Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2023.245516.2989 Volume 30, Issue 1.1, - January 2024, Supplement Issue
Manuscript ID ZUMJ-2310-2989 (R2)
DOI 10.21608/ZUMJ.2023.245516.2989

ORIGINAL ARTICLE

Effects of Quercetin and Vitamin D3 on Klotho Gene Expression in Relation to Neurological Functions in Chronic Kidney Disease Rat Model

Eman R.Abozaid, Dalia I. Abd-Alaleem, Kholoud Hasan Abd-Elsalam* , Sherein F.El-sayed.
Physiology department, Faculty of Medicine, Zagazig University.

***Corresponding author:**
Kholoud Hasan Abd- Elsalam

Email:
drkholoudhassan.kh@gmail.com

Submit Date 2023-10-31
Revise Date 2023-11-24
Accept Date 2023-11-20

ABSTRACT

Background: Chronic kidney disease (CKD) is a gradual damage to kidneys, featured by tubular atrophy, glomerulosclerosis and interstitial fibrosis. It is associated with cognitive dysfunctions. Moreover, inflammation and oxidative stress play vital roles in pathophysiology and progression of both CKD and accompanying neurobehavioral changes. This study aimed to detect possible beneficial effects of quercetin and vitamin D on progression of CKD and its associated neurological complications and to detect their possible mechanism/s in relation to klotho gene expression in CKD rat model.

Methods: Thirty adult male albino rats were allocated into 5 groups as the following: Group 1 (Control group), Group 2 (CKD group), Group 3 (CKD+ quercetin group), Group 4 (CKD+VIT D3), and Group 5 (CKD+ quercetin+ VIT D3). Kidney function tests, some inflammatory, apoptotic and oxidative stress markers, BDNF, Klotho gene expression in kidney and brain and behavioral tests were assessed for all groups.

Results: There was a significant deterioration in all parameters in CKD group. However, treated groups showed a significant improvement, especially the combined treated group. These results were supported by the significant improvement in histopathological examination.

Conclusion: Quercetin and vitamin D supplementation caused elevation of klotho gene expression which in turn decreases oxidative stress, fibrosis, and apoptosis. They also increase BDNF which attenuates the associated neurobehavioral complications. So, they had a prophylactic role in CKD, prevented its progression and attenuated the associated neurobehavioral complications.

Keywords: Chronic Kidney Disease; Neurodegenerative diseases; Klotho gene; Quercetin; Vitamin D.

Introduction

Chronic kidney disease (CKD) is a serious disorder featured by tubular atrophy, glomerulosclerosis, and interstitial fibrosis of the kidney. Moreover, inflammation and oxidative stress play vital roles in its pathophysiology and they become worse with its progression [1].

CKD is associated with cognitive dysfunctions. Even though albuminuria and a low estimated glomerular filtration rate (GFR) are risk factors for the occurrence of cognitive impairment, cognitive function alterations can happen early in the CKD course [2].

Klotho gene expression is frequently low in CKD cases, and the lack of it has been linked to the progression and development of CKD. A rise in Klotho levels, on the other hand, improves kidney function and inhibits CKD development, lending credence to the idea that modifying Klotho levels could be a potential therapeutic method for CKD management [3]. Furthermore, in animal models, the onset and development of CKD is related to a decrease in Klotho [4].

Vegetables and fruits contain a flavonoid called quercetin, which has special biological activities that include antiviral, anti-inflammatory, antioxidant, anti-carcinogenic, and psychostimulant effects. It can also restrict lipid peroxidation, and capillary permeability while promoting mitochondrial biogenesis [5].

Vitamin D or calcitriol is a steroid hormone that has long been known for its important role in regulating body levels of calcium and phosphorus, and in mineralization of bone. It is involved also in brain functions as it passes through the blood-brain barrier and was given the name of neurosteroid. Active vitamin D has antifibrotic effects, antiapoptotic and antioxidant potential that ameliorates against oxidative damage [6].

Interestingly, this is the first study to examine the combined effect of quercetin and vitamin D3 on neurobehavior changes that accompany CKD and Klotho gene expression in the folic acid-induced CKD rat model.

This study aimed to detect the possible beneficial effects of quercetin and vitamin D on CKD progression and the associated neurological complications and to detect their possible mechanism in relation to the Klotho gene expression in the CKD rat model.

Methods

This study was performed on 30 adult male albino rats of a local strain, aged from 10 to 12 weeks with body weight about 200– 260 g, bought from the laboratory animal house, Faculty of Veterinary Medicine, Zagazig University. The animals were kept in plastic rodent small cages at normal density (24 × 40 × 20 cm at 160 cm^2/rat (6 rats per cage)) under completely hygienic conditions, at room temperature (24 -26) ℃.

They fed on standard commercial rat chow, obtained from the faculty of Agriculture- Zagazig University, with free access to water, and were maintained on a normal light cycle. The rats were housed in animal houses for two weeks prior to the experiment.

Approval for this study was obtained from the Physiology Department and Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC). The approval number is; ZU-IACUC/3/F/424/2022.

Rats were randomly allocated into 5 groups as the following: Group 1 (Control group) (n= 6): treated with Vehicle (0.3M NaHCO3 (EL Gomhoureya-Egypt) (5ml/ Kg) intraperitoneal (I.P.) injection and 1ml of normal saline by oral gavage daily for one month. Group 2 (CKD group) (n=6): Folic acid (FA) induced CKD group. rats received 1ml of normal saline by oral gavage daily for one month and were treated with I.P. injection of FA (EPICO-Egypt) (250 mg/kg) dissolved in 0.3M $NaHCO_3$ every third day during the induction phase (days 0–9), followed by a maintenance phase (from day 10 to the end of the experiment (one month), during which the animals in this group received the same I.P. dose of FA (250 mg /kg) daily [7]. Group 3(CKD+ quercetin group) (n= 6): chronic kidney disease group treated with quercetin (XPRS Nutra, Jordan). It was administered 24 hours after the first dose of folic acid injection (300mg/kg) 'dissolved in normal saline solution' by oral gavage once daily for one month) [8]. Group 4(CKD+VIT D3) (n= 6): chronic kidney disease group treated with vitamin D3. It was administered 24 hours after the first dose of folic acid injection (500IU/kg by oral gavage once daily for one month) [9]. Group 5(CKD+ quercetin+ VIT D3) (n= 6): chronic kidney disease group treated with both quercetin (300mg/kg, orally once daily) and vitamin D3 (500IU/kg by oral gavage once daily) for one month.

Blood pressure measurement: Rat blood pressure was assessed at the end of the study period and after the neurobehavioral tests by a non-invasive blood pressure monitoring system (Kent Scientific, Torrington, CT, USA) which assesses tail blood pressure utilizing volume pressure.

Evaluation of neurobehavioral functions: The following tests were evaluated: open field maze test (OFM) for evaluation of Behavior [10], modified forced swim test (MFST) depressive-like behavior testing [11], novel object recognition test (NORT) used to evaluate animal memory, learning, and exploratory behaviors [12].

