Research Article

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Hydroxyquinoline sulfanilamide ameliorates STZ-induced hyperglycemia-mediated amyleoid beta burden and memory impairment in adult mice

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Abstract: The aim of the current study was to evaluate therapeutic potentials of Hydroxyquinoline Sulfanilamide (HSM) for Alzheimer's disease in a mice model of Streptozotocin (STZ)-induced diabetes. The antioxidant analysis of mice brain homogenates was carried out by catalase (CAT) assay, reduced glutathione (GSH) assay, and lipid peroxidation (LPO) assay. Biochemical analysis of blood plasma was conducted by peroxidase (POD) assay and superoxide dismutase (SOD) assay. The expression of mice brain proteins was evaluated using Western Blotting analysis. The administration of HSM increased the activity of antioxidant enzymes including CAT, SOD, GSH, and POD and lowered LPO actions. These findings indicate that STZ may lead to diabetes in mice, followed by hypercholesterolemia, expressed as triglycerides in mice blood. However, HSM significantly decreased STZ-induced hyperglycemia and hypercholesterolemia in adult albino mice. The data confirmed that HSM lowered STZ-induced oxidative stress, inhibited phosphorylated JNK, NF-kB, and upregulated Nrf-2 to improve and restore the synapse and memory defects in adult albino mice. Conclusively, the current findings suggested that HSM is a safe, novel, and potential drug candidate against metabolic dysfunction and induced cognitive impairment.

Keywords: diabetes mellitus, streptozotocin, neuroinflammation, Morris water maze, hydroxyquinoline sulfanilamide

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1 Introduction

Diabetes mellitus (DM) is a major chronic condition caused by abnormal blood glucose balance negatively affecting health. Diabetes patients encounter several complications that considerably raise morbidity and mortality [1]. Alzheimer's disease (AD) induces a neurological disorder named dementia. It is thought that the progression of neurodegeneration in the hippocampus, including activation of microglia, neuronal inflammation, neuronal degeneration, loss of metabolic energy, and oxidative stress, contributes to the development of AD [2]. There is significant evidence that DM adversely affects the central nervous system (CNS) [3]. Neurological disorders like dementia, sadness, anxiety, and AD are common in diabetic animals [4]. The primary recognized mechanisms underlying the brain-damaging effects of

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DM include oxidative stress, inflammation, and altered signaling pathways in the cells which are the major hallmarks associated with DM [4]. Streptozotocin (STZ), a diabetes-inducing chemical, causes the accumulation of amyloid-β (Aβ) in the brain of diabetic animals. In the case of the AD animal model, lipid deposition and inflammation of the cerebrovascular system were enhanced by hyperglycemia, which also caused memory loss. Similar mechanisms, such as protease-resistant misfolding, elevated oxidative stress, persistent neuroinflammation, as well as the development of protein aggregation, underline most of neurodegenerative disorders [5,6].

The prevalence of diabetes is rising to the level that 592 million adults have been estimated to be affected by 2035 [7]. The increased production of free radicals, e.g., hydroxyl (OH) radicals and superoxide (O^{2-}), as well as the resulting decrease in the activity of antioxidant defense systems, are correlated with oxidative stress and DM [8]. Hyperglycemia produces reactive oxygen species (ROS) through various processes, such as polyol pathways, non-enzymatic protein glycation, and glucose autoxidation [9]. Free radicals can degrade vital biomolecules like DNA, carbohydrates, and proteins [10]. Oxidative damage to different parts of the brain follows memory loss, long-term difficulties, and morphological defects [11]. Recent research indicates that oxidative damage caused by DM may adversely affect CNS, causing cognitive impairment and issues with the peripheral nervous system [7]. The hippocampus is considered a particular target in the CNS for alterations induced by diabetes [12]. As part of the brain's limbic system, the hippocampus is regarded as a center for integrating cognitive processes such as memory and learning [13]. Diabetes has been linked to memory and cognitive impairments, suggesting that the disease may damage the hippocampus [14]. In the experimental mice model induced with STZ, the hippocampus has exhibited increased astrocyte proliferation and neuronal synaptic rearrangement due to insufficient insulin supply and/or poor glucose management [15]. Hippocampal neuronal cell death caused by hyperglycemia may be attributed to apoptosis. Apoptotic morphological features include cell shrinkage and blebbing of the nuclear membrane [16]. Antioxidants' neuroprotective capabilities have been associated with the therapy of experimental neurodegenerative animal models [7].

Sulfanilamides are pharmacological compounds widely used to treat specific infections caused by certain fungi, gram-positive and negative bacteria, and some protozoa. Although sulfanilamide medications were initially introduced as antibiotics, some are also effective against epilepsy. Sulfanilamides are now being employed as anticonvulsants in treating epilepsy and other neurological disorders [17].

8-Hydroxyquinoline (8HQ) is a quinoline derivative, naturally obtained from plants and synthetically available, with good metal recognition and strong coordinating property. Metal deficiency and overload result from inappropriate metal absorption or metal metabolism that originate from the loss of homeostasis. 8HQ is one of the most exciting hydroxyquinoline derivatives yet to be investigated due to its multiple bioactivities and medicinal potentials. Most of the bioactivities of 8HQ and its derivatives result from its chelating properties. 8HQ is an effective chelator that could help restore metal balance and be effective in treating metal-associated disorders. Moreover, for many neurodegenerative disorders, the production of ROS in the body is the primary contributing factor. An 8HQ derivative, i.e., hydroxyquinoline sulfanilamide (HSM), exhibits a scavenging property to diminish ROS [18]. For this reason, the current work aims to evaluate the antioxidative and antidiabetic characteristics of a newly synthesized 8HQ-based molecule, i.e., HSM, in STZ-treated diabetic mice.

