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RESEARCH REPORT

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Pre-exposure of voglibose exerts cerebroprotective effects through attenuating activation of the polyol pathway and inflammation

Vishal Chavda | **Pooja Shah** | **Snehal S. Patel** | **Shraddha Bhadada**

Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmadabad, Gujarat, India

Correspondence
Snehal S. Patel, Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, 382 481, India.
Email:

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Abstract

Chronic hyperglycemia induces activation of the polyol-sorbitol pathway, which is a major contributor to microvascular complications like stroke. The current study was designed to elucidate the therapeutic role of α-glucose inhibitor in chronic hyperglycemia-induced impaired polyol pathway and associated micro-complications. Male albino-Wistar rats (200–250 g) were treated with voglibose 10 mg kg^{-1} day^{-1}/p.o. for 2 weeks before middle cerebral artery occlusion; 72 hr after surgery, neurological score was evaluated and blood was collected for the assessment of various serum biochemical parameters like CRP, CK-MB, LDH, lipid profile, and blood glucose levels. In the end, brain samples were excised for determination of brain infarct volume, brain hemisphere weight difference, Na+-K+ ATPase activity oxidative stress-related parameters, aldose reductase activity, and gene expression studies. Results from the present study indicate that pre-treatment with voglibose showed significant improvement in lipid parameters but did not impact glucose levels. Voglibose has shown a statistically significant ($p < .05$) reduction in neurological score and brain infarct volume, and the difference in brain hemisphere weight as compared to the disease control group. Voglibose significantly ($p < .05$) improve all biochemical parameters and reduced Na+-K+ ATPase and aldose reductase activity. Moreover, voglibose produced a significant reduction in oxidative stress and down-regulation of TNF-α and BCl-2 gene expression which reduces the risk of factors related to stroke. In conclusion, the pleiotropic effect of voglibose on cerebrovascular complications may be due to inhibition of aldose reductase or anti-inflammatory pathways.

KEYWORDS

cerebroprotective, stroke, transient middle cerebral artery occlusion (tMCAo), Voglibose, α-glycosidase inhibitor

*These authors contributed equally.

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1 | INTRODUCTION

Stroke is a serious cerebrovascular event, characterized by permanent neuronal damage leading to paralysis and loss of cerebral function in the affected area (Bederson et al., 1986). The prevalence of stroke was found to be the second-most frequent cause of death around the globe. Approximately 17 million people have a stroke every year (Kissela et al., 2004). Oxidative stress plays an important role in the pathogenesis of cerebrovascular complications and can result from multiple mechanisms (Hardigan et al., 2016). Previous studies have shown that the polyol-sorbitol pathway is an important source of hyperglycemia-induced oxidative stress. An excessive amount of glucose shunted to the polyol pathway, where aldose reductase (AR) reduces glucose into sorbitol at the expense of nicotinamide adenine dinucleotide phosphate (NADPH). As NADPH is essential for the generation of intracellular antioxidants, the depletion of NADPH by the AR impairs intracellular antioxidant defense. Moreover, sorbitol is further converted into fructose with the formation of reduced nicotinamide adenine dinucleotide (NADH). Further studies showed that aldose reductase inhibition contributes to a protective mechanism by the maintenance of the NADH/NAD+ ratio (Shinmura et al., 2002; Ho et al., 2006). Thus, aldose reductases act as an important mediator in the pathogenesis of cerebrovascular complications. Therefore, we believed that the inhibition of AR can be beneficial in hyperglycemia-induced oxidative stress and can be used in treatment of ischemic brain disease.

Recent evidence demonstrates that postprandial hyperglycemia not only exacerbates vascular endothelial dysfunction, inflammatory reactions, and oxidative stress in individuals with type 2 diabetes but also transiently impairs vascular function in healthy individuals (Mah & Bruno, 2012). Oxidative stress-induced deregulation of nitric oxide contributes to postprandial hyperglycemia-mediated vascular endothelial dysfunction. Compelling evidence exists suggesting that controlling postprandial hyperglycemia can be a potential target for preventing cerebrovascular complications (Node & Inoue, 2009). Voglibose, an anti-diabetic agent that belongs to the class of α-glycosidase inhibitor, showed a significant decrease in blood sugar level by acting on the absorption and catabolism of oligosaccharide in the brush border of the small intestine (Satoh et al., 2006). Its current clinical research reports support the use of voglibose as monotherapy in type 2 diabetes, as well as a healthy individual can reduce the incidence of a cardiovascular event by decreasing postprandial hyperglycemia (Chavda and Patel, 2019; Shah et al., 2020; Zeymer, 2006). Several studies have reported that treatment with voglibose also results in a significant decline in triglyceride levels and prevents atherosclerosis (Beckman et al., 2002). Based on accumulating evidence, which suggests that voglibose is an ideal cardioprotective agent, may exert cerebroprotective effects. Therefore, we have aimed to evaluate the cerebroprotective effect of voglibose in an animal model of middle cerebral artery occlusion (MCAO).

2 | MATERIALS AND METHODS

Voglibose was obtained from Torrent Pharmaceuticals Ltd, Ahmadabad, India, as gift sample. Various biochemical estimation kits for serum glucose, triglyceride, total cholesterol, high-density lipoprotein (HDL), C reactive protein (CRP), lactate dehydrogenase (LDH), and creatinine kinase (CK-MB) were obtained from Labcare Diagnostics, India. Other chemicals used were of analytical grade.

2.1 | Experimental animals

Healthy Sprague–Dawley rats weighing 200–250 g were used for the study. They were bred under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%), and 12 hr/12 hr light-dark cycle. Conventional laboratory diet and filtered water were provided ad libitum. Institutional Animal Ethics Committee (IAEC) has approved the protocol for the experiment as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (IPS/PCOL/MPH 11-12/1007). There were five groups with eight animals in each group: normal control, sham operated, normally treated with voglibose (10 mg kg^{-1} day^{-1}, p.o.), MCAO operated, and MCAO operated animals treated with voglibose (10 mg kg^{-1} day^{-1}, p.o). Treatment was carried out for 2 weeks before MCAO surgery.