Biochemical assay: 24hours urine collection was done at the end of the experiment from all rats for estimation of creatinine, calculation of Albumin-to-Creatine Ratio (ACR) according to Sreemantula et al. [13] and GFR according to Cockcroft and Gault [14]

At the end of the experiment and after measuring blood pressure (between 9:00-11:00 a.m.), blood samples (about 6 ml/rat) were taken from each rat's retro-orbital plexus. The blood samples were permitted to coagulate at room temperature prior to being centrifuged for 20 minutes at 4000 rpm. The serum was kept at a temperature of -80° C.

Blood urea nitrogen (BUN) and creatinine (Spinreact, S.A.U.ctra. Santa Colona), malondialdehyde (MDA) and superoxide dismutase (SOD) (Egyptian Company for Biotechnology

(SAE), Obour city, Egypt), tumor necrosis factor (TNF-α) (Cat. NO. MBS355371.Sigma-USA), Caspase 3 (Casp-3) (Cat. NO. CSB-E08857r; USA), brain-derived neurotrophic factor (BDNF) (ELISA kits. ZellBio GmbH, Germany) levels were assessed in serum.

Histopathological examination: After blood samples collection, all rats were decapitated under Urethane 1.2 g/kg i.p [15] Both kidneys and hippocampus samples were dissected. Right kidneys and right hippocampus samples were fixed for 24 hours in 4% paraformaldehyde solution and 10% formal saline respectively then processed to make paraffin blocks. Paraffin sections of 5-6 µm thickness were stained with Hematoxylin and Eosin (H&E) for examination [16]. Scoring of histopathological lesions of kidney was done by pathologist according to the following scales: Inflammatory infiltrate in the kidney, tubular cell damage and tubule-interstitial fibrosis were graded according to the area of tubular lesion or fibrosis: grade 0 (0%), grade 1 (<10%), grade 2 (10–30%), grade 3 (30–50%), grade 4 (>50%), grade 5 (almost 100%) [7]. The left kidney and left hippocampus specimens were frozen in liquid nitrogen and stored at -80°C for later gene expression of klotho protein.

Klotho gene expression: Total RNA was extracted from the tissue using Trizol (Invitrogen; Thermo Fisher Scientific, Inc., UK). For assessing the RNA quality, the NanoDrop® ND–1000 Spectrophotometer (NanoDrop Technologies; Wilmington, Delaware, United States) was utilized for 1.5 µl of the RNA. For cDNA synthesis, a High-Capacity cDNA Reverse Transcription Kit cDNA Kit; (Applied Biosystems™, USA) was used. Klotho primer: forward 5' -CACGCCGAGCAAGACTCACTG-3', the reverse: 5' -TTGATGTCGTCCAACACGTAGGC-3'. The real-time RT-PCR was carried out using a Mx3005P Real-Time PCR System (Agilent Stratagene, USA) using TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725 or P750) (Enzynomics, Korea) following the manufacturer's instructions.

Statistical Analysis

The sample size was estimated using the Open Epi program (Dean AG, Sullivan KM, Soe MM) assuming the mean MDA was 0.27±0.05. Vs. 0.34±0.06 in the control vs disease group [17]. The statistical analysis is done by using SPSS 25 Software (Inc. Chicago, IL, USA) using mean ± SD for data analysis and the difference was compared using One-way Analysis of Variance (ANOVA) followed by Post hoc (LSD) test; p value < 0.05 was considered statistically significant.

Results

Blood pressure assessment: Arterial blood pressure showed high significant increase in CKD group compared to control (p< 0.001) while showed high significant decrease in all treated groups compared to CKD group (p< 0.001) (Table 1).

Neurobehavioral examination: Regarding OFM, there was a high significant increase (P<0.001) in the number of fecal boli, rear latency, and latency to move associated with high significant decrease (P<0.001) in number of crossed squares and rear number in CKD group compared to control. There was a significant reduction (P<0.05) in the number of fecal boli, rear latency, and latency to move associated with high significant increase (P<0.001) in number of crossed squares and rear number in (CKD + quercetin group), (CKD+ VIT D3 group) and (CKD + quercetin and VIT D3 group) when compared with CKD group (Figure 1).

Concerning NORT and modified forced swim, there was a significant decrease in discrimination index, exploration time of a novel object, visit numbers to a novel object (P<0.05) climbing time, and swimming time (P<0.001) associated with a high significant increase (P<0.001) in immobility time in CKD group compared to control. However a high significant improvement (P<0.001) in these parameters was observed in (CKD + quercetin group), (CKD+ VIT D3 group) and (CKD + quercetin and VIT D3 group) when compared with that of CKD group (Figure 2).

Biochemical measures: Deterioration of kidney function tests in CKD group in form of high significant increase (P<0.001) in the levels of BUN, serum creatinine and albumin creatinine ratio (ACR) in urine while there was a high significant decrease (P<0.001) in GFR compared to other groups. The serum levels of apoptotic marker “caspase-3”, the inflammatory marker “TNF-α” and the oxidant “MDA” showed a high significant elevation (P<0.001) but the antioxidant marker “serum SOD” showed high significant decrease (P<0.001) in CKD group when compared to other groups. while these parameters were significantly reversed in (CKD + quercetin group), (CKD+ VIT D3 group), and (CKD + quercetin and VIT D3 group) compared to CKD group (P<0.001) (Table 1).

There was a high significant elevation in serum BDNF level (P<0.001) in (CKD + quercetin group), (CKD+ VIT D3 group), and (CKD + quercetin and

VIT D3 group) when compared with that of CKD group (Figure 3).

Klotho gene expression: In both kidney and brain tissue, there were high significant decrease in klotho gene expressions (P<0.001) in CKD group compared to control. However high significant elevation in klotho gene expressions (P<0.001) in (CKD + quercetin group), (CKD+ VIT D3 group), and (CKD + quercetin and VIT D3 group) when compared with CKD group. Interestingly, there were high significant upregulation in klotho gene expressions (P<0.001) in (CKD + quercetin and VIT D3 group) when compared with other groups (Figure 3).

Histopathological examination: The hippocampus of the control group revealed normal viable neurons with centrally placed nuclei and normal thickness of CA3 and dentate gyrus. CKD group showed a marked decrease in the number of normal viable neurons with centrally placed nuclei and a decreased thickness dentate gyrus with numerous degenerated cells with pyknotic dark stained nuclei. Quercetin group showed a moderate increase in the number of normal viable neurons with centrally placed nuclei and a reduced number of degenerated cells with pyknotic dark-stained nuclei. The Vit D3 treated group showed a moderate increase in several normal viable neurons with centrally placed nuclei and a decrease in the number of degenerated cells with pyknotic dark stained nuclei. Quercetin + Vit D3 treated group: exhibiting marked increased number of normal viable neurons with centrally placed nuclei and disappearance of degenerated cells with pyknotic dark stained nuclei in DG (Figure 4).

The kidney tissues of the control group exhibited normal architecture of glomeruli and tubules. CKD group revealed marked vascular congestion, tubular dilatation with many tubular casts, and marked interstitial inflammatory cell infiltrate. quercetin-treated group revealed mild improvement with a decrease in vascular congestion and decreased interstitial inflammatory cell infiltrate. Vit D3 treated group revealed mild improvement with a decrease in vascular congestion, and decreased interstitial inflammatory cell infiltrate. Quercetin + Vit D3 treated group: revealed near normal glomerular and tubular architecture with the absence of vascular congestion, tubular casts, and interstitial inflammation. There was marked variance between CKD group and the treatment groups concerning inflammatory infiltrate percentage in renal tissues (Figure 5).

