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Research Article
Chlorogenic Acid Improves Cognitive Deficits in Diabetic Rats

[1]Liu Xianchu, [1]Cheng Changhao, [1]Deng Beiwang, [2]Peng Huan and [3]Liu Ming

[1]Department of Human Movement Science, Institute of Physical Culture, Hunan University of Arts and Science, 415000 Changde, China
[2]Department of Hematology, Xiangya Changde Hospital, 415000 Changde, Hunan, China
[3]Department of Biochemistry, College of Life and Environmental Sciences, Hunan University of Arts and Science, 415000 Changde, China

Abstract

Background and Objective: Diabetes is fundamentally connected with the inability of cognition. Chlorogenic acid (CGA) has multiple biologic functions and is diffusely utilized in diabetic complications. The current study aimed to explore the improvement effect of CGA on cognitive deficits in diabetic rats. **Materials and Methods:** The model of the diabetic rat was constituted by STZ (50 mg kg^{-1}). The experiment rats were treated with CGA (30 mg kg^{-1}/day) by gastric perfusion for 8 weeks. After the last treatment, Morris water maze was examined to estimate cognitive function. In hippocampus tissue, the spectrophotometer was performed to evaluate SOD, CAT, GSH and MDA levels. The qRT-PCR and ELISA were utilized to analyze TNF-α and IL-1β contents. Western blot was used to detect the protein expressions of BDNF, GFAP Nrf-2 and HO-1. **Results:** Current data demonstrated that CGA reduced escape latency and increased times of crossing platform in Morris water maze test to improve diabetic-induced learning and memory impairments. CGA inhibited AChE and GFAP expressions, while augmented ChAT, BDNF, Nrf-2 and HO-1 expressions in the hippocampus. Moreover, CGA promoted SOD, CAT and GSH levels and suppressed MDA concentration to mitigate oxidative stress. Meanwhile, CGA inhibited TNF-a and IL-1β contents to relieve inflammatory response. Lastly, CGA restrained Bax/Bcl-2 ratio to alleviate apoptosis. **Conclusion:** CGA protected against diabetic-induced learning and memory impairments via improvement of oxidative stress, inflammation and apoptosis and could be used as a novel therapeutic in the prevention and treatment of DACD.

Key words: DACD, chlorogenic acid, oxidative stress, inflammation, apoptosis

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Corresponding Author: Liu Xianchu, Department of Human Movement Science, Institute of Physical Culture, Hunan University of Arts and Science, 415000 Changde, China Tel: +8619936907699

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) has become an epidemic disease in the current world. The number of diabetes mellitus will reach substantially 629 M in 2045[1]. The DM is a common glucose metabolism dysregulation with aberrant secretion and action of insulin. Its outstanding characteristic is high blood sugar and chronic hyperglycemia. Poorly controlled hyperglycemia brings about various complications, such as nephropathy, retinopathy, neuropathy and encephalopathy[2,3]. Abnormality of cognitive function is the central characteristic of diabetic encephalopathy[4]. Nowadays, more and more patients with hyperglycemia will be affected by diabetes-associated Cognitive Decline (DACD), the pathogenesis and curative mechanism of which is an urgent problem of research.

Long-term hyperglycemia facilitates the development of cerebral diseases like DACD because the hippocampus is vulnerable to diabetes-associated nervous toxicology[5]. Clinic pathologically, diabetes is fundamentally related to the inability of learning and memorizing[6]. The etiology and pathogenesis of DACD are multifarious and complex. There is a powerful witness that diabetes perturbs the antioxidation system *in vivo*. In the hippocampus, the activities of SOD, CAT and GSH as antioxidant factors are decreased in STZ-induced diabetic rats, while the level of MDA as an end product of LPO is increased. In addition, TNF-α and IL1-β, which belong to inflammatory cytokines are activated to induce inflammatory response[7,8]. Such anomalies likely contribute to apoptosis as represented by mediating BAX and BCL-2 expressions. These results peculiarly demonstrated that the consequences of DACD were significantly due to the impairment of anti-oxidation, anti-inflammation and anti-apoptosis[9]. Hence, it is considered to develop new therapeutic schedules to hinder the above abnormal phenomena.

Chlorogenic acid (CGA) is a kind of polyphenolic component which is widely recognized as a therapeutic drug. A previous study showed that CGA exhibited multiple pharmacological and biological activities to improve the varied physiologic function of organism[10]. In diabetes, CGA was involved in the regulation of blood sugar by attenuating insulin resistance and modulating glucose uptake[11]. In addition, CGA was proved to relieve the risk of learning and memory deterioration in many pathological models, such as Aβ25-35, alcohol, lead and scopolamine-induced brain damage[12,13]. However, no researches have implied the improvement effects of CGA on cognitive ability in diabetes. This study investigated the CGA exerted improvement on diabetic-induced learning and memory impairments via ameliorating oxidative stress, inflammation and apoptosis.

MATERIALS AND METHODS

Study area: The study was carried out at the Human Movement Science Laboratory, Hunan University of Arts and Science (January-April, 2021).

Animals and experimental design: Three-month-old Sprague-Dawley rats (male, 230±20 g) were housed throughout the experiment at SPF environment on temperature (23±2°C), humidity (50±10%), 12:12 hrs light-dark cycle. All rats were allowed to drink water and eat food ad libitum. Forty five rats were randomly divided into 3 groups: Control group (CON, n = 15), Diabetes mellitus (DM, n = 15), Diabetes treated with chlorogenic acid (DM+CGA, n = 15). The STZ (50 mg kg^{-1}) was an intraperitoneal injection to induce diabetic rats. Blood from the tail vein was collected to appraise glycemia. The blood glucose concentration was more than 16.7 mmol L^{-1} in rats, which could be ascertained as an available diabetic model. The CGA was orally fed at 30 mg kg^{-1}/day for 8 weeks. In this study, animal experiments were inspected according to the Ethics Committee of Hunan University of Arts and Science (No. HUAS-2021-TY-127).