2 Materials and methods

2.1 Chemicals

STZ, phosphate buffer saline (PBS) tablets (Cat. No; P4417-50TAB), sulfosalicylic acid, Ellman's reagent (DTNB), ascorbic acid, ferric chloride, trichloroacetic acid, thiobarbituric acid, dextrose, guaiacol, sodium pyrophosphate buffer, phenazine methosulfate, NADH, Tri base, glacial acetic acid, ammonium per sulfate (APS), H_2O_2 bisacrylamide, sodium chloride, sodium dodecyl sulfate (SDS), potassium chloride, and acrylamide were supplied by Daejung Chemicals & Metals Co. Ltd (Gyeonggi-do, Shiheung, South Korea and Sigma Chemicals Co. St. Louis, MO, USA).

2.2 Mice and their grouping

The current work relied exclusively on male albino mice as experimental animal model, so as to avoid the physiological variability linked with the estrous cycle of female mice [19]. For this purpose, eight-week-old adult male albino mice were used, supplied by the Veterinary Research Institute, Peshawar. The animals ($n = 16$) were divided into four groups and kept in the laboratory in suitable cages

(Biobase China). Prior to experiments, mice were given enough time to adjust to their new environment.

The groups were as follows:

1. Normal (n = 4) mice (untreated control group)
2. STZ-injected (n = 4) mice (90 mg/kg)
3. STZ-injected (n = 4) mice (90 mg/kg) + HSM-treated mice (10 mg/kg)
4. HSM-injected (n = 4) mice (10 mg/kg)

The male mice with 30–32 g body weights were housed in a room where suitable food and water were available. A temperature of 25°C was maintained in the chamber with a 12/12-h light/dark cycle. The animals were treated carefully per the committee's instructions for animal ethics in the laboratory.

2.3 Antioxidant analysis of brain homogenates

For catalase assay, an earlier developed method was employed [20] with little modifications. The 3 mL reaction mixture comprised of 400 µL of 5.9 mM H_2O_2, 2,500 µL of 50 mM phosphate buffer (at pH 5.0), and 100 mL of brain supernatant was analyzed for the change in the absorbance at 240 nm at one-minute intervals. A 0.01 unit/minute change in absorbance was regarded as one unit of activity.

The glutathione assay involved the precipitation of proteins contained in 1,000 µL of brain homogenate by adding 4% sulfosalicylic acid solution in an equal volume as per published research [21]. After 1 hour of 4°C incubation, the reaction mixture was centrifuged for 20 min at 1,200×g. The reaction mixture was comprised of 200 µL of 100 mM DTNB, 100 µL of centrifuged aliquot, and 2,700 µL of 0.1 M phosphate buffer at pH 7.4. The change in the absorbance of the reaction mixture was immediately measured at 412 nm. Reduced glutathione results were expressed as µM/g tissue.

A reported method was employed [22] with little modification to perform a lipid peroxidation assay. This experiment was conducted using a 1,000 µL of the reaction mixture that contained 20 µL (100 mM) ferric chloride, 200 µL (100 mM) ascorbic acid, 580 µL (0.1 M, pH 7.4) phosphate buffer, and 200 µL brain homogenate supernatant. After 1 h of incubation at 37°C, the reaction stopped by adding 1,000 µL trichloroacetic acid solution (10%) as a stopping reagent. Then, a 1,000 µL thiobarbituric acid solution was added to the tubes, heated to 95 °C for 20 min in a water bath, and immediately shifted to a crushed ice bath. Subsequent centrifugation for about 10 min (at 25×g) produced lipid peroxidation, calculated from absorbance measured at 535 nm on a spectrophotometer. Results were indicated as nM TBARS/min/mg of tissue at a temperature of 37°C (the TBARS molar extinction coefficient is $1.56 \times 105\,M^{-1}\,cm^{-1}$).

For glucose tolerance test (GTT), mice were kept fasting for 6–8 h, and their blood glucose level was measured at zero. Following the injection of 200 mg/kg dextrose into each of the four groups, blood glucose level was measured using a glucometer at intervals of 15, 30, 60, 120, and 180 min, respectively.

2.4 Biochemical analysis of plasma

After finishing the drug administration, the animals were butchered, followed by their blood collection for biochemical analysis, e.g., the measurement of total cholesterol, TGL, VLDL, LDL, and HDL.

The POD activity was measured using the previously described method [20], with little modifications. The mixture used in the current experiment consists of 100 µL of 20 mM guaiacol, 300 µL of 40 mM H_2O_2, 1,000 µL brain homogenate supernatant, and 2,500 µL of 50 mM phosphate buffer (pH, 5.0). The absorbance of the mixture was noted at 470 nm at minute intervals. A change of 0.01 unit/minute in absorbance was taken as one unit of POD activity.

An earlier developed method was employed [23] with slight modification to perform a superoxide dismutase (SOD) assay. For this purpose, the reaction mixture comprised 300 µL brain homogenate supernatant, 1,200 µL of 0.052 mM sodium pyrophosphate buffer (pH, 7.0), and 100 µL (186 M) phenazine methosulfate. The enzymatic process was initiated after adding 200 µL of 780 mM NADH to the reacting mixture. The reaction stopped after adding 1,000 µL of glacial acetic acid at an interval of 1 minute. The absorbance of the mixture was noted at 560 nm, and the amount of chromogen produced was quantified in units per mg of protein.

