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# Berberine Ameliorate Oxidative Stress and Astrogliosis in the Hippocampus of STZ-Induced Diabetic Rats

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**Abstract** Diabetes mellitus increases the risk of central nervous system (CNS) disorders such as stroke, seizures, dementia, and cognitive impairment. Berberine, a natural isoquinoline alkaloid, is reported to exhibit beneficial effect in various neurodegenerative and neuropsychiatric disorders. Moreover, astrocytes are proving critical for normal CNS function, and alterations in their activity and impaired oxidative stress could contribute to diabetes-related cognitive dysfunction. Metabolic and oxidative insults often cause rapid changes in glial cells. Key indicators of this response are increased synthesis of glial fibrillary acidic protein (GFAP) as an astrocytic marker. Therefore, we examined the effects of berberine on glial reactivity of hippocampus in streptozotocin (STZ)-induced diabetic rats, using GFAP immunohistochemistry. Lipid peroxidation, superoxide dismutase (SOD) activity, and nitrite levels were assessed as the parameters of oxidative stress. Eight weeks after diabetes induction, we observed increased numbers of $GFAP^+$ astrocytes immunostaining associated with increased lipid peroxidation, decreased superoxide dismutase activity, and elevated nitrite levels in the hippocampus of STZ-diabetic rats. In contrast, chronic treatment with berberine (50 and 100 mg/kg p.o. once daily) lowered hyperglycemia, reduced oxidative stress, and prevented the upregulation of GFAP in the brain of diabetic rats. In conclusion, the present study demonstrated that the treatment with berberine resulted in an obvious reduction of oxidative stress and GFAP-immunoreactive astrocytes in the hippocampus of STZ-induced diabetic rats.

**Keywords** Berberine · Diabetes · Oxidative stress · Gliosis · GFAP · Glial fibrillary acidic protein

## Introduction

Diabetes mellitus (DM) is strongly associated with degenerative and functional disorders of the central nervous system [1]. Increasing evidence shows that oxidative stress is the final common pathway through which risk factors of several diseases, including diabetes, exert their deleterious effects [2]. Reactive oxygen species (ROS) and other chemical entities can result in the development of oxidative stress in the body and consequently lead to neuronal death, which contributes to the neuropathology associated with diabetes [3]. Furthermore, hyperglycemia reduces the levels of superoxide dismutase (SOD), a key antioxidative enzyme. It also increases lipid peroxidation and free radicals such as nitric oxide (NO) [4].

Astrocytes play critical roles in a number of central nervous system (CNS) activities including synaptic activity and synaptogenesis, production of growth factors, and regulation of the cerebral microcirculation protection against toxic episodes such as excitotoxicity and oxidative stress [5, 6]. Moreover, astrocytes preserve neuronal survival through inactivation of ROS [7–9].

In response to any kind of injury to CNS, astrocytes change their appearance and undergo a characteristic hypertrophy of their cellular processes. This phenomenon is known as reactive gliosis or astrogliosis [10]. A key indicator of glial reactivity is the increased synthesis of glial fibrillary acidic protein (GFAP), an intermediate filament cytoskeletal protein [11].

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Increases in GFAP are commonly used to examine the distribution of glial cells in response to neural injury [7, 12].

Berberine is an isoquinoline alkaloid reported to exhibit anxiolytic, analgesic, anti-inflammatory, antipsychotic, antidepressant, and antiamnesic effects [13–15]. A number of clinical and preclinical investigations have shown beneficial effects of berberine on diabetes [16–19] which are mainly attributed to enhanced insulin expression, B cell regeneration and potential as an antioxidant [20, 21]. Moreover, berberine is also reported to inhibit acetylcholinesterase enzyme activity and play an important role in metabolic syndrome [22]. In addition, Peng et al. showed that the antiamnesic effect of berberine is related to an increase in peripheral and central cholinergic neuronal system activity [23]. Recently, berberine has been reported to have beneficial effects on neural health. It can also protect neurons from various brain insults [14, 24, 25]. This study set out to determine whether chronic oral administration of berberine could improve oxidative stress and astrogliosis in the hippocampus of the streptozotocin-induced diabetic rats.