Chemicals and reagents: Chlorogenic acid (purity: ≥98%), streptozotocin and qRT-PCR primer sequences were obtained from Sangon Biotech (Shanghai, China). The assay kits of SOD, CAT, GSH, MDA, AChE and ChAT were obtained from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). The ELISA kits of TNF-α and IL-1β were obtained from BOSTER Biological Technology (Wuhan, China). The antibodies of Nrf2, HO-1 and β-actin were obtained from protein tech (Wuhan, China). The antibodies of BDNF and GFAP were obtained from service bio (Wuhan, China).

Morris water maze: Rats were examined by Morris water maze after CGA treatments to assess learning and memory abilities. Before the experiment proper starts, the rat swam freely to adapt water environment. In training periods, rats were guided to locate the hidden platform. In escape latency tests, the average time of seeking the hidden platform was recorded to estimate learning capacity for 4 consecutive days. In probe trial tests, rats were tested to look for the previous platform, which was removed on the fifth day. The frequency of the crossing platform was checked to estimate memory retention.

Biochemical assay: After Morris water maze experiments, rats were killed by chloral hydrate. Hippocampus was separated

rapidly, washed by PBS and stored in liquid nitrogen. In biochemical assay, hippocampus samples were ground and centrifuged for protein extraction. The contents of SOD, CAT, GSH, MDA, AChE and ChAT were detected with commercial kits, while TNF-α and IL-1β levels were tested by ELISA kits.

qRT-PCR analysis: Hippocampus was split, homogenized and centrifuged (4°C, 12,000 g, 15 min) for RNA extraction by trizol reagent. Then extractive RNA was changed into cDNA by MBI Revert Aid. The mRNA expression was detected by qRT-PCR. The qRT-PCR was carried out with SYBR through Bio-Rad real-time PCR systems[14]. TNF-α and IL-1β mRNA expressions were normalised to β-actin. The signal was exhibited by comparative CT methods.

Western blot: The hippocampus was cut apart, homogenized and lysed gradually by RIPA and proteinase inhibitor to acquire protein. The protein content was detected by the BCA method. The extractive supernatant with dithiothreitol and loading buffer was boiled to eliminate the stereoscopic secondary structure of proteins. The SDS-PAGE was carried out to separate detected proteins. Then, a wet electro blotting system was performed to transfer the different molecular weights of proteins onto the PVDF membrane. The membrane was incubated with 5% milk sealant and appropriate concentrations of primary antibodies and secondary antibodies labelled with HRP. The band detection was carried out by the ECL system. The protein expressions were analyzed by Image J. The signal was described as protein relative expression which was normalization to β-actin.

Statistical analysis: All data were shown as Mean±SD. The results were analyzed with SPSS 16.0 software. Statistical difference was demonstrated by the ANOVA test with Tukey's post hoc test. $p<0.05$ was deemed statistically significant.

RESULTS

Properties of CGA on diabetic-induced cognitive deficit: Morris water maze was used to evaluate the ameliorations of CGA against diabetic-induced learning and memory dysfunction. In the CON group, the escape latency of the first, second, third and fourth day were 46.21±2.12, 39.35±2.49, 34.24±2.30 and 29.85±2.04, respectively. Compared with the CON group, diabetes caused a significant elevation in the escape latency to reach 59.48±1.64, 55.27±1.92, 51.29±2.05 and 47.18±2.31, respectively. The CGA dramatically reduced escape latency to reach 53.34±2.09, 45.41±2.41, 40.32±2.72 and 35.92±2.11 compared with the DM group in Fig. 1a. In the probe tests, diabetes caused a significant decrease in the frequency of crossing the removed platform to reach 2.70±1.16, compared with the CON group 6.50±1.35. The CGA dramatically augmented the frequency of crossing the removed platform to reach 4.90±1.52, compared with DM group 2.70±1.16 in Fig. 1b.

Properties of CGA on cholinergic dysfunction: To evaluate the regulation of CGA on diabetic-induced synaptic dysfunction, the concentrations of AChE and ChAT were examined in the hippocampus. Compared with the CON group (0.37±0.12), the DM group showed a significantly high

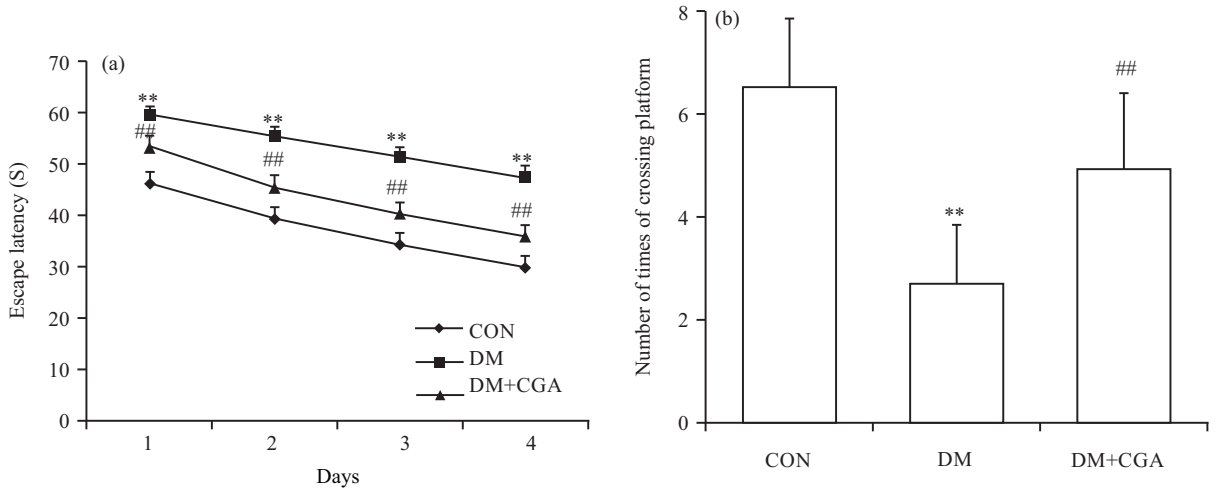

Fig. 1(a-b): Properties of CGA on diabetic-induced cognitive deficit

(a) Property on escape latency and (b) Property on platform crossings , **p<0.01 compared with CON group, ##p<0.01 compared with DM group