2.2 | Experimental protocol

Rats underwent focal ischemia by transient middle cerebral artery occlusion (tMCAo) using the method described by Bederson et al., 1986. The animals were anaesthetized using combination of ketamine and diazepam (20 mg/kg i.m. and 0.5 mg/kg i.m., respectively). Animals are placed on the homoeothermic operating table. The fur of the neck region was shaved to expose the skin. The surgical skin area was disinfected using a 5% chlorhexidine solution. The ocular lubricant was applied to both eyes, to prevent them from drying during the surgery. After removal of fur, a 1.5 cm midline incision was made. The left common carotid artery (CCA) was exposed and freed from the surrounding tissue and vagal nerve. The occipital artery that arises from the external carotid artery (ECA) was then isolated and tied using a cotton thread. The ECA dissected further distally and tied using cotton thread along with the terminal lingual and maxillary artery branches. Polyamide-coated monofilaments (Ethicon

NW-3318 size 4.0) were inserted into the ECA stump to block the origin of the MCA. After the occlusion, the midline incision is closed, and analgesics and antibiotics are given to the animal. The animal was placed into a pre-warmed recovery cage with free access to food and water. After 24, 48, and 72 hr of MCAO, measurement of a neurological score was carried out for assessment of the degree of damage over a period as per the method described by Bederson et al. and scored as per Table 1. Blood samples were collected and serum was separated for the determination of various biochemical parameters. The animals were killed and the whole brain was removed immediately and rinsed in chilled 20% sucrose. Both the hemispheres were weighed on the digital balance, and thereby difference is calculated between both the coordinating brain hemisphere regions of each animal.

2.3 | Measurement of brain infarct volume using TTC staining

The infarct volume measurement was carried out from six coronal forebrain sections which were prepared from fresh cold brain stained in % 2,3,5-triphenyl tetrazolium chloride (TTC) in phosphate buffer (pH 7.4) at 37°C for 10–15 min followed by 10% formalin for 24–48 hr to determine viable and non-viable tissue. Brain sections scanned into the computer using Image J software and indirect infarct volume were determined.

The brain samples were dried using blotting paper and wrapped into an aluminum foil and stored in a deep freezer maintaining a temperature of −15 to −20°C for estimation of parameters like Na+-K+ ATPase activity, AR activity, and oxidative stress parameters such as total protein, malondialdehyde (MDA), reduced glutathione (GSH), nitrite, and superoxide dismutase (SOD).

2.4 | Evaluation of biochemical parameters in serum

The serum was analyzed colorimetrically for serum glucose (catalog number GLU500), triglyceride (catalog number TGLSLR125), total cholesterol (catalog number CHOLSLR125), HDL (catalog number DHDL40), LDH (catalog number LDH25), and CK-MB (catalog number MBSLR25) levels using commercially available kits and spectrophotometer (Shimadzu Analytical India Pvt. Ltd).

TABLE 1 Scoring of neurological behavior

Score	Neurological behavior
Score-1	Contra-lateral forelimb flexion when suspended by tail
Score-2	Decreased grip of the contra-lateral forelimb while tail pulled
Score-3	Spontaneous movement in all directions or contra-lateral circling only if pulled by the tail
Score-4	Spontaneous contra-lateral circling
Score-5	Death after recovery from the anesthesia

2.5 | Preparation of brain homogenate from brain samples

Rat brain samples were subjected to perfusion transcardially with ice-cold saline. Whole brain samples were subjected to homogenization with 10% ice-cold phosphate-buffered saline and were centrifuged at 10,000 rpm at −4°C for 15 min. The supernatants were collected in a centrifuge tubes and were kept at 4°C temperature. Obtained supernatants were subjected to various antioxidant parameters and Na+ K+ ATPase activity.

2.6 | Estimation of Na+-K+ ATPase activity

Na+-K+ ATPase activity is measured as a marker of ischemic damage. The supernatant from brain homogenate was used for the determination of Na+ K+ ATPase activity by the method described by Singh et al. (2015). Na+-K+ ATPase was expressed as nmol of inorganic phosphate (Pi) released per min. per mg of protein (Ahmad et al., 2006; Singh et al., 2015).

2.7 | Estimation of aldose reductase activity in brain homogenate

The determination of AR activity assayed from the supernatant from brain homogenate as per the method described by Ao et al. (1991). Brain homogenate was mixed with 100 mM sodium phosphate EDTA buffer and centrifuged. NADPH of 0.1 ml and supernatant of 0.1 ml were mixed with 0.1 ml of DL-glyceraldehyde and the final volume was made to 1 ml. The absorbance was recorded at 340 nm for 3 min at 30-s interval. Percentage of aldose reductase activity was calculated concerning the activity of normal control animals (Ao et al., 1991).

2.8 | Evaluation of antioxidant activity in the brain

One gram of brain tissue was homogenized in 10 ml ice-cold tris hydrochloride buffer. The prepared homogenates of each group were centrifuged and the supernatants were used for the determination of total protein and antioxidant parameters.

2.9 | Measurement of total protein level

The total protein was estimated by the method of Lowry et al., 1951 (Lowry et al., 1991). A total volume of 200 µl ml^{-1} of supernatant was mixed with 0.5 ml of alkaline copper solution and 0.5 ml of folic-ciocalteu reagent. The absorbance was read against blank at 600 nm. The protein concentration was calculated using the standard curve of albumin.

2.10 | Measurement of malondialdehyde level

Brain sample supernatants were subjected to assessment of antioxidant parameters like an estimation of MDA level by the method described by Alam et al. (2013). Briefly, 200 µl of supernatant was mixed with 0.2 ml of 8% W/V sodium dodecyl sulfate, 1.5 ml of 20% acetic acid, and 1.5ml freshly prepared of thiobarbituric acid (1%W/V). The mixture was heated in a water bath at 95°C for 45 min, cooled, and 2 ml of the mixture mixed with 2 ml of 10% trichloroacetic acid. The resulting mixture was centrifuged at 1,000 rpm for 5 min. The developed intense pink color was read against blank at 532 nm. The amount of MDA was reported as nmol of MDA/mg protein.