## Materials and Methods

### Animals

Male albino Wistar rats (Pasteur's Institute, Tehran, Iran) weighing 225–285 g were housed in an air-conditioned colony room on a light/dark cycle (21–23 °C and a humidity of 30–40 %) and supplied with standard pelleted diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the NIH Guidelines for the Care and Use of Laboratory Animals.

### Experimental Procedure

The rats ($n=60$) were randomly selected and allocated to five groups: control, control berberine-treated (100 mg/kg), diabetic, and diabetic berberine-treated (50 and 100 mg/kg) groups. Berberine hydrochloride and streptozotocin (Sigma-Aldrich Co., St. Louis, MO, USA) were used in the present study. All the drugs were dissolved in double-distilled water except streptozotocin (STZ), which was dissolved in citrate buffer (pH 4.4). Drug solutions were prepared fresh, and their doses were expressed in terms of their free bases. Diabetes was induced in rats using an earlier reported method [26]. In brief, STZ was dissolved in 0.1 M sodium citrate buffer, pH 4.4, and administered at the dose of 55 mg/kg through i.p. route. Streptozotocin-treated rats received 5 % of glucose solution instead of water for 24 h after injection of STZ in order to reduce the death due to hypoglycemic shock. Blood samples were taken from the tail vein 72 h after STZ injection to measure blood glucose levels. Control animals received an injection of an equivalent volume of normal saline. One week after STZ injection, overnight fasting blood samples were collected and serum glucose concentrations were measured using the glucose oxidation method (Zistshimi, Tehran). Only those animals with a fasting serum glucose level higher than 250 mg/dl were selected as diabetic for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. Berberine chloride was administered p.o. at doses of 50 and 100 mg/kg/day 1 week after STZ injection for a period of 8 weeks. Biochemical and immunohistochemical assessment was performed at the end of the study as described below.

### Hippocampal Malondialdehyde Measurements

The rats were anesthetized with ketamine (100 mg/kg); decapitated brains were removed, and the anterior third left of the left midbrain block was blotted dry, weighed, made into a 10 % tissue homogenate in ice-cold 0.9 % saline solution, and centrifuged (1,000×$g$, 4 °C, 10 min) to remove particulates. The obtained supernatant was aliquoted and stored at −80 °C until assayed. Malondialdehyde (MDA), a marker of lipid peroxidation, was measured by a commercial colorimetric assay kit (BioVision, Milpitas, CA, USA) following the manufacturer's instructions. MDA levels in hippocampal sample homogenates were expressed as nanomoles of MDA per milligram of protein.

### Measurement of Hippocampal SOD Activity

The supernatant of hippocampal homogenate was obtained as described earlier. SOD activity measurement was according to the previous works. Briefly, the supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min, and nitroblue-tetrazolium (NBT) was added. Blue formazan was then monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50 % maximum was defined as 1 nitrite unit (NU) of SOD activity.

### Assay of Hippocampal NO Concentration

Supernatant NO content was assayed by the Griess method. NO is a compound with a short half-life, and it is rapidly converted to the stable end products of nitrate ($NO_3^-$) and nitrite ($NO_2^-$); consequently, the principle of the assay is the conversion of nitrate into nitrite by cadmium followed by color development with Griess reagent (sulfanylamide and *N*-naphthyl ethylenediamine) in acidic medium. The total nitrite was measured by Griess reaction. The absorbance was determined at 540 nm with a spectrophotometer.

### Protein Assay

The protein content of the supernatant was measured with the Bradford method using bovine serum albumin (Sigma Chemical, St. Louis, MO, USA) as the standard [5].

### Immunohistochemistry

Five rats in each group were anesthetized with sodium pentobarbital (40 mg/kg i.p.), and they were perfused with ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4). Unfixed tissues of the hippocampus were snap frozen in liquid nitrogen.