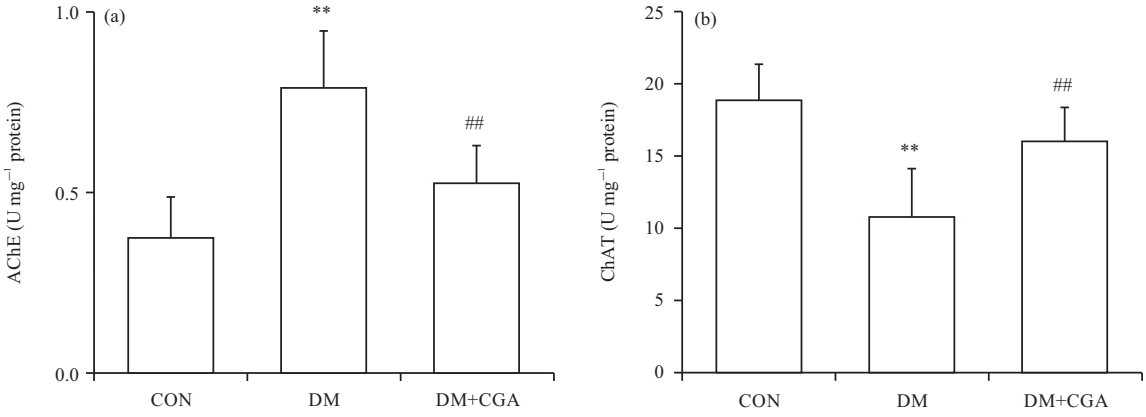

Fig. 2(a-b): Properties of CGA on the concentrations of cholinergic system-mediated enzymes

(a) Property on the concentration of AChE, (b) Property on the concentration of ChAT, **p<0.01 compared with CON group, ##p<0.01 compared with DM group

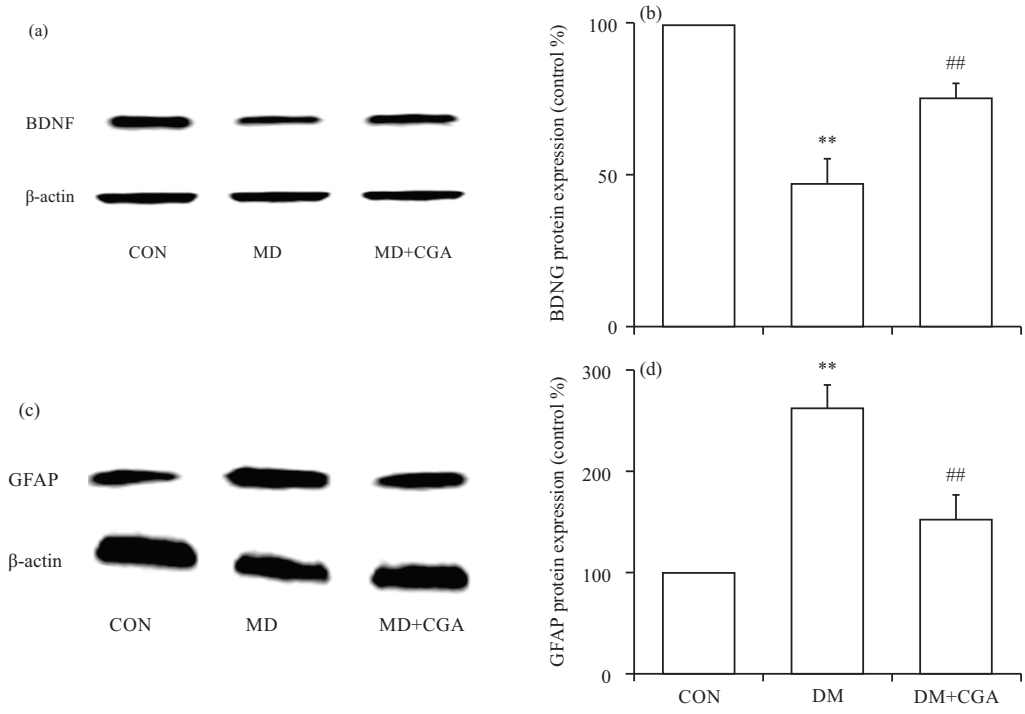

Fig. 3(a-d): Properties of CGA on diabetic-induced nervous system disorder

(a) BDNF expression was examined by Western blot, (b) Quantifications of BDNF protein level in hippocampus, (c) GFAP expression was examined by Western blot and (d) Quantifications of GFAP protein level in hippocampus

concentration of AChE which reached 0.79±0.17. The concentration of AChE in the DM group treated with CGA showed a marked decrease reaching 0.52±0.11 in Fig. 2a. In contrast, diabetes caused a significant decrease in ChAT concentration to reach 10.79±3.43, compared with the CON group (18.92±2.57). The CGA caused a significant increase of ChAT (16.03±2.40) compared with the DM group (10.79±3.43) in Fig. 2b.

