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Research Paper

Voglibose and saxagliptin ameliorate the post-surgical stress and cognitive dysfunction in chronic anaesthesia exposed diabetic MCAo induced ischemic rats

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ABSTRACT

Background: Chronic surgical anaesthesia and uncontrolled hyperglycemia are bidirectional risk factors for the development of psychiatric, cerebrovascular, and cardiovascular diseases.
Objective: The current study was designed to elucidate the neuroprotective effects of anti-diabetic agents in pre and post-surgical anaesthesia exposure on diabetic ischemic rats.
Methods: The diabetes type-2 was induced and rats having more than 250 gm/dl blood glucose levels were considered for study. Administration of anaesthetic agents (ketamine 100 mg/kg IP, xylazine 10 mg/kg IP) were done pre and post MCAo surgery for 7 days. The treatment with anti-diabetic agents (voglibose, saxagliptin, repaglinide, dapagliflozin) was carried out after 7 days of Post MCAo surgery for one week. After treatment, assessment of neurobehavioral function was carried out using Morris Water Maze. After that, brains were excised and bloods were collected from all groups subjected for assessment of neuromodulator levels, oxidative stress parameters, serum biochemical biomarkers.
Results: The treatment with voglibose and saxagliptin not only improved neuromodulator levels statistically significant ($p < 0.001$) and cognitive profile but also significantly improved ($p < 0.01$) overall stroke serum biomarkers (Serum Glucose, GGT, CRP, CK-MB, LDH).
Conclusion: The results of the present study, suggested that chronic exposure of anaesthesia worsens the cognition and increases risk of stroke biomarkers in diabetic conditions. We can conclude that voglibose, saxagliptin, and dapagliflozin can significantly improve the postoperative mortality, morbidity, and cognitive dysfunction caused by post-surgical stress and chronic anaesthesia-induced cognitive dysfunction.

1. Introduction

Diabetes is the most common chronic metabolic syndrome in the world, and it is characterized by a number of increasing systemic dysfunctions as well as an increase in risk factors. Diabetics are four times more likely than non-diabetics to suffer from a stroke and the resulting cognitive deterioration. It has also been commonly noted that binge drinking and psychiatric illnesses are linked to diabetes (Munukutla et al., 2016; Kessler et al., 1997). Previous research has demonstrated that the kind and length of anaesthesia have an impact on systemic perioperative hyperglycemia after surgical stress, as well as play a role in post-surgical recovery (Xiong et al., 2020). During surgery, anaesthesia and operation might aggravate diabetic patients' symptoms. Patients with diabetes who have poor blood glucose control and a history of stroke or other brain injury are at risk of developing life-threatening consequences such multi-organ failure, diabetic ketoacidosis, post-operative inflammation, and permanent renal impairment (Wang et al., 2017, Kotagal et al., 2015). Pre-existing hyperglycemias, as well as acute or chronic anaesthetic exposure during surgery, are all known risk factors for post-operative cognitive impairment and delirium, according to previous studies. Poorly Controlled Hyperglycaemia is an independent risk factor post-operative cognitive dysfunction, neurological complications like stroke or vascular encephalitis (Steinmetz and Rasmussen, 2016; Bilotta, Qeva, and Matot, 2016; Sanders, et al., 2011; Wei et al, 2008). Sympathetic nerve activation, which releases catecholamines, cortisol, and glucagon into the bloodstream, can be caused by post-operative stress. This is followed by gluconeogenesis and glycogen breakdown, both of which result in an excess of free sugar in the blood,

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leading to hyperglycemia, which is further fueled by insulin signaling failure (Finnerty et al., 2013). According to previous research, diabetic patients have a poor post-operative prognosis and can cause a variety of secondary pathologies such as wound infection, pneumonia, and a series of cardiac and cerebrovascular events (Finnerty et al., 2013; Palermo et al., 2016). Strict blood glucose control helps to reduce the side effects of surgery, such as cognitive impairment, wound infection, and systemic secondary diseases (Garg et al., 2018; Simha and Shah, 2019). Diabetes risk factors can be reduced by maintaining rigorous glycemic control (Momen et al., 2020; Semenkovich et al., 2015). In a nested case-control study, pioglitazone dramatically reduced cardiovascular events in diabetic stroke patients (Woo et al., 2019). In our previous research reports, Voglibose, a -glucosidase inhibitor, has shown excellent control over postprandial glucose levels, which has a significant impact on stroke risk and outcome (Chavda et al., 2021; Shah et al., 2020). Saxagliptin, a DPP-4 inhibitor, displayed rigorous control of hyperglycemia and stroke risk variables in recent pre-clinical investigations (Vashi et al., 2020; Dhillon, 2015). Repaglinide and dapagliflozin have shown significant reductions in hyperglycemia and associated co-morbidities in pre-clinical investigations (Pishdad et al., 2020). As a result, the goal of this study was to look into the neuroprotective potential of these selected class of anti-diabetic drugs in the context of continuous anaesthetic exposure in hyperglycaemic situations with stroke co-morbidity.