2.11 | Measurement of reduced glutathione level

GSH level was estimated by the method described by Forman et al. (2009). The supernatant (200 µl) was mixed with 10% chilled trichloroacetic acid. The mixture was kept in an ice bath for 30 min and centrifuged at 1,000 g for 10 min. The 0.5 ml supernatant was mixed with 2 ml 0.3 M disodium hydrogen phosphate and 0.25 ml 5, 5′-dithiobis-2-nitrobenzoic acid just before measuring the absorbance at 412 nm. Results were expressed as µmole of GSH/g tissue.

2.12 | Measurement of nitrite level

The measurement of nitrite content was done by methods described by Topcu Ali et al. (2014). A total volume of 100 µl of Griess reagent was added to 100 µl of supernatant and absorbance was measured at 550 nm. Obtained nitrite concentration was expressed as µmol/mg protein.

2.13 | Measurement of superoxide dismutase level

The SOD was estimated by the method of Madi et al. (2016). In brief, the supernatant (0.1 ml) was mixed with 0.1 ml of Ethylenediaminetetraacetic acid, 0.5 ml of carbonate buffer (pH 9.7), and 1 ml of Epinephrine. The optical density of formed adrenochrome was read at 480 nm for 3 min at an interval of 30 s. The enzyme activity was expressed in terms of U/min/mg protein.

2.14 | Measurement of C-reactive protein Levels (CRP)

The CRP levels were estimated by Latex Turbidimetry method using the commercially available kits (Accucare, Labcare Diagnostics (HS-CRP TURBILATEX)) from serum samples of each group of rats. Ten µl serum sample was added to working reagent and absorbance was taken immediately after 4 min of incubation at 540 nm.

2.15 | Gene expression

The gene expression studies for BCL-2 and TNF-α were carried out from brain samples using triazole reagent as described in the manufacturer's instructions. The isolated RNA was purified using alcohol and quantified with

TABLE 2 Primer sequences used for qPCR reaction

Gene	Sence, anti-sence	Tm Annealing
Bcl−2	Forward: 5′-GAG TAC CTG AAC CGG CAT CT-3′ Reverse: 5′-GAA ATC AAA CAG AGG TCG CA-3′	53
TNF-α	Forward 5′-AAATGGGCTCCCTCTCATCAGTTC-3′ Reverse: 5′-TCTGCTTGGTGGTTTGCTACGAC-3′	53
β -actin	Forward 5′-TATGCCAACACAGTGCTGTCTGG-3′ Reverse: 5′-TACTCCTGCTTGCTGATCCACAT-3′	49
PCR conditions used	**For TNF-α**: denaturation at 95°C, 33 cycles for 30 s, annealing for 30 s at 53°C, extension for 30°C seconds at 37°C. **For Bcl−2**: denaturation at 95°C, 35 cycles for 30s, annealing for 30 s at 53°C, extension for 34°C seconds at 38°C. The PCR products were electrophoresed on a 2% agarose gel.	

a nanodrop spectrophotometer. The RNA was reversibly quantified with single-stranded cDNA using a cDNA synthesis kit (Thermo-Fisher) as per the manufacturer's manual. The gene primer sequences acquired from ILS-Delhi were used in the qPCR amplification are provided in Table 2. The quantitative polymerase chain reaction for Bcl-2 and TNF-α was performed on the BIO-RAD Thermal cycler 100 system. First-strand cDNA was used as a template for quantitative PCR with a pair of both specific primers (reverse and forward) in a 25-μl reaction volume. The sequences of specific primer and PCR conditions are listed in Table 2. The relative quantitative differences in gene expression between various groups were carried out with gel documentation system (BIO-RAD) against housekeeping gene β-actin.

2.16 | Statistical analysis

The data were expressed as mean ± *SEM*. Comparisons among groups were performed by one-way ANOVA followed by Tukey's multiple comparisons post-test. Significant differences were established at $p < .05$. All statistics were done using Graph pad prism (Version 5.0).

3 | RESULTS

3.1 | Effect of voglibose on body weight, food intake, and water intake

Pre-treatment with voglibose did not produce any significant change in body weight, food intake, and water intake in any groups before MCAO induction. After MCAO induction, the sham and MCAO control animals showed a significant ($p < .05$) decrease in body weight, food, and water intake after surgery as compared to normal control animals. Animals treated with voglibose (10 mg kg^{-1} day^{-1}, p.o.) showed a significant ($p < .05$) improvement in body weight, food intake, and water intake as compared to MCAO operated animals after surgery (Figure 1).

3.2 | Effect of voglibose on weight difference of brain hemisphere

The MCAO operated animals showed a significant ($p <. 05$) increase in weight difference of brain hemispheres as compared to the normal control animals. Pre-exposure of voglibose (10 mg kg^{-1} day^{-1}, p.o.) in MCAO animals showed a