Properties of CGA on the regulation of BDNF and GFAP: To evaluate the improvement of CGA on diabetic-induced nervous system disorder, the protein expressions of BDNF and GFAP were examined in the hippocampus. Compared with the CON group, diabetes remarkably decreased BDNF expression by about 47% (46.83±8.99). CGA resulted in a significant elevation of BDNF by about 160% (74.80±5.51), compared with the DM group (46.83±8.99) in Fig. 3a, b. In contrast, the

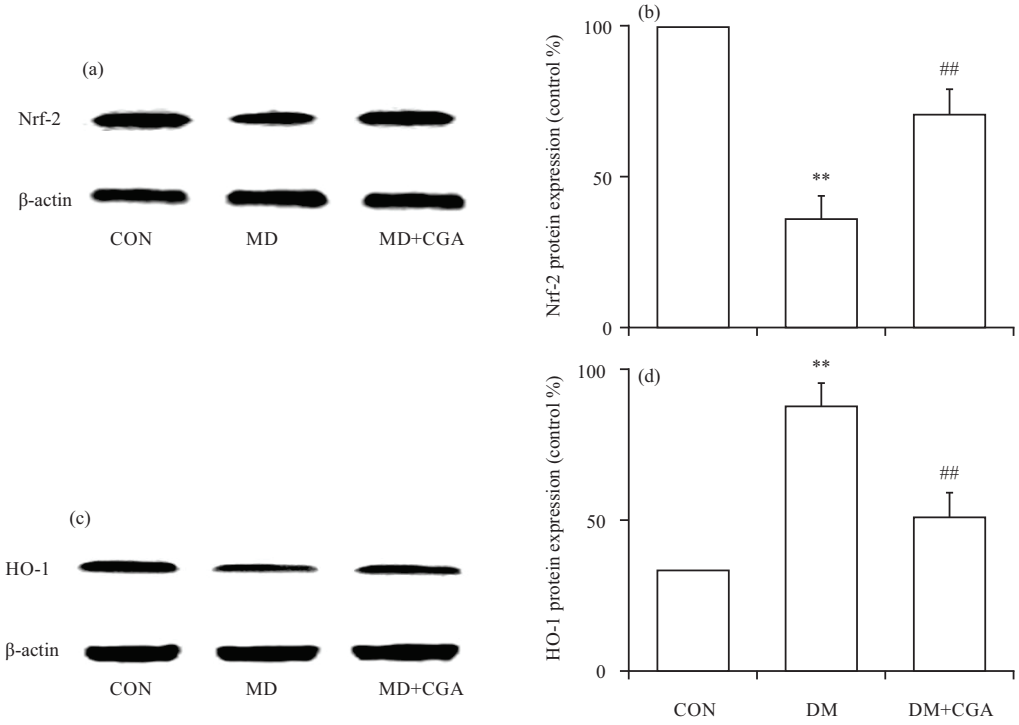

Fig. 4(a-d): Properties of CGA on the regulation of Nrf-2/HO-1 pathway in the hippocampus

(a) Nrf-2 expression was examined by Western blot, (b) Quantifications of Nrf-2 protein level in hippocampus, (c) HO-1 expression was examined by Western blot and (d) Quantifications of HO-1 protein level in hippocampus, **p<0.01 compared with CON group, ##p<0.01 compared with DM group

GFAP expression was remarkably elevated by about 261% (261.23±26.34) in the DM group when compared with the CON group. CGA observably inhibited GFAP expression by about 42% (152.17±26.61), compared with the DM group (261.23±26.34) in Fig. 3c and d.

Properties of CGA on the regulation of Nrf-2/HO-1 pathway: Nrf-2/HO-1 pathway is known to the function of modulating hyperglycemic-related complications. Compared with the control group, diabetes-induced a reduction in the protein expression of Nrf-2 by about 35% (35.07±8.57). CGA dramatically strengthened Nrf-2 expression by about 198% (69.50±8.71), compared with the DM group (35.07±8.57) in Fig. 4a, b. Similarly, the protein expression of HO-1 was remarkably restricted by about 42% (42.04±7.01) in the DM group when compared with the CON group. CGA dramatically enhanced HO-1 expression by about 186% (78.11±9.74), compared with the DM group (42.04±7.01) in Fig. 4c and d.

Properties of CGA on oxidative stress: Compared with the CON group (9.29±3.28), the DM group showed significant high activity of SOD which reached 3.62±1.91. The activity of SOD in the DM group treated with CGA showed a marked decrease reaching 6.81±2.37 in Fig. 5a. Additionally, CAT activity was remarkably decreased to reach 5.48±3.03 in the DM group when compared with the CON group (12.12±3.46). The CGA observably elevated the activity of CAT (9.47±2.77), compared with the DM group (5.48±3.03) in Fig. 5b. Similarly, diabetes caused a significant decrease in GSH level to reach 7.64±2.72, compared with the CON group (17.73±1.89). CGA caused a significant increase of GSH (13.13±3.63), compared with the DM group (7.64±2.72) in Fig. 5c. In contrast, diabetes caused a significant increase in MDA content to reach 1.83±0.44, compared CON group (0.61±0.23). CGA caused a significant decrease of MDA (1.19±0.30), compared with the DM group (1.83±1.44) in Fig. 5d.

