower horns of the lateral ventricles. It is considered one of the key components of the limbic system. The three distinct sections of the hippocampal formation are the hippocampus, dentate gyrus, and subiculum. The pyramidal cells, which have efferent and afferent axons and dendrites, characterize the cellular architecture of the hippocampus. The basal dendrites face the ventricle surface, while the apical dendrites point away from the lateral ventricles and toward the dentate gyrus. Axons convey information from the hippocampus to other brain regions. According to Siegel and Sapru [53], distinct sections of the hippocampus are known as the cornu ammonis (CA): CA1, CA2, CA3, and CA4 regions.

The pyramidal cells closest to the subiculum were found in CA1, whereas those closest to the hilum of the dentate gyrus were found in CA4. According to Siegel and Sapru [53], CA1 and CA4 surround CA2 and CA3. As they return to the CA1 area, the CA3 axons that stretch from their pyramidal cells are known to be recurrent, which explains why any pathological insults specifically affect CA1 and CA3, consistent with the findings from the present study. According to long-term potentiation theory, the hippocampus collects and organizes information, creating long-term memories [54].

The neurological sequelae of AKI include encephalopathy, seizures, and a decline in mental status. Neuronal pyknosis and microgliosis in the hippocampus are two notable consequences in animal models [55]. In the present study, we assessed the impact of AKI on the microstructure of the CA1 and CA3 regions of the hippocampus. We detected pyramidal cell degeneration and disorganization with pyknosis, microglial cell infiltration, and blood capillary congestion. Koehler *et al*. [56] reported that the hippocampal regions that appeared more sensitive to AKI included CA1 and CA3, consistent with the findings of the present study. According to Liu *et al*. [55], a higher level of inflammatory mediators in the hippocampus and cortex accounts for the increase in pyknotic neurons and microglia in the hippocampal CA1 and CA3 following AKI. Following AKI, the hippocampus exhibits an increase in inflammation, which is reflected by the accumulation of microglia and essential mediators of inflammatory cascades.

According to research by Chou *et al*. [57], substantial amounts of reactive oxygen species, cytokines, and NO in the brain after AKI are associated with apoptosis and neuronal cytotoxicity. Pyknotic neurons and pyknosis in the hippocampus indicate that the hippocampal nuclei have undergone necrosis or apoptosis, as evidenced by chromatin condensation [55]. These findings might explain the results reported in the present study of microglial cell infiltration and increased degenerating neuronal cells in the CA1 region of the hippocampus. According to the earlier study, the number of pyknotic neurons, microglia, and brain macrophages in the hippocampal CA1 region increases after AKI [58].

Structural deterioration results from an imbalance between antioxidant enzymes and free radicals triggered by oxidant accumulation after AKI. However, the results of the present study contrast with those of Karimi *et al*. [59], who showed that 45 minutes of renal ischemia after 24 hours of reperfusion did not cause apoptosis or neuronal loss in hippocampal neuronal cells. As observed in rats with AKI treated with saline, the results of the present study revealed disorganization and degeneration of pyramidal cells in CA1 and CA3 in addition to the formation of congested blood capillaries. Fluids containing excessive chloride have been linked to an increased risk of AKI [60]. Saline has been linked to metabolic acidosis, inflammation, hypotension, and AKI according to Semler and Kellum [61]. This finding explains why the effects of AKI in the hippocampus were exacerbated after receiving normal saline therapy. In the present study, vildagliptin reduced the number of microglia and restored the architecture of pyramidal cells at CA1 and CA3, reducing the impact of AKI on hippocampal tissue. Because they significantly reduce oxidative stress in the brain and enhance cognition and hippocampal synaptic plasticity in a neurodegenerative rat model, DPP-4 inhibitors, such as vildagliptin, have neuroprotective effects on animal models of neurodegenerative diseases [62].

Conclusions

The present study demonstrated that vildagliptin has a protective effect on uremic encephalopathy caused by

AKI, as evidenced by histological analysis of the CA1 and CA3 hippocampal regions, social behavior, and biochemical blood test results. This amelioration was believed to be prompted by improved mitochondrial function, which also altered the expression of synaptic proteins and positively impacted the energetics of the brain. Additional ultrastructural and immunohistochemical evaluations must be performed to determine the mitochondrial mechanism involved, mitophagy markers, and mitochondrial biogenesis. In addition, further investigations are needed to determine other control brain areas and hippocampal microglial alterations.

Availability of Data and Materials

The data are available upon reasonable request from the corresponding author.

Author Contributions

SNA designed the research study. SNA, MFME, WBEG, and SAS performed the research. SNA analyzed the data. MFME, SNA, WBEG, SAS, SSH, DAE and HIS acquired data and wrote the manuscript draft. All the authors contributed to editorial changes in the manuscript. All the authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Hashemite University's Institutional Review Board granted clearance for the study's protocols, with approval number 5/7/2020/2021 received on 8/3/2021.

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Conflict of Interest

The authors declare no conflict of interest.

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