How to Cite:
Duaibel, A.- karrar K. abdul J., Alnaji, H. A., Abdulhadi, A. M. H., Almulla, A. F., AL-attraqchi, A. M. M., & Rahim, I. Q. (2022). The role of MCP-1 in the developments of diabetic nephropathy in patients with type 2 diabetes mellitus patients. *International Journal of Health Sciences*, *6*(S2), 8158–8166. https://doi.org/10.53730/ijhs.v6nS2.7049

The role of MCP-1 in the developments of diabetic nephropathy in patients with type 2 diabetes mellitus patients

Al-karrar Kais abdul Jaleel Duaibel
Medical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq
Corresponding author email: Alkkais50@gmail.com

Haider A. Alnaji
Medical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq & Biology Department, College of Science, Babylon University, Babylon, Iraq
Email: Hgeoda995@gmail.com

Ali Mohammed Hussein Abdulhadi
Alkafeel University, College of Medical Technologies, Najaf, Iraq
Email: alialrubayee@alkafeel.edu.iq

Abbas F. Almulla
Medical Laboratory Technology Department, College of Medical Technology, The Islamic University, Najaf, Iraq & Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
Email: abbass.chem.almulla1991@gmail.com

Asmaa M. Mohammed AL-attraqchi
Middle-Euphrates-Neuroscience-Center in Al najaf Al-Ashraf/Iraq
Email: Night_wind_16@yahoo.com

Inas Qasim Rahim
Al-Furat Al-Awsat Technical University, Medical Technology department, Najaf, Iraq
Email: inas.al-hajmi@atu.edu.iq

***Abstract*---**Several complications may be developed during infection with type 2 diabetes mellitus. One of the most is diabetic nephropathy, leading to end-stage renal disease in more than 30% of diabetes mellitus. Monocyte, which is derived from blood circulation to the tissue the maintains an inflammatory state. Several factors and chemokines regulate the activity of monocytes. One of them is

Monocyte chemoattractant protein-1 (MCP-1) which is released by several adipocytes that help attract monocytes during inflammation. Patients and methods: the samples were collected from patients who attended diabetes and endocrinology center in Al-Sader Teaching Hospital in Najaf between January 2019 to April 2019. This study involves 300 subjects. One hundred fifty of them were diagnosed with type 2 diabetes mellitus. According to their albumin/creatinine ratio (ACR), these patients were classified into three groups, including 50 patients with severe nephropathy, 50 patients with moderate nephropathy, and 50 patients with mild nephropathy. The control group involved 150 subjects who appeared healthy and the same age as the patients' group. Result: a significant increase of MCP-1 level was found in patients with mild, moderate and severe diabetic patients compared with healthy control. Conclusion: Most of the biomarkers above may be highly related to the degree of proteinuria, such as MCP-1. This immunological parameter was significantly correlated with patients who had diabetic nephropathy, so there is a susceptibility association between these interleukins and the incidence of this disease among Iraqi patients.

***Keywords*---**diabetic nephropathy, diabetes mellitus patients, MCP-1.

Introduction

Diabetes mellitus is a chronic illness that occurs either when the pancreatic beta cells cannot manufacture a suitable amount of insulin or when the body cannot exploit the insulin effectively, leading to the accumulation of sugar in the blood (Gadsby, 2002). Diabetic prevalence globally has climbed from 171 million persons in 2006 to 285 million people in 2010, an increase of 6.4 percent. By 2030, this is expected to reach 439 million people, or a 7.7 percent prevalence. Between 2010 and 2030, the proportion of DM in industrialized nations will rise by 20%, whereas the percentage of DM in developing countries will rise by 69% (Nguyen et al.,2012). Insulin is a hormone that is manufactured by beta cells of the pancreas. Insulin helps glucose to get into the cells of the body. During DM, sugar will be built up in the blood; a condition referred to as hyperglycemia (Nguyen et al.,2012). Chronic hyperglycemia is related to macrovascular and microvascular complications, which may cause visual weakness, nerve damage, renal and cardiac illnesses, amputation and stroke (Soumya and Srilatha 2011), which cause the morbidity and mortality of the disease. There are three main types of DM, according to WHO. The first one is Diabetes mellitus type 1 (most commonly occurs in children) is an autoimmune disease. During T1DM, the immune system attacks the beta cells in the pancreas. It damages their results from the body's inability to synthesize insulin. The patient must inject insulin (also called Juvenile diabetes and insulin-dependent DM, IDDM for pithiness. The second type is Diabetes mellitus type 2 (adult diabetes mellitus), the most common type of DM. It occurs due to body cells showing insulin resistance, a case when body cells fail to exploit insulin properly, usually associated with insulin insufficiency previously called non-insulin-dependent DM (NIDDM) (Hall, 2011), and the third type is Gestation diabetes mellitus occurs during gestation to