Properties of CGA on inflammatory response: Compared with the CON group (99.64±9.75), diabetes caused a significant elevation in TNF-α level to reach 466.93±33.27. The CGA observably reduced TNF-α level to reach 309.75±48.06, compared with the DM group (466.93±33.27) in Fig. 6a. The mRNA expression of TNF-α was remarkably enhanced by about 267% (2.67±0.21) in the DM group when compared with the CON group. CGA dramatically restricted TNF-α mRNA expression by about 28% (1.92±0.16), compared with the DM group (2.67±0.21) in Fig. 6b. Similarly, diabetes caused a significant elevation in IL-1β level to reach

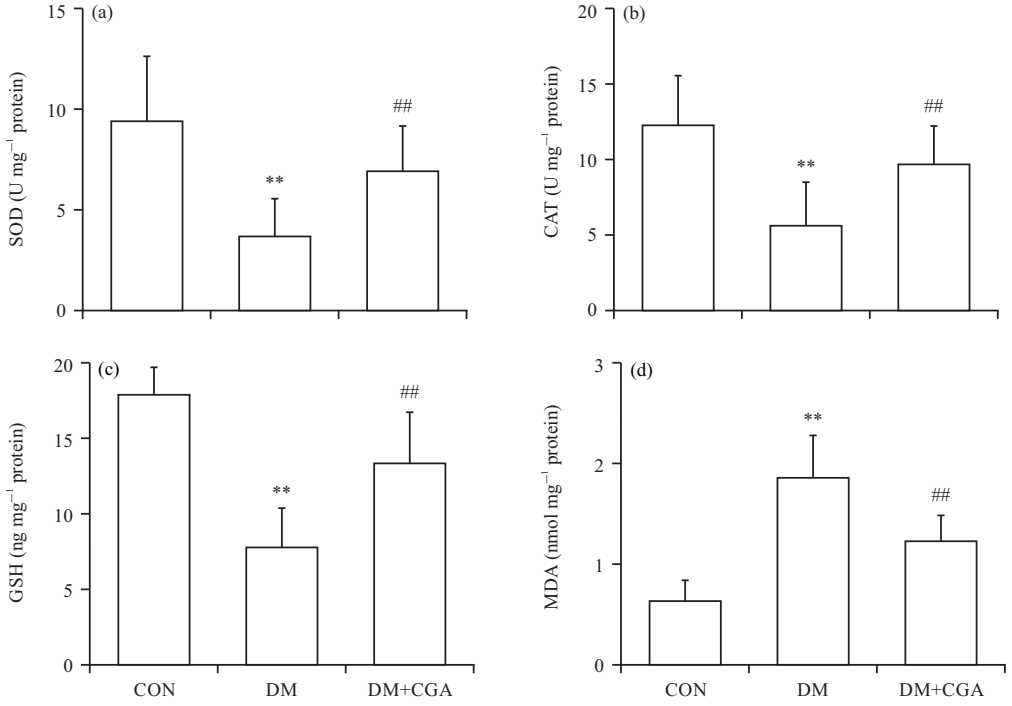

Fig. 5(a-d): Properties of CGA on diabetic-induced alterations of oxidative stress

(a) Property on the activity of SOD, (b) Property on the activity of CAT, (c) Property on the level of GSH and (d) Property on the content of MDA, **p<0.01 compared with CON group, ##p<0.01 compared with DM group

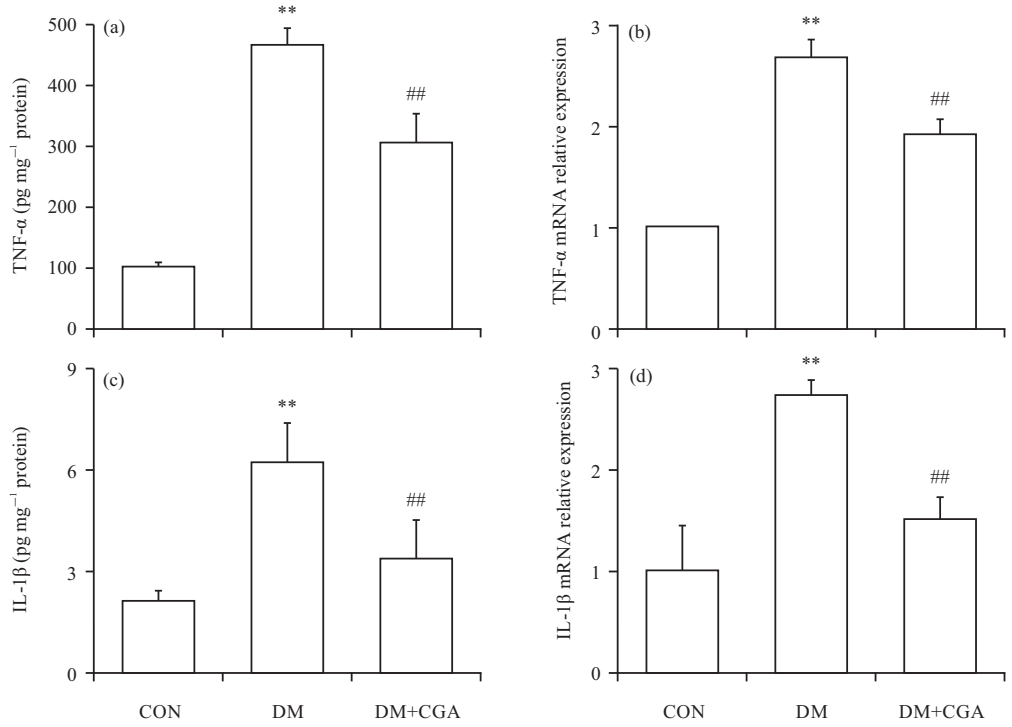

Fig. 6(a-d): Properties of CGA on diabetic-induced alterations of inflammatory response

(a) Property on the protein expression of TNF-α, (b) Property on the mRNA expression of TNF-α, (c) Property on the protein expression of IL-1β and (d) Property on the mRNA expression of IL-1β, **p<0.01 compared with CON group, ##p<0.01 compared with DM group