2.5 Behavioral tests

Behavioral tests were conducted to check the positive effects of HSM on STZ-induced memory impairment. Adult albino mice were administered STZ intraperitonealy (i.p.) with or without HSM. Mice species were divided into four

groups randomly, and the observer performing the behavioral test was fully unaware of the various mice groups throughout this practice.

The Morris water maze (MWM) test was conducted to check the hippocampus-based long-term spatial learning capabilities. The device for MWM testing is described in detail in the recent study [24]. For the first 3 days, the mice were trained twice daily. The escape latency of the animals to search for the hidden disc was observed. If the mice could not locate the platform, they were manually guided and made to stay there for 10 s. This procedure was followed for 5 days; each day, the various experimental groups had a specific set of data (seconds). After receiving two days of rest, the mice were subjected to a probe test to search for the hidden disc, and the duration of time they stayed in the target area was recorded.

The procedure Y-Maze test was carried out as per the already reported method [24]. The Y-Maze is a device with three arms that measure $50 \times 10 \times 20\,\text{cm}^3 (L \times W \times H)$ and are oriented at 120°. Each time, the animals were given 10 min to acclimatize to their new environment. After that, the animals were kept at the center of the maze and given 8 minutes to explore the maze. The overall arm entries of the mice and the frequency of the successive triplets were monitored using the software. The formula used to calculate the alternations percentage is as follows:

[Spontaneous alternation/Total arm entries − 2] × 100.

2.6 Western blotting analysis

Following the duration of treatment, the animals were sacrificed to conduct the western blot analysis, as previously reported [24]. The mice brains were immediately recovered, and the hippocampus was carefully separated and immersed in a 1:1 RNA-to-PBS solution on ice. Afterward, the hippocampus component was blended with a T-PER (Thermo Scientific) reagent to extract the protein from the tissue. To determine the protein concentration, Bio-Rad protein assay tests were performed to measure absorbance at 595 nm. The protein of each sample was standardized to 30 µg/group, and an electrophoresis gel of 12 to 15% SDS PAGE was used. It was then followed by transferring protein samples onto the polyvinylidene difluoride membrane (Santa Cruz Biotechnology, Santa Cruz, CA, USA) using a semi-dry transblot (Bio-Rad). Various primary antibodies, i.e., mouse-derived (anti-Aβ, anti-actin, anti-BACE1, anti-SYP, anti-PSDF95, anti-NF-kB, anti-NRF-2, anti-TNF-α, anti-p-JNK, and anti-HO-1) monoclonal antibodies (Santa Cruz, CA, USA) were used. Finally, HRP-conjugated anti-mouse secondary antibody (Santa Cruz, CA, USA) was used, and the X-ray-based results were developed.

2.7 Statistical analysis

The results obtained as X-rays film were scanned, organized, and analyzed statistically using specific computer software, i.e., Adobe Photoshop, Prism 5 graph pad, and Image J. Protein densities indicated by the mean S.E.M. in arbitrary units (A.U.s). The symbol # means significantly different from normal and * is significantly different from STZ-administered mice, respectively.*,**,*** & $^{\#,\ \#\#,\ \#\#\#}$ $p < 0.05$, 0.01, and 0.001.

3 Results

3.1 HSM-suppressed STZ-induced hyperglycemia and hypercholesterolemia in adult albino mice

A single dose of STZ caused hyperglycemia and hypercholesterolemia in mice. The blood of all experimental animals was tested. The results demonstrated that STZ considerably raised the random blood glucose level of these animals, thereby suppressing their capability to tolerate the oral glucose (Figure 1) that they were supplied during the GTT. These findings indicate that STZ may lead to diabetes in mice, followed by hypercholesterolemia, which is expressed as triglycerides, LDL, VLDL, and relatively low levels of HDL in mice blood. However, HSM significantly decreased random and GTT elevated blood sugar levels and STZ-induced hyperglycemia and hypercholesterolemia in adult albino mice (Table 1 and Figure 1).

3.2 HSM lowered STZ-induced oxidative stress-mediated AD in adult albino mice brain

We injected STZ intraperitonealy once into adult albino mice since prior research has suggested that STZ causes oxidative stress-mediated AD in the brain [25]. Adult albino mice were administered the novel HSM intraperitonealy at a dose of 10 mg/kg a week later. To determine the antioxidant potential of HSM against STZ-induced oxidative stress, several antioxidant assays, including

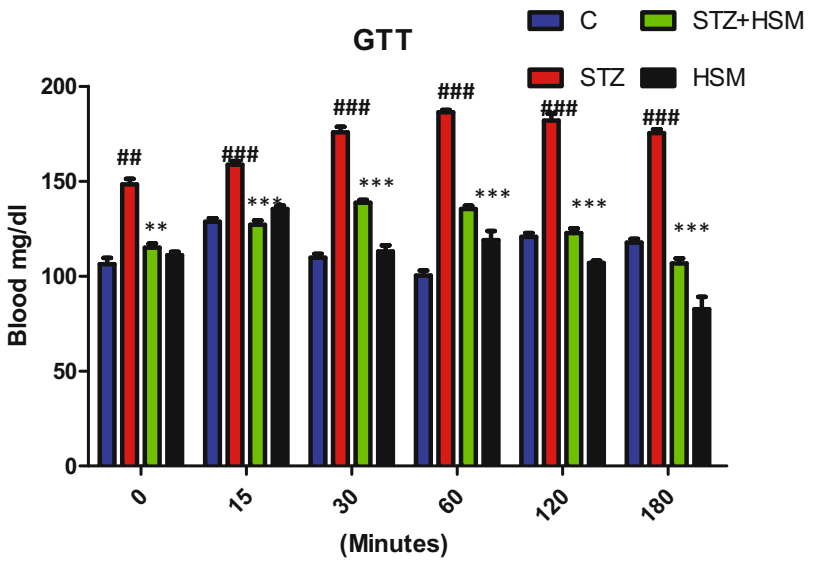

Figure 1: HSM-suppressed STZ-induced hyperglycemia and hypercholesterolemia in adult Albino Mice. Shown are the results of GTT in the serum of experimental animals. These experiments were replicated three times. Significance of control vs STZ is expressed as #, while * denotes STZ vs STZ + HSM$^{**, \#\#}p \leq 0.01$ &$^{***, \#\#\#}p \leq 0.001$.

