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Neuroprotective Effect of Barbaloin on Streptozotocin-Induced Cognitive Dysfunction in Rats via Inhibiting Cholinergic and Neuroinflammatory Cytokines Pathway—TNF-α/IL-1β/IL-6/NF-κB

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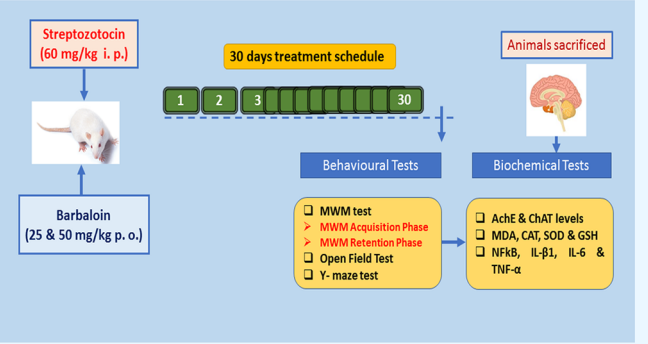
ABSTRACT: Streptozotocin (STZ) impairs memory in rats through altering the central nervous systems (CNS) as a result of impaired cholinergic dysfunction, oxidative stress, persistent hyperglycemia, and alterations in the glucagon-like peptide (GLP). In this model cholinergic agonist, antioxidant and antihyperglycemic treatment has been shown to have positive effects. Barbaloin has a variety of pharmacological effects. However, there is no evidence on how barbaloin improves memory dysfunction caused by STZ. Thus, we examined its effectiveness against cognitive damage caused by STZ at a dose of 60 mg/kg i.p. in Wistar rats. Blood glucose levels (BGL) and body weight (BW) were assessed. To assess learning and memory skills, the Y-maze test and Morris water maze (MWM) test were utilized. Superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione (GSH) as oxidative stress markers were regulated to reverse the cognitive deterioration, and choline-acetyltransferase (ChAT) and acetyl-cholinesterase (AChE) as indicators of cholinergic dysfunction, nuclear factor kappa-B (NF-κB), IL-1β (interleukin-1β), IL-6, and tumor necrosis factor-α (TNF-α) contents were used. Barbaloin treatment thereby significantly decreased the BW and learning and memory capacities, resulting in substantial behavioral improvement in the Y-maze and MWM test. BGL, SOD, CAT, MDA, GSH, AChE, ChAT, NF-κB, IL-6, TNF-α, and IL-1β levels were also altered. In conclusion, the findings revealed that barbaloin had a protective impact against cognitive dysfunction caused by STZ.



1. INTRODUCTION

Diabetes mellitus (DM), which has been around for a while, is regarded as a prevalent metabolic illness that has a negative influence on people's quality of life. Brain atrophy and cognitive decline are two neurological problems that are recurrently seen in the CNS and peripheral system.[1−3] Multiple organs, including the brain, eyes, heart, lower limb blood vessels, and lungs, may have complications as a result of DM. There is growing evidence that DM causes memory loss and cognitive dysfunction in diabetic (DM) animal models. Although the precise mechanism is unknown, a major risk factor for cognitive decline is DM. The hippocampus is a crucial part of the brain that regulates learning and memory, and it has been shown that chronic hyperglycemia can cause ultrastructural destruction of the hippocampus.[4,5]

There are a number of things that seem to contribute to cognitive decline in diabetics.[6] Many investigations have shown that hyperlipidemia and persistent hyperglycemia are important initiating and developing factors for diabetes-related cognitive impairments.[7−9] Additionally, deposition of amyloid-β (Aβ), aberrant insulin signaling, and a strong inflammatory reaction can all result from a disruption of protein, carbohydrate, and lipid metabolism under diabetic conditions, which also contributes to diabetes-related neuronal injury and cognitive deficiencies.[9−11]

The cerebrovascular changes,[12−14] oxidative stress,[15,16] enhanced advanced-glycation end products,[17,18] and underlying causes of diabetic dementias are assumed to be dysfunctions in brain insulin signaling systems.[19] Additionally, it has been suggested that antioxidants,[20,21] hypoglycemics, and insulin sensitizing medications[22] can decreased DM-related cognitive decline. However, no specific medications are offered at this time to address or prevent cognitive impairment in DM.[23]

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Furthermore, in rats, diabetes caused by streptozotocin (STZ) is a well-established paradigm for experimental diabetes. According to past findings, STZ caused severe weight loss, and berberine treatment reversed the same.[20,24] It is widely known from the literature that enhanced expression of pro-inflammatory cytokines is closely related to neuronal damage.[25] Despite the fact that the pathophysiology of the cognitive decline caused by STZ has not yet been completely established, the idea that cognitive decline induced by diabetes is linked to the enhanced inflammatory mediators like IL-6, TNF-α, and IL-1β has been supported by a number of studies.[1,26]