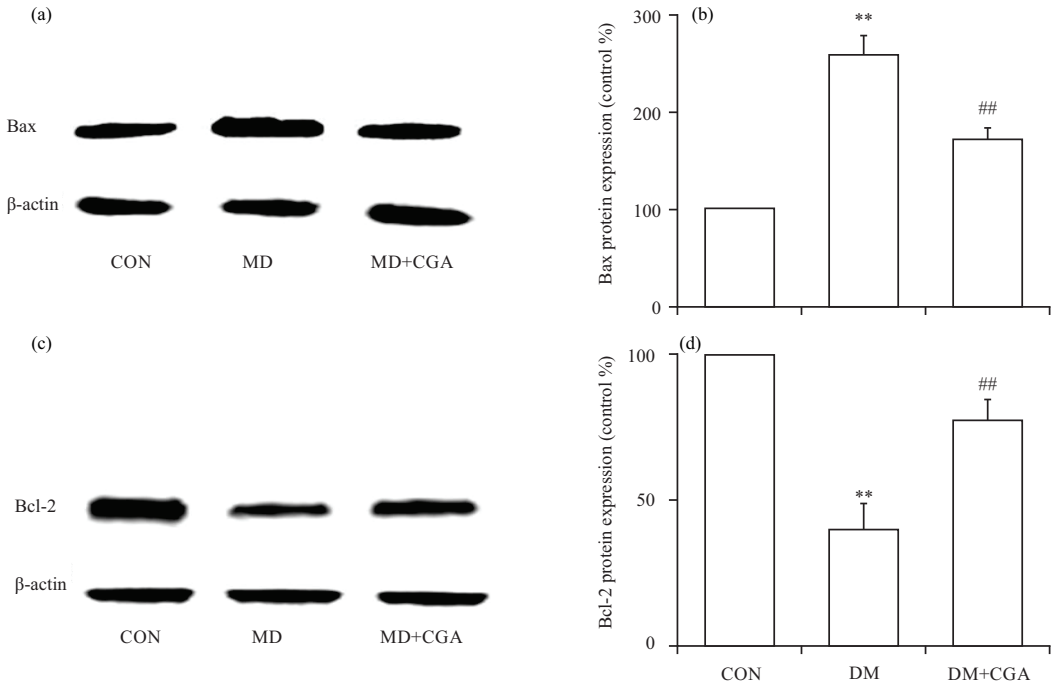

Fig. 7(a-d): Properties of CGA on diabetic-induced alterations of apoptosis

(a) Bax expression was examined by Western blot, (b) Quantifications of Bax protein level in hippocampus, (c) Bcl-2 expression was examined by Western blot and (d) Quantifications of Bcl-2 protein level in hippocampus, **p<0.01 compared with CON group, ##p<0.01 compared with DM group

6.23±1.19, compared with the CON group (2.13±0.35). CGA observably reduced IL-1β level to reach 3.38±1.19, compared with DM group (6.23±1.19) in Fig. 6c. The mRNA expression of IL-1β was remarkably enhanced by about 273% (2.73±0.15) in the DM group when compared with the CON group. CGA dramatically restricted IL-1β mRNA expression by about 45% (1.50±0.21), compared with the DM group (2.73±0.15) in Fig. 6d.

Properties of CGA on apoptosis: Compared with the control group, diabetes-induced a significant increase in protein expression of Bax by about 256% (255.40±23.01). CGA observably reduced diabetic-induced Bax expression by about 151% (169.12±13.15), compared with the DM group (255.40±23.01) in Fig. 7a, b. In contrast, the protein expression of Bcl-2 was remarkably reduced by about 40% (39.68±9.60) in the DM group when compared with the CON group. CGA dramatically promoted Bcl-2 expression by about 48% (76.71±8.06), compared with the DM group (39.68±9.60) in Fig. 7c and d.

DISCUSSION

DACD is a neurogenic disease induced by hyperglycemia, which is hard to remedy and can stimulate various negative influences on the brain of patients. CGA with biological efficacies of anti-oxidation, anti-inflammation and anti-apoptosis is widely applied to therapy in diabetes and cognitive impairment[15]. Previous studies showed that CGA was involved in preventing brain injury to relieve cognitive impairments due to its neuroprotective effects[16]. In experimental diabetes, CGA was proved to alleviate nephropathy, retinopathy, neuropathy and encephalopathy[17]. The present study demonstrated CGA reduced escape latency and increased times of crossing platform in the Morris water maze test, which suggested CGA had a protective effect on diabetes-related cognitive deficits.

Cholinergic dysfunction is closely associated with cognitive impairments and uncontrolled hyperglycemia is a risk factor in the regulation of synaptic function[12]. Previous studies showed that CGA was involved in the modulation of AChE activity to improve memory function in Pb-induced cognitive decline[18]. The levels of AChE and ChAT, important participants in diabetes-induced cholinergic dysfunction were determined in this study. The present study showed CGA mitigated AChE activity and enhanced ChAT activity in the hippocampus, suggesting CGA could modulate the cholinergic system to improve diabetes-related cognitive decline.

Long-term hyperglycemia exacerbates neurotoxicity and brain injury which is to the disadvantage of neuronal metabolism. In diabetes-induced hippocampus impairments, the expression of BDNF as a neurotrophin, was decreased, while the expression of GFAP as an astrocyte marker was increased[19,20]. CGA was proved to alleviate cerebral ischemia/reperfusion-related nerve damage by promoting BDNF expression[21]. In addition, CGA attenuated astrocyte activation by reducing GFAP staining to reoccur motor coordination in MPTP-intoxicated mice[22]. The present study showed CGA moderated neuronal metabolism by strengthening BDNF expression and weakening GFAP expression in the hippocampus to alleviate diabetes-related cognitive deficits.