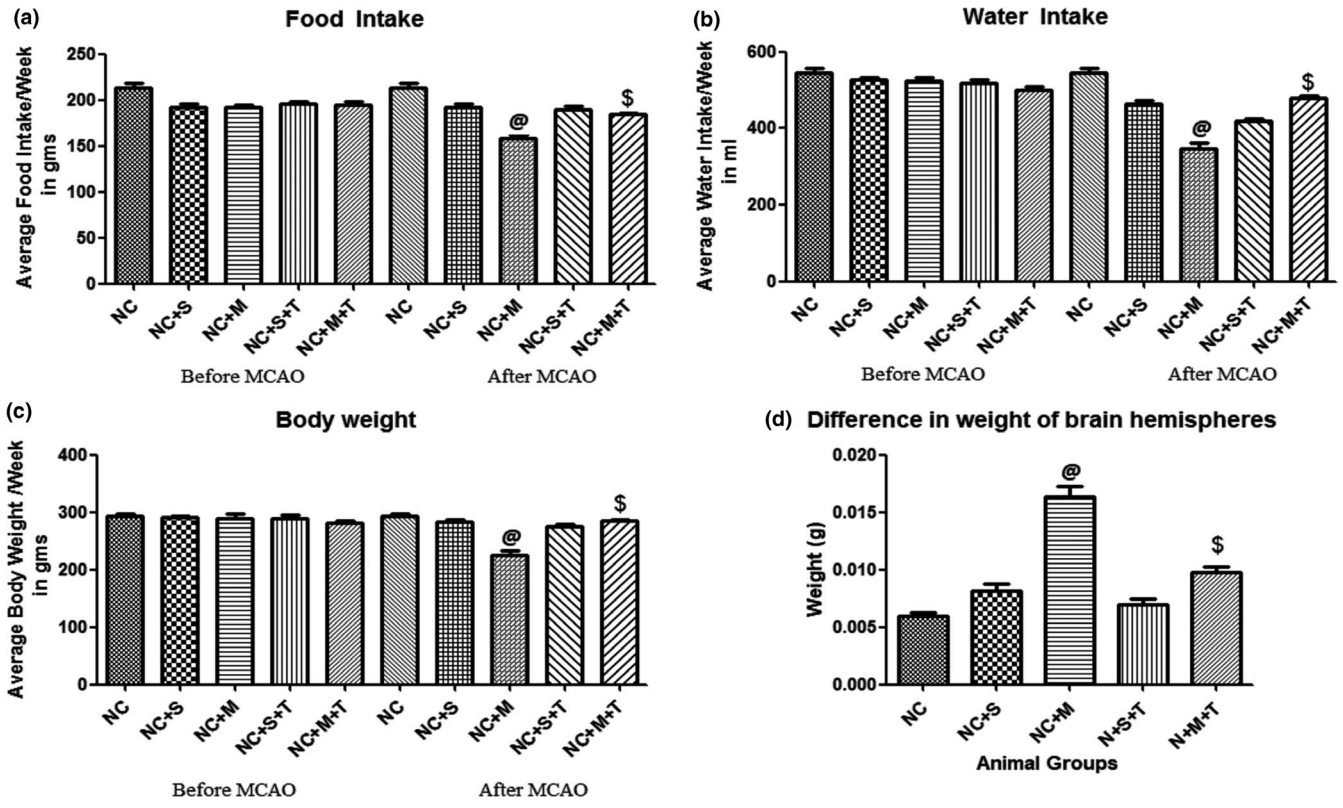

FIGURE 1 Effect of voglibose on morphological parameters. (a) Effect on food intake, (b) Effect on water intake, (c) Effect on weight gain, (d) Effect on Difference in weight of brain Hemisphere. Values are expressed as Mean ± *SEM* of 8 animals. $^{\$}$Significantly different from sham control ($p < .05$), $^{\#}$Significantly different from MCAO control ($p < .05$). NC, normal control; NC+S, sham-operated; NC+M, MCAO induced; N+S+T, sham-operated treated with voglibose; N+M+T, MCAO induced treated with voglibose

significant ($p < .05$) reduction in a difference of brain hemispheres as compared to MCAO operated animals (Figure 1).

3.3 | Effect of voglibose on the neurological score

The neurological score was determined at 24, 48, and 72 hr after ischemia induction in respective groups based on a 4-point scale method. The MCAO control animals showed severe injuries and neurological deficits on all observation points. They showed significantly ($p < .05$) increased neurological scores compared to normal and sham control animals. Treatment with voglibose (10 mg kg day^{-1}, p.o.) in MCAO operated animals showed a significant ($p < .05$) decrease in neurological score compared to the MCAO operated animals (Figure 2).

3.4 | Effect of voglibose on brain infarct volume

The MCAO operated animals showed a significant ($p < .05$) increase in brain infarct volume as compared to the normal control animals. Pre-treatment with voglibose (10 mg kg^{-1} day^{-1}, p.o.) in MCAO animals showed significant ($p < .05$) reduction in brain infarct volume or neuroprotection as compared to the MCAO operated animals (Figure 2).

3.5 | Effect of voglibose on glucose and lipid profile

Normal and sham control rats were found to have normal serum glucose levels and no significant increase was observed in MCAO operated group. Treatment with voglibose (10 mg kg^{-1} day^{-1} p.o.) did not alter serum glucose levels in MCAO operated group. Normal and sham control rats were found to have normal serum lipid profiles and no significant influence was observed due to MCAO or sham surgery. The MCAO operated group treated with voglibose (10 mg kg^{-1} day^{-1}, p.o.) did not show any significant difference in serum lipid profile (Table 3).

3.6 | Effect of voglibose on CK-MB and LDH levels

In our study, MCAO operated animals showed a significant ($p < .05$) increase in CK-MB levels as