Table 1: Blood pressure,Kidney function, oxidative stress, inflammatory and apoptotic markers among the studied groups:

	Control		CKD	CKD + quercetin	CKD+ VIT D3	CKD + quercetin+ VIT D3
MABP (mmHg)	**Mean ± SD** **P value of LSD**	82.60±4.76	123.80±14.99 $P<0.001^{a}$	98.33±7.89 $P<0.05^{a}$ $P<0.001^{b}$	102.70±6.8 $P<0.05^{a}$ $P<0.001^{b}$	90.36±5.53 $P<0.001^{b}$
BUN (mg/dl)	**Mean ± SD** **P value of LSD**	9.74±1.18	56.43±7.39 $P<0.001^{a}$	34.33±4.34 $P<0.001^{ab}$	35.41±5.46 $P<0.001^{ab}$	14.14±3.90 $P<0.001^{bcd}$
Creatinine (mg/dl)	**Mean ± SD** **P value of LSD**	0.62±0.18 (0.2-1)	3.93±0.56 $P<0.001^{a}$	2.41±0.38 $P<0.001^{ab}$	2.61±0.32 $P<0.001^{ab}$	1.61±0.42 $P<0.001^{bcd}$
ACR	**Mean ± SD** **P value of LSD**	27.46±6.80	199.98±58.21 $P<0.001^{a}$	100.56±7.68 $P<0.001^{ab}$	104.20±31.12 $P<0.001^{ab}$	62.98±11.20 $P<0.001^{b}$ $P<0.05^{acd}$
GFR (ml/min)	**Mean ± SD** **P value of LSD**	0.63±0.077	0.07±0.012 $P<0.001^{a}$	0.22±0.043 $P<0.001^{ab}$	0.22±0.036 $P<0.001^{ab}$	0.54±0.055 $P<0.001^{bcd}$ $P<0.05^{a}$
MDA (nmol/ml)	**Mean ± SD** **P value of LSD**	0.61±0.20	9.87±1.69 $P<0.001^{a}$	3.78±1.02 $P<0.001^{ab}$	3.75±0.96 $P<0.001^{ab}$	1.44±0.49 $P<0.001^{bcd}$
SOD (unit/ml)	**Mean ± SD** **P value of LSD**	264.20±68.89	46.76±14.59 $P<0.001^{a}$	117.63±21.85 $P<0.001^{ab}$	103.62±14.31 $P<0.001^{ab}$	139.22±30.67 $P<0.001^{abcd}$
TNF-α (pg/ml)	**Mean ± SD** **P value of LSD**	6.96±1.48	67.63±8.56 $P<0.001^{a}$	30.86±5.77 $P<0.001^{ab}$	33.75±3.94 $P<0.001^{ab}$	15.51±5.49 $P<0.001^{bcd}$ $P<0.05^{a}$
Caspase-3 (pg/ml)	**Mean ± SD** **P value of LSD**	0.59±0.106	10.94±1.64 $P<0.001^{a}$	3.63±0.75 $P<0.001^{ab}$	3.96±1.04 $P<0.001^{ab}$	1.94±0.76 $P<0.001^{b}$ $P<0.05^{acd}$

MABP: mean arterial blood pressure(mmHg), BUN: blood urea nitrogen(mg/dl), GFR: glomerular filtration rate(ml/min), MDA: malondialdehyde(nmol/ml),ACR: Albumin/creatinine ratio, SOD: superoxide dismutase(unit/ml), TNF-α: tumor necrosis factor(pg/ml).
a: significant versus control group.
b: significant versus CKD group.
c: significant versus CKD + quercetin group.
d: significant versus CKD + VIT D3 group.

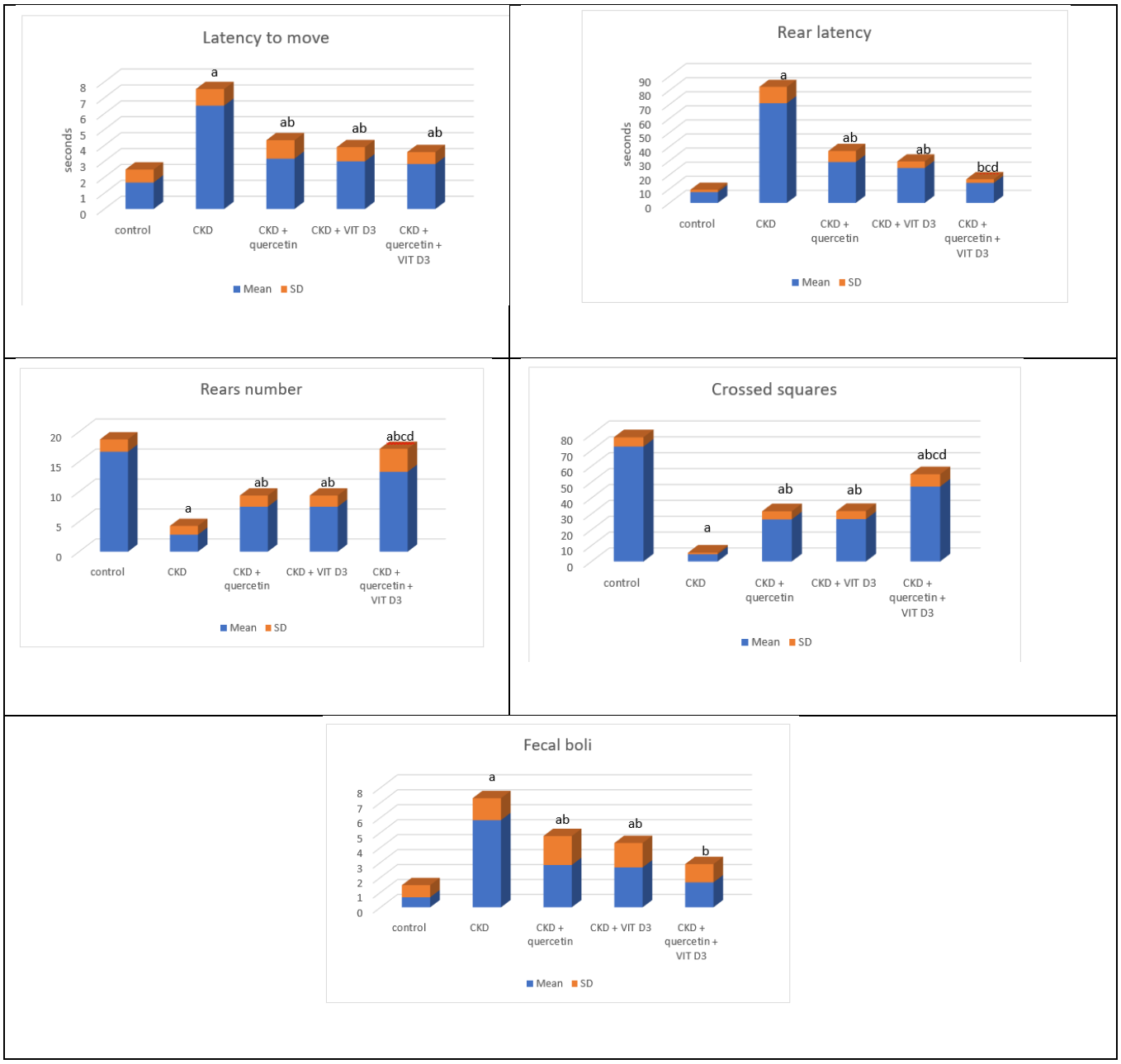

Figure 1: Comparison between all study groups regarding OFM (latency to move, rear latency, rears number, crossed squares, and fecal boli). a: significant versus control group, b: significant versus CKD group, c: significant versus CKD + quercetin group, d: significant versus CKD + VIT D3 group.