the women who do not have a history with diabetes previously and who have elevated glucose level during pregnancy. It may lead to the development of type 2 DM. (ADA. 2020). Diabetes is the most common cause of chronic renal replacement therapy (RRT) in patients with end-stage renal disease (ESRD). According to published research, the prevalence of diabetes among individuals initiating RRT varied between 24% and 51%. (Poretsky, 2010). End-stage renal disease (ESRD) is the last stage of chronic kidney disease (CKD) which is irreversible and progressive to reach its final stage in 10 to 20 years. Patients with ESRD cannot control their normal kidney function, resulting in the accumulation of urea and other nitrogenous compounds in the blood (uremia and azotemia, respectively) (Imani et al., 2018). Renal failure Dialysis-dependent diabetes is the most prevalent cause of end-stage renal failure. (Lok et al., 2004). Renal replacement treatment (RRT) is required for ESRD patients with an estimated glomerular filtration rate (eGFR) greater than or equal to 6 mL/min per 1.73 m2. (Lopez-Giacoman and Madero 2015). Many studies recorded that inflammation plays a major role in ESRD. Evidence has shown that type II diabetes is associated with a subclinical systemic inflammation attributable to the dysregulation of the innate immune system. This immune response is characterized by elevated blood levels of certain acute-phase markers (Zhong et al., 2020). The inflammatory cytokines have been found in the blood of CKD patients and related to its complications. The cytokines caused the secretion of C-reactive protein (CRP) as acute-phase protein (the most common sed inflammation marker) from the hepatocytes to begin the immune response. Diabetic nephropathy is characterized by the deposition of extracellular matrix, thickening of the glomerular basement membrane, and glomerulosclerosis. (Zoungas et al., 2014)**.** Futagami et al. 2008 observed that diabetic nephropathy patients' urine MCP-1 levels were higher than healthy controls. The frequency of interstitial macrophages and MCP-1 positive cells in kidney biopsy tissue associated favorably with urine MCP-1 levels. Therefore, it was postulated that MCP-1 had a key role in inflammatory renal diseases, including diabetic nephropathy, and macrophages potentially mediated this. Subsequent studies in diabetic mouse models confirmed that macrophages mediate renal injury and progression of nephropathy. MCP-1 knockout mice with streptozotocin-induced diabetic nephropathy had reduced macrophage recruitment and activation in the kidneys. They were protected from glomerular damage. Thus, the evidence that MCP-1 plays a role in this inflammatory process is confirmed. The number of glomerular and interstitial macrophages correlates strongly with the number of glomerular macrophages. accumulation in patients with diabetic nephropathy and the rate of progression of kidney failure, as measured by serum creatinine, degree of proteinuria and interstitial fibrosis (Futagami et al. 2008). It was more predictive in macroalbuminuric individuals. Levels predicted eGFR decrease better than the commonly used urine protein/creatinine ratio at time zero. Subsequently, a larger sample of 190 patients with continuous drop in eGFR over 5 years was matched to 190 controls with minor decline (Tam et al., 2009; Montero et al., 2016).

Subjects

In this case-control study, about one hundred fifty patients from diabetes and endocrinology center in Al-Sader Teaching Hospital. The patients were of both

sex, and their ages ranged between(23-70) years. One hundred fifty healthy volunteers have been chosen in this study; this group was collected from people not who have diabetes, through measuring Fasting and Random blood sugar ≤ 135 mg/dl, and measuring HbA1c ≤ 6% with measure ACR less than 3 mg/mmol. Their ages ranged between 28-66 years from both genders.