Nrf-2/HO-1 pathway plays a vital function in regulating apoptosis triggered via oxidative stress and inflammation. In diabetes, the Nrf-2/HO-1 pathway is inactive in the hippocampus[23,24]. In diabetic nephropathy, CGA was proved to inhibit oxidative stress and inflammation by modulating Nrf-2 and HO-1 expressions[17]. Moreover, CGA is a main component of coffee, which promotes the Nrf-2-ARE anti-oxidative pathway to ameliorate proper cognitive functionality[25]. Besides, CGA was involved in the regulation of the Nrf-2/HO-1 pathway to attenuate mitochondrial apoptosis in dexamethasone-induced osteoblasts cytotoxicity[26]. The present study showed CGA ameliorated diabetes-related cognitive deficits by promoting Nrf-2 and HO-1 expressions in the hippocampus, which implied that its protective effects were closely relevant to anti-oxidation, anti-inflammation and anti-apoptosis.

Hippocampus is one of the sensitive brain regions during oxidative stress. In this study, diabetes reduced the levels of SOD, CAT and GSH, whereas the level of MDA was increased in the hippocampus. It was shown that chronic hyperglycemia aggravated the disequilibrium of oxidation and antioxidation in DACD. Previous research showed CGA possessed its oxidation resistance through increasing SOD activity and inhibiting MDA generation to alleviate glucotoxicity in H9c2[27]. Moreover, CGA relieved MDA concentration in the hippocampus to reverse learning and memory impairment in scopolamine-induced amnesia[28]. The present study demonstrated the potential protective function of CGA was linked to the augment of antioxidant mediated status against STZ-induced cognitive dissonance by strengthening SOD and CAT activities, elevating GSH content and mitigating MDA concentration in the hippocampus.

Chronic hyperglycemia may induce inflammatory reaction and lead to the increase of TNF-α and IL1-β, which serves an important role in the pathogenesis of cognitive decline. Previous research showed CGA alleviated high glucose-induced activation of TNF-α and IL1-β in diabetic nephropathy[29]. In alcohol-induced cognitive dysfunction, CGA ameliorated inflammatory mediators by inhibiting TNF-α and IL1-β levels in the hippocampus of neonatal rat[16]. In this study, there was an increase in TNF-α and IL-1β levels in the DM group, expressly implying that inflammatory reaction was triggered. Interestingly, treatment with AA relieved these pro-inflammatory factors levels in the hippocampus. Therefore, AA possessed an anti-inflammatory effect against STZ-induced cognitive injury.

Apoptotic signals were activated leading to cognitive decline during hyperglycemia. Besides, oxidative stress and inflammatory stimulation are key factors that lead to cell injury. Current results showed the apoptosis process was raised in DACD as represented by preventing Bax expression and enhancing Bcl-2 expression. Hence, STZ-induced apoptosis must accelerate the development of cognitive decline. Previous research showed CGA was involved in preventing pericyte apoptosis through TUNEL staining in diabetic retinopathy[30]. In the Pb-induced toxicant experiment, CGA was proved to improve memory function by step-down inhibitory avoidance task and suppress Bax/Bcl-2 ratio to relieve hepato-renal injury[18]. In this study, CGA reduced Bax expression and increased Bcl-2 expression in the hippocampus of diabetic rats. These data showed that CGA exhibited its suppression of apoptosis to improve STZ-induced cognitive impairment.

CONCLUSION

Current research indicated that CGA improved diabetic-induced learning and memory impairments which contributed to its modulation of oxidative stress, prevention of inflammation and suppression of apoptosis. The above results demonstrated CGA may be a novel therapeutic approach to alleviating cognitive decline in diabetes.

SIGNIFICANCE STATEMENT

This study demonstrated that the ameliorative efficacy of CGA on diabetic-induced learning and memory impairments. Treatment with reduced escape latency and increased times of crossing platform in Morris water maze test. In the hippocampus, diabetes increased the levels of AChE and GFAP and led to a decrease in the contents of ChAT and BDNF. However, Treatment with CGA reversed these changes. Concurrently, CGA was proved to strengthen diabetic-induced inactivation of Nrf-2 and HO-1 expressions. Furthermore, CGA

decreased oxidative stress, inflammatory response and apoptosis. These results revealed that CGA possessed multiple biological and pharmacological properties in the hippocampus of diabetic mice and could be regarded as a novel agent for the diabetes-induced cognitive defect.