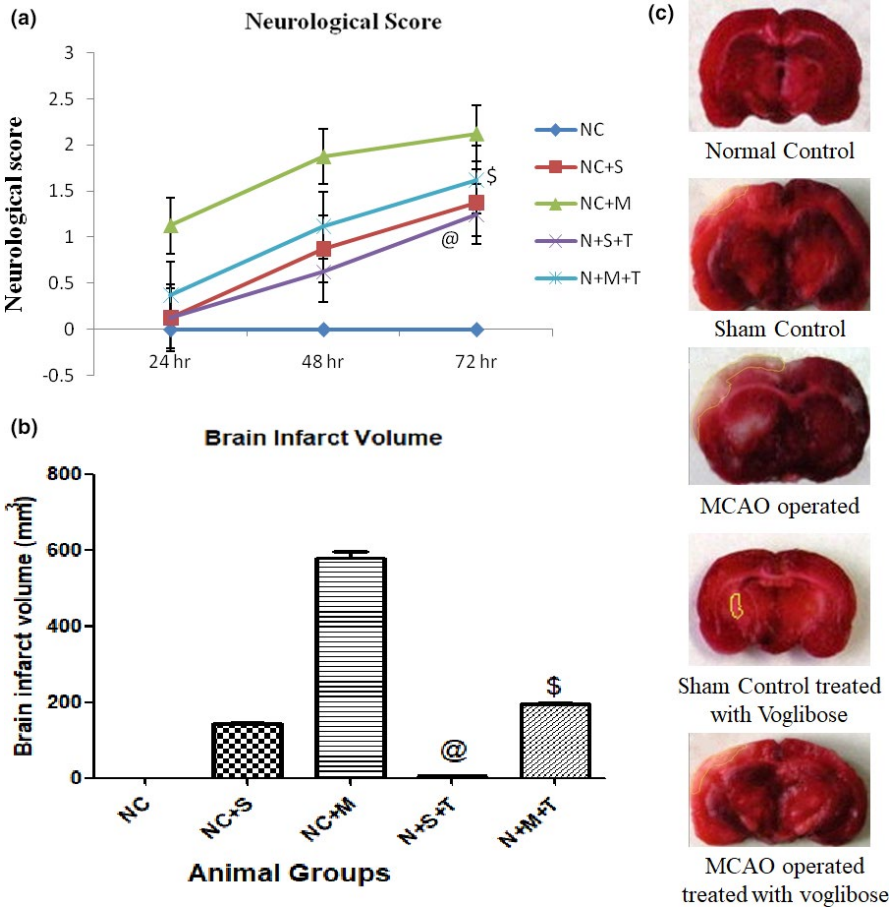

FIGURE 2 Effect of voglibose on neurological parameter. (a) Effect on the neurological score, (b) Effect on Brain Infarct Volume, (c) Brain sections stained with 2,3,5-triphenyl tetrazolium chloride. Values are expressed as Mean ± *SEM* of 8 animals. [@]Significantly different from sham control ($p < .05$), [$]Significantly different from MCAO control ($p < .05$). NC, normal control; NC+S, sham-operated; NC+M, MCAO induced; N+S+T, sham-operated treated with voglibose; N+M+T, MCAO induced treated with voglibose

TABLE 3 Effect of voglibose on serum biochemical parameters

Parameters	NC	NC + S	NC + M	N + S + T	N + M + T
Serum glucose (mg/dl)	129.6 ± 4.1	130.4 ± 5.4	130.6 ± 5.6	122.2 ± 2.9	121.1 ± 3.3
Serum cholesterol (mg/dl)	45.1 ± 0.9	45.9 ± 1.4	46.2 ± 1.4	45.5 ± 1.5	46.3 ± 1.6
Serum LDL (mg/dl)	14.8 ± 0.2	15.6 ± 0.2	15.9 ± 0.3	15.4 ± 0.3	16.1 ± 0.2
Serum triglyceride (mg/dl)	80.6 ± 3.9	81.6 ± 5.6	82.8 ± 10.8	81.4 ± 5.9	81.6 ± 4.7
Serum HDL (mg/dl)	24.1 ± 1.9	23.4 ± 1.4	23.08 ± 1.4	23.9 ± 1.9	23.5 ± 1.3
Serum LDH (U/L)	846.2 ± 38.9	975.6 ± 32.1	1,066.3 ± 27.6	786.1 ± 43.1[@]	877.5 ± 50.5[$]
Serum CK-MB (U/L)	285.6 ± 14.5	335.1 ± 12.3	363.7 ± 11.5	270.5 ± 13.0[@]	299.6 ± 11.9[$]

Note: Values are expressed as Mean ± *SEM* of 8 animals.

Abbreviations: NC, normal control; NC+S, sham operated; NC+M, MCAO induced; N+S+T, sham operated, treated with voglibose; N+M+T, MCAO induced, treated with voglibose.

[@]Significantly different from sham control ($p < .05$).

[$]Significantly different from MCAO control ($p < .05$).

compared to sham control animals. Treatment with voglibose (10 mg kg^{-1} day^{-1}, p.o.) showed a significant ($p < .05$) reduction in the CK-MB levels as compared to the MCAO operated groups. Similarly, MCAO operated animals showed a significant ($p < .05$) increase in LDH levels as compared to sham control animals. Pre-treatment with voglibose (10 mg kg^{-1} day^{-1}, p.o.) showed a significant ($p < .05$) reduction in the LDH levels as compared to the MCAO operated groups (Table 3).

3.7 | Effect of voglibose on Na+-K+ ATPase activity

Ischemic stroke influences the activity of the sodium–potassium pump with the inhibition of mitochondrial respiration. In the present study, MCAO operated animals showed a significant ($p < .05$) reduction in Na+-K+ ATPase enzyme activity as compared to normal control animals. Treatment with Voglibose (10 mg kg^{-1} day^{-1}, p.o.) showed a significant ($p < .05$) increase in Na+-K+ ATPase enzyme activity as compared to MCAO operated animals (Figure 3).

3.8 | Effect of voglibose on AR activity

Evaluation of aldose reductase activity was carried out in the brain homogenate to check the mechanism of action of the cerebroprotective effect. MCAO operated animals showed a significant ($p < .05$) increase in aldose reductase activity as compared to normal control animals. Treatment with Voglibose (10 mg kg^{-1} day^{-1}, p.o.) showed a significant ($p < .05$) decrease in aldose reductase activity in MCAO-induced animals as compared to MCAO operated animals (Figure 3).