Samples collections

About 5ml of blood was collected from each subject in this study, 2ml was transferred to EDTA tube for HbA1c measurements, and the remaining 3ml were transferred to gel activator tube and the serum separated by centrifugation at 5000 rpm for 5 minutes for serological study (FBS, urea, creatinine and MCP-1). The urine samples were collected from each subject to measurements albumin and creatinine.

Results and Discussion

Characterization features of the parameters

The characteristics of the participants are shown in table (1). It includes all the diabetic patients and the healthy group; the number of patients, sex, age, FBs, creatinine, urea, HbA1C were tabulated.

Table 1: Comparison of some socio-demographic data, clinical and biochemical characteristics of studied subjects

Parameter	Control(n=150)	Patients(n=150)	P-value
Age/years	44.72±9.08	48.14±13.74	0.264
Gender Male Female	 48% 52%	 48% 52%	 1
FBS	97.4±19.5	191.3±54.6	<0.0001
HbA1C %	5.2±0.6	9.03±1.4	<0.0001
Creatinine mg/dl	0.79±0.28	2.3±0.7	<0.0001
Urea mg/dl	22.7±11.7	77.09±36.7	<0.0001
BMI	26±1.8	28±1.3	0.3

No significant difference was found between the patients' group and healthy control, enforcing the comparison of the two independent samples at the age variable. It is recommended and highly reliable for the studied samples. In the same context, age, gender and BMI variables did not report any source of variation between the two groups. In addition, regarding gender, the highest percentage of the studied samples (52%) were females in both patients and the control group. The vast majority of the patients' group for the age factor were reported at (34 - 61 yrs.) with a mean age of 48.14 years (SD±13.74) for the study sample, while the control group the age ranged from 35 to 53 yrs., and mean age of 44.72 years (SD±9.08). The results of estimation of the values of the biochemical parameters did not appear to be very different (except sugar) in diabetic patients compared to their values in the serum of control (Table 1).

In contrast, the serum sugar in diabetic nephropathy patients was higher than in control, with a significant difference ($p<0.05$). Results in values were expressed as mean± SD; *: $p< 0.05$ or significant differences between values of parameters; normal reference values of parameters were predefined by Gharib *et al.* 2015**.** The diabetic patient group had a slightly higher urea level (statistically not significant). The rise of plasma concentrations of serum creatinine and urea resulted from the diminishing of glomerular filtration rate in both groups of patients because all results represented the mean of the serum biochemical parameters values which evaluated in diabetic and healthy control, and all these renal parameters were highest in comparison within normal limits. A study conducted by Dhanya *et al.* 2017 found that the mean urea level in patients with CRF undergoing dialysis was (134.33 mg/dl). Also, the same study found that the mean creatinine level in patients with CRF undergoing dialysis was (9.7 mg/dl). Kiritoshi et al. 2003 have reported that an increase in the mitochondrial reactive oxygen species production due to hyperglycemia will induce cyclooxygenase-2 gene expression by activating nuclear factor κB . These results may participate in the pathogenesis of diabetic complications, particularly diabetic nephropathy. In this study, we have also noted a statistically significant elevated ($p<0.0001$) in the HbA1c level in the subject with diabetic nephropathy when compared with control subjects in agree with Grams et al., 2016) who found that FBS, fasting serum insulin and $HbA1_c$ level was significantly increased in the T2DM subjects.

Distribution of the Study Groups According to the Albumin to Creatinine Ratio

Table 2: comparison of urine albumin –creatinine ratio between control and patient groups

	Control	Patients			P-value
Mean±SD	0.45± 0.43	19.13± 15.62			**< 0.0001
		Mild	Moderate	Sever	
		3.2±0.75	17.44±7.04	38.05±4.61	
Sig		**	**	**	
P-value		< 0.0001	< 0.0001	< 0.0001	
Sig		**			
P value	< 0.0001				

** $p \leq 0.01$ NS = Non-significant

Regarding the urine albumin to creatinine ratio, the result of this study has shown that there is a significant difference ($P <0.001$) between the patients and the control group, being higher in the patients' group. This result agrees with wang *et al.* (2016) $p \leq 0.01$. Regarding the albuminuria stage, the result of this study has shown that urine ACR was significantly highest in the severe diabetic nephropathy group followed by the moderate group and then by mild and the control groups (P less than 0.001). This result agrees with several studies (Rabellino et al., 2010, HE ., 2013, El-Shimi et al., 2015). The DN progression is a gradual process that begins with the initial stage prior to any kidney injury.