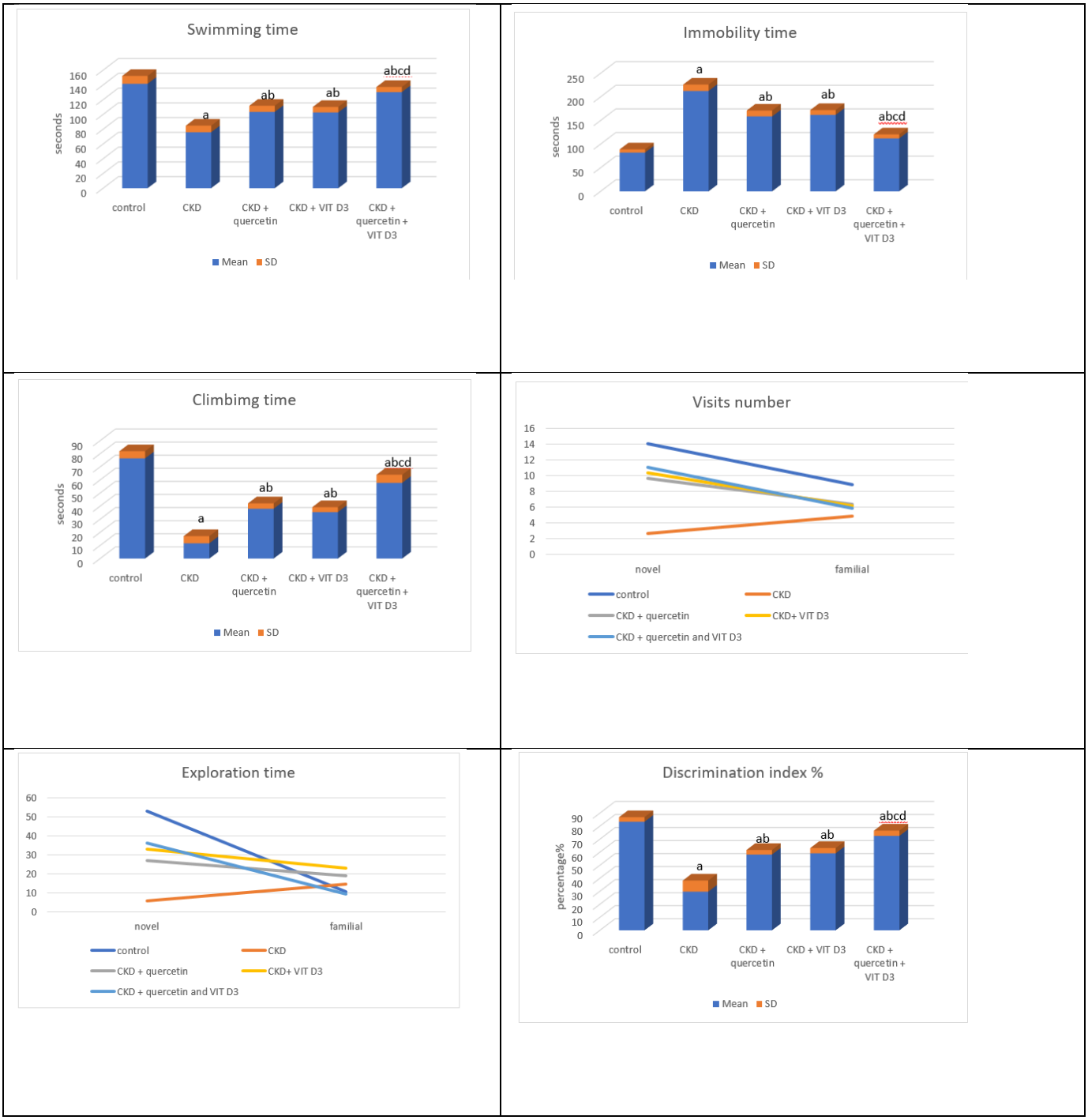

Figure 2: Comparison between all study groups regarding(swimming time, immobility time, climbing time) and NORT (visits number, exploration time , discrimination index %). a: significant versus control group, b: significant versus CKD group, c: significant versus CKD + quercetin group, d: significant versus CKD + VIT D3 group.