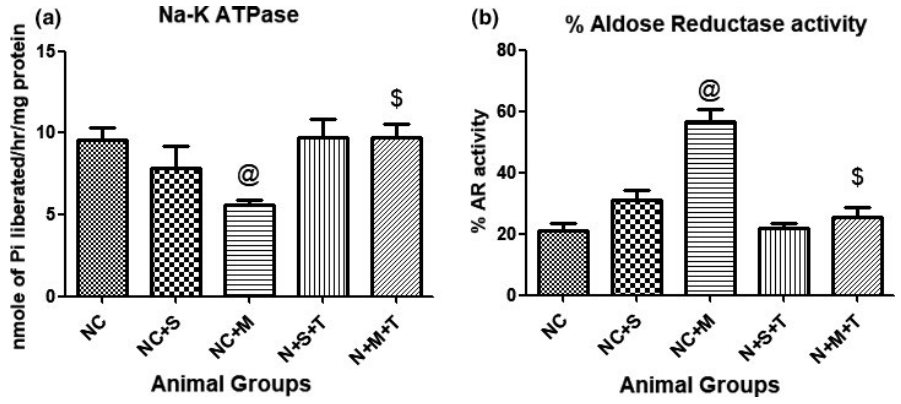

FIGURE 3 Effect of voglibose on biochemical parameters in rain homogenate. (a) Effect on Na-K ATPase. (b) Effect on % Aldose Reductase Activity. Values are expressed as Mean ± *SEM* of 8 animals. [@]Significantly different from sham control ($p < .05$), [$]Significantly different from MCAO control ($p<0.05$). NC, normal control; NC+S, sham-operated; NC+M, MCAO induced; N+S+T, sham-operated treated with voglibose; N+M+T, MCAO induced treated with voglibose

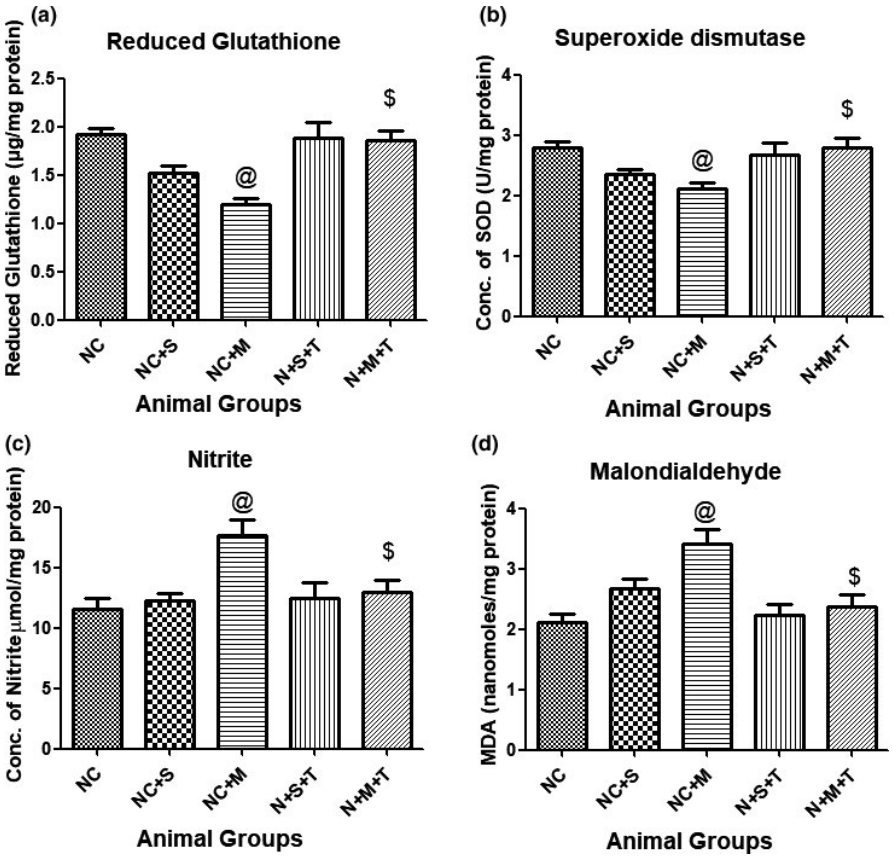

FIGURE 4 Effect of voglibose on antioxidant parameters in brain homogenate. Effect on GSH level. (b) Effect on SOD. (c) Effect on MDA. (d) Effect on nitrite level Values are expressed as Mean ± *SEM* of 8 animals. [@]Significantly different from sham control ($p < .05$), [$]Significantly different from MCAO control ($p < .05$). NC, normal control; NC+S, sham-operated; NC+M, MCAO induced; N+S+T, sham-operated treated with voglibose; N+M+T, MCAO induced treated with voglibose

3.9 | Effect of voglibose on oxidative stress parameters

A significant ($p < .05$) reduction was seen in the levels of antioxidant enzymes such as GSH, catalase, and SOD in MCAO operated group as compared to the normal control group. Whereas the levels of MDA and nitrite were increased significantly ($p < .05$). Pre-treatment with voglibose (10 mg kg^{-1} day^{-1}, p.o.) showed a significant ($p < .05$) increase in GSH and SOD levels and decrease in MDA and nitrite levels as compared to MCAO operated group (Figure 4).

3.10 | Effect of pre-exposure of voglibose on CRP levels

A significant ($p < .05$) elevation in the CRP levels was found in MCAO operated group as compared to sham-operated group. The pre-treatment with voglibose to MCAO operated group showed significant reduction in serum CRP levels, which indicates remarkable protection (Figure 5a).

3.11 | Effect of pre-exposure of voglibose on Bcl-2 and TNF-α gene expression

A notable up-regulation was observed in Bcl-2 and TNF-α gene expression in MCAO operated group. The pre-treatment with voglibose showed significant down-regulation of Bcl-2 and TNF-α inflammatory markers in MCAO operated groups (Figure 5b,c).

4 | DISCUSSION

Postprandial hyperglycemia is predictor of future cerebrovascular mortality compared with fasting glucose in both diabetic and normoglycemic individuals. Focal ischemia that results from occlusion of a fundamental artery in the brain generally produces tissue infarction, in which affected components of the brain showcase a non-selective loss of all cells such as neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells (Stokum et al., 2016). The animal model of MCAO enables the study of stroke pathophysiology and assessment of the latest healing approaches (Zhang et al., 2015).