SOD, POD, CAT, GSH, and LPO, i.e., TBARS, were conducted on all experimental groups of mice brains (hippocampus) after being subjected to homogenization. The data confirmed that HSM considerably increased the activity of antioxidant enzymes, e.g., SOD, POD, CAT, GSH, and lowered LPO actions in the homogenized sample of adult mice brains as indicated in Table 2.

3.3 HSM-abrogated STZ-induced amyloidogenic pathway of Aβ production in adult albino mice

As previously reported STZ treatment results in the production of Aβ-based AD pathology in mice brains [26]. We observed that STZ treatment was a contributing factor to activating the beta-secretase enzymes, upregulating the BACE1 expression in the homogenized brain samples of experimental mice and rendering it one of the best animal AD models. The amyloid precursor protein (APP) was fragmented into harmful Aβ fragments as a result of this rise in BACE1 protein expression observed, as depicted in Figure 2. As a result, amyloidogenic pathway is triggered, leading to the production of Aβ in the mouse brain. The results of the western blot for both BACE-1 and Aβ can be seen in Figure 2. Contrary to this, HSM considerably lowered the expression of toxic Aβ in the homogenized brain samples of adult mice, suppressing the expression of BACE1 protein, as given in Figure 2.

3.4 HSM-restored STZ-induced synaptotoxicity in adult albino mice

Elegant investigations have shown that STZ administration causes AD-like intoxication at neuronal synapses [27]. Both pre-synapse proteins (synaptophysin-SYP) and post-synapse density proteins (PSD95) were determined using

Table 1: Effect of different groups on antioxidant enzymes and lipid peroxidation

Groups (mg/dL)	Total Cholesterol (mg/dL)	TGL (mg/dL)	VDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)
Control	74 ± 2	40 ± 2	53 ± 2	6 ± 1	43 ± 2
STZ	145 ± 2$^{\#\#\#}$	80 ± 2$^{\#\#\#}$	125 ± 2$^{\#\#\#}$	13 ± 2$^{\#\#\#}$	32 ± 2$^{\#\#\#}$
STZ + HSM	80 ± 2***	50 ± 2***	64 ± 2***	8 ± 2***	39 ± 2***
HSM	75.33 ± 1.5***	41 ± 2***	54.67 ± 1.5***	7 ± 1***	44 ± 2***

Shown are the results of total cholesterol, TGL, VDL, VLDL, and HDL in the serum of experimental animals. These experiments were replicated three times (n = 3). Significance of control vs STZ is expressed as $^{\#}$, while * denotes STZ vs STZ + HSM. $^{**,\#\#}p \leq 0.01$ &$^{***,\#\#\#}p \leq 0.001$.

Table 2: Effect of different groups on antioxidant enzymes and lipid peroxidation

Groups (µmol/min/mL)	SOD (µmol/min/mL)	POD (µmol/min/mL)	Catalase (µmol H_2O_2/min/mg of protein)	GSH (µmol/mg of protein)	LPO (nM/min/mg of protein)
Control	1.083 ± 0.07	1.04 ± 0.06	76 ± 2	8.40 ± 1.31	43.67 ± 3.05
STZ	0.64 ± 0.06$^{###}$	0.54 ± 0.05$^{###}$	55 ± 2$^{###}$	4.63 ± 0.611$^{###}$	76 ± 2$^{###}$
STZ + HSM	0.91 ± 0.05***	0.90 ± 0.04***	73 ± 2***	9.13 ± 0.60***	63 ± 2 ***
HSM	0.95 ± 0.05***	0.93 ± 0.03***	72 ± 3.51***	7.83 ± 0.45***	53.67 ± 2.51***

Symbols $^{###}$ and *** show a significance difference at $p < 0.001$. Data are shown as mean ± SEM ($n = 5$). Abbreviations: SOD, POD, peroxidase; GSH, glutathione and LPO, lipid peroxidation. The results of each mice group are indicated as Mean ± SEM. # indicates the significance of control vs STZ, whereas * denotes STZ vs STZ + HSM. $^{**,##}p \leq 0.01$ & $^{***,###}p \leq 0.001$.

the immunoblot technique. The western blot analysis data shown in Figure 4 indicate that STZ injection suppressed pre- and post-synapse protein in adult male mice brains. We employed HSM to determine its protective effects on the neural synapse. The outcomes demonstrated that HSM greatly enhanced the level of SYP and PSD95 protein in the homogenized samples of mice brains (Figure 3).

3.5 HSM-improved STZ-induced memory and behavior impairment in adult albino mice

Earlier research suggested STZ as the cause of behavior and memory impairment in animal models. All the mice injected with STZ, STZ plus HSM, and the control groups were subjected to the two most common behavior tests, i.e., the Y-maze test and the MWM test. During the MWM test, the animals were trained twice daily for two days. Following one day of rest, the mean escape latencies of mice were measured over the course of 5 days. According to daily observations, control mice were found to have much lower mean escape between day 1 and day 5. Moreover, the mean escape latencies of these animals were consistently dropping, as given in (Figure 3).