Aloe vera L. is the source of the barbaloin, a naturally occurring bioactive anthracycline.[27] Barbaloin possesses numerous pharmacological actions, including antiviral, anti-inflammatory, cathartic and antioxidant,[28−30] laxative,[31] and antitumor, similar to *Aloe vera* L.[32] Additionally, recent research has shown that barbaloin provides protective benefits against liver damage caused by ethanol in mice by reducing inflammation and oxidative stress.[33] Prior studies have discovered that barbaloin showed NF-κB-dependent overexpression of IL-6, IL-1β, and TNF-α, which was likewise severely reduced after blocking PI3K/AKT. These findings offer concrete proof that barbaloin decreases cellular generation of reactive oxygen species (ROS) by inhibiting PI3K and AKT phosphorylation, which in turn prevents NF-κB activation.[34]

These data proposed that barbaloin might have an impact on the cognitive impairment caused by diabetes. There are, however, no reports mentioning this problem. The current study investigates the effects barbaloin has against STZ-induced cognitive dysfunction in rodents by evaluation using a behavioral test including the Morris water maze (MWM), Y-maze test, open field test (OFT), and biochemical alterations, i.e., oxidative stress markers—superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and glutathione (GSH)—and cholinergic indicators—choline-acetyltransferase (ChAT) and acetyl-cholinesterase (AChE)—and inflammatory markers—nuclear factor kappa-B (NF-κB), IL-1β (interleukin-1β), IL-6, and tumor necrosis factor-α (TNF-α).

2. EXPERIMENTS

2.1. Subjects. We utilized male Wistar rats that weighed 200 ± 25 g. The animals were kept in a typical lab atmosphere with pellet food and access to unlimited water during natural hours (cycle: light and dark). As per the "CPCSEA Guidelines for Laboratory Animal Facilities," the proper approvals were obtained (IAEC/TRS/PT/022/018).

2.2. Drugs. STZ, metformin (Sigma-Aldrich, USA), and barbaloin (SKL supplier, Maharashtra, India) were used to measure. The neuroinflammatory cytokines IL-6, IL-1β, and TNF-α as well as the neuroinflammatory indicator NF-κB were analyzed (MyBioSource, USA). All other chemicals used in this investigation were of high-grade quality.

2.3. Diabetes Induction. An earlier described approach was used to cause diabetes in rats. Briefly stated, STZ was administered intraperitoneally 60 mg/kg after being prepared in sodium citrate buffer (0.1 M) with a pH of 4.4. In order to decrease deaths from hypoglycemic shock, STZ-administered rats were given solution of 5% glucose rather than water for 24 h following injection of STZ. Then, 48 h after injection of STZ, blood samples were collected from the tail to analyze blood glucose levels (BGL). For the following research, only diabetic rats with a fasting BGL > 250 mg/dL were employed.[35]

2.4. Experimental Protocol. Thirty Wistar rats were randomly divided into five groups with six animals in each group assigned as such:

Group I: Nondiabetic control
Group II: Diabetic control
Group III: Diabetic + barbaloin 25 mg/kg per oral
Group IV: Diabetic + barbaloin 50 mg/kg per oral
Group V: Diabetic +500 mg/kg per oral metformin

The treatment groups received the test or standard, and the nondiabetic control group received saline or a vehicle dose scheduling from 1 to 30 days. Animal weight and BGL were monitored at the beginning and end of the experiment.

2.5. Cognitive Task. *2.5.1. MWM Test.* The MWM test, as previously described, was used to analyze the cognitive performance of rats.[35−37] The test device was a round, dark gray plastic water tank that had a circumference of 180 and 60 cm height and was partially occupied with water at a temperature of 24 °C. By adding creamy milk, the water became opaque. In the tank, there were four equally spaced quadrants. The platform (12.5 cm circumference and 38 cm height) was positioned in one quadrant out of four and placed into the water surface at a depth of 2.0 cm. Throughout the whole experiment, the platform was placed in the same quadrant. The only distal spatial clues present in the testing environment were required to help the rats locate the platform. Through the course of the test, the cues remained unchanged. For 5 days, the rats underwent four daily training sessions in tandem. Each session had a maximum time of 60 s and a session interval of roughly 30 s. To get onto the platform buried beneath the water, the rat had to swim. The animal reached the platform and stayed there for 20 s until the next session started. The distal cues' distance from the escape platform remained constant. If the rat did not get to the rescue platform in the allotted maximum time of 60 s, it was placed softly upon the rescue platform and left there for the same period of time. It was measured how long it took to get to the platform (latency in seconds). On the last day of training, rats were put to the test in a MWM with a visual platform to rule out any sensorimotor processing deficiencies.[38] There is no special alignment needed for the test using the visible platform,[35,39] and the test was employed to demonstrate potential sensorimotor processing deficiencies. The platform was positioned 1 cm above the water's surface inside the tank for the test. For 60 s, rats were permitted to swim. Rescue latency was measured as the time it took to get to the platform. Rats were gently dried with a towel after the final trial, kept warm for 1 h, and then put back in their original cages.

