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Research article

Co-administration of berberine/gypenosides/bifendate ameliorates metabolic disturbance but not memory impairment in type 2 diabetic mice

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ABSTRACT

Cognitive impairment is a well-known complication of Type 2 diabetes mellitus (T2DM) characterized by cellular insulin resistance, chronic inflammation, and metabolic disturbances. Berberine, gypenosides and bifendate are traditional Chinese herbal medicines with multiple pharmacological activities including anti-inflammation, anti-oxidant, metabolism improvement and memory improvement. To investigate whether they have synergistic effect on T2DM metabolic syndrome and associated memory impairment, we measured in this study the effect of a low dose of berberine/gypenosides/bifendate (BGB) co-administration on metabolism and memory performance of T2DM model mice. We found that BGB co-administration ameliorated metabolic abnormalities of both high-fat diet/streptozotocin (STZ)-induced T2DM mice and db/db mice. However, it did not alleviate memory impairment in either type of T2DM model mice. Since neither berberine, gypenosides nor bifendate alone at the low dose is effective, we presume that BGB co-administration has synergistic action on T2DM metabolic syndrome. In addition, our findings suggest that higher doses of BGB might be required to ameliorate memory impairment than metabolic disturbance associated with T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is the most common chronic metabolic disease characterized by insulin resistance and chronic inflammation, leading to hyperglycemia and accelerated aging [1, 2]. Cognitive impairment is a well-known complication of T2DM; however, the precise mechanism through which T2DM metabolic disturbances contribute to cognitive decline remains uncertain.

Growing evidences have demonstrated a close relationship between T2DM and neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) [3, 4]. In particular, T2DM is known to be a major risk factor for AD since they share several important features causing brain damage, including impaired insulin sensitivity, Aβ accumulation, tau hyper-phosphorylation, vascular damage, inflammation, and oxidative stress [5]. In addition, diabetic AD patients exhibit robust pathological changes compared to AD patients without DM [6]. Studies have suggested that pharmacological strategies targeting T2DM could have potential benefits to prevent or delay neuronal damage and cognitive decline associated with metabolic disorders [5]. Metformin, a first-line drug in T2DM treatment, was recently shown to produce neuroprotection and to improve memory deficits [7].

Berberine, gypenosides and bifendate are traditional Chinese OTC herbal medicines that possess multiple pharmacological activities like anti-inflammation, anti-oxidant, and anti-tumor [8, 9, 10]. Studies have demonstrated the potential of berberine, gypenosides and bifendate in treatment of interconnected diseases such as diabetes, obesity and cognitive deficits. Berberine is an ancient herbal medicine in treating diarrhea. Recent experimental and clinical studies have illuminated its therapeutic effects on diabetes, obesity and cardiovascular diseases. In particular, it was reported that berberine and metformin exhibit similar potential to treat T2DM and ameliorate associated memory impairment [11]. Gypenosides were also reported to regulate lipid metabolism, reduce hyperlipidemia [12], and attenuate memory impairment associated with various pathological conditions including lipopolysaccharide (LPS)-induced neuroinflammation and 1-methyl-4-phenyl-1,2,3,6-tetrahy- Dropyridine (MPTP) lesion [9, 13]. Bifendate is clinically used for the treatment of hepatitis and as a hepatoprotective against drug-induced liver injury. A very recent study showed that AB-38b, a novel bifendate derivative, improves nephropathy [10] and cognitive decline in diabetic mice [14]. Since the pharmacological effects of berberine, gypenosides and bifendate are multitarget, we wondered

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whether they have synergistic effects on metabolic disturbance and memory impairment in T2DM.

The *db/db* mice is a well-adopted genetic model of T2DM, which displays moderate obesity and severe diabetes caused by a spontaneous mutation in leptin receptor. The combination of high-fat diet (HFD) feeding with subsequent injection of a low dose of streptozotocin (STZ) is a popular, non-genetic approach to induce T2DM, which mimic the transition from the pre-diabetic insulin-resistant obese state to overt T2DM [15]. Previous studies have reported that both *db/db* mice and HFD/STZ-induced diabetic mice exhibit hyperglycemia and memory loss [15, 16]. In this study, we propose to use both *db/db* mice and HFD/STZ-induced diabetic mice to examine whether a low dose of berberine/gypenosides/bifendate (BGB) co-administration ameliorates metabolic dysfunction as well as memory deficit associated with T2DM. Meanwhile, this study may help us to understand the correlation between metabolic disturbance and cognitive impairment in T2DM.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (4 weeks of age) were purchased from the Vital River Laboratory Animal Technology (China). Homozygous male *db/db* mice in C57BL/6J background (4 weeks of age) were purchased from Model Animal Research Center of Nanjing University [17]. All animals were group-housed under a 12h/12h light/dark cycle with free access to water and standard chow (Jiangsu Xietong Pharmaceutical Biology, China) for two weeks to acclimate to new environment after transportation. The Chancellor's Animal Research Committee at Qingdao University approved animal protocols in accordance with National Institutes of Health guidelines. The ethical approval number is MECAH-QDU-20180518001.