The STZ-induced mice exhibited increased mean escape latencies between days 1 and 5. The mean escape latencies were on the higher side, despite a slight decrease from day 1 to day 5 in those latencies. It is interesting to note that animals treated with HSM have significantly decreased

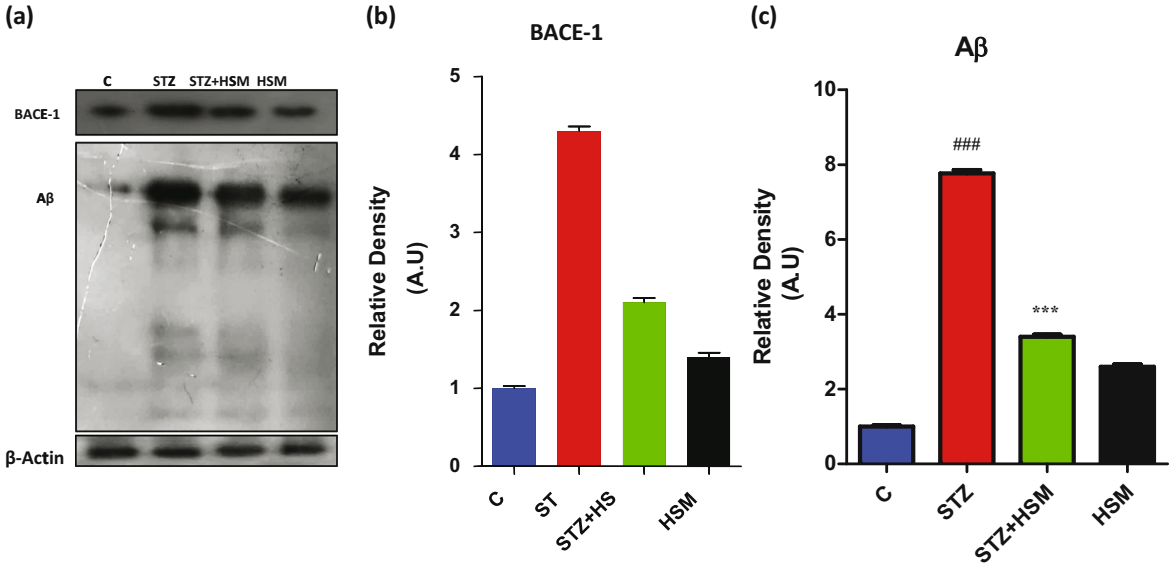

Figure 2: HSM-abrogated STZ-induced amyloidogenic mechanism of Aβ formation in Albino mice. Results of the amyloidogenic pathway markers based on immune blot analysis i.e., BACE-1 and Aβ of control vs STZ and STZ + HSM administration, are displayed as (a), whereas corresponding histograms are given as (b) and (c). The results were computed by Image J software and displayed in arbitrary units (A. U). The histogram displays the mean as A.U. ± SEM. # denotes the significance of control vs STZ, and * expresses STZ vs STZ + HSM. $^{**,##}p \leq 0.01$ and $^{***,###}p \leq 0.001$.