2. Material and methods

2.1. Materials

Voglibose, saxagliptin, dapagliflozin and repaglinide were obtained from Pharmaceutical Industry as a gift sample. Labcare Diagnostics, India, provided various biochemical estimation kits for serum glucose, C reactive protein (CRP), lactate dehydrogenase (LDH), serum creatinine kinase (CK-MB), and Gamma glutamyl transferase (GGT). The other compounds employed in the experiment were of analytical grade.

3. Methods

3.1. Experimental animals

Healthy albino Wistar male rats, 9-10 weeks old, weighing 200–250 gm were used for the study. They were brought from a registered licensed breeder and kept under well-controlled conditions of temperature (22 ± 2 °C), humidity (55 ± 5 %) and 12 h/12 h light-dark cycle keeping three males in a cage. Conventional laboratory diet and filtered water were provided ad libitum. The experiment protocol was authorized by the Institutional Animal Ethics Committee (IAEC) in accordance with the guidelines of the Government of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (IP/PCOL/PHD/27/2020/042). The animals were carried out in 8 groups (n = 6) as normal control, diabetic control, MCAO control, diabetic MCAO control, MCAO operated Voglibose treated, MCAO operated saxagliptin treated, MCAO operated repaglinide treated and MCAO operated saxagliptin treated. All groups were subjected to one week treatment after anesthesia exposure.

3.2. Induction of diabetes and chronic exposure of anesthesia agents

3.2.1. Selection and induction of chronic anaesthesia exposure

Ketamine induction has neuroprotective effects in normal people following ischemic circumstances by applying anti-excitotoxic and anti-inflammatory actions, but it might cause substantial cognitive impairment in diabetic people after orthopaedic or heart surgery (Hudetz et al., 2009). The anesthetic drugs (ketamine 100 mg/kg IP, xylazine 10 mg/kg IP) were given to all groups once a day for 7 days to see how they affected them before and after MCAO surgery.

Induction of diabetes type-2: Dexamethasone injections (1 mg/kg i.m.) were used to induce diabetes for 7 days. The male rats were split into normal, disease control, and therapy groups after diabetes was confirmed (>250 gm/dl glucose).

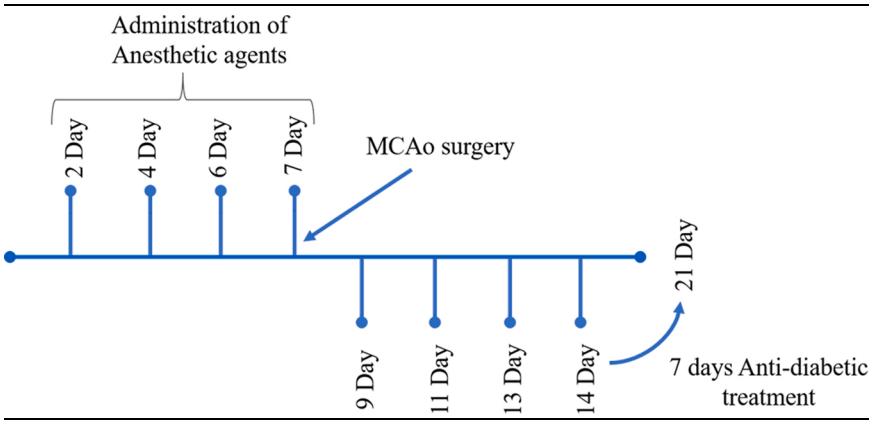
Induction of Ischemia: Rats underwent focal ischemia by transient middle cerebral artery occlusion (tMCAo) by the method described by (Bederson et al., 1986). The animals were anaesthetized using combination of ketamine 100 mg/kg IP, xylazine 10 mg/kg IP, respectively). Animals were placed on the homoeothermic operating table and body temperature was measured using rectal anal probe prior to surgery. The fur of the neck region was shaved to expose the skin. The surgical skin area was disinfected using a 5 % chlorhexidine solution and ethanol 70%. 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 arises from the external carotid artery (ECA) were 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 was closed with sutures (Ethicon-4-0, Absorbable) for 90 minutes followed by reperfusion and monofilament removal. The animals were closed with Absorbable sutures (Ethion-3-4). As a Part of Post operative care, the animals were given 1cc normal saline by oral gavage, analgesics through i.p route and Neomycin ointment at surgical site topically to avoid secondary infection. The animals were placed into a pre-warmed recovery cage with free access to food and water.