2.5.2. Probe Test. The degree of memory retention was measured during a probe test.[21,38] The proportion of memory retention that has occurred after learning is indicated by the time being spent in the target quadrant. On day 36, the probe test was conducted, and a separate rat was dropped into the tank similar to the training session, except that the tank's concealed platform had been taken out. For 60 s, the time that was spent in the target quadrant was recorded. Additionally, each rat's frequency of passing the platform site was counted and measured.

2.5.3. Y-Maze Test. The formerly reported, Y-maze test was preferred to measure the behavioral parameters.[40] The working memory of the animal was observed with the help of the Y-maze test, which observed random rearrangements. A wooden maze has three separate arms that were each spaced with a 120° inclination and were measured as 40 cm in length, 12 cm in width, and 35 cm in height. The edges of each arm were

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Table 1. Effect of Barbaloin on Body Weight and Blood Glucose

	body weight (g)		blood glucose (mg/dL)	
group	onset of study	end of study	onset of study	end of study
nondiabetic control	217.21 ± 2.82	203.7 ± 5.23	98.65.00 ± 2.42	102.10 ± 1.75
diabetic control	217.75 ± 3.43	180.7 ± 4.64[a]	101.20 ± 3.37	295.6 ± 6.78[a]
diabetic + barbaloin 25	219.21 ± 3.24	215.8 ± 5.58[b]	106.42 ± 3.00	270.7 ± 6.58[b]
diabetic + barbaloin 50	219.15 ± 3.36	2192 ± 5.62[b]	97.25 ± 3.57	217.6 ± 2.51[b]
diabetic + metformin 500	219.00 ± 3.00	214.8 ± 6.52[b]	102.21 ± 4.14	275.2 ± 6.90[b]

[a]$P < 0.001$ vs nondiabetic control group (one-way ANOVA followed by Tukey's *post hoc* test). [b]$P < 0.001$ vs diabetic control (one-way ANOVA followed by Tukey's *post hoc* test).

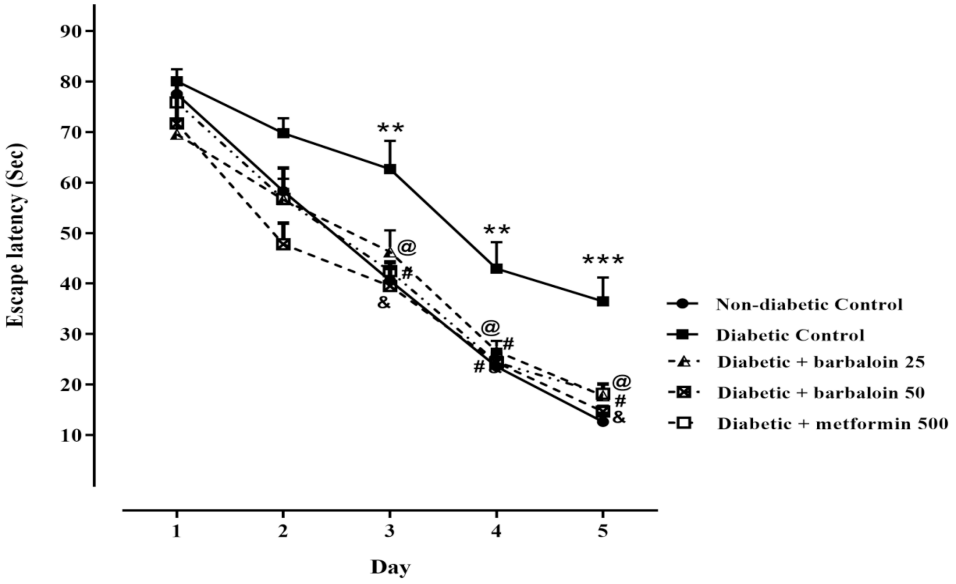

Figure 1. Effect of barbaloin on MWM tests in the acquisition trial. Mean ± SEM ($n = 6$). **$P < 0.01$ and ***$P < 0.001$ vs nondiabetic control and [@]$P < 0.05$, [#]$P < 0.01$, and [&]$P < 0.001$ vs diabetic control. Two-way ANOVA followed by Bonferroni post analytic test.

embellished with varied patterns and given the names a, b, and c. Individual rats were left to go free at the end of the maze. Over a period of 5 min, the total number of visits to each arm was noted. The apparatus was sanitized with ethanol (10%) following each exercise to reduce odors. abc, cab, or bca were examples of three consecutive entries in three different mazes that were considered to be a random rotation. This led to the calculation of the SA% and spontaneous alternation performance (SAP) score.