2.2. Diets and treatment

After acclimation, 6-week-old wild-type C57BL/6J mice were randomly assigned to HFD/STZ group or control (CON) group. The HFD/STZ mice (n = 20) were fed with high-fat diet containing (w/w) 59% standard chow, 20% sugar, 18% animal fat, and 3% egg yolk [18] for 4 weeks. The CON mice (n = 7) and *db/db* mice (n = 14) were provided with normal diet. On the first and the 6th day of 11 weeks of age, HFD/STZ group mice received intraperitoneal injection with a low dose of STZ (85 mg/kg, Sigma, dissolved in 0.1 M citrate buffer) after overnight fasting. The blood glucose levels of all mice were assessed using Yuyue type II (560) blood glucose monitoring system (China). Fourteen out of 20 HFD/STZ group mice with blood glucose levels higher than
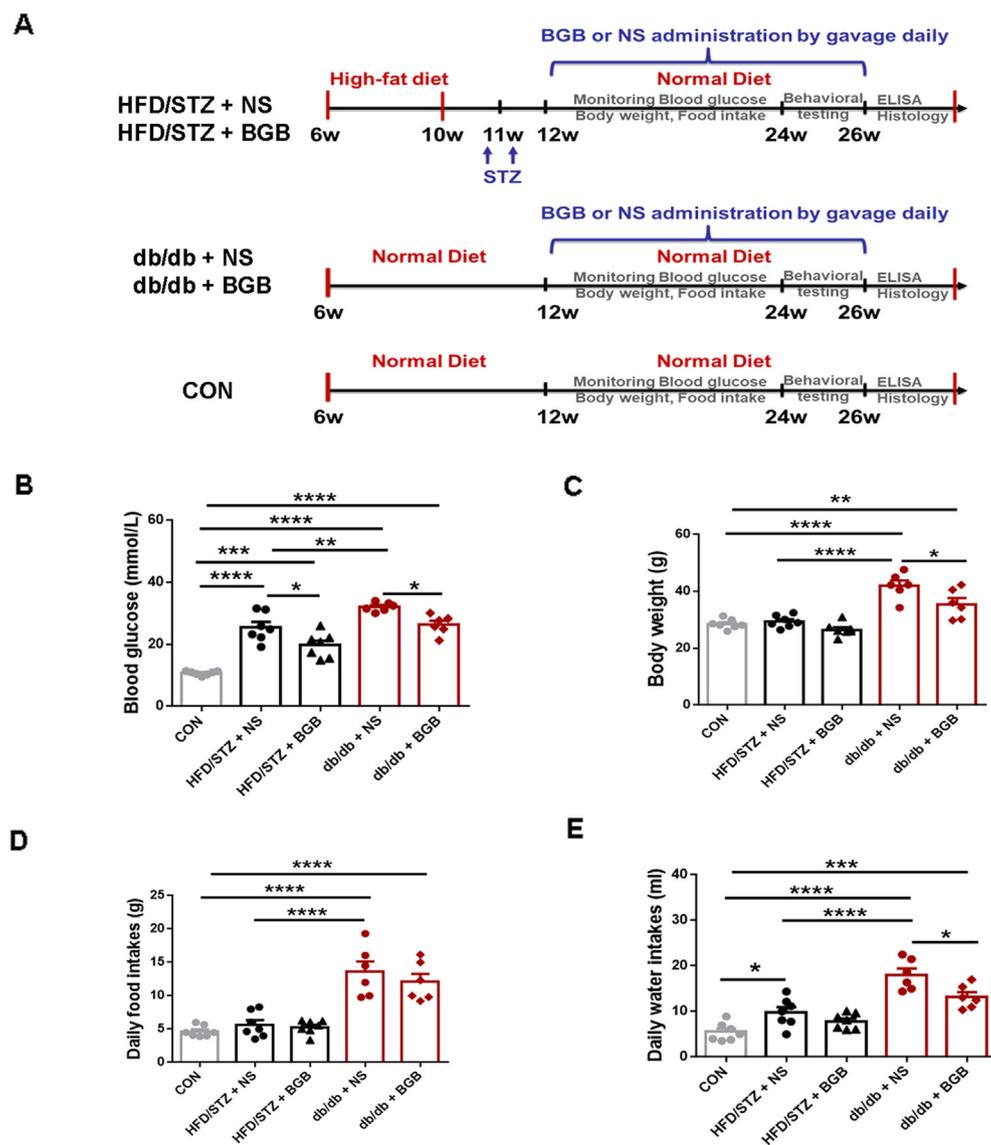