Microalbuminuria is considered to be predictive of progression to nephropathy in T2DM. Overt nephropathy is characterized by persistent albuminuria (UAE > 300 mg/d that usually accompanies a decrease in GFR (Butler et al., 2017). Moreover, in T2DM patients with microalbuminuria, the elevated levels of urinary albumin and ACR, together with the reduced levels of GFR, fulfill the characteristics of microalbuminuria (Almutairi et al.,2017).

Monocyte Chemoattracting Protein -1 (MCP-1) Level

Table 3: MCP-1serum vs. Patient Groups

	Control	Patients			P-value
Mean±SD	77.1 ±61.4	311.5±191.3			**< 0.001
		Mild	Moderate	Sever	
		89.55±84.05	244.7±152.8	474.6±343.8	
Sig		NS	**	**	
P-value		0.0903	< 0.001	< 0.001	
Sig			**		
P-value			0.001		

* p ≤ 0.05

The current study demonstrated a significant increase ($p<0.05$) in serum levels of MCP-1 in diabetic nephropathy patient groups in compared within the control group (healthy persons) respectively table (3). Individuals with larger severe diabetic nephropathy typically have elevated pro-inflammatory factors like monocyte-chemotactic-protein-1 (MCP-1) table (3) in concordance with Gürkan *et al.*,2016. Kidney cells produce MCP-1 in response to a variety of pro-inflammatory stimuli, and predictably, its expression has been identified in kidney diseases that involve significant inflammation, which include diabetic nephropathy (Motawi et al., 2018). In agreement with the other study demonstrate that activation of PKC, higher levels of oxidative stress, and nuclear translocation of the transcription component nuclear factor-B (NF-B) all contribute to an increase in MCP-1 production from human and mouse mesangial cells during diabetes mellitus (Giunti et al.,2008, Gao et al.,2009). This stimulatory effect of high glucose in mesangial cells is further enhanced by the presence of advanced glycation end products (AGEs) or mechanical stretch (Klöting and Blüher, 2014). A variety of AGEs are also capable of stimulating MCP-1 production by mesangial cells, which involves interaction with the receptor for AGEs (RAGE) with subsequent generation of oxidative stress via activation of peroxisome proliferator-activated receptor-γ (PPARγ) (Zieger et al.,2018, Roussel et al, 2020). MCP-1 is also produced by kidney epithelial cells, including glomerular podocytes and tubular cells, in response to elevated glucose and AGEs. High glucose treatment immediately stimulates the release of MCP-1 mRNA and protein from cultured mouse podocytes, which is blocked by all-trans retinoic acid therapy (Lam et al.,2014). Also, glycated BSA and carboxymethyl lysine (CML)-BSA promote MCP-1 gene and protein expression in mouse podocytes via RAGE, oxidative stress and activation of ERK/NF-B, which may be blocked by pravastatin (Pippias et al., 2017). Similarly, cultured rat proximal tubular cells secrete increased levels of

MCP-1 in response to both high glucose and AGE-BSA (Tam et al., 2009). The initial studies of MCP-1-deficient diabetic kidneys showed that a lack of MCP-1 reduces the accumulation of interstitial myofibroblasts and the deposition of glomerular and interstitial collagen type IV (Menzies et al., 2017). Subsequent examination has shown that MCP-1 deficiency in type 1 diabetes also reduces glomerular deposition of fibronectin and mRNA and protein expression and transforming growth factor-β1 (TGF-β1) in the total kidney (Yin et al.,2015). Furthermore, therapeutic MCP-1 inhibition in type 1 diabetic mice lowers glomerular deposition of TGF-1 and collagen IV, as well as the mesangial matrix fraction. These investigations found that MCP-1 deficit or blockage was related with a significant reduction of kidney macrophages, which suggests that MCP-1-mediated fibrosis may be caused by the recruitment of macrophages (Wang et al., 2017).