2.6. Open Field Test—OFT. After the probe session, for 1 h, the rats were moved to an OFT device measuring 60 × 40 × 28 cm with a floor separated into 12 squares. An observer manually counted the rearing activity (vertical) and number of crossing (horizontal) responses during the 5 min open field session. The test was run to rule out any potential motor impairment.[35]

2.7. Measurement of Body Weight and BGL. A handy glucometer (manufactured by Bayer, USA) was used to measure BGL. In a summary, blood was drawn from the rat's tail using a vein rupture technique, and the blood drop was put on the strip of a glucometer that had been loaded into the glucometer for the purpose of determining BGL. Body weight and BGL were periodically checked during the study (10, 20, 30, and 36 days following the start of the treatment).

2.8. Neurochemical Estimation. *2.8.1. Brain Tissue.* Following the behavioral test, the complete brains of sacrificed animals were removed and kept at a temperature under −50 °C.[41]

2.8.2. Forming Brain Tissue Homogenate. Physiological saline was used to thoroughly rinse the animals' brains. The consolidation of brain tissues was done using a neutral pH phosphate buffer. The samples were centrifuged, and biochemical testing was done on the supernatant.[41]

2.8.3. Acetyl-Cholinesterase (AChE) and Choline-Acetyltransferase (ChAT). An approach related to that described by Ellman was used to measure AChE level expressed as μmol AcSCh/min/mg of protein.[40,42] Commercial kits and hydroxylamine procedures were used to measure the amounts of brain ChAT activity.

2.8.4. Oxidative Stress Indicators: MDA, CAT, SOD, and GSH. The in-brain MDA level was calculated using the technique of Wills et al. The expression for the MDA concentration was nmol of MDA/mg of protein.[43] Using a previously published technique, Ellman assessed GSH.[44] SOD was assessed using the Misra and Frodvich method.[43−45] The process described by Afzal et al. was used to measure the CAT activity.[44,46]

2.8.5. Neuromodulatory Cytokines. The pro-inflammatory indicators NF-κB, IL-6, IL-1β, and TNF-α were checked using an immunoassay kit. Using calibration curves, indicator concentrations were computed and expressed as pg/mL protein.

2.9. Statistical Analysis. The values of results were analyzed using GraphPad Prism-9 (GraphPad Software Inc., USA) and represented as mean ± SEM. The data were examined for the MWM test using a two-way analysis of variance

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(ANOVA) followed by a Bonferroni *post hoc* test and one-way ANOVA followed by a Tukey's *post hoc* test.

3. RESULTS

3.1. Influence of Barbaloin on BGL and Body Weight. Thirty-seven days after STZ administration, BGLs were significantly higher in diabetic control rats in comparison to nondiabetic control rats. In addition, there was a significant decrease in the body weight of diabetic control rats in comparison to nondiabetic control rats. Barbaloin treatment (25 and 50 mg/kg) significantly lowered the BGL [F (4, 25) = 115.8, (P < 0.0001)] and enhanced the body weight of diabetic control rats [F (4, 25) = 8.038, (P < 0.0003)]. Metformin treatment (500 mg/kg) significantly (P < 0.0001) reduced BGL and increased body weight (P > 0.01) in comparison to diabetic control rats (Table 1).

3.2. Influence of Barbaloin on Performance of MWM Test. In the MWM test, cognitive performance was analyzed. Throughout the 20 learning tries in each group, the trained rats' mean rescue latency was reduced. Diabetic control rats showed significantly longer rescue latency on days 3, 4, and 5 during course trials in comparison to nondiabetic control rats (P < 0.05). Barbaloin (25 and 50 mg/kg) treatment significantly reduced rescue latency in diabetic control rats [F (4, 125) = 215.9, (P < 0.0001)]. Similar effects with metformin treatment (500 mg/kg) were seen in diabetic control rats (Figure 1).

The probe trial evaluates how effectively the animals retained and learned the platform position throughout the five-day training. Diabetic control rats spent a shorter time in the target quadrant in comparison to nondiabetic control rats, and barbaloin and metformin treatment greatly affected the same [F (4, 25) = 16.55, (P < 0.0001)] (Figure 2).

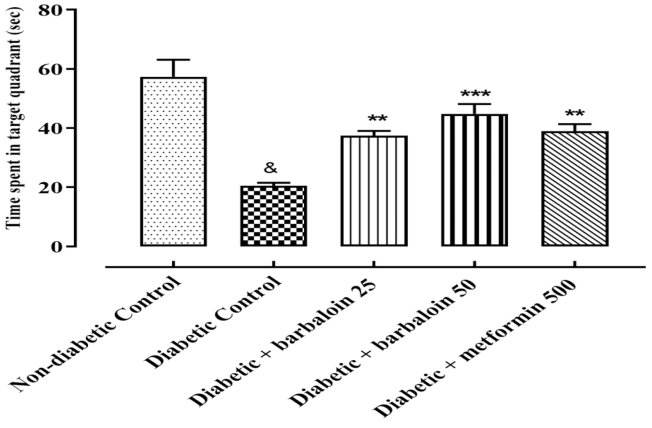

Figure 2. Effect of barbaloin on MWM tests in probe trial. Mean ± SEM (n = 6). $^{\&}P$ < 0.001 vs nondiabetic control and **P < 0.01 and ***P < 0.001 vs diabetic control. One-way ANOVA followed by Tukey's test.