Figure 1. Comparing blood glucose level, body weight, and food and water intake among all groups of mice. (A) Experimental design. (B) Blood glucose levels. (C) Body weight. (D) Daily food intake. (E) Daily water consumption. One-way ANOVA and Tukey's multiple comparisons test, $****P < 0.0001$, $***P < 0.001$, $**P < 0.01$ or $*P < 0.05$ means significant difference between groups. Control C57BL6 mice (CON), n = 7; HFD/STZ-induced diabetic mice with intragastric administration of normal saline (HFD/STZ + NS), n = 7; HFD/STZ-induced diabetic mice with intragastric co-administration of BGB (HFD/STZ + BGB), n = 7; *db/db* mice with saline treatment (*db/db* + NS), n = 6; *db/db* mice with BGB administration (*db/db* + BGB), n = 6. All data are shown as means ± SEM.

16.7 mmol/L by the end of 12 weeks of age were considered diabetic and were used for further analysis [18]. All mice had free access to water and standard chow until all experiments were done (Figure 1A). The body weight, food and water intake, and blood glucose level were monitored every 2 weeks.

Berberine and gypenosides were purchased from Sigma (USA). Bifendate was purchased from Beijing Union Pharmaceutical Factory (China). Intragastric co-administration of berberine (10 mg/kg), gypenosides (1 mg/kg) and bifendate (0.3 mg/kg) were carried out daily for 14 weeks until all experiments were done (Figure 1A). Drug doses were chosen based on both previous reports and our preliminary studies designed to find out the minimal effective dose of each drug to suppress LPS-triggered inflammation in cultured BV-2 cells. In addition, we tried different doses of BGB co-administration in preliminary experiment, and found no acute (24 h) or chronic toxicity (2 weeks) of BGB co-administration in C57BL/6J mice, even at maximal dose used (50 mg/kg berberine, 50 mg/kg gypenosides, 6 mg/kg bifendate).

2.3. Experimental groups

Five groups of mice were investigated in the following experiments. The control mice (CON, n = 7) received intragastric administration of 0.9% saline (NS) once a day. The HFD/STZ-induced diabetic mice received an intragastric co-administration of either BGB (HFD/STZ + BGB, n = 7) or NS (HFD/STZ + NS, n = 7) every day. The *db/db* mice were also treated daily with either BGB (*db/db* + BGB, n = 6) or NS (*db/db* + BGB, n = 6).

2.4. Behavioral test

Behavioral experiment began when all five groups of mice were 24 weeks of age. All behavioral tests were done between 9:00 am and 6:00 pm without handler's awareness of experimental design. Animal behaviors were video-tracked and analyzed independently with Noldus Etho-Vision XT software.

2.4.1. Novel object recognition (NOR) and novel object-place recognition (NPR)

NOR and NPR training and testing were performed in a square, non-transparent plastic chamber (27.3 × 27.3 × 20.3 cm) with visual cues on the wall [19]. Mice were handled 3 min/day for 3 days and then habituated in the empty chamber for additional 3 days (10 min/day) prior to training to reduce anxiety. During training, mice freely explored two identical objects inside the chamber for 10 min/trial and three trials with an inter-trial interval of 30 min. NOR testing was done 1 hr after training with one object replaced by a new one. NPR testing was done 24 h after training with one object transferred to a new location. Percentage of time exploring novel object versus old object (NOR), or object in new place versus in old place (NPR) was measured during a 5-min testing. Recognition Memory Index (%) was calculated as (exploration time for novel object or object in new place/total objects exploration time) x 100%. The higher index indicates the better recognition memory. Memory index around or lower than 50% means memory impairment.

2.4.2. Morris water maze (MWM)

MWM is used to evaluate spatial learning and memory. The water maze pool was divided into four quadrants. During training, an invisible, 10 cm-in-diameter escape platform was anchored in one quadrant and 0.8 cm below the surface of opaque water. Mice were released facing the wall from one of six pesudo-random starting points and allowed to search the platform for 60 s. A training trial ended whenever mice climbed onto the platform within 60 s, or when time exceeded 60 s. Mice were trained two trials/day for consecutive 6 days with an inter-trial-interval of 1 h. Spatial memory was tested on day 3 or day 5 after training, and also on day 7, during which the hidden platform was removed. The percentage of time spending in each quadrant during the probe test was analyzed [20].