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REFERENCES

1. Nna, V.U., A.B.A. Bakar, A. Ahmad and M. Mohamed, 2020. Diabetes-induced testicular oxidative stress, inflammation and caspase-dependent apoptosis: The protective role of metformin. Arch. Physiol. Biochem., 126: 377-388.
2. Harding, J.L., M.E. Pavkov, D.J. Magliano, J.E. Shaw and E.W. Gregg, 2019. Global trends in diabetes complications: A review of current evidence. Diabetologia, 62: 3-16.
3. Cole, J.B. and J.C. Florez, 2020. Genetics of diabetes mellitus and diabetes complications. Nat. Rev. Nephrol., 16: 377-390.
4. Chen, R., J. Shi, Q. Yin, X. Li and Y. Sheng *et al*., 2018. Morphological and pathological characteristics of brain in diabetic encephalopathy. J. Alzheimer's Dis., 65: 15-28.
5. Choi, S.H., R. Lee, S.M. Nam, D.G. Kim and I.H. Cho, 2021. Ginseng gintonin, aging societies and geriatric brain diseases. Integr. Med. Res., 10.1016/j.imr.2020.100450
6. Biessels, G.J. and F. Despa, 2018. Cognitive decline and dementia in diabetes mellitus: Mechanisms and clinical implications. Nat. Rev. Endocrinol., 14: 591-604.
7. Farbood, Y., S. Ghaderi, M. Rashno, S.E. Khoshnam, L. Khorsandi, A. Sarkaki and M. Rashno, 2019. Sesamin: A promising protective agent against diabetes-associated cognitive decline in rats. Life Sci., 230: 169-177.
8. Mahmoudi, N., Z. Kiasalari, T. Rahmani, A. Sanaierad and S. Afshin-Majd *et al*., 2021. Diosgenin attenuates cognitive impairment in streptozotocin-induced diabetic rats: Underlying mechanisms. Neuropsychobiology, 80: 25-35.
9. Ma, W.X., J. Tang, Z.W. Lei, C.Y. Li and L.Q. Zhao *et al*., 2020. Potential biochemical mechanisms of brain injury in diabetes mellitus. Aging disease, 11: 978-987.
10. Bhandarkar, N.S., L. Brown and S.K. Panchal, 2019. Chlorogenic acid attenuates high-carbohydrate, high-fat diet–induced cardiovascular, liver, and metabolic changes in rats. Nutr. Res., 62: 78-88.
11. Chen, L., H. Teng and H. Cao, 2019. Chlorogenic acid and caffeic acid from *Sonchus oleraceus* Linn synergistically attenuate insulin resistance and modulate glucose uptake in hepG2 cells. Food Chem. Toxicol., 127: 182-187.
12. Heitman, E. and D.K. Ingram, 2017. Cognitive and neuroprotective effects of chlorogenic acid. Nutr. Neurosci., 20: 32-39.
13. Wasim, S., V. Kukkar, V.M. Awad, S. Sakhamuru and B.H. Malik, 2020. Neuroprotective and neurodegenerative aspects of coffee and its active ingredients in view of scientific literature. Cureus, Vol. 12. 10.7759/cureus.9578.
14. Kaur, H., I. Patro, K. Tikoo and R. Sandhir, 2015. Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy. Neurochem. Int., 89: 40-50.
15. Miao, M. and L. Xiang, 2020. Pharmacological action and potential targets of chlorogenic acid. Pharmacol. Adv. Nat. Prod. Drug Discovery, 87: 71-88.
16. Guo, Z. and J. Li, 2017. Chlorogenic acid prevents alcohol-induced brain damage in neonatal rat. Transl. Neurosci., 8: 176-181.
17. Yan, Y., X. Zhou, K. Guo, F. Zhou and H. Yang, 2020. Use of chlorogenic acid against diabetes mellitus and its complications. J. Immunol. Res., Vol. 2020. 10.1155/2020/9680508.
18. Cheng, D., H. Li, J. Zhou and S. Wang, 2019. Chlorogenic acid relieves lead-induced cognitive impairments and hepato-renal damage *via* regulating the dysbiosis of the gut microbiota in mice. Food Funct., 10: 681-690.
19. Wang, K., F. Song, K. Xu, Z. Liu, S. Han, F. Li and Y. Sun, 2019. Irisin attenuates neuroinflammation and prevents the memory and cognitive deterioration in streptozotocin-induced diabetic mice. Mediators Inflammation, Vol. 2019. 10.1155/2019/1567179.
20. Huang, L., S. Yan, L. Luo and L. Yang, 2019. Irisin regulates the expression of BDNF and glycometabolism in diabetic rats. Mol. Med. Rep., 19: 1074-1082.
21. Liu, D., H. Wang, Y. Zhang and Z. Zhang, 2020. Protective effects of chlorogenic acid on cerebral ischemia/reperfusion injury rats by regulating oxidative stress-related Nrf2 pathway. Drug Des., Dev. Ther., 14: 51-60.
22. Singh, S.S., S.N. Rai, H. Birla, W. Zahra and G. Kumar *et al*., 2018. Effect of chlorogenic acid supplementation in MPTP-intoxicated mouse. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.00757.
23. Chen, Y.J., Z.Z. Tang, L. Du, Y. Liu, Q. Lu, T.F. Ma and Y.W. Liu, 2019. A novel compound AB-38b improves diabetes-associated cognitive decline in mice via activation of Nrf2/ARE pathway. Brain Res. Bull., 10.1016/j.brainresbull.2019.05.010.
24. Wang, B.N., C.B. Wu, Z.M. Chen, P.P. Zheng and Y.Q. Liu *et al*., 2021. DL-3-n-butylphthalide ameliorates diabetes-associated cognitive decline by enhancing PI3K/AKT signaling and suppressing oxidative stress. Acta Pharmacol. Sinica, 42: 347-360.
25. Butt, M.S. and M.T. Sultan, 2011. Coffee and its consumption: Benefits and risks. Crit. Rev. Food Sci. Nutr., 51: 363-373.

26. Han, D., X. Gu, J. Gao, Z. Wang, G. Liu, H.W. Barkema and B. Han, 2019. Chlorogenic acid promotes the Nrf2/HO-1 anti-oxidative pathway by activating $p21^{Waf1/cip1}$ to resist dexamethasone-induced apoptosis in osteoblastic cells. Free Radical Biol. Med., 137: 1-12.
27. Rani, M.R.P., N. Anupama, M. Sreelekshmi and K.G. Raghu, 2018. Chlorogenic acid attenuates glucotoxicity in H9c2 cells via inhibition of glycation and PKC α upregulation and safeguarding innate antioxidant status. Biomed. Pharmacother., 100: 467-477.
28. Kwon, S.H., H.K. Lee, J.A. Kim, S.I. Hong and H.C. Kim *et al*., 2010. Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. Eur. J. Pharmacol., 649: 210-217.
29. Bao, L., J. Li, D. Zha, L. Zhang, P. Gao, T. Yao and X. Wu, 2018. Chlorogenic acid prevents diabetic nephropathy by inhibiting oxidative stress and inflammation through modulation of the Nrf2/HO-1 and Nf-κB pathways. Int. Immunopharmacol., 54: 245-253.
30. Kim, J., K. Jo, I.S. Lee, C.S. Kim and J. Kim, 2016. The extract of *Aster koraiensis* prevents retinal pericyte apoptosis in diabetic rats and its active compound, chlorogenic acid inhibits AGE formation and AGE/RAGE interaction. Nutrients, Vol. 8., 10.3390/nu8090585