3.3. Influence of Barbaloin on Y-maze Test. In the Y-maze test, diabetic control rats showed a significant reduction in SAP% in comparison to the nondiabetic control rats (P < 0.001). Barbaloin treatment (25 and 50 mg/kg) enhanced SAP%, when compared to diabetic control rats [F (4, 25) = 8.163, (P < 0.0002)], and metformin treatment also influenced the same (Figure 3).

3.4. Influence of Barbaloin on Locomotion Activity in Open Field Test—OFT. Motor impairments may be connected to barbaloin and metformin's effects on cognitive impairments in diabetic control rats. An OFT was conducted to rule out such possibilities. None of the treatments significantly affected the locomotor activity, according to a one-way-ANOVA [F (4, 25) = 1.062, (P = 0.3961)] (Figure 4).

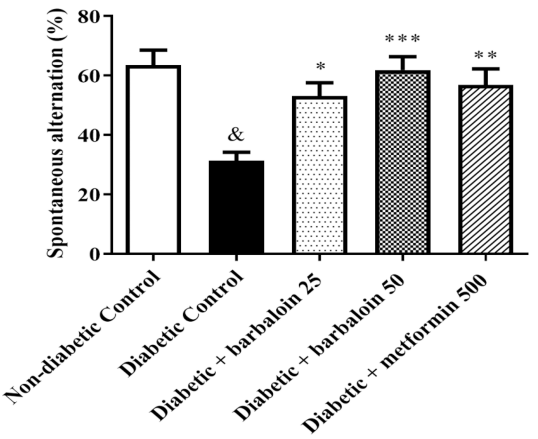

Figure 3. Effect of barbaloin on Y-maze tests. Mean ± SEM (n = 6). $^{\&}P$ < 0.001 vs nondiabetic control and *P < 0.05, **P < 0.01, and ***P < 0.001 vs diabetic control. One-way ANOVA followed by Tukey's test.

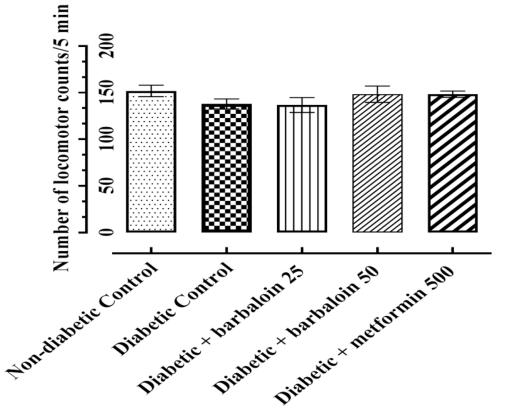

Figure 4. Effect of barbaloin in the open field test. Mean ± SEM (n = 6). One-way ANOVA followed by Tukey's test. No significant effect shown in open field test.

3.5. Influence of Barbaloin on Changes in AchE and ChAT Activity. AchE level was highly enhanced in the brain region of diabetic control rats when compared to nondiabetic control rats. Barbaloin (25 and 50 mg/kg) treatment significantly lowered the AchE activity in comparison to diabetic control rats [F (4, 25) = 14.36, (P < 0.0001). These outcomes were similar to those of metformin (Figure 5A). Whereas ChAT activity was in high decline in the brain region of diabetic control rats in comparison to the nondiabetic control rats, barbaloin (25 and 50 mg/kg) treatment significantly enhanced the ChAT activity in comparison to diabetic control rats [F (4, 25) = 12.72, (P < 0.0001)]. These outcomes were similar to those of metformin (Figure 5B).

3.6. Influence of Barbaloin on Oxidative Stress Indicators. Figure 6A showed that diabetic control rats highly enhanced MDA levels in the brain region in comparison to nondiabetic control rats (P < 0.001). However, barbaloin treatment significantly reduced MDA content in the brain region of diabetic control rats [F (4, 25) = 8.783, (P = 0.0001). Divergently, it was noticed that the CAT, SOD activity, and the levels of GSH were all drastically lower in the brain region of

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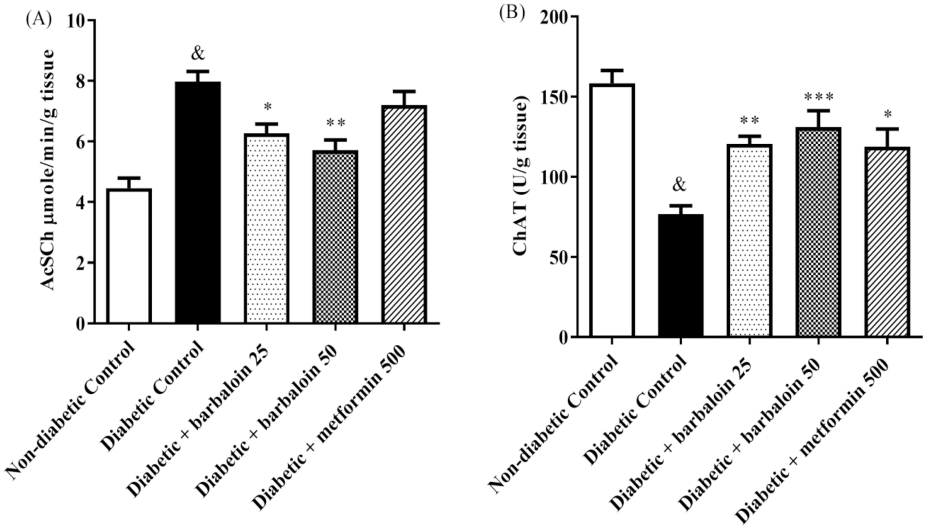