2.5. Blood serum collection and enzyme-linked immunosorbent assay (ELISA)

Around 200μl of whole blood was freshly taken from each mouse and kept in a sterile PE tube without any anticoagulant. Blood sample was left at room temperature for 30 min to form a clot. Immediately after centrifuging at 1500 x g for 10 min at 4 °C, the resulting supernatant serum was quickly transferred into a clean PE tube using a Pasteur pipette. Samples were kept at 4 °C while handling and stored at -80 °C until use. The concentration of triglyceride (TG) and low-density lipoprotein (LDL) in serum were measured with corresponding mouse ELISA kits according to the manufacturer's instruction (Wuhan Colorful Gene biological technology, China). Absorbance value for each sample running in triplicates was measured at 450 nm using a 96-well microplate spectrophotometer. Protein concentration was calculated from the standard curve plotted by absorbance values of a diluted series of standards provided in each kit.

2.6. Paraffin section preparation, hematoxylin and eosin staining

The liver and pancreas tissues were removed, washed, and fixed overnight in 4% neutral formalin solution. After trimming, small tissue blocks (1.5 × 1.5 × 0.3cm) were incubated in 4% neutral formalin solution for an additional 3 h, then dehydrated using 50%, 75%, 80%, 95%, and 100% gradient alcohol (2 × 30 min per gradient) followed by incubation in xylene for 20 min and 3 times. Paraffin embedding was performed by placing transparent tissue blocks in molten paraffin wax for 3 h at 60 °C and then solidification at 4 °C. Tissue sections (4 μm) were mounted on slides, subjected to rehydrate with gradient alcohol and then hematoxylin and eosin (H&E) staining. The slides were visualized using an inverted microscope (Leica, USA) at 4x, 10x, and 40x objective lens.

2.7. Statistical analysis

Results were expressed as mean ± SEM. Statistical analyses were performed with GraphPad Prism 6.0 software. One-way or Two-way ANOVA and following multiple comparisons were used as described in the main context. The significance level was set to $P < 0.05$.

3. Results

3.1. BGB co-administration ameliorated hyperglycemia in T2DM model mice

We compared averaged blood glucose level, body weight, and food and water intake among five groups of mice at 24 weeks of age (Figure 1A). In consistence with numerous previous studies, we found that the blood glucose level of HFD/STZ-induced diabetic mice (HFD/STZ + NS, n = 7) and *db/db* mice (*db/db* + NS, n = 6) were significantly higher than control mice (CON, n = 7) (Figure 1B; one-way ANOVA, $F_{(4, 28)} = 43.02$, $P < 0.0001$; Tukey's multiple comparisons test, $P < 0.0001$). Hyperglycemia in *db/db* mice is more severe than in HFD/STZ-induced diabetic mice (Figure 1B; *db/db* + NS mice vs. HDF/STZ + NS mice, $P < 0.01$). Importantly, we found that BGB co-administration for 12 weeks reduced blood glucose levels of HFD/STZ mice (HFD/STZ + BGB, n = 7) and *db/db* mice (*db/db* + BGB, n = 6), compared to saline-treated T2DM model mice (Figure 1B; HFD/STZ + BGB mice vs. HFD/STZ + NS mice, $P < 0.05$; *db/db* + BGB mice vs. *db/db* + NS mice, $P < 0.05$). Since the blood glucose levels of BGB-treated T2DM mice were higher than C57BL/6 control mice (Figure 1B; $P < 0.0001$), we concluded that a low dose of berberine/gypenosides/bifendate co-administration partially ameliorates hyperglycemia in T2DM model mice.

The *db/db* mice were more obese than HFD/STZ mice or CON mice at 24 weeks of age (Figure 1C; one-way ANOVA, $F_{(4, 28)} = 22.68$, $P < 0.0001$; Tukey's multiple comparisons test, $P < 0.0001$). BGB treatment for 12 weeks suppressed body weight gain of *db/db* mice (Figure 1C; *db/*

db + BGB mice vs. *db/db* + NS mice, *P* < 0.05), although treated *db/db* mice remain obese compared to control mice (*db/db* + BGB mice vs. CON, *P* < 0.01). The daily food intake and water consumption of *db/db* mice were also much more than HFD/STZ mice or CON mice (Figure 1D and 1E, one-way ANOVA and Tukey's multiple comparisons test; *P* < 0.0001). BGB treatment reduced daily water consumption of *db/db* mice (Figure 1D; *db/db* + BGB mice vs. db/db + NS mice, *P* < 0.05). HFD/STZ mice showed normal body weight, normal food intake and slightly increased water consumption in comparison to CON mice (Figure 1E; *P* < 0.05). We did not observe significant impact of BGB treatment on body weight, food or water intake of HFD/STZ mice. Altogether, our results indicate that *db/db* mutation causes more severe metabolic disturbance than HFD/STZ treatment, and a low dose of BGB co-administration partially ameliorates metabolic disturbance in T2DM model mice.