Cryostat sections were cut with a thickness of 5 μm at −20 °C. Sections were incubated in 1 % $H_2O_2$ for 20 min to inhibit endogenous peroxidase activity and then incubated in a blocking solution of 3 % dry milk at room temperature followed by overnight incubation at 4 °C with primary monoclonal antibody against GFAP. Primary antibody was diluted (1/400) in PBS containing nonspecific goat serum in the presence of 0.2 % Triton X-100. After rinsing in PBS, the sections were incubated for 1 h at room temperature with a 1/300 diluted secondary antibody (goat anti-rabbit antibody). Antibody–antigen complex was revealed by 0.1 % 3,3′-diaminobenzidine in the presence of 0.03 % $H_2O_2$. After final rinse with PBS, the sections were mounted onto cresyl gel (0.1 %*w*/*v* gelatin in 8 % ethyl alcohol) and coverslipped with Eukit balsam.

### Statistical Analysis

All data were expressed as mean±S.E.M. For the histological and biochemical assessments, one-way ANOVA test followed by Tukey's post hoc test were applied. In all analyses, the null hypothesis was rejected at 0.05 level.

## Results

At the end of the study, the weight of the diabetic control rats significantly decreased as compared to that of control rats. Moreover, chronic treatment with berberine (50 and 100 mg/kg) significantly increased body weights in diabetic rats (Table 1).

In addition, diabetic rats had also an elevated serum glucose level over those of control rats ($p<0.001$), and treatment of diabetic rats with berberine at both doses of 50 and 100 mg/kg for 8 weeks caused a significant decrease in the serum glucose ($P<0.01$) as compared to diabetic control group. Moreover, control berberine-treated (100 mg/kg) rats had also a reduction in the weight and serum glucose level over those of control (Table 1).

STZ-induced diabetes led to a marked elevation in the levels of MDA in the hippocampus region compared to those of the control group (Fig. 4). Administration of berberine (50 and 100 mg/kg) significantly reduced the levels of MDA in hippocampal homogenates as compared to diabetic control rats (Fig. 1).

Similarly, nitrite levels in the homogenates of hippocampus significantly increased in diabetic rats, and chronic treatment with berberine also significantly decreased nitrite levels in berberine-treated group (50 and 100 mg/kg) as compared to diabetic group (Fig. 2).

Effects of chronic administration of berberine on SOD levels are depicted in Fig. 3. There was a significant fall in SOD levels in hippocampal homogenates of diabetic rats as compared to control ones. Berberine treatment (50 and 100 mg/kg) significantly increased SOD levels as compared to diabetic rats ($P<0.05$) (Fig. 3).

Immunohistochemical studies used GFAP monoclonal antibody to examine glial reactivity in the hippocampus of STZ-induced diabetic rats with and without berberine treatment. Increased GFAP immunostaining was detected in the hippocampus of STZ-diabetic rats as compared to control. Hypertrophic astrocytes were apparent in the hippocampus of the diabetic animals, and berberine treatment (50 and 100 mg/kg/day) reduced the number of $GFAP^+$-immunoreactive astrocytes (Fig. 4).

## Discussion

In this study, astrocyte in the hippocampus became reactive in diabetic rats 8 weeks after STZ injection. Increased activity of glia is a common feature of brain injury [9, 11], and it is commonly seen after a variety of insults, including oxidative stress [27, 28]. Thus, we evaluated the effects of STZ-induced oxidative stress on glial reactivity of rats and examined the potential protective effects of berberine against oxidative stress and glial reactivity.

Due to the hyperglycemia associated with diabetes, enhanced formation of reactive oxygen and nitrogen species

**Table 1** Effect of chronic treatment with berberine on body weights and blood glucose levels (mean ± S.E.M.) in different groups of rats at the onset and at the end of the experiments

| Treatment | Body weight (g) | | Blood glucose (mg/dl) | |
|---|---|---|---|---|
| | Onset of study | End of study | Onset of study | End of study |
| Control | 242.3±23.6 | 334.4±32.7 | 102.4±11.6 | 97.5±7.3 |
| Control+Br (100 mg/kg) | 233±15.9 | 265.4±22.2 | 84.75±5.9 | 73.5±3.9 |
| Diabetes | 232±17.6 | 152±19# | 474.3±35.8 | 567.3±43.8# |
| Diabetes+Br (50 mg/kg) | 246.4±16.7 | 197.62±21* | 475.9±47.9 | 430.8±43.3* |
| Diabetes+Br (100 mg/kg) | 250±22.8 | 217.1±27* | 470.4±42.2 | 395±38.9* |