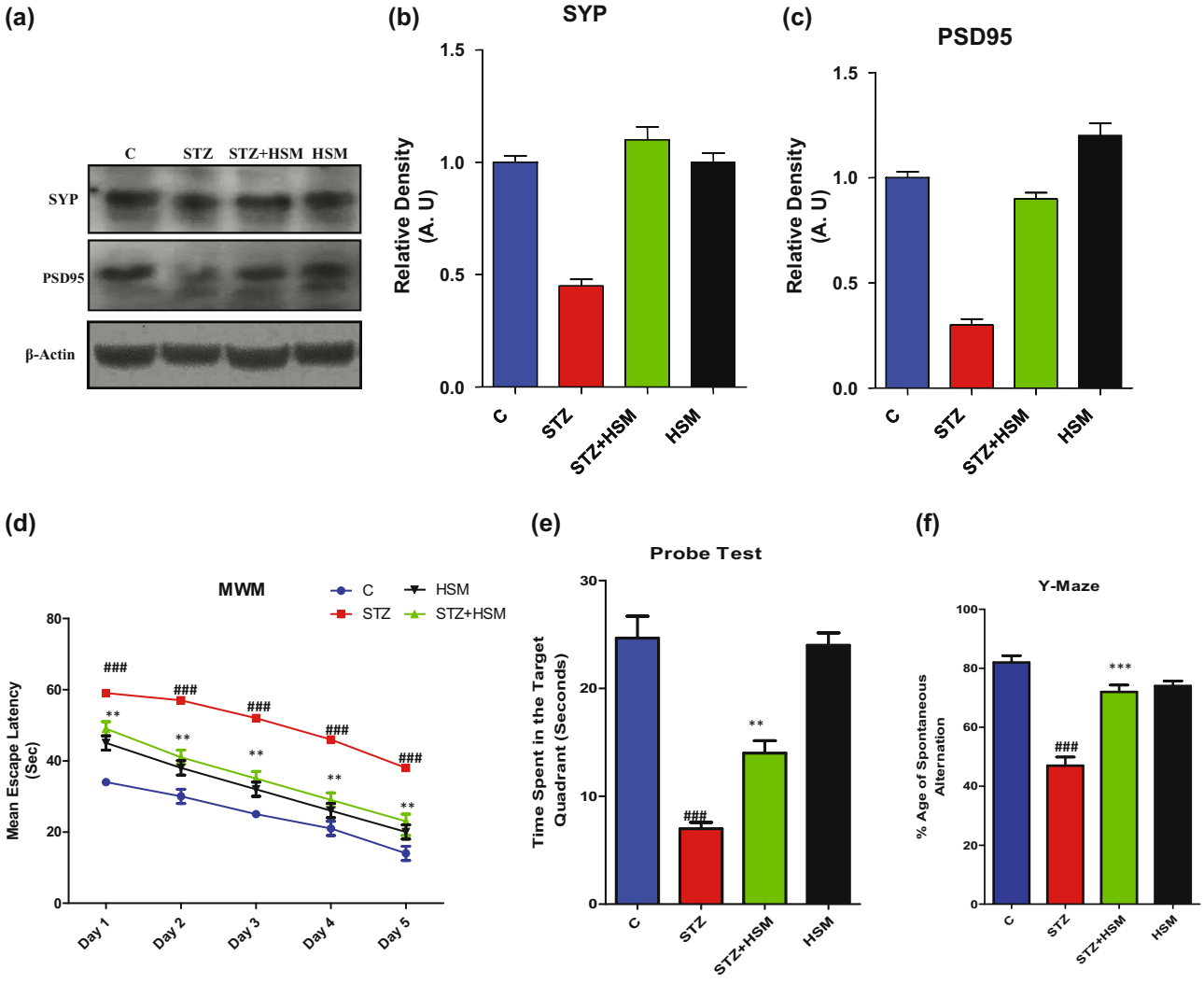

Figure 3: HSM-improved STZ-induced memory and behavior impairment in adult albino mice. The SYP and PSD95 upregulation in response to HSM treatment coupled with STZ compared to the control group and STZ is given as (a), while corresponding relative densities are plotted in the histogram at (b and c). The data of the behavior test are shown as (d) MWM-based mean escape latency as (e), probe test, and (f) % age of spontaneous alternation based on the Y-Maze test. The results were generated based on Image J and were indicated in arbitrary units (A. U). The histogram expresses the mean as A.U ± SEM. # denotes the significance of control vs STZ, and * expresses STZ vs STZ + HSM. $^{**,\#\#}p \leq 0.01$ and $^{***,\#\#\#}p \leq 0.001$.

mean escape latencies between day 1 and day 5 and consistently declined to reach the platform. Even though HSM improves behavior and lowers mean escape latencies, their escaping times remained longer than those of the control animals, as shown in Figure 3.

The mice received one day of rest, and then, the probe test was conducted where the disc was kept hidden in water to search which animals moved freely in the water. The duration for which animals stayed around the disc area was noted. It was found that control group animals remained in the target quadrants for a more extended period of time than the STZ-treated animals, which stayed there for a much shorter period. In contrast, animals treated with HSM stayed in the target area for longer, but overall, they stayed shorter compared to control group animals (Figure 3).

Finally, the Y-maze test was performed on these animals, and the % age at which spontaneous alternation occurred was documented, which depends on the spatial memory. The control mice in the Y-maze test were again found to have a high % age of spontaneous alternation, whereas mice treated with STZ spent considerably short periods of time, as indicated in Figure 4. On the other hand, HSM and STZ-administered animals during Y-Maze practice demonstrated a high % age of spontaneous alteration, as shown in Figure 3.