3.2. BGB co-administration failed to improve memory impairment in T2DM model mice

Next, we assessed whether chronic BGB co-administration, which partially ameliorate metabolic disturbance, could improve memory impairment of T2DM model mice. We found that, both HFD/STZ mice (HFD/STZ + NS, n = 7) and *db/db* mice (*db/db* + NS, n = 6) spent less percentage of time exploring novel object (Figure 2A) or object in novel place (Figure 2B) than control C57BL/6 mice (CON, n = 7) (Figure 2A and 2B; one-way ANOVA and Tukey's multiple comparisons test, *P* < 0.01 to *P* < 0.001). Their poor memory index indicates that both HFD/STZ mice and *db/db* mice have recognition memory impairment. The low

dose of BGB co-administration for >12 weeks did not improve memory impairment in either HFD/STZ mice or *db/db* mice (Figure 2A and 2B; BGB vs. NS, *P* > 0.05). In addition, we noticed that the total object exploration time was significantly decreased in *db/db* mice compared to HFD/STZ or control mice (Figure 2A and 2B; *P* < 0.0001), indicating that *db/db* mutation affect mice exploration activities while HFD/STZ treatment did not.

We also evaluated spatial learning and memory performance of these five groups of mice using a hidden version of Morris water maze. The latency to platform curve across six consecutive training days revealed that only *db/db* mice (*db/db* + NS, n = 6) had difficulties to quickly locate the hidden platform after training (Figure 2C). This result thus indicated spatial learning deficit in *db/db* mice, not HFD/STZ mice (Figure 2C; two-way ANOVA and Tukey's multiple comparisons test, day 6, *db/db* + NS vs. CON, *P* < 0.01; HFD/STZ + NS vs. CON, *P* > 0.05). BGB treatment did not ameliorate spatial learning deficit of *db/db* mice (Figure 2C; *db/db* + BGB vs. *db/db* + NS, *P* > 0.05; *db/db* + BGB vs. CON, *P* < 0.01). Further probe test at day 7 revealed that both *db/db* mice and HFD/STZ mice had spatial memory deficit (Figure 2C). Specifically, the control mice (CON, n = 7) spent significant more percentage of time searching the target (training) quadrant than the other three quadrants (Figure 2C; two-way repeated measure ANOVA and Bonferroni post-tests, *P* < 0.05 to *P* < 0.001, n = 7), while *db/db* mice (n = 6) and HFD/STZ model mice (n = 7) spent similar time in four quadrants (*P* > 0.05). Similarly, we found that BGB treatment did not improve spatial memory impairment of either *db/db* mice or HFD/STZ mice (Figure 2C; one-way ANOVA and Newman-Keuls Multiple Comparison Test, training quadrant searching, BGB vs.
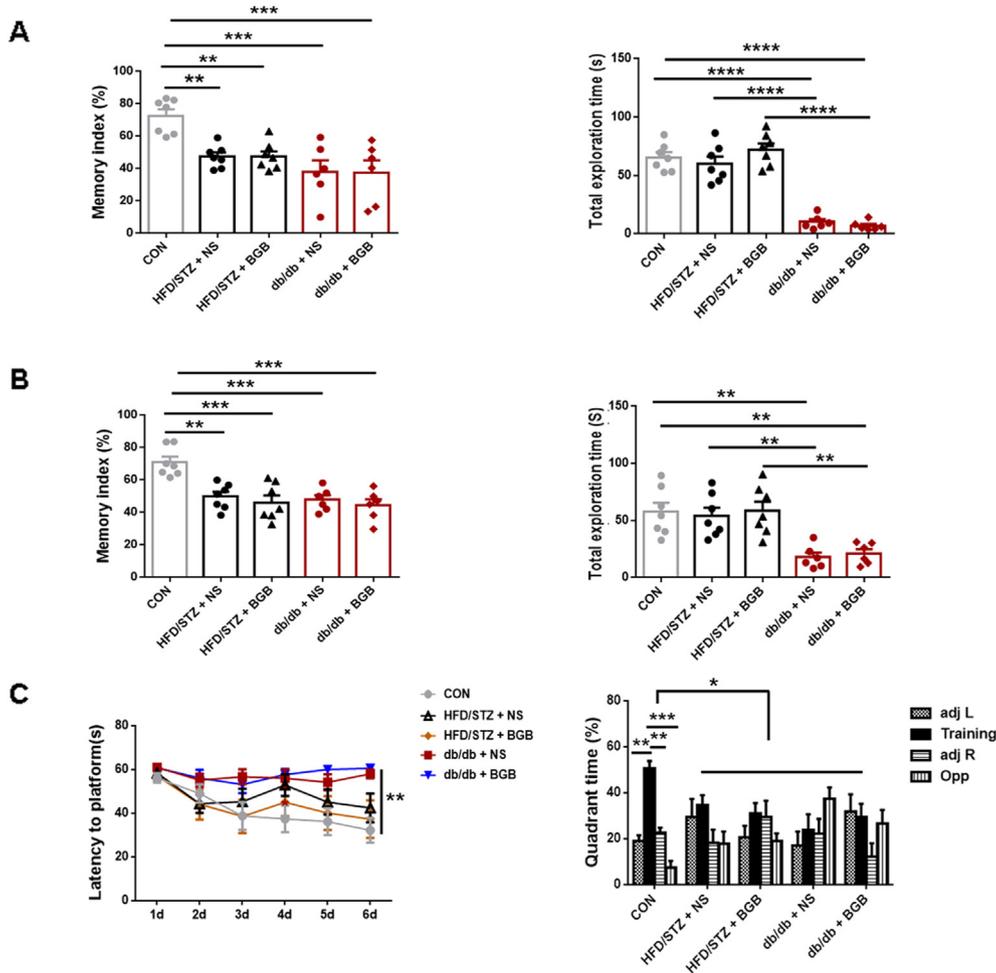