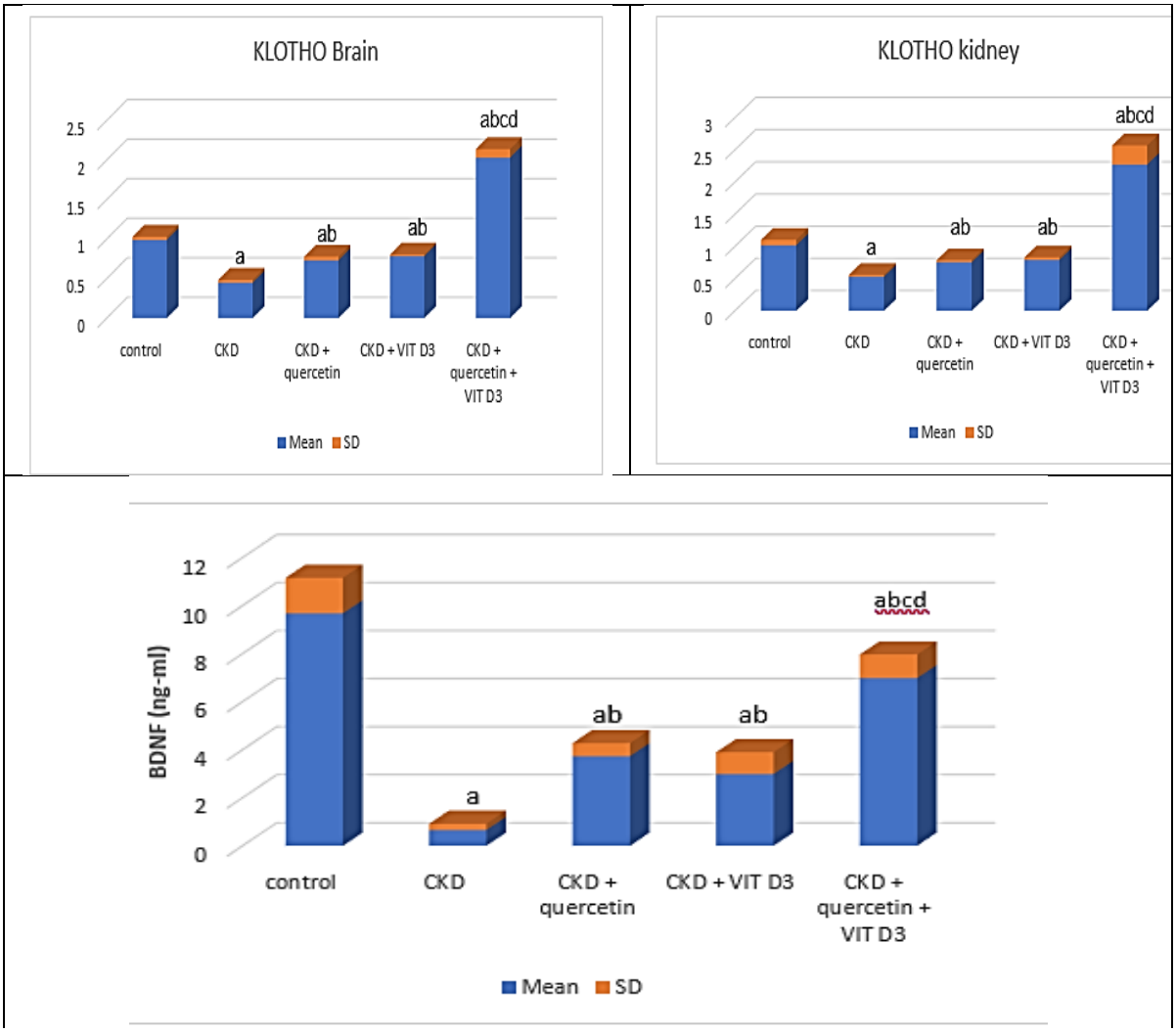

Figure 3: comparison between all studied groups regarding serum BDNF(ng/ml) and KLOTHO gene expression in brain and kidney tissues. a: significant versus control group, b: significant versus CKD group, c: significant versus CKD + quercetin group, d: significant versus CKD + VIT D3 group

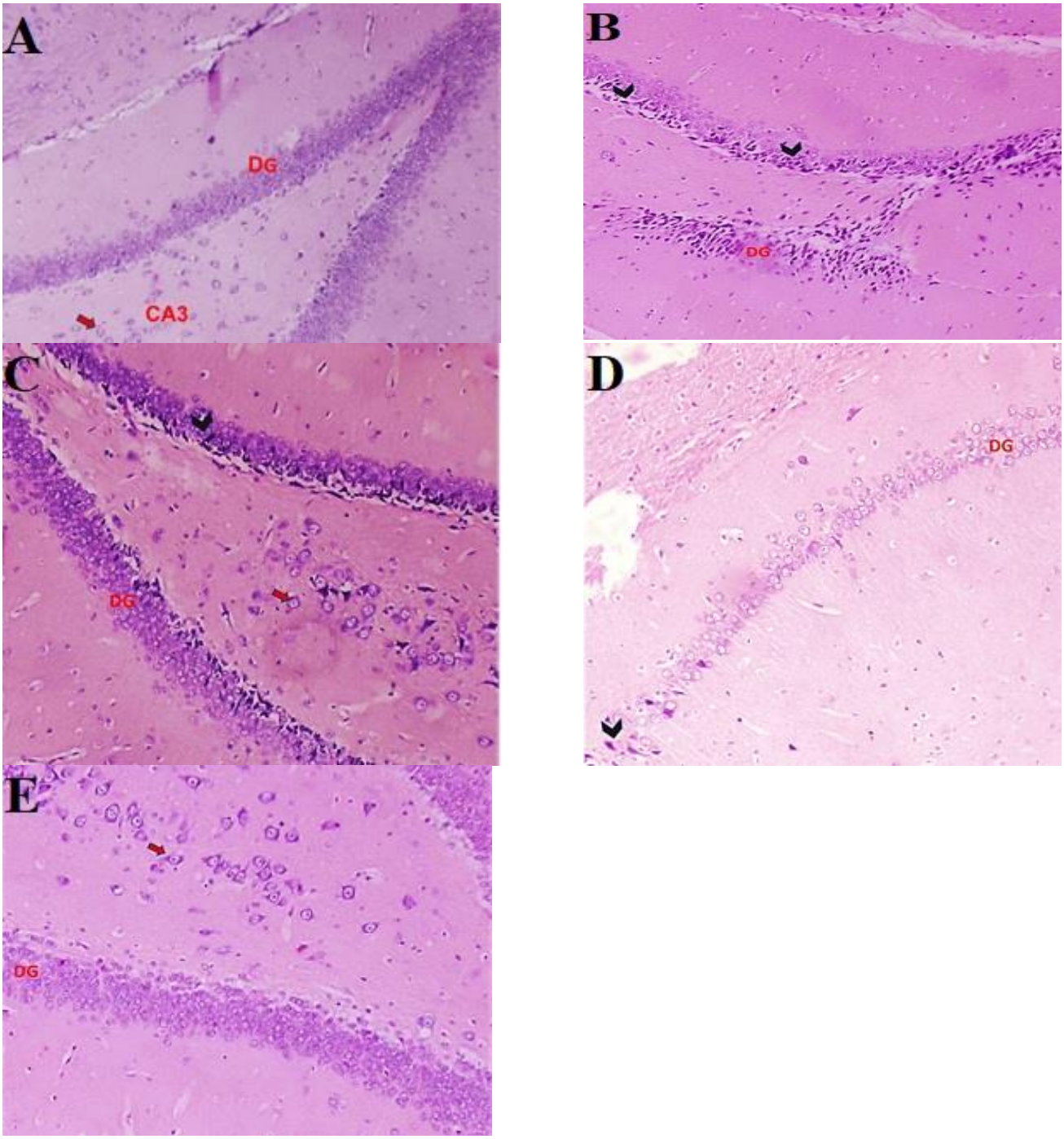

Figure 4: Photomicrograph of hippocampus tissues of the studied groups. (A) control group: showing showing normal viable neurons with central placed nuclei (red arrows) and normal thickness of CA3 and dentate gyrus. (B) CKD group: showing marked decrease of the thickness of dentate gyrus with numerous degenerated cells with pyknotic dark stained nuclei (black arrow head). (C) CKD+quercetin group: showing viable neurons with central placed nuclei (red arrows) and decrease in number of degenerated cells with pyknotic dark stained nuclei (black arrow head). (D) CKD+ VIT D3 group: showing decrease in number of degenerated cells with pyknotic dark stained nuclei (black arrow head). (E) CKD+quercetin+ VIT D3 group: showing showing marked increase in number of normal viable neurons with central placed nuclei (red arrows) and disappearance of degenerated cells with pyknotic dark stained nuclei in DG

