

## Original Research Article

# Ursolic acid mitigates cognitive dysfunction through amelioration of oxidative stress, inflammation and apoptosis in diabetic rats

Liu Xianchu<sup>1,2</sup>, Liu Kang<sup>1</sup>, Peng Huan<sup>3</sup>, Liu Ming<sup>1\*</sup>

<sup>1</sup>College of Life Science, Hunan University of Arts and Science, 415000, Changde, <sup>2</sup>Key Laboratory of Physical Fitness and Exercise Rehabilitation of Hunan Province, Hunan Normal University, Changsha 410081, <sup>3</sup>Department of Hematology, Xiangya Changde Hospital, Changde 415000, PR China

\*For correspondence: **Email:** [liuminghappy36@163.com](mailto:liuminghappy36@163.com); **Tel:** +86-736-7186030; **Fax:** +86-736-7186030

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### Abstract

**Purpose:** To determine the effect of ursolic acid (UA) on diabetes-induced cognitive defect, as well as its mechanism of action in streptozotocin (STZ)-induced diabetic rats.

**Methods:** A rat model of diabetes was established by administration of STZ. The rats received UA via gastric perfusion for 56 successive days. Learning and memory functions were assessed using Morris water maze. Superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels in hippocampus tissues were determined spectrophotometrically. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-6 (IL-6) levels were assayed by quantitative real-time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA). The protein expression levels of nuclear factor erythroid-2-related factor-2 (Nrf-2), heme oxygenase-1 (HO-1), Bcl-2 and Bax were evaluated by western blotting.

**Results:** Learning and memory impairment in STZ-induced diabetic rats was mitigated by UA ( $p < 0.05$ ). In hippocampus tissue, UA reduced oxidative stress by enhancing SOD activity and reducing MDA levels. Furthermore, UA reduced inflammatory response by downregulating TNF- $\alpha$ , IL-1 $\beta$  and IL-6 levels ( $p < 0.05$ ). Concomitantly, the lower protein concentrations of Nrf-2 and HO-1 were elevated by administration of UA. Furthermore, UA suppressed Bax/Bcl-2 ratio to ameliorate apoptosis ( $p < 0.05$ ).

**Conclusion:** UA reduces diabetes-induced hippocampal oxidative stress, inflammation and apoptosis. Thus, it might be a potential drug candidate for delaying diabetes-associated cognitive decline (DACD).

**Keywords:** Apoptosis, Cognitive dysfunction, Inflammation, Oxidative stress, Ursolic acid

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## INTRODUCTION

Long-term uncontrollable hyperglycemia has negative effects on multiple organs, resulting in diabetic complications [1]. Diabetes has become a prominent health problem in many countries of

the world. Diabetic complications constitute a complex syndrome which is closely related to a variety of diseases such as nephropathy, cardiomyopathy and neurodegeneration [2]. Cognitive defect is regarded as the most prevalent complication of DM. Diabetic

individuals may easily suffer from peripheral and central nervous neuropathy which are associated with impairments in learning and memory [3]. Thus, there is need to prevent diabetes-induced impairment of cognitive function.

Diabetes-associated cognitive dysfunction (DACD) is a neurogenic disease characterized by learning and memory impairments in patients with poorly controlled hyperglycemia. Streptozotocin (STZ) is widely used for establishment of animal model of diabetes [4]. It is also involved in the development of brain disease, particularly in cognitive decline [5]. The cause of DACD is multifactorial. Oxidative damage, inflammatory responses and apoptosis-induced dysfunctions are essentially consequences of STZ-induced learning and memory impairments [6]. Consistent with these findings, anti-oxidation, anti-inflammation and anti-apoptosis may be effective approaches for prevention and therapy of DACD.

Ursolic acid is present in various plants, flowers, fruits and berries [8]. It is widely used as food additive and medicine in health and disease because of its biological activities [7]. UA is effective against diverse diseases such as obesity and diabetes [8]. However, not much is known about the effect of UA on DACD. Our aim was to determine the effect of UA and its mechanism on cognitive dysfunction in diabetic rats.