Figure 2. Comparing memory performance among all groups of mice. (A) NOR. Left, summary of NOR memory index of five groups of mice. Right, total object exploration time during a NOR test. (B) NPR. Left, summary of NPR memory index. Right, total object exploration time during a NPR test. A-B, one-way ANOVA and Tukey's multiple comparisons test, ****P < 0.0001, ***P < 0.001 or **P < 0.01 means significant difference between groups. (C) Hidden version of Morris water maze. Left, latency time to reach platform across 6 training days. Right, spatial memory performance at day 7 probe test. Two-way ANOVA and Tukey's multiple comparisons test (left) or Bonferroni post-tests (right). ***P < 0.001, **P < 0.01 or *P < 0.05 means significant difference between groups. CON, n = 7; HFD/STZ + NS, n = 7; HFD/STZ + BGB, n = 7; *db/db* + NS, n = 6; *db/db* + BGB, n = 6. All data are shown as means ± SEM.

NS, $P > 0.05$; CON vs. others, $P < 0.05$). Altogether, our results demonstrated that a low dose of BGB co-administration could not improve learning and memory deficit inT2DM model mice.

3.3. BGB co-administration ameliorated dyslipidemia and tissue degeneration in T2DM model mice

Since dyslipidemia is closely associated with T2DM, we compared serum lipid levels among five groups of mice. Serum ELISA analysis revealed that *db/db* mice (*db/db* + NS, n = 6) exhibited higher level of TG than HFD/STZ mice (HFD/STZ + NS, n = 7) or control mice (CON, n = 7) (Figure 3A; One-way ANOVA and Tukey's multiple comparisons test, *db/db* + NS vs. HFD/STZ + NS, $P < 0.001$; *db/db* + NS vs. CON, $P < 0.0001$). BGB co-administration for >12 weeks reduced serum TG level of *db/db* mice (Figure 3A; *db/db* + BGB vs. *db/db* + NS, $P < 0.05$; *db/db* + BGB vs. CON, $P > 0.05$). HFD/STZ mice showed similar level of TG as control mice ($P > 0.05$). The levels of LDL in *db/db* mice and in HFD/STZ mice were comparable, and both of them were higher than control mice (Figure 3A; One-way ANOVA and Tukey's multiple comparisons test; LDL, HFD/STZ + NS vs. CON, $P < 0.0001$; *db/db* + NS vs. CON, $P < 0.001$). BGB co-administration for >12 weeks significantly reduced serum LDL concentration in both HFD/STZ mice and *db/db* mice (Figure 3A; BGB vs. NS, $P < 0.05$).

We also determined the effect of BGB treatment on the liver and the pancreas histology of T2DM mice using H&E staining. A normal architecture of the hepatic lobules and the central veins were observed in control mice. The hepatocytes showed normal nests and bands of arrangement. In contrast, the hepatic lobules of *db/db* mice (*db/db* + NS) were poorly structured. Many hepatocytes were enlarged with foam-like lipid vacuoles in the cytoplasm and nuclei squeezed to one side. Only small lipid vacuoles were observed in a few hepatocytes of HFD/STZ mice (Figure 3B). Therefore, the overall steatosis of the hepatocytes was more severe in *db/db* mice than in HFD/STZ mice. Clearly, BGB treatment ameliorated liver damage of both HFD/STZ mice and *db/db* mice. Similarly, reduced blood sinuses were remarkable in the pancreas islets of *db/db* mouse, but not in HFD/STZ mice. Some islet cells in *db/db* mice

exhibited amyloidosis, which was not observed in HFD/STZ mice or control mice. BGB treatment also improved pancreas pathology of *db/db* mice (Figure 3B). These findings thus indicated that *db/db* mutation causes more severe degenerative changes in both the hepatocytes and pancreatic islet cells. More importantly, our results demonstrated that chronic BGB co-administration ameliorates hyperglycemia and hyperlipidemia, meanwhile improves pathological changes of the liver, and therefore contributes to the treatment of metabolic disorders in T2DM mice.