*Br* berberine

#$P<0.001$ vs. control group; *$P<0.05$ vs. diabetic group

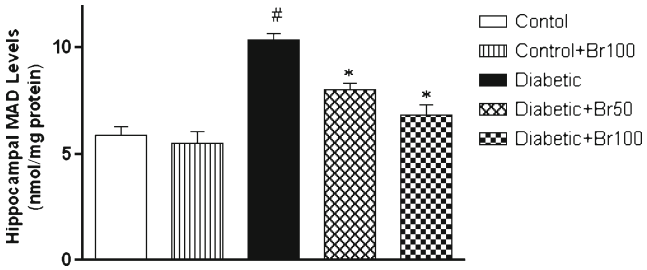


**Fig. 1** Malondialdehyde (MDA) levels in hippocampal homogenates from control, diabetic, and diabetic berberine-treated groups (50 and 100 mg/kg p.o.) of rats. #$P<0.01$ vs. control and *$P<0.05$ vs. STZ-diabetic rats

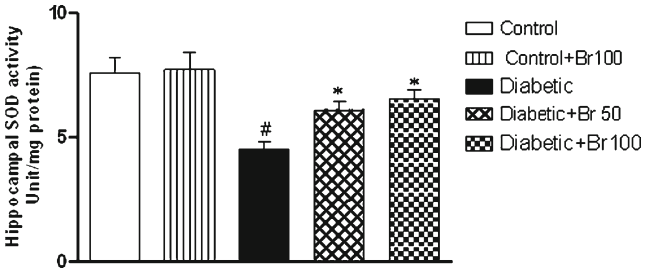


**Fig. 3** SOD activity in hippocampal homogenates from control, diabetic, and diabetic berberine-treated groups (50 and 100 mg/kg p.o.) of rats. #$P<0.01$ vs. control and *$P<0.05$ vs. STZ-diabetic rats

occurs; this leads to increased neuronal death by oxidizing proteins, damaged DNA, and augmented levels of lipid peroxidation products in cellular membranes [6, 28]. Thus, we hypothesized that glial reactivity in diabetic rats would reduce if they were treated with the antioxidant berberine.

In the present study, administration of berberine to diabetic treated rats (50 and 100 mg/kg) lowered GFAP immunoreactivity, as the most common manifestation of glial hyperactivity. There was also a significant correlation between GFAP reactivity and nitrite and peroxidized lipid levels. Furthermore, chronic treatment with berberine (50–100 mg/kg) reduced blood glucose levels, and it increased body weights in diabetic rats which is well in accordance to earlier studies [16, 19].

In diabetic rats MDA and nitrite levels significantly increased in the hippocampus region of the brain. These results confirm previous reports that STZ-induced diabetes is accompanied by an increased generation of reactive species [29–31]. One reason for the elevated lipid peroxidation and nitrite in diabetic rats is the reduction in the levels of SOD, a potent endogenous antioxidant. In accordance with previous publications [32], here in, we found that untreated diabetes caused generally lower levels of SOD in the hippocampus region [16, 33]. Administration of berberine to diabetic berberine-treated rats significantly reduced the levels of nitrite and lipid peroxidation products and increased SOD activity. Even though the exact mechanisms of neuroprotective effects of berberine in the hippocampus of STZ-diabetic animals is not clear yet, several hypotheses have been suggested [24, 34].