## EXPERIMENTAL

### Animals

Male Sprague-Dawley rats (mean weight = 230 ± 2 g) were procured from Animal Center of Hunan Normal University (Changsha, China). Rats were grown in a specific pathogen-free (SPF) environment at a relative constant temperature, fixed humidity and unchanging dark cycle. They were randomly raised in groups of 3-4 animals per cage. The study received approval from the Ethics Committee of Hunan Normal University (approval no. 2019057), and it was performed according to World Health Organization (WHO) guidelines [9].

### Experimental design

UA (purity ≥95%) and STZ were purchased from Sangon Biotech (Shanghai, China). The rat model of diabetes was established by STZ at a dose of 50 mg/kg. Only rats with glycerin above 16.7 mM were considered diabetic. Three groups were established: control (CON) group, DM group, and DM+UA group, with 15 rats in

each group. UA was kept at 2-8°C. In this study, ursolic acid was dissolved in corn oil, and a dose of 35 mg/kg was fed by gastric perfusion for 56 successive days. The control group were treated at the same dose of corn oil amounted to individual body weight.

### Morris water maze

The experimental rats were subjected to Morris water maze for 5 consecutive days to appraise their cognitive function. Before the experiments, the rats were permitted to swim freely for 5min. From the first to the fourth day, the rats swam from different placement points in an attempt to locate a circular platform below the water level. The time taken for a rat to locate the platform was taken as the escape latency. At day 5, the platform was removed. Experimental rats swam at a fixed starting point to search for it. The number of platform crossings was recorded.

### Biochemical assays

After Morris water maze experiment, the rats were anesthetized with chloralhydrate. Hippocampal tissue of each rat was separated from brain matter and homogenized under liquid nitrogen, and the homogenate was centrifuged. Then, SOD activity was assayed in the supernatant using the hydroxylamine method. The concentration of MDA in the supernatant was measured with the thiobarbituric acid (TBA) method, while TNF- $\alpha$ , IL-1 $\beta$  and IL-6 levels were measured using ELISA.

### Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA was extracted from hippocampal tissues using TRIzol reagent. RNA was transformed into cDNA with MBI Revert Aid. RNA and cDNA were stored at -80 °C. The gene expressions were detected using qRT-PCR. Primer sequences were used as previously reported [10]. mRNA expressional values were corrected by comparison with the control group and normalization to  $\beta$ -actin expression.

### Western blot assay

Hippocampus tissue was ground with RIPA to acquire protein. Samples were separated using 12 % SDS-PAGE. Then, protein was transferred onto a PVDF membrane, which was incubated with a solution of non-fat milk in PBS at 4°C for 2 h to block non-specific binding of the blot. Thereafter, the blot was incubated with primary antibodies overnight at 4°C and secondary peroxidase-conjugated antibody at 25°C for 2 h.

The band was observed by ECL system. Protein expression levels were normalized to  $\beta$ -actin.

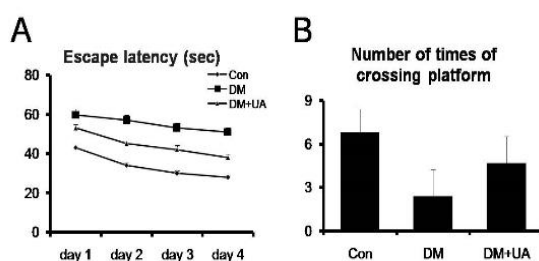
### Statistical analysis

Data are expressed as mean  $\pm$  SD and analyzed with SPSS16.0 software. Statistical significance was done with ANOVA and Post hoc test. Values of  $p < 0.05$  were taken as significant.