4. Discussion

There is substantial evidence that inflammatory processes play a crucial role in both T2DM and AD pathogenesis [21]. The elevated synthesis of pro-inflammatory cytokines, such as interleukin-6 (IL-6), characterizes the early or preclinical stages of T2DM [22]. These inflammatory mediators can activate brain-residing microglia and astrocytes, leading to CNS inflammation and synaptic dysfunction [23]. Recent study further demonstrated that insulin-signaling dysregulation and tau pathology are critical for T1DM-associated cognitive/synaptic deficits, while chronic inflammation plays an important role in T2DM-associated cognitive/synaptic impairments [2]. Therefore, alleviating inflammation may represent a promising therapeutic option to ameliorate cognitive dysfunction and dementia in both T2DM and AD.

Anti-inflammation activity is one of the common pharmacological properties shared by berberine, gypenosides and bifendate [8, 9, 10]. Besides anti-inflammation and anti-oxidant activities in common, these three traditional herbal medicines also exhibit diverse biological effects and wide clinical applications, including anti-diabetes, anti-hyperlipidemia, and hepatoprotection. Particularly, berberine was reported to share many features with metformin in treating T2DM, obesity, as well as inflammation [11]. A clinical study showed that combined use of berberine and metformin showed better efficacy than metformin alone in treating T2DM patients [24]. It is also worth to be mentioned that berberine has much lower cost than metformin. Gypenosides is well known for its effect in regulating lipid metabolism, anti-hyperlipidemia
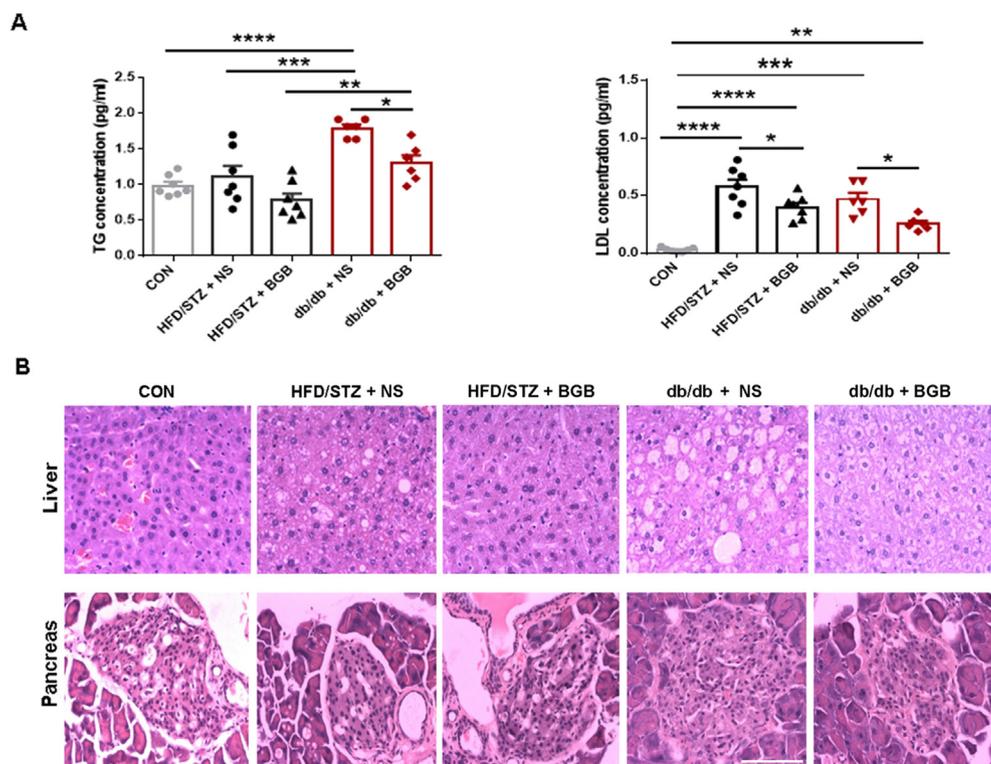
Figure 3. Comparing dyslipidemia and tissue degenerative changes among all groups of mice. (A) Summary of serum TG (left) and LDL (right) concentrations. One-way ANOVA and Tukey's multiple comparisons test, ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$ or *$P < 0.05$ means significant difference between groups. (B) Sample H&E staining of the liver (upper) and pancreas (lower). Final magnification 200 x. CON, n = 7; HFD/STZ + NS, n = 7; HFD/STZ + BGB, n = 7; *db/db* + NS, n = 6; *db/db* + BGB, n = 6. All data are shown as means ± SEM.