References

1. Nguyen, V. Lynn, C., Shaw, Maria, B., Grant. (2012). Inflammation in the pathogenesis of microvascular complications in diabetes Dung. MINI REVIEW ARTICLE published: 21 December.
2. Soumya, D., & Srilatha, B. (2011). Late stage complications of diabetes and insulin resistance. *J Diabetes Metab*, *2*(9), 1000167.
3. Hall, J. E. (2011). *Guyton y Hall*. Elsevier Health Sciences Spain.
4. American Diabetes Association. (2020). 15. Diabetes care in the hospital: standards of medical care in diabetes—2020. *Diabetes Care*, *43*(Supplement 1), S193-S202.
5. Poretsky, L. (Ed.). (2010). *Principles of diabetes mellitus* (Vol. 21). Springer Science & Business Media.
6. Imani, P. D., Carpenter, J. L., Bell, C. S., Brandt, M. L., Braun, M. C., & Swartz, S. J. (2018). Peritoneal dialysis catheter outcomes in infants initiating peritoneal dialysis for end-stage renal disease. *BMC nephrology*, *19*(1), 1-7.
7. Lopez-Giacoman, S., & Madero, M. (2015). Biomarkers in chronic kidney disease, from kidney function to kidney damage. *World journal of nephrology*, *4*(1), 57.
8. Zhong, Z. X., Cui, Z. H., Li, X. J., Tang, T., Zheng, Z. J., Ni, W. N., ... & Sun, J. (2020). Nitrofurantoin combined with amikacin: A promising alternative strategy for combating MDR uropathogenic Escherichia coli. *Frontiers in cellular and infection microbiology*, *10*, 811.
9. Zoungas, S., Chalmers, J., Neal, B., Billot, L., Li, Q., Hirakawa, Y., ... & Woodward, M. (2014). Follow-up of blood-pressure lowering and glucose control in type 2 diabetes. *New England Journal of Medicine*, *371*(15), 1392-1406.
10. Futagami, S., Hiratsuka, T., Shindo, T., Hamamoto, T., Tatsuguchi, A., Nobue, U., ... & Sakamoto, C. (2008). COX-2 and CCR2 induced by CD40 ligand and MCP-1 are linked to VEGF production in endothelial cells. *Prostaglandins, leukotrienes and essential fatty acids*, *78*(2), 137-146.
11. Montero, R. M., Covic, A., Gnudi, L., & Goldsmith, D. (2016). Diabetic nephropathy: What does the future hold?. *International urology and nephrology*, *48*(1), 99-113.

12. Tam, F. W., Riser, B. L., Meeran, K., Rambow, J., Pusey, C. D., & Frankel, A. H. (2009). Urinary monocyte chemoattractant protein-1 (MCP-1) and connective tissue growth factor (CCN2) as prognostic markers for progression of diabetic nephropathy. *Cytokine*, *47*(1), 37-42.
13. Gharib, M. A. A. K., Bakrand, E. S. H., & Baz, S. M. (2016). Hypoglycemic efficacy of date kernels coffee on diabetic and nephrodiabetic patients. *Life Science Journal*, *13*(4), 10-18.
14. Dhanya, R., Arya, A. D., Nisha, P., & Jayamurthy, P. (2017). Quercetin, a lead compound against type 2 diabetes ameliorates glucose uptake via AMPK pathway in skeletal muscle cell line. *Frontiers in Pharmacology*, *8*, 336.
15. Kiritoshi, S., Nishikawa, T., Sonoda, K., Kukidome, D., Senokuchi, T., Matsuo, T., ... & Araki, E. (2003). Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. *Diabetes*, *52*(10), 2570-2577.
16. Grams, M. E., Sang, Y., Levey, A. S., Matsushita, K., Ballew, S., Chang, A. R., ... & Garg, A. X. (2016). Kidney-failure risk projection for the living kidney-donor candidate. *New England Journal of Medicine*, *374*(5), 411-421.
17. Wang, S. Y., Zang, X. Y., Fu, S. H., Bai, J., Liu, J. D., Tian, L., ... & Zhao, Y. (2016). Factors related to fatigue in Chinese patients with end-stage renal disease receiving maintenance hemodialysis: a multi-center cross-sectional study. *Renal failure*, *38*(3), 442-450.
18. Rabellino, M., Aragón-Sánchez, J., González, G., Zander, T., Baldi, S., Garcia-Nielsen, L., ... & Maynar, M. (2010). Is endovascular revascularisation worthwhile in diabetic patients with critical limb ischemia who also have end-stage renal disease?. *Diabetes research and clinical practice*, *90*(3), e79-e81.
19. HE, F., Xia, X., Wu, X. F., Yu, X. Q., & Huang, F. X. (2013). Diabetic retinopathy in predicting diabetic nephropathy in patients with type 2 diabetes and renal disease: a meta-analysis.
20. El-Shimi, M. S., El-Farrash, R. A., Ismail, E. A., El-Safty, A., Nada, A. S., El-Gamel, O. A., ... & Shoukry, S. M. (2015). Renal functional and structural integrity in infants with iron deficiency anemia: relation to oxidative stress and response to iron therapy. *Pediatric Nephrology*, *30*(10), 1835-1842.
21. Gürkan, A., Eren, G., Çetinkalp, Ş., Akçay, Y. D., Emingil, G., & Atilla, G. (2016). Monocyte chemotactic protein-1, RANTES and macrophage migration inhibitory factor levels in gingival crevicular fluid of metabolic syndrome patients with gingivitis. *Archives of oral biology*, *69*, 82-88.
22. Motawi, T. K., Shehata, N. I., ElNokeety, M. M., & El-Emady, Y. F. (2018). Potential serum biomarkers for early detection of diabetic nephropathy. *diabetes research and clinical practice*, *136*, 150-158.
23. Giunti, S., Tesch, G. H., Pinach, S., Burt, D. J., Cooper, M. E., Cavallo-Perin, P., ... & Gruden, G. (2008). Monocyte chemoattractant protein-1 has prosclerotic effects both in a mouse model of experimental diabetes and in vitro in human mesangial cells. *Diabetologia*, *51*(1), 198-207.
24. Gao, Y. J., Zhang, L., Samad, O. A., Suter, M. R., Yasuhiko, K., Xu, Z. Z., ... & Ji, R. R. (2009). JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. *Journal of Neuroscience*, *29*(13), 4096-4108.
25. Klöting, N., & Blüher, M. (2014). Adipocyte dysfunction, inflammation and metabolic syndrome. *Reviews in Endocrine and Metabolic Disorders*, *15*(4), 277-287.