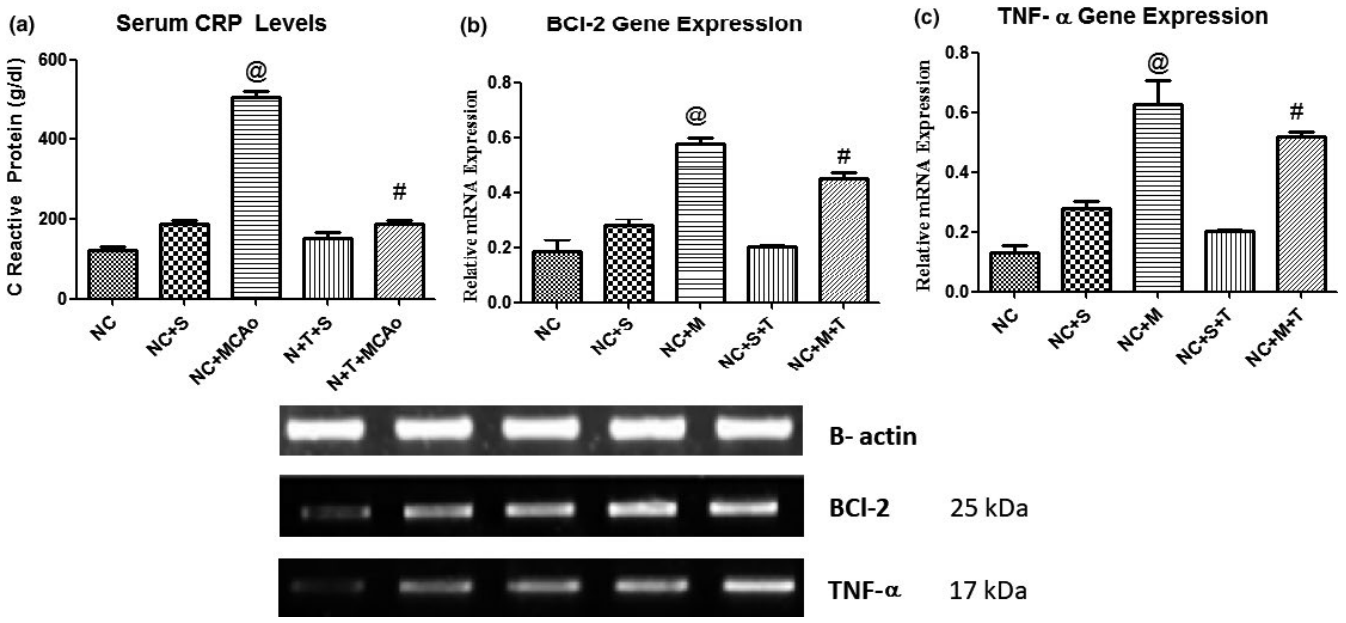

FIGURE 5 Effect if voglibose on Serum CRP levels and post-stroke gene expression on TNF-α and Bcl-2 genes. Values are expressed as the Mean ± *SEM* of 8 animals. [@]Significantly different from sham control ($p < .05$), [$]Significantly different from MCAO control ($p < .05$). NC, normal control; NC+S, sham operated; NC+M, MCAO induced; N+S+T, sham-operated treated with voglibose; N+M+T, MCAO induced treated with voglibose

Clinical observations suggest that malnutrition and dehydration were observed after cerebral ischemia. MCAO surgery presumably leads to pain, impairment of mastication, and swallowing in affected rats. It also produces postoperative catabolism, which probably results in reduced food and water consumption, and consequently delay body weight regain after surgery (Ojo & Brooke, 2016). In the present study, food and water intake decreased in the immediate post-operative period in MCAO operated rats. Improvement in food, water intake, and weight gain after surgery was observed in a treatment group. This could be due to a protective effect by voglibose.

The evaluation of neurological function allows the assessment of the degree of damage. The intended aim of stroke treatment is the restoration of behavioral functional characteristics. A motor disturbance was observed as a result of lesions in the frontal cortex and striatum due to ischemic stroke (Kaji, et al., 2004). In our study, a significant reduction in neurological score was found in voglibose-treated animals. The administration of α-glycosidase inhibitors has reported a reduced development of neuropathy by lowering of postprandial hyperglycemia. Thus, a positive correlation was observed with the neurological outcome by treatment with voglibose.

It is evident that sudden flux via polyol pathway results in the elevation of intracellular sorbitol that consequently increases intracellular osmotic pressure in the neurons, which in turn consequently results in cerebral edema, neuronal death, and increase in brain infarct volume (Giacco and Brownlee, 2010). Furthermore, it is reported that α-glucosidase inhibitors exert beneficial effects in both autonomic and somatic polyneuropathy (Creutzfeldt, 1999). In the current study, treatment with voglibose showed a significant reduction in brain infarct volume and difference in the brain hemisphere weight. These favorable effects explain that reduced brain infarct volume in the ischemic brain may due to cerebral edema by voglibose.

The brain tissue that deprives oxygen acts as the serious ransom paid by the brain due to inefficient control on the metabolism of glucose and lipoprotein levels (Benchenane et al., 2005). No significant changes observed in the serum glucose and lipid profile between the normal control and MCAO operated animals. Treatment with voglibose also did not produce any significant change in lipid levels in MCAO operated animals.

CK-MB levels proved to increase in acute brain injury during ischemic stroke, which further leads to edema and inflammation (Ay et al., 2002). In our study, a significant reduction in serum CK-MB levels was observed by treatment with voglibose. The α-glucosidase inhibitor was reported to have the protective effect in inflammation induced by postprandial hyperglycemia and CKMB levels which are likely to implicate during inflammation (Fukaya et al., 2009). Thus, the positive cerebroprotective effect of voglibose may be due to the prevention of swelling and inflammation, which positively correlates with the decrease in CK-MB levels.