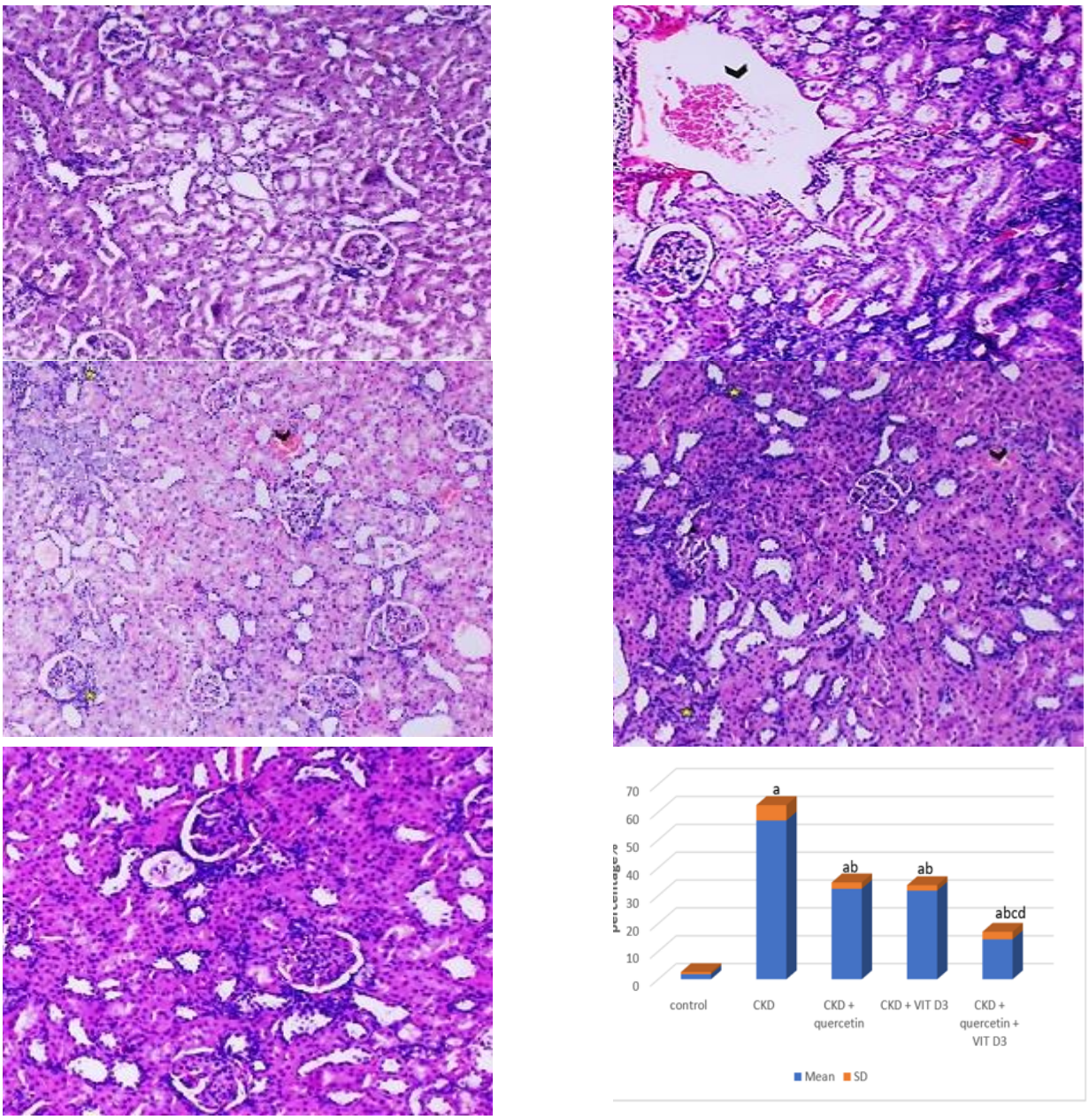

Figure 5: Photomicrograph of kidney tissues of the studied groups. (A) Control group revealed normal architecture of glomeruli and tubules. (B) CKD group revealed marked vascular congestion (black arrow head), tubular dilatation with many tubular cast (red arrows),and marked interstitial inflammatory cell infiltrate (yellow star). (C) CKD +quercetin group revealed mild improvement with decrease in vascular congestion (black arrow head), and decreased interstitial inflammatory cell infiltrate (yellow star). (D) CKD +VIT D3 group revealed mild improvement with decrease in vascular congestion (black arrow head) ,and decreased interstitial inflammatory cell infiltrate (yellow star). (E)CKD+ quercetin + VIT D3 group: revealed near normal glomerular and tubular architecture with absence of vascular congestion, tubular casts and interstitial inflammation. (F) Inflammatory infiltrate percentage in histopathological examination of kidney tissue in all studied groups. a: significant versus control group. b: significant versus CKD group. c: significant versus CKD + quercetin group. d: significant versus CKD + VIT D3 group.

Discussion

Large doses of Folic acid administration previously was proved to deteriorate kidney functions [7]. In the present study, folic acid induced CKD model showed a significant deterioration in kidney function in form of: elevation in the levels of arterial blood pressure, serum BUN, serum creatinine, increased albumin creatinine ratio

(ACR) in urine with a marked reduction in GFR compared with that of control group. Yan et al. [18] and Renczés et al. [19] reported a significant increase in the serum level of BUN and creatinine as well as increased ACR in urine in CKD rats.

Histopathological analysis of renal tissue in the present study revealed a significant tubular dilatation, tubular necrosis, tubular cast, shedded intratubular cells, necrotic cells, marked interstitial inflammatory cell infiltrate and interstitial fibrosis this comes in FA induced CKD group.

According to these results, multiple researchers interpreted these alterations by pointing out that the kidneys' high folate receptor capacity allows FA accumulation there more than in other organs. Nearly, 40% of the folate pool that is kept in renal cells can influence mitochondria leading to aberrations and oxidative stress. Furthermore, excessive folate levels in the kidneys can seriously impair cellular antioxidative mechanisms that also need NADPH since folate reduction takes a lot of this reducing energy. This can exacerbate oxidative stress and redox imbalance in the kidneys [18].

Since FA is poorly soluble and forms crystals in the renal lumen, injecting large doses of FA has been shown to be an effective model for inducing AKI. This has been proposed to cause a change in the architecture of cells, oxidative stress, and fibrosis, which lead to CKD [7,20]. Moreover, Fu et al. [21] proposed that the development from AKI to CKD could be provoked by repeated damage caused by several FA treatments.

In the present study, quercetin and vitamin D3 treated groups showed a significant improvement in kidney function tests in comparison with that of CKD group. Moreover, quercetin and vitamin D3 treated groups revealed significant improvement of renal histopathology.

In line with our results, Yang et al. [22] reported that quercetin improves kidney function in the form of a significant decrease in the levels of BUN, creatinine, and uric acid. They reported also, that treatment of the rats, in the chronic kidney model, with quercetin reduced the alterations in renal tissues' architecture including interstitial chronic inflammatory reactions.

Furthermore, according to Layal et al. [23], the administration of quercetin had no effect on plasma creatinine, urea, or the amount of protein in urine. However, in their CKD model, it tended to lower the MDA level and the degree of fibrosis in the kidney.

Furthermore, Al-Sroji et al. [24] reported that vitamin D3 treatment prevented acute renal damage caused by lipopolysaccharide (LPS) and lowered urea and creatinine levels. Moreover, oxidative stress decreased—damage of renal tissue and glomeruli damage was mitigated by active vitamin D3. These consequences might result from upregulation of SOD and downregulation of the expression of NADPH oxidase.

In our investigation, inflammatory, oxidative stress status and apoptosis were increased in the folic acid-induced CKD group as there were a significant increase of TNF-α, MDA and Caspase-3 due to the marked inflammation and the increased lipid peroxidation by ROS and a significant decrease in SOD due to its consumption as a defense factor in CKD as reported in previous studies [7,18,20,21].

In our study, quercetin and vitamin D3 treated groups showed a significant decrease in serum TNF-α, MDA, and Caspase-3 and a significant increase in the level of serum SOD in comparison with the CKD group. Bagheri et al. [25] evaluated the reno-protective advantages of quercetin in rats with induced ischemia/reperfusion injury through the modulation of inflammatory cytokines, NF-kB, and apoptotic proteins.