Figure 5. (A, B) Effect of barbaloin on AChE and ChAT levels. Mean ± SEM ($n = 6$). Mean ± SEM ($n = 6$). $^{\&}P < 0.001$ vs nondiabetic control and $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs diabetic control. One-way ANOVA followed by Tukey's test.

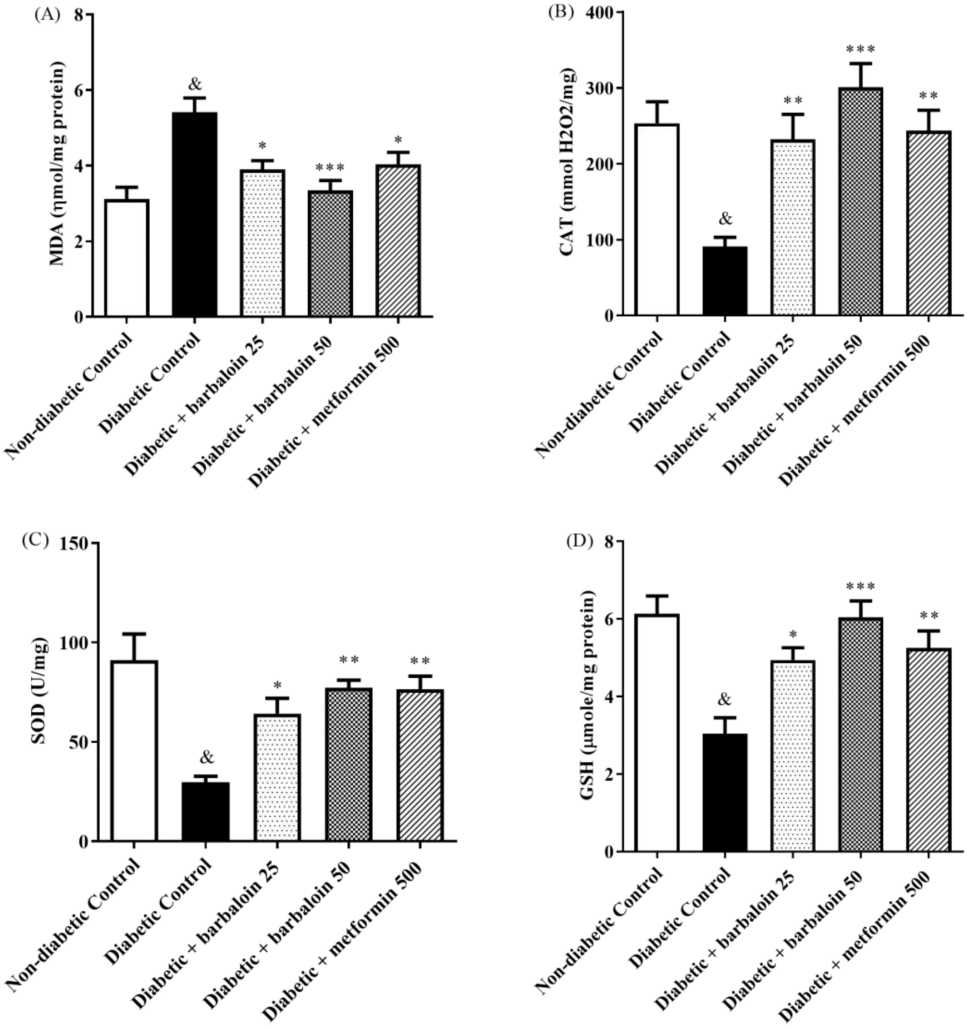

Figure 6. (A–D) Effect of barbaloin on MDA, CAT, SOD, and GSH level. Mean ± SEM ($n = 6$). Mean ± SEM ($n = 6$). $^{\&}P < 0.001$ vs nondiabetic control and $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs diabetic control. One-way ANOVA followed by Tukey's test.

diabetes control rats in comparison to nondiabetic control rats ($P < 0.01$ and $P < 0.001$, respectively). Barbaloin treatment significantly reversed these decreased levels in the brain regions of diabetic control rats' CAT [F (4, 25) = 8.325, $P = 0.0002$], SOD [F (4, 25) = 8.955, $P = 0.0001$], and GSH [F (4, 25) =