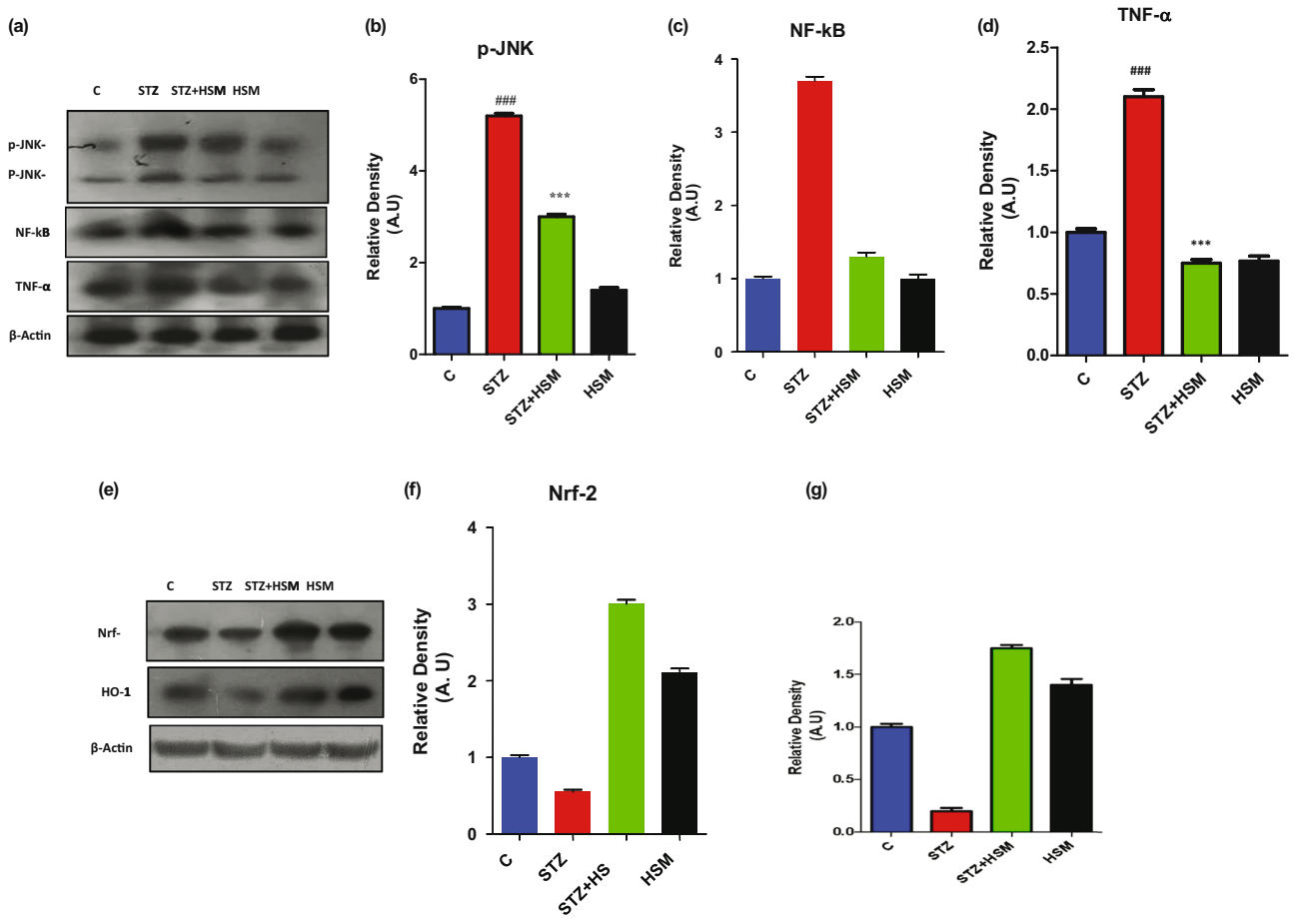

Figure 4: HSM-inhibited JNK and stimulated Nrf-2/HO-1 proteins to abrogate STZ-induced neuroinflammation in mice. The results of neuroinflammatory markers based on immune-blot analysis are shown in (a), while p-JNK, NF-kB, and TNF-α, along with histograms of corresponding relative densities, are given in (b–d). The results of immune-blot analysis of Nrf-2 and HO-1 are given as (e), and the histograms of corresponding relative densities are expressed in (f and g). In all these experiments, the loading control was β-actin. Image J software was used to determine the results, indicated in arbitrary units (A. U). Histogram expresses mean in A.U ± SEM. # denotes the significance of control vs STZ, and * expresses STZ vs STZ + HSM. Significance: $^{**,\#\#}p \leq 0.01$ and $^{***,\#\#\#}p \leq 0.001$.

3.6 HSM via p-JNK/Nrf-2/NF-kB signaling attenuated STZ-induced multiple AD neuropathological features in adult albino mice

All experimental animals' brain homogenates were analyzed using the western blot method. Literature reveals that oxidative stress enhances the phosphorylation of JNK protein, consequently inhibiting Nrf-2 and HO-1 expression similar to that of AD-like pathology, thereby upregulating NF-kB and TNF-αprotein. Similarly, in the present work, our immunoblot results further reveal that STZ significantly induced phosphorylation at JNK accompanied by suppression of both Nrf-2 and HO-1 protein expression in adult mice brains. These events ultimately enhanced NF-kB and TNF-α expression, as shown in (Figure 4). On the other hand, HSM significantly inhibited p-JNK and NF-kB protein and stimulated Nrf-2/HO-1 proteins expression and hence, attenuated STZ-induced multiple AD neuropathological features in adult albino mice. The proposed mechanism of HSM is shown in Figure 4.

4 Discussion

In the current study, we have used a chemical approach based on STZ injection to cause diabetes in mice. The hyperglycemic effects of STZ (2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose), produced by Streptomycete sachromogenes, have been extensively employed in animals, either as a single dose injection or in multiple doses. When STZ enters pancreatic β-cells through the GLUT2

glucose transporter, it induces DNA alkylation. This stimulates the initiation of poly ADP-ribosylation, resulting in a decline in cellular NAD + and ATP and a suppression of insulin production and discharge. It is also widely recognized that nitric oxide (NO), a chemical produced during STZ cellular metabolism, may be associated with STZ-administered DNA damage [28–30]. Literature reveals that systemic treatment of STZ activates neurodegeneration and neuroinflammation, leading to memory and synaptic deficits [27].

In accordance with previous studies [31], the results demonstrated that animals given STZ injections had higher plasma glucose levels, while diabetic mice groups given HSM experienced considerably lower blood glucose levels. Furthermore, our findings were consistent with earlier research, demonstrating the effectiveness of 8-hydroxyquinoline and its derivatives as hypoglycemic agents [32].

One of the pathways activated to reduce the excessive production of free radicals during inflammation and oxidative stress is Nrf-2 activation. The enhanced expression and activation of Nrf-2 in diabetic mice were triggered by elevated oxidative stress induced by hyperglycemia.