Respecting Vitamin D3 findings in our study, Al-Sroji et al. [24] reported that active vitamin D3 lowers oxidative stress and may mitigate renal and glomerular damage. These effects might result from upregulation of the cytosolic SOD enzyme and downregulation of the expression of NADPH oxidase.

CKD affected the brain tissues, our study showed that hippocampal histopathology of CKD group has marked neurodegenerative changes associated with impaired cognition with increase depressive and anxiety like behavior in NORT, MFST and OFM respectively. All of these changes were improved in quercetin and/or vitamin D3 treated groups.

In line with our result, Huang et al. [26] noted in their study that the CKD group had hippocampus structural alterations, neuronal degeneration, and necrosis. Also, Ruiz et al. [27] found that apoptosis of hippocampal neurons was observed in uremic animal models.

Also, Bobot et al. [12] reported an impairment of both cognitive function and short-term learning performance in the NORT in three models of CKD in rats. Experimental models of severe and moderate renal deficiency led to reduced exploratory and directional movement ability in rats

[28]. Moreover, Renczés et al. [19] reported an observed depression in CKD animal model.

Interestingly, the hippocampal histopathology of the group treated with both quercetin and vitamin D3 revealed a marked increase in several normal viable neurons with centrally placed nuclei and the disappearance of degenerated cells with pyknotic dark stained nuclei in DG.

In line with our result, many studies reported quercetin anti-depression potentiality in many animal models. Demir et al. [29] demonstrated that quercetin can attenuate depression in diabetes.

Concerning vitamin D3 neuroprotective effect proved in the present study, similar findings were observed by other researchers in animal models, Mokhtari-Zaer et al. [30], reported that LPS-impaired cognitive functions were improved in Vit D3 group elevated SOD. In addition, Rastegar et al. [31] reported that, vitamin D_3 helped in restoring the impaired neurogenesis of hippocampus and decreasing apoptosis of neurons in their study.

Interestingly, the current study showed a significant decrease in serum level of BDNF in CKD group, suggesting that downregulation of BDNF is a possible mechanism that mediates the effect of CKD on depression-like behaviors.

In line with these findings, Kielstein et al. [28] found lower BDNF levels were linked to greater depression scores, and it was shown that in rats with chronic renal failure, a significant decrease in BDNF occurs in the event of a 5/6 nephrectomy, which significantly reduces locomotion and exploratory behavior.

In our result, Quercetin and vitamin D3 increase the serum level of BDNF. Moreover, Naghizadeh et al. [32] showed that quercetin administration increased downregulated cytochrome C levels and caspase-3 in diabetic rat retina.

In addition, Khairy & Attia [33] reported that, raising vitamin D levels is a helpful approach for brain ageing in a healthy way. Enhancing the brain is an important way that vitamin D prevents age-related brain impairment appears to be through BDNF.

The anti-aging protein “klotho” has antioxidant and pro survival properties, in our study CKD group showed a significant decrease in its expression in both kidney and neural tissues in comparison with control group. While there was a significant increase in klotho gene expression in quercetin and vitamin D3 treated groups in comparison with CKD group. Interestingly, klotho gene expression in both kidney and neural tissues in the group treated with both quercetin and vitamin D3 showed a significant increase in comparison with control group.

Correspondingly, other investigations have agreed with our findings showing that the pathogenesis and development of CKD are associated with Klotho downregulations in animal models [3,34]. In addition, Huang et al. [26] reported that since α klotho expression appears to have an impact on the cognition of CKD rats, it could be a useful target for the prevention and therapy of impaired cognitive functions linked to CKD.

Interestingly, Klotho expression level was significantly increased in the quercetin group in Bhatiya et al. [35] study, which is in line with our finding.

Moreover, Forster et al. [36] reported that in human and mouse kidney cells, the expression of the anti-aging Klotho gene is maintained by the Vitamin D receptor (VDR). In addition, Lau et al. [37] reported that VDR agonists increase klotho in CKD-mice model with diet of high phosphate. So that evidence might be the reason for increasing klotho gene expression in response to quercetin and vitamin D3.

Quercetin and vitamin D3 have different structures. But quercetin and vitamin D3 have similar functions, such as sharing networks of hydrogen bonds between the coordinated hydroxyl groups of the six-membered ring legend and docking with similar amino acid sequences when binding with VDR [38]. This suggests that quercetin may have a role in maintaining different genes expression by VDR activation and causing it to bind VDR elements.

Limitations

Fibrosis evaluation of kidney by histopathology was not clarified and fibrotic markers as TGF- α was not assessed in this study.

Conclusion

CKD is an inflammatory process that is correlated with increased fibrosis, oxidative stress, apoptosis, and decline in Klotho gene expression. Quercetin and vitamin D supplementation caused elevation of klotho gene expression which in turn decreases oxidative stress, fibrosis, and apoptosis. They also increase BDNF which attenuates the associated neurobehavioral complications. Both have synergistic prophylactic role when taken together in CKD, prevented its progression, and attenuated the associated neurobehavioral complications.

Conflicts of interest:

No potential conflict of interest was reported by the authors.

Funding:
No funding was declared.