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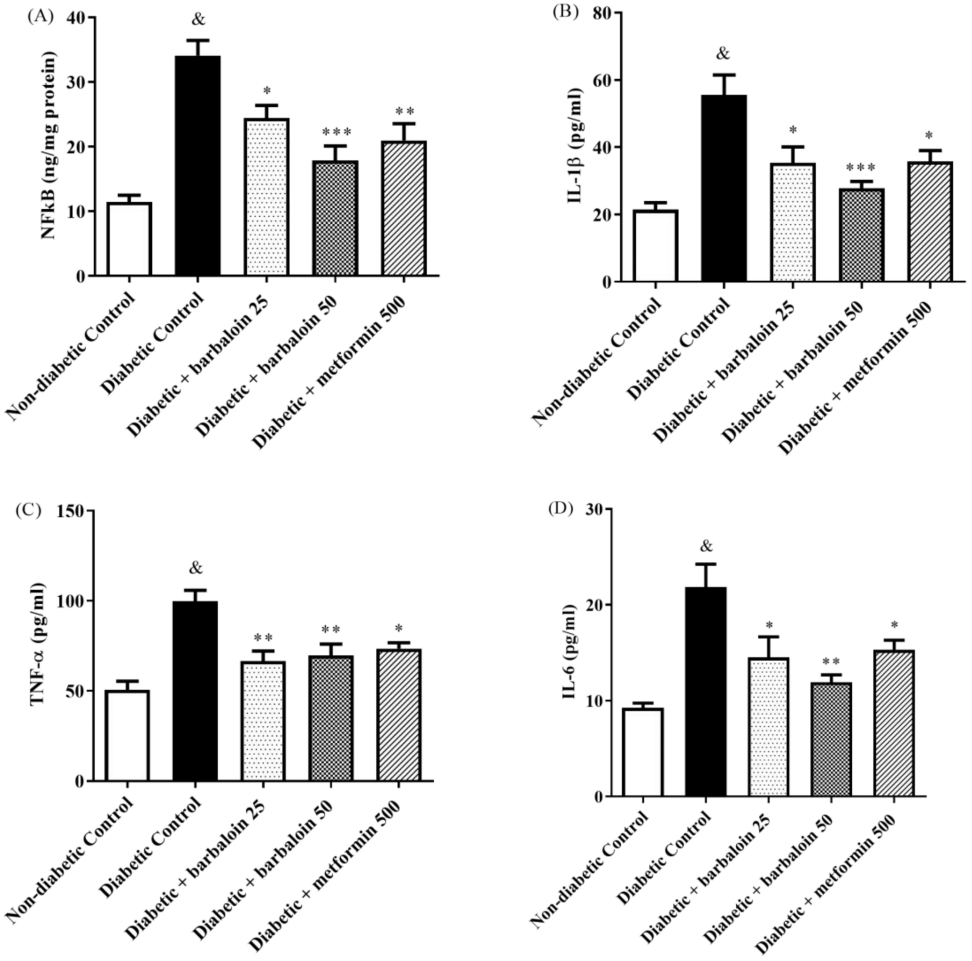

Figure 7. (A–D) Effect of fustin on cytokine NF-κB, IL-1β, TNF-α, and IL-6. Mean ± SEM ($n = 6$). Mean ± SEM ($n = 6$). $^{\&}P < 0.001$ vs nondiabetic control and $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs diabetic control. One-way ANOVA followed by Tukey's test.

9.128, $P = 0.0001$] (Figure 6B, C, and D). These results are comparable to those with metformin treatment.

3.7. Influence of Barbaloin on Neuronflammatory Cytokines in the Brain. The influence of barbaloin on the inflammation caused by diabetes was also observed in our current investigation. It is interesting to note that the actions of key inflammatory factors, such as NF-κB, IL-1β, IL-6, and TNF-α, were significantly enhanced in the brain region of diabetic control rats in comparison to nondiabetic control rats ($P < 0.001$). Barbaloin (25 and 50 mg/kg) treatment significantly decreased inflammatory responses in the brain region of diabetic control rats' NF-κB [F (4, 25) = 15.84, $P < 0.0001$)], IL-1β [F (4, 25) = 10.65, $P < 0.0001$)], IL-6 [F (4, 25) = 9.115, $P = 0.0001$)], and TNF-α [F (4, 25) = 11.13, $P < 0.0001$)] (Figure 7A–D).