Previous experiments have shown that the efflux of LDH operated by hypoxia is proportional to the number of neurons damaged or destroyed and neuronal cell injury is quantitatively assessed by the measurement of LDH after cerebral ischemia. Therapeutic paradigms that reduce LDH levels

can prevent neuronal damage (Cai et al., 2016). In our study, treatment with voglibose significantly reduced the serum LDH levels in ischemic rats, which indicates a neuroprotective effect of voglibose.

It has been demonstrated from previous research reports that the polyol pathway leads to a decrease in Na+-K+ ATPase activity in the central nervous system. Also, Na+-K+ ATPase levels and activity are down-regulated in focal ischemia and traumatic brain (Huang et al., 2015). In our study, a significant decrease in Na+-K+ ATPase activity was observed in MCAO operated animals. However, treatment with voglibose significantly increased the Na+-K+ ATPase activity. Recent studies showed that α-glucosidase inhibitors restrain the activity of Na+/K+-ATPase by inhibition of glucose absorption from small intestines, which regulate the polyol pathway (Arbeloa et al., 2012). This indicates that voglibose produces a shrink in the production of polyol through inhibition of the Na+-K+ ATPase activity during ischemic injury.

Approximately 30% of absorbed glucose is channeled into the AR-dependent polyol pathway, which depletes NADPH and consequently reduces the antioxidant level. Furthermore, the polyol pathway also plays a crucial role in elevating advanced glycation end-product formation, which contributes to neurotoxicity and brain damage during ischemic stroke (Ramana et al., 2004). Thus, there is crosstalk between AR activity and vascular function. AR activity is linked with overexpression of astrocytic endothelin, which brought excessive neurological deflects with multiple cerebral edema and infarct (Lo et al., 2007). In our study, we found reduced levels of AR activity in the MCAO operated group which insulted brain morphology and normal physiological function. Treatment with voglibose prevented this rise in AR activity in the MCAO operated group. As voglibose was reported to exhibit a decrease in postprandial glucose level, thereby levels of advanced glycation end products. The results indicates that neuroprotection offered by voglibose may be due to reduction in AR activity.

The ischemic brain initiates a series of pathological reactions within the brain cell membrane (Flamm et al., 1978). During cerebral ischemia, rapid overproduction of free radicals overwhelms the detoxification and scavenging capacity of cellular antioxidant enzymes like superoxide dismutase (SOD) and reduces glutathione. Several studies also revealed increased MDA concentrations in acute stroke (Smith et al., 2005). In our study, we reported a substantial reduction in SOD and GSH levels, and increase in levels of MDA in MCAO operated animals which was reversed significantly in animals treated with voglibose. Voglibose reported to have decreased in glucotoxicity, which leads to a decrease in oxidative stress (Frantz et al., 2005). Therefore, combating effect of voglibose probably due to its free radicals scavenging activity could contribute to the neuroprotection.

Besides, during cerebral ischemia, the generation of peroxy nitrate (ONOO-) in an endothelial cell is known to increase transcription of the plasminogen activator inhibitor-I (PAI-1). Fibrinolysis was impaired by increased levels of PAI-1 that would contribute to vascular plugging (Singh and Trigun, 2010). In the present study, we have observed high nitrate content in the brain homogenate of MCAO operated animals. Shinoda et al. have reported that acarbose markedly reduces the plasma PAI-1 and fibrinogen levels induced by peroxy nitrate (Shinoda et al., 2006). In our study, we observed a remarkable reduction in nitrate content by voglibose pre-treatment in MCAO operated group, which signifies voglibose may have a cerebroprotective effect by reducing nitrate levels that down-regulates PAI-I-like transcription factors and reduce the chances of vascular plugging, which would further contribute to inhibition of fibrinolytic property.

The protection from inflammation is one of the major mechanisms thought to underlie neuroprotection (Stokum et al., 2016). CRP levels are the important biomarkers of brain damage (Di Napoli et al., 2018). In the present study, the pre-treated group showed reduced CRP levels in MCAO operated animals. This result manifests that voglibose exerts neuroprotective action in stroke-like conditions by reducing levels of inflammatory markers. In the previously reported study, the α-glucosidase inhibitor reported decreased levels of NFκB (Patel, 2016). Moreover, the expression of the Bcl-2 gene and TNF-α gene was found to be up-regulated in the MCAO operated group but it was significantly down-regulated in the voglibose-treated groups which indicates the anti-inflammatory effects of voglibose in cerebral ischemia. In support, treatment with voglibose significantly attenuated the increase in brain hemisphere weight difference in the MCAO operated, voglibose-treated group. Thus, the cerebroprotective effect of voglibose may attribute to the inhibitory activity of pro-inflammatory cytokines that would result in a decrease in cell swelling and associated apoptotic cell death.

5 | CONCLUSION

Our data suggest that voglibose shows the protective effect in cerebrovascular complications as evident from the reduction in serum stroke markers, brain infarct volume, and neurological score, and LDH and CKMB levels. Voglibose also exerts its neuroprotective effect by producing an antioxidant effect by increasing antioxidant enzymes, which are implicated in cerebral ischemia. Voglibose also reduces the C-reactive protein level, a marker of inflammation and tissue injury. Mechanistically, voglibose induces neuroprotection by decreasing Na+-K+ ATPase and aldose reductase activity. The pre-exposure of voglibose in the MCAO operated group implements the down-regulation of Bcl-2 and TNF-α, which plays a vital role in neurodegeneration. In conclusion,

pre-exposure to voglibose attenuates the stroke-induced oxidative stress and neurodegeneration by attenuating activation of the inflammatory and polyol pathway.

CONFLICT OF INTEREST

Authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Vishal Chavda and Pooja Shah (designed, performed, and written drafted manuscript), Snehal Patel (supervised the experiment, edited and revised the final Manuscript), and Shraddha Bhadada (supervised the experiment).

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All supporting data and materials can be accessed at the corresponding author's host institution at Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India-382481.

ORCID

Vishal Chavda https://orcid.org/0000-0002-5812-3412
Snehal S. Patel https://orcid.org/0000-0002-5002-7530

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