## RESULTS

### Effect of UA on STZ-induced cognitive deficit

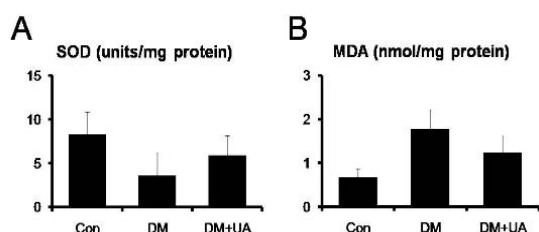
The escape latency was significantly enhanced in DM group, while administration of UA markedly weakened escape latency ( $p < 0.05$ ; Figure 1 A). The frequency of platform crossing was significantly decreased in the DM group. In contrast, administration of UA markedly increased the frequency of platform crossings ( $p < 0.05$ ; Figure 1 B).



**Figure 1:** Effect of UA on escape latency (A) and frequency of platform crossings (B) in diabetic rats

### Effect of UA on STZ-induced changes in oxidative stress

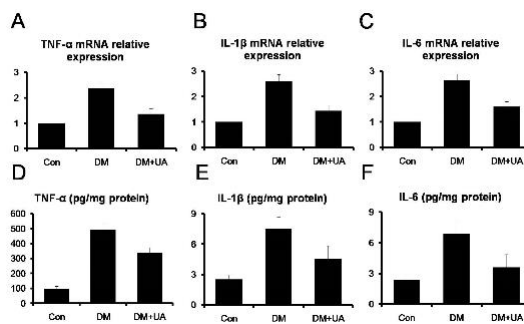
SOD activity was significantly reduced in DM group, while administration of UA markedly increased hippocampal SOD activity ( $p < 0.05$ ; Figure 2 A). In contrast, STZ-induced significant increase in hippocampal MDA content was markedly weakened by administration of UA ( $p < 0.05$ ; Figure 2 B).



**Figure 2:** Effect of UA on hippocampal SOD (A) and MDA (B) levels in hippocampus

### Effect of UA on STZ-induced inflammatory response

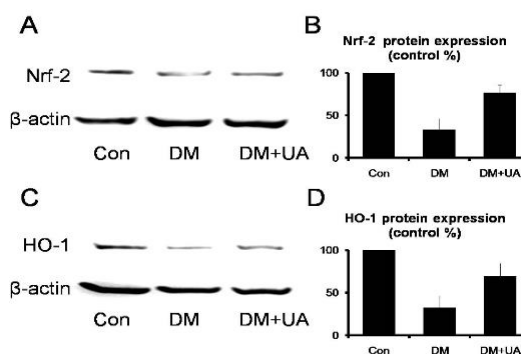
mRNA and protein expressions of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 were significantly enhanced in DM group. In contrast, administration of UA markedly resulted in downregulation of the expressions of these inflammatory cytokines in hippocampus ( $p < 0.05$ ; Figure 3).



**Figure 3:** Effect of UA on hippocampal mRNA and protein expressions of TNF- $\alpha$  (A and D), IL-1 $\beta$  (B and E) and IL-6 (C and F)

### Effect of UA on STZ-induced changes in Nrf-2/HO-1 pathway

To elucidate the effect of UA on oxidant damage in STZ-induced diabetes rats, Nrf-2/HO-1 pathway was measured in hippocampus. The protein expressions of Nrf-2 and HO-1 were significantly suppressed in DM group. However, administration of UA markedly resulted in upregulation of Nrf-2/HO-1 pathway in hippocampus ( $p < 0.05$ ; Figure 4).