References

1. Huang R, Fu P, Ma L. Kidney fibrosis: from mechanisms to therapeutic medicines. Signal Transduct Target Ther. 2023;8:129
2. Pépin M, Klimkowicz-Mrowiec A, Godefroy O, Delgado P, Carriazo S, Ferreira AC, et al. Cognitive disorders in patients with chronic kidney disease: Approaches to prevention and treatment. Eur J Neurol. 2023;30:2899–911.
3. Li S-S, Sheng M-J, Sun Z-Y, Liang Y, Yu L-X, Liu Q-F. Upstream and downstream regulators of Klotho expression in chronic kidney disease. Metabolism. 2023;142:155530.
4. Buchanan S, Combet E, Stenvinkel P, Shiels PG. Klotho, Aging, and the Failing Kidney. Front Endocrinol (Lausanne). 2020;11:560.
5. Shabbir U, Rubab M, Daliri EB-M, Chelliah R, Javed A, Oh D-H. Curcumin, Quercetin, Catechins, and Metabolic Diseases: The Role of Gut Microbiota. Nutrients. 2021;13:206.
6. Berretta M, Quagliariello V, Bignucolo A, Facchini S, Maurea N, Di Francia R, et al. The Multiple Effects of Vitamin D against Chronic Diseases: From Reduction of Lipid Peroxidation to Updated Evidence from Clinical Studies. Antioxidants (Basel). 2022;11:1090.
7. Perales M, Saucedo A, Lucio J, Waksman N, Alarcon G, Govea G, et al. Metabolomic and biochemical characterization of a new model of the transition of acute kidney injury to chronic kidney disease induced by folic acid. PeerJ. 2019;7:e7113.
8. Sriraksa N, Wattanathorn J, Muchimapura S, Tiamkao S, Brown K, Chaisiwamongkol K. Cognitive-Enhancing Effect of Quercetin in a Rat Model of Parkinson's Disease Induced by 6-Hydroxydopamine. eCAM. 2012;2012:1–9.
9. Hajiluian G, Nameni G, Shahabi P, Mesgari M, Sadigh S, Farhangi MA. Vitamin D administration, cognitive function, BBB permeability and neuroinflammatory factors in high-fat diet-induced obese rats. Int J Obes (Lond). 2017;41:639–44.
10. Feyissa D, Aher Y, Engidawork E, Höger H, Lubec G, Korz V. Individual Differences in Male Rats in a Behavioral Test Battery: A Multivariate Statistical Approach. Front Behav Neurosci. 2017;11:26.
11. Slattery D, Cryan J. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat Protoc. 2012;7:1009–14.
12. Bobot M, Thomas L, Moyon A, Fernandez S, McKay N, Balasse L, et al. Uremic Toxic Blood-Brain Barrier Disruption Mediated by AhR Activation Leads to Cognitive Impairment during Experimental Renal Dysfunction. J Am Soc Nephrol. 2020;31:1509–21.
13. Sreemantula S, Boini K, Nammi S. Reserpine methonitrate, a novel quaternary analogue of reserpine augments urinary excretion of VMA and 5-HIAA without affecting HVA in rats. BMC pharmacology. 2004;4:30.
14. Cockcroft D, Gault M. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16:31–41.
15. Field K, Lang C. Hazards of urethane (ethyl carbamate): a review of the literature. Lab Anim. 1988;22(3):255-62.
16. Suvarna S, Layton C, Bancroft J, editors. Bancroft's theory and practice of histological techniques. Eighth edition. Amsterdam: Elsevier; 2018.
17. Garrido P, Reis F, Costa E, Teixeira E, Parada B, Alves R, et al. Characterization of a rat model of moderate chronic renal failure--focus on hematological, biochemical, and cardio-renal profiles. Ren Fail. 2009;31:833–42.
18. Yan L-J. NADH/NAD+ Redox Imbalance and Diabetic Kidney Disease. Biomolecules. 2021;11:730.
19. Renczés E, Marônek M, Gaál A, Vavrincová D, Tóthová L, Hodosy J. Behavioral Changes During Development of Chronic Kidney Disease in Rats. Front Med (Lausanne). 2019;6:311.
20. Stallons L, Whitaker R, Schnellmann R. Suppressed mitochondrial biogenesis in folic acid-induced acute kidney injury and early fibrosis. Toxicol Lett. 2014;224:326–32.
21. Fu Y, Tang C, Cai J, Chen G, Zhang D, Dong Z. Rodent models of AKI-CKD transition. Am J Physiol Renal Physiol. 2018;315:F1098–106.
22. Yang H, Song Y, Liang Y, Li R. Quercetin Treatment Improves Renal Function and Protects the Kidney in a Rat Model of Adenine-Induced Chronic Kidney Disease. Med Sci Monit. 2018;24:4760–6.
23. Layal K, Perdhana I, Louisa M, Estuningtyas A, Soetikno V. The effects of quercetin on oxidative stress and fibrosis markers in chronic kidney disease rat model. MJI. 2017;26:169–77.
24. Al-Sroji R, Al-Laham S, Almandili A. Protective effects of vitamin D3 (cholecalciferol) on vancomycin-induced oxidative nephrotoxic damage in rats. Pharm Biol. 2023;61:755–66.

25. Bagheri A, Radman G, Aria N, Rezaei F, Khajenouri M, Ghiabi S, et al. The Effects of Quercetin on Apoptosis and Antioxidant Activity in a Renal Ischemia/Reperfusion Injury Animal Model. Drug Res (Stuttg). 2023;73:255–62.
26. Huang M, Li G, Tan J, Huang M, Huang F, Ma R, et al. Effects of chronic kidney disease on cognitive function and α-klotho expression in hippocampus. Transl Androl Urol. 2022;11:1157–68.
27. Ruiz S, Pergola P, Zager R, Vaziri N. Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. Kidney Int. 2013;83:1029–41.
28. Kielstein H, Suntharalingam M, Perthel R, Song R, Schneider S, Martens J, et al. Role of the endogenous nitric oxide inhibitor asymmetric dimethylarginine (ADMA) and brain-derived neurotrophic factor (BDNF) in depression and behavioural changes: clinical and preclinical data in chronic kidney disease. Nephrol Dial Transplant. 2015;30:1699–705.
29. Demir E, Gergerlioglu H, Oz M. Antidepressant-like effects of quercetin in diabetic rats are independent of hypothalamic-pituitary-adrenal axis. Acta Neuropsychiatr. 2016;28:23–30.
30. Mokhtari A, Hosseini M, Salmani H, Arab Z, Zareian P. Vitamin D3 attenuates lipopolysaccharide-induced cognitive impairment in rats by inhibiting inflammation and oxidative stress. Life Sci. 2020;253:117703.
31. Rastegar-Moghaddam S, Alipour F, Hosseini M, Ebrahimzadeh A. Anti-apoptotic and neurogenic properties in the hippocampus as possible mechanisms for learning and memory improving impacts of vitamin D in hypothyroid rats during the growth period. Life Sci. 2023;312:121209.
32. Naghizadeh M, Mirshekar M, Montazerifar F, Saadat S, Shamsi A, Jafari S, et al. Effects of quercetin on spatial memory, hippocampal antioxidant defense and BDNF concentration in a rat model of Parkinson's disease: An electrophysiological study. Avicenna J Phytomed. 2021;11:599–609.
33. Khairy E, Attia M. Protective effects of vitamin D on neurophysiologic alterations in brain aging: role of brain-derived neurotrophic factor (BDNF). Nutr Neurosci. 2021;24:650–9.
34. Muñoz J, Rodelo C, Pendon M, Martin A, Santamaria R, Rodriguez M. Klotho/FGF23 and Wnt Signaling as Important Players in the Comorbidities Associated with Chronic Kidney Disease. Toxins (Basel). 2020;12:185.
35. Bhatiya M, Pathak S, Jothimani G, Duttaroy A, Banerjee A. A Comprehensive Study on the Anti-cancer Effects of Quercetin and Its Epigenetic Modifications in Arresting Progression of Colon Cancer Cell Proliferation. Arch Immunol Ther Exp (Warsz). 2023;71:6.
36. Forster R, Jurutka P, Hsieh J, Haussler C, Lowmiller C, Kaneko I, et al. Vitamin D receptor controls expression of the anti-aging klotho gene in mouse and human renal cells. Biochem Biophys Res Commun. 2011;414:557–62.
37. Lau W, Leaf E, Hu M, Takeno M, Kuro M, Moe O, et al. Vitamin D receptor agonists increase klotho and osteopontin while decreasing aortic calcification in mice with chronic kidney disease fed a high phosphate diet. Kidney Int. 2012;82:1261–70.
38. Sannappa N, Shiragannavar D, Puttahanumantharayappa D, Shivakumar T, Dallavalasa S, Basavaraju G, et al. Quercetin activates vitamin D receptor and ameliorates breast cancer induced hepatic inflammation and fibrosis. Front Nutr. 2023;10:1158633.

To Cite

Abozaid, E., Abd-Alaleem, D., Abd-Elsalam, K., El-sayed, S. Effects Of Quercetin and Vitamin D3 on Klotho Gene Expression in Relation to Neurological Functions in Chronic Kidney Disease Rat Model. Zagazig University Medical Journal, 2024; (396-408): -. doi: 10.21608/zumj.2023.245516.2989