It's interesting to note that several research studies found lower levels of Nrf-2 in diabetic cardiac cells and other related vascular issues. Comparing treated diabetic mice to untreated diabetic mice, AD medication restored the expression of Nrf-2. The capability of AD to restore Nrf-2 expression can be linked to antioxidant compounds, scavenging free radicals and suppressing oxidative stress. Similarly to this mechanism, Nrf-2 is important in regulating the inflammatory processes and may promote neuroinflammation and other neurological disorders [33]. It has been reported that in the Nrf-2 mutant mice, this transcription factor controls the expression of prostanoids, chemokines, and cytokines, as well as the immune cell responses to multiple insults. For instance, after receiving the bacterial endotoxin LPS, mice lacking Nrf-2 displayed increased gene expression of interferon-inducible transcripts, pro-inflammatory cytokines, and NF-kB binding activity in peritoneal macrophages [34]. Furthermore, along with Nrf-2, NF-kB is also thought to control cellular reactions to inflammation and oxidative stress, both being activated by the same stimuli. NF-kB can control Nrf-2 transcription and activity, having both positive as well as negative impacts on the target gene expression. According to scientific research, the lack of Nrf-2 can boost NF-kB action and cause an increase in cytokine production [35]. Hence, improved treatment approaches that can control the Nrf-2/NF-kB interaction response in both healthy and pathological settings may be developed as a result of the functional cross-talk between the Nrf-2 and NF-kB pathways [36].

The current study reveals for the first time that HSM exhibits neuroprotective potential in an animal model of AD triggered by oxidative stress and STZ-mediated hyperglycemia. During the investigation, we found that HSM can ameliorate neuroinflammation, oxidative stress, and Aβ production induced by STZ. Moreover, it can also improve memory impairment and pre- and post-synapse in adult male albino mice. Interestingly, HSM in the current work suppressed p-JNK, subsequently stimulating Nrf-2 and HO-1 proteins to reduce the oxidative stress burden.

Studies have demonstrated that Nrf-2 regulates the enhancement of body's CAT, SOD, and other antioxidant capacities [37]. The expression of Nrf-2 may be responsible for the observed rise in CAT and SOD activities. When compared to treated diabetic and normal mice, the untreated diabetic mice's modest reduction in lipid peroxidation is an indication of Nrf-2 expression. According to research, Nrf-2 suppresses lipid peroxidation and increases the transcription of anti-ferroptotic genes, involved in combating lipid peroxides, and inhibits ferroptotic cell death [38].

The findings of our investigation showed that STZ treatment elevated the oxidative stress indicators in the hyperglycemic mice's brains. As a result, we noticed decreased GSH in the hippocampus. These findings were in line with research showing elevated protein carbonyl content, decreased glutathione peroxidase, SOD, and catalase activity, as well as increased lipid peroxidation in the hippocampus, hypothalamus, and frontal cortical lysate of STZ-induced mice [39]. According to scientific data, DM is consistently linked to decreased cognitive function, particularly with aging. Although the precise association between DM and cognitive deficits is not fully understood, some studies have shown that insulin and the insulin-like growth factor (IGF) control neuronal and glial cellular functions, crucial for enhancing cognitive performance, such as survival, growth, metabolism, protein synthesis, gene expression, plasticity, and synapse formation [40,41]. On the other hand, brain insulin/IGF resistance causes neuroinflammation, oxidative stress, mitochondrial dysfunction, decreased cell survival, dysregulated lipid metabolism, and endoplasmic reticulum stress, leading to a mild neurodegenerative process comparable to AD [42]. In the CNS, NF-kB transcription factors may have a significant regulatory role in physiological processes like neurotogenesis, synaptic plasticity, and neurogenesis, being linked to memory and learning.

Hydroxyquinolines, found in large amounts in nature, possess a wide range of functions; their supplementation activates the Nrf-2 signaling pathway, which encounters neurodegenerative disorders [43]. 8-Hydroxyquinolines have been reported in several studies for restoring learning and memory dysfunctions in neurotoxic conditions[18]. Previous reports have shown that STZ injection decreases the expression of synaptophysin and post-synaptic protein (PSD-95) known as pre- and post-synaptic proteins [27]. Additionally, our findings demonstrated that systemic STZ administration reduced the expression of synaptophysin as well as PSD-95 in the mice's brain homogenates. Our results also show that HSM treatment abrogates the STZ-mediated synaptic impairment in mice brains. Furthermore, our research also revealed that STZ injection causes memory impairment which HSM subsequently improved. It, therefore, confirmed HSM as a therapeutic agent to improve the memory functions involved in pre- and post-synaptic pathways in the adverse effects of CNS insult.

STZ-stimulated high generation of ROS induces oxidative stress, which mediates neuroinflammation via triggering p-JNK and NF-kB(as transcription factors) signaling. NF-kB, a heterodimer, is an essential transcription factor comprising the proteins p50 and p65.

Most importantly, the current work demonstrates that HSM can lower STZ-mediated oxidative stress stimulating NRF-2/HO-1 signaling pathway to rescue adult mice against STZ insult. Furthermore, it also inhibited NF-kB and TNF-α (which is one of the NF-kB downstream signaling molecules) to reduce STZ-induced neuroinflammation in mice brains which is attributed to its antioxidant property to improve memory and neuronal synapse impairment in mice brains.

Several studies correlate Aβ production and deposition to neurodegeneration, neuroinflammation, and memory impairment [44]. This study suggests that HSM therapy suppressed the pathways leading to the production of amyloidogenic Aβ, thereby decreasing APP protein level and BACE1 (β-secratase) activity. HSM significantly reduced the level of Aβ in adult mice brains.

5 Conclusion

In the current study, we have demonstrated that HSM is a safe and novel neurotherapeutic agent in the treatment of diabetic mouse models. It significantly restored memory impairment and neuronal synapse in adult albino mice. This study reported that HSM significantly improved pre- and post-synapse and memory defects in adult mice via stimulation of the Nrf-2 signaling pathway suppressing NF-kB and its downstream signaling. Although it has been used in the present study where diabetes was induced through chemicals, it is still warranted to study its efficacy in detail in transgenic mouse models and cell culturing to confirm its mechanism.

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Conflict of interest: The authors declare no conflict of interest.

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