MCAO surgery was done to MCAO group, Diabetic MCAO group, Voglibose MCAO group, Saxagliptin MCAO group, Repaglinide MCAO group, Dapagliflozin MCAO group. The treatment with various anti-diabetic agents in respective groups was carried out for 7 days post-surgery (Voglibose 10 mg/kg; Saxagliptin 10 mg/kg; Repaglinide 1 mg/kg; Dapagliflozin 0.5 mg/kg). The treatments were carried out orally once a day. After the treatment, the neurological and neurobehavioral assessment was carried out through manual neurobehavioral scoring. The blood was collected from all groups for various brain biomarkers and biochemical estimation like CRP, GGT, Serum CK-MB, Glucose and LDH. All the animals were sacrificed and brains were excised. Brain homogenates were subjected to evaluation of oxidative stress and neuromodulators levels.

3.2.2. Assessment of neurocognitive performance

Neurobehavioral assessment was made with water maze paradigm. The Mean Escape Latency (MEL) was assessed to evaluate the cognitive deficit. The analysis was divided into 3 phases: Pre-screening, Training and Assessment Phase. Before initiation of each phase of the trials, the maze (Water Maze) was cleaned using 70 % isopropanol prior to the trials to wipe out olfactory stimuli(Davis et al., 2017). The behavioural tracking and MEL score analysis was done with the help of ANY-maze software (Trial Version 6.05).

The Chronic Anesthesia induction in MCAo Set-up and Treatment with Anti-diabetics



3.3. Morris water maze paradigm

The water maze comprises of plastic tank (1.40-meter diameter, 0.60 m of height). It was constructed on a stage in such a manner to form a rectangular shape with size (10 cm x 8 cm). It was filled with water having 25–30 °C to prevent hypothermia in rats(Vorhees and Williams, 2006).

Pre-Screening: The rats were allowed to swim at their liberty into the pool with one platform to be familiar with the pool to improve their swimming speeds as a novel learning phase.

Training Phase: The rats were given 5 days of training in a semi-random sequence of release positions to customize them from the task and also for they do not recognize their definite release order into a pool. The platform was kept 1 cm above the water level in the pool and visual cues were used for the facilitation of learning. Rats that were not able to perform the task on the fifth day were excluded from the study.

Assessment Phase: At the end of the training phase, the rats were subjected to the learning and memory assessment with different simultaneous platforms in the pool. The assessment was made by ANY-maze software with a video tracking device mounted at 250 cm above the centre maze. The rats were released from different four opposite directions into the pool at the intervals of 15 s and were legitimate to locate the platform. The utmost time allowable was one minute subsequent to which rats immobilized to find the platform were tenderly hand-guided to the platform and taken for the next trial. The average time is taken for them to climb a platform was considered as the mean escape latency (MEL) and the average distance covered by the rat to find a platform was considered as the mean path length (MPL). The behavioural analysis was done with the help of ANY-maze software trial version (6.05). The immobile time of rat into MWM was recorded of each group.

3.4. Preparation of brain homogenate

The rats were sacrificed under an overdose of diethyl ether as euthanasia. After confirmation of no palpation and no respiratory activity, rat brains were subjected to excised and perfused with ice-cold saline. Whole brains were subjected to homogenize with 10 % ice-cold phosphate-buffered saline and were centrifuged at 10,000 rpm at –4 °C for 15 min. The supernatant and homogenate was collected in a centrifuge tube and were kept at 4 °C temperature. Obtained supernatant and homogenate were subjected to various antioxidant parameters and neuromodulators assessment.

4. Assessment of neuromodulators levels

4.1. Assessment of dopamine and serotonin level

4.1.1. Dopamine level

Estimation of dopamine from each rat brain tissue was carried out as per standard protocol from previous research studies. 100 mg tissue of each brain rat was weighed and homogenized in 5 ml HCl-butanol for 1 min followed by centrifugation at 2000 rpm for 10 min 1 ml supernatant was added to the mixture of 2.5 ml heptane and 0.31 ml 0.1 M HCl followed by centrifugation at 2000 rpm for 2 min. Form the centrifugation, 0.2 ml of the aqueous phase was aliquoted and added into a mixture of 0.05 ml 0.4 M HCl and 0.1 ml acetate buffer followed by the addition of iodine solution (0.1 ml 0.1 M in ethanol) for the oxidation process. After 2 min of incubation time, 0.1 ml of Na_2SO_3 solution and 0.1 ml Acetic acid was added after 