and neuroprotection [12, 25]. Previous studies have demonstrated that both berberine and gypenosides at a daily dose of 50 mg/kg/day could improve memory impairment associated with neuroinflammation, such as in diabetes [8, 9, 13]. Bifendate is used widely in clinical fields as a hepatoprotective drug. It was reported that bifendate (6 mg/kg/day) could protect against drug-induced liver injury and hepatitis [26]. Interestingly, a recent study showed that AB-38b, a novel bifendate derivative, improves nephropathy [10] and cognitive decline in diabetic mice [14]. Considering that the pharmacological actions of berberine, gypenosides and bifendate are multitarget with a common effect of anti-inflammation, we postulated that combination use of these drugs might allow reduction in dosage of individual drugs, therefore help to solve problems, such as oral bioavailability and tolerance, and minimize the side effects of each alone. Therefore, we co-administrated in this study a low dose of berberine (10 mg/kg/day), gypenosides (1 mg/kg/day) and bifendate (0.3 mg/kg/day) for 14 weeks, the minimal dose for each drug to suppress LPS-induced inflammation in cultured BV-2 cells (preliminary data not shown).

We found that hyperglycemia, hyperlipidemia and liver damage, but not cognitive impairment, were ameliorated in both *db/db* mice and HFD/STZ-induced diabetic mice. Since neither such dosage of berberine, gypenosides, nor bifendate alone was effective in our preliminary study; we postulated that BGB co-administration might have synergistic action on T2DM metabolic syndrome. In addition, our results suggest that higher doses of BGB might be required to ameliorate memory impairment than metabolic disturbance associated with T2DM. The reason why BGB co-administration that ameliorates metabolic disturbance fails to improve memory impairment in type 2 diabetic mice is uncertain. Further analyses of pro-inflammatory factors, such as IL-6 and NF-κB levels [11], both in the blood and in the hippocampus, and microglia morphology in the hippocampus of T2DM mice as well are required to elucidate the possible mechanism underlying the ameliorating effect of BGB co-administration on metabolic disturbance associated with T2DM. Further investigations about the activities of cholinergic and insulin signaling pathways [8], SIRT1/ER stress pathway [27], Nrf2/ARE signaling pathway [10, 14], and BDNF/TrkB signaling pathway [9] in the hippocampus may help to explain why BGB co-administration in our study ameliorates metabolic disturbance but not memory impairment in T2DM mice.

Hyperglycemia is the most important feature of diabetes and the principal cause of its complications including cognitive deficits. Consistent with abundant previous reports, we found in this study that both *db/db* mutant mice and HFD/STZ-treated mice exhibit hyperglycemia and memory deficit. While these two types of T2DM model mice showed identical recognition and spatial memory deficits, the metabolic syndrome of *db/db* mice are obviously more severe than age-matched HFD/STZ mice, which includes hyperglycemia, hyperlipidemia, degenerative changes of the liver and the pancreas. In addition, we noticed that *db/db* mice exhibited significantly reduced exploration activities, which may have intricate effect on learning and memory performance. From this point of view, we suggest that diabetic mice induced by HFD and low-dose STZ treatment is a better model to study T2DM-associated cognitive impairment. In addition, its characteristics of low cost, easy operation and high success rate enables HFD/STZ-treated mice a well-adopted animal model to study T2DM [28]. Since HFD/STZ mice showed only mild metabolic disturbances compared to *db/db* mice, it might be more applicable in the study of early diabetes. The disadvantages of HFD/STZ-induced T2DM mice include lack of genetic impact on the pathogenesis of diabetes, and large individual variability. The renal toxicity of STZ may also have an impact on the experimental results, so that low dose of STZ is required to avoid other tissue damage besides partial β-cell loss. Different doses (~30–100 mg/kg) of single or multiple STZ injection(s) have been reported in previous studies [28, 29]. In this study, we chose a dose of 85 mg/kg and twice injections with an interval of 5 days based on previous reports and our preliminary findings. It is interesting to refine

the dose of STZ and test whether lower STZ correlates with less memory impairment.

In conclusion, we demonstrate in this study that chronic, co-administration of a low dose of BGB synergistically ameliorates metabolic disturbance in T2DM model mice. Higher doses of BGB may be required to rescue cognitive impairment associated with T2DM.

Declarations

Author contribution statement

M. Zhang: Performed the experiments; Wrote the paper.

X. Wang, X. Guo, J. Li: Performed the experiments.

D. Shi, L. Cui: Analyzed and interpreted the data.

Y. Zhou: Conceived and designed the experiments; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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