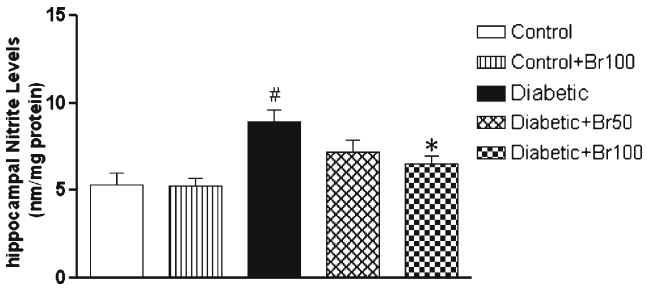


**Fig. 2** Nitrite levels in hippocampal homogenates from control, diabetic, and diabetic berberine-treated groups (50 and 100 mg/kg p.o.) of rats. #$P<0.01$ vs. control and *$P<0.05$ vs. STZ-diabetic rats

Berberine can easily cross the blood–brain barrier, transport into the neurons, having a slow elimination rate, which suggests that it has a direct action on neurons, and accumulate in the hippocampus [35]. Other mechanisms that have been suggested in the neuroprotective effect of berberine include the inhibitory effects on norepinephrine, $H_2O_2$-induced [$Ca^{2+}$] elevation and neurotransmitters induced [$Ca^{2+}$] elevation [36, 37]. Moreover, berberine functions as a radical scavenger and antioxidant agent in cells [38]. In addition to neutralizing a variety of oxidizing species, berberine, as noted above, protects cells from oxidative damage by stimulating GSH synthesis and by promoting the activity of several antioxidative enzymes, including GSH-Px [39–41]. Even though we showed that berberine treatment significantly decreased diabetes-induced neuronal apoptosis and improved synaptic dysfunction and memory impairment as compared to diabetic control rats [26, 41], there was no evidence indicating the protection effect of berberine on chronic oxidative stress and astrocyte reactivity. Several mechanisms may account for the astrocyte reaction in streptozotocin-induced diabetes, including increases in the polyol pathway, protein glycation, disturbed calcium homeostasis, and oxidative stress [11, 42]. Moreover, it is obvious that glial cells express a variety of neurotrophic factors and cytokines that protect neurons from reactive oxygen species-induced neurotoxicity [43, 44]. Astrocytes are also known to have more antioxidant capacity than do neurons [45, 46]. Astrocytes contain high levels of GSH and GSH-Px activity [46]. Maintenance of glial GSH levels and high activity of GSH-Px is essential in the protection of the CNS from oxidative insults. Thus, they protect neurons against oxidative stress and promote neuronal survival [9]. Moreover, there is some evidence that berberine can modulate NO synthesis, and it also has cyclooxygenase (COX)-2 inhibiting property [47]. Thereby, it may have anti-inflammatory and neuroprotective properties due to balance in the NO system [24, 47–49]. In addition, berberine suppresses neuroinflammatory responses through AMP-activated protein kinase activation in BV-2 microglia and astrocyte [50]. Activated AMPK deactivates gluconeogenic enzymes and increase glucose uptake by stimulating GLUT4 and GLUT1 [50, 51]. GLUT1 present at a high concentration at the