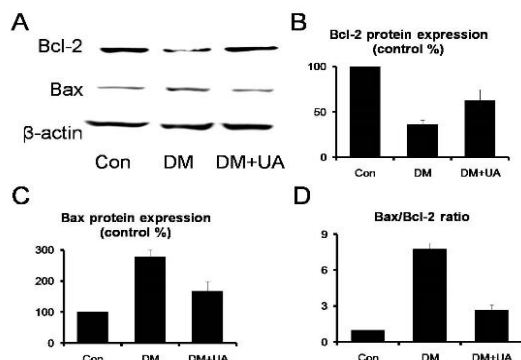


**Figure 4:** Effect of UA on protein expressions of Nrf-2 (A and B) and HO-1 (C and D) in hippocampus

### Effect of UA on STZ-induced apoptotic response

Bcl-2 and Bax expressions in hippocampus were measured using western blotting assay to elucidate the anti-apoptosis effect of UA. Bcl-2

expression was decreased, whereas Bax expression was elevated in DM group. However, UA markedly enhanced Bcl-2 expression and reduced Bax expression in hippocampus ( $p < 0.05$ ; Figure 5A - 5C). Moreover, the ratio of Bax/Bcl-2 was significantly enhanced in DM group, while administration of UA markedly mitigated Bax/Bcl-2 ratio to restrain apoptosis in hippocampus ( $p < 0.05$ ; Figure 5A and D).



**Figure 5:** Effect of UA on protein expressions of Bax (A and B) and Bcl-2 (A and C), and Bax/Bcl-2 ratio (A and D) in hippocampus

## DISCUSSION

UA as a pentacyclic triterpene is well known for its multiple pharmacological and biological effects. Recently, it was reported that UA exerted a potential therapeutic effect on diabetes mellitus [11]. STZ is widely used for induction of diabetes mellitus in animals. Excess hyperglycemia is harmful to the nervous system, and it damages the hippocampus [12]. This study revealed the mitigating effect of UA on cognitive disorder in diabetic rats. The results revealed that UA alleviated DACD via ameliorating oxidative stress, inflammation and apoptosis.

In hippocampus, any disturbance in redox homeostasis may lead to nerve disease [13]. In this study, significant decrease in SOD activity and significant enhancement in MDA content were observed in diabetic rats. SOD is a key antioxidant enzyme involved in redox reactions, and it is beneficial in the prevention of oxidative stress. MDA is an indicator of lipid peroxidation due to oxidative stress. Thus, increased levels of MDA indicate aggravated oxidative damage. This study demonstrated that UA elevated SOD activity and weakened MDA level, suggesting UA might improve cognitive disorders by inhibiting oxidative damage. In addition, UA exerted anti-inflammatory effect in diabetic rats [14]. Inflammatory response has negative effects on neurological function, while reduced levels of

inflammatory cytokines mitigate brain injury and relieve cognitive dysfunction. In our study, UA reduced TNF- $\alpha$ , IL-1 $\beta$  and IL-6 levels in hippocampus, indicating that UA alleviated inflammatory response, thereby mitigating cognitive impairment.

Nrf-2/HO-1 pathway is closely involved in resistance to oxidation and inflammatory response [15]. In cellular redox homeostasis, Nrf-2 is an endogenous transcription factor with various regulatory roles in response to oxidative damage [16]. Studies have revealed HO-1, a target gene of Nrf-2, ameliorates diabetic complications by suppressing the expressions of pro-inflammatory cytokines [17]. In addition, oxidative stress regulates inflammatory reactions. In our study, UA enhanced Nrf-2 and HO-1 protein expressions. Thus, UA displayed anti-oxidative and anti-inflammatory effects for prevention and treatment of cognitive deficits.

Apoptosis is also one of the mechanisms involved in cognitive deficits. In DM, hyperglycemia destabilizes apoptotic homeostasis by activating apoptotic signals [18]. Indeed, targeting of apoptosis and participant pathways is a vital strategy for reversal of cognitive deficits. This study showed that UA altered the Bax/Bcl-2 expression ratio in hippocampus of diabetic rats. This suggests that UA ameliorated diabetes-induced cognitive deficit through its regulating effect on apoptosis.

## CONCLUSION

This research has demonstrated that UA mitigates hyperglycemia-related learning and memory impairment by modulating oxidative stress, inflammation and apoptosis in the hippocampus. Thus, UA may be a potential drug for improvement of diabetes-induced cognitive dysfunction.

## DECLARATIONS

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### Conflict of interest statement

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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