26. Zieger, K., Weiner, J., Kunath, A., Gericke, M., Krause, K., Kern, M., ... & Heiker, J. T. (2018). Ablation of kallikrein 7 (KLK7) in adipose tissue ameliorates metabolic consequences of high fat diet-induced obesity by counteracting adipose tissue inflammation in vivo. *Cellular and molecular life sciences*, *75*(4), 727-742.
27. Roussel, E., Verbiest, A., Milenkovic, U., Van Cleynenbreugel, B., Van Poppel, H., Joniau, S., ... & Albersen, M. (2020). Too good for CARMENA: criteria associated with long systemic therapy free intervals post cytoreductive nephrectomy for metastatic clear cell renal cell carcinoma. *Scandinavian Journal of Urology*, *54*(6), 493-499.
28. Lam, N. N., Lentine, K. L., & Garg, A. X. (2014). End-stage renal disease risk in live kidney donors: what have we learned from two recent studies?. *Current opinion in nephrology and hypertension*, *23*(6), 592.
29. Pippias, M., Kramer, A., Noordzij, M., Afentakis, N., Alonso de la Torre, R., Ambühl, P. M., ... & Stel, V. S. (2017). The European renal association–European dialysis and transplant association registry annual report 2014: a summary. *Clinical kidney journal*, *10*(2), 154-169.
30. Menzies, R. I., Booth, J. W., Mullins, J. J., Bailey, M. A., Tam, F. W., Norman, J. T., & Unwin, R. J. (2017). Hyperglycemia-induced renal P2X7 receptor activation enhances diabetes-related injury. *EBioMedicine*, *19*, 73-83.
31. Yin, J., Gu, L., Wang, Y., Fan, N., Ma, Y., & Peng, Y. (2015). Rapamycin improves palmitate-induced ER stress/NF κ B pathways associated with stimulating autophagy in adipocytes. *Mediators of inflammation*, *2015*.
32. Wang, Y., Zhao, L., Wang, C., Hu, J., Guo, X., Zhang, D., ... & Ji, B. (2017). Protective effect of quercetin and chlorogenic acid, two polyphenols widely present in edible plant varieties, on visible light-induced retinal degeneration in vivo. *Journal of Functional Foods*, *33*, 103-111.