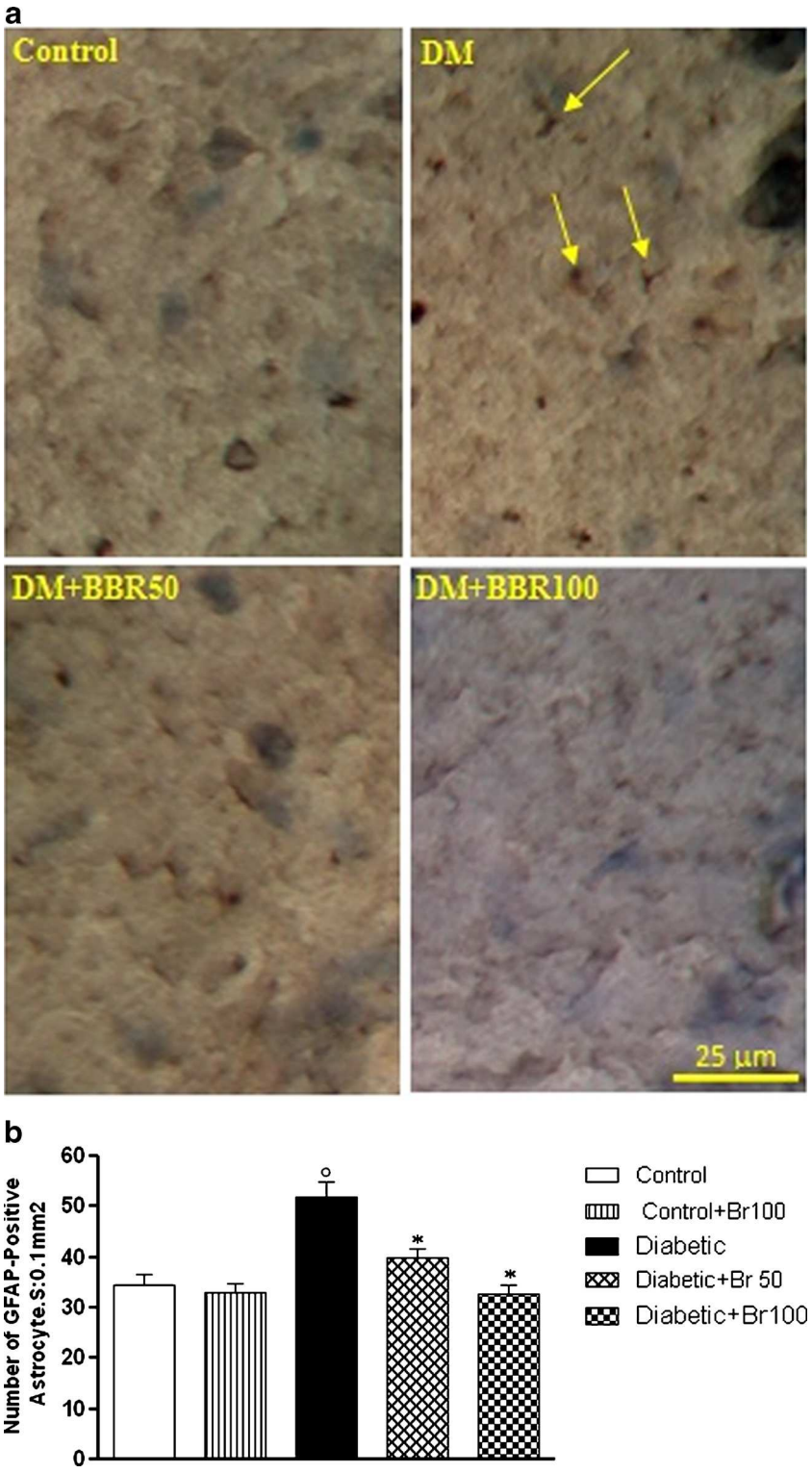


**Fig. 4** **a** Photomicrographs stained immunohistochemically for GFAP$^+$ astroglial cells in the hippocampus of control and diabetic, diabetic berberine-treated (50 and 100 mg/kg) rats 8 weeks after STZ injection. Scale bar=25 μm (for all figures). **b** The number of all GFAP$^+$ astroglial cells per 0.1 mm$^2$ was estimated. Following induction of DM in long term, reactive astrocytes are often identified by increased immunoreactivity for GFAP. In the normal brain, only a few astrocytes expresses GFAP, and the highest numbers of activated astrocytes were only seen in the diabetic rats. Values in the figure were the means±SEM. °$P$<0.001 vs. control, *$P$<0.05, and $P$<0.01 vs. STZ-diabetic rats

blood–brain barrier as well as in parenchymal cells, most likely in astrocytes, could be relevant to the antidiabetic effects of berberine in the hippocampus [52].

The current study showed that glial cells respond to the diabetes by overexpression of GFAP a few weeks after the onset of diabetes induced with streptozotocin. Moreover, administration of berberine showed beneficial effects via decreasing lipid peroxidation and preventing reactive gliosis. The present findings demonstrate that, in addition to its direct protective effects on neurons, berberine also has beneficial effects on glial cell against chemical and/or metabolic insults to the brain.

## Conclusion

The authors suggest that the effect of berberine in restoring astrogliosis and ameliorating oxidative stress was probably one of the potential mechanisms by which berberine plays neuroprotective activity in the hippocampus of STZ-induced diabetic rats. Further studies are warranted to investigate involved mechanisms in detail.

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