4. DISCUSSION

This research investigated the influence of barbaloin on the physiological and behavioral processes in STZ-induced diabetic rats. In the brain, AChE, ChAT activity, oxidative stress, and neuroinflammatory cytokines were markedly enhanced in diabetes caused by STZ, which led to a significant deterioration in cognitive performance. The effects of barbaloin treatment on cognitive impairments, cholinergic impairment, oxidative stress indicators, and neuroinflammatory cytokines were significantly improved in STZ-induced diabetic rats. A well-known diabetes experiment model in rats is STZ-induced diabetes. Significant weight loss was induced by STZ, although it was reversed after barbitaloin therapy. Clinical and experimental data are mounting showing diabetes may be associated with cognitive impairment. In the present research, diabetes produced substantial memory and learning deficits in the Y-maze test and MWM test, which is in accord with earlier studies.[47,48] Patients with diabetes frequently have neuropathy, which has a major impact on neuroplasticity and memory retention.[48,49] The findings of this investigation demonstrated that learning, memory, and locomotor deficits caused by diabetes were improved after receiving barbaloin treatment. Barbaloin-treated rats showed significantly reduced rescue latency and enhanced percentage alternation, which may indicate increased spatial learning and memory. A lot of research suggested that performances in the passive avoidance test (PAT) and MWM test were significantly different between control and STZ-induced diabetic rats. These findings were similar to earlier research showing that DM can impair neurogenesis and cognitive performance.[48−51] Barbaloin may have multiple variables contributing to the positive benefits seen in the current study. An increasing number of studies indicate that hyperglycemia may be a factor in diabetic patients' cognitive deterioration,[9,52] suggesting that strict blood glucose management is essential for reducing diabetes-related cognitive impairments. According to a prior study, hypoglycemic drugs and insulin sensitizers lower the incidence of cognitive

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deterioration in diabetics.[9,22] In the present investigation, barbaloin treatment significantly lowered BGL. Therefore, the potential of barbaloin to lower hyperglycemia may be responsible for the recovery of cognitive performance seen in STZ-induced diabetic rats in the present investigation. This is further confirmed by the finding from the current study that the antihyperglycemic drug metformin enhanced the efficiency of STZ-induced diabetic rats in the MWM.

It was discovered that DM causes oxidative damage caused by elevated BGL.[53,54] This is in line with previously published findings showing that diabetes significantly altered hippocampus oxidative stress indicators, as shown by reduced enzymatic antioxidant properties, specifically GPx, SOD, and CAT as well as elevated levels of MDA.[24,55−57] According to reports, antioxidants protect against the cognitive damage caused by diabetes.[20,21] In the current investigation, MDA levels were dramatically elevated, although SOD, CAT, and GSH activity was noticeably lowered in STZ-induced diabetic rat brains, which is congruent with past results.[21] Barbaloin treatment restored the MDA and antioxidant enzymes SOD, CAT, and GSH levels in STZ-induced diabetic rats back to normal. Therefore, barbaloin may prevent diabetes-related memory loss in rats by lowering oxidative stress.

An essential mechanism supporting memory and cognitive performance is cholinergic neurotransmission. Learning and memory are formed by cholinergic basal forebrain neurons in the nucleus basalis magnocellularis, which activate the amygdaloid complex, hippocampus, and cortex.[58,59] Learning and memory deficiencies have been linked to cholinergic impairment in diabetic rats.[60] ChAT and AChE levels are two distinct indicators of cholinergic neurons in the brain regions under normal circumstances. They are essential in the cholinergic pathway's regulation. According to the findings of our present investigation, barbaloin administration to STZ-induced diabetic rats increased ChAT activity and lowered AChE activity. Barbaloin may thereby improve the pathology of cognitive impairments caused by diabetes through reducing AChE activity and enhancing the ChAT activity.

Pro-inflammatory cytokines are essential for the development of neuropathological conditions linked with cognition. Evidence has accumulated to show that rats with diabetes-caused cognitive decline experienced substantial discharges of inflammatory mediators.[61,62] Notably, IL-6, TNF-α, and IL-1β are significant pro-inflammatory cytokines in the development of inflammation. Additionally, it has been established that inflammation-related mediators are strongly linked to cognitive decline.[63] The p65 subunit has a favorable correlation with the NF-κB signaling pathway, and NF-κB might also be thought of as one of the essential components influencing the inflammatory response.[64] NF-κB signaling and caspase-3 activation were previously found to be modulated by IL-1β, TNF-α, and IL-6 in the brain of diabetic rats.[65] According to a recent study, diabetes-associated cognitive damage was caused by the NF-κB-signaling pathway being activated.[66] Our present studies showed that barbaloin treatment inhibited inflammation by substantially suppressing NF-κB signaling and markedly reduced the levels of the proinflammatory cytokines IL-1β and IL-6 and TNF-α in brain regions, showing its anti-inflammatory impact on a model of cognitive decline linked to diabetes. This study has limitations due to its short duration and the limited number of animals used. To better understand and confirm the mechanism of barbaloin, future studies will be required using cellular investigation with immunochemistry and Western blot.

In conclusion, the results of this study indicate that the antioxidant, antidiabetic, AChE-inhibiting and neuroinflammatory cytokine inhibitory properties of barbaloin may be responsible for its protective influence on STZ-caused cognitive decline; it may be clinically useful in treating neurological and cognitive impairment in diabetics.

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Notes

The authors declare no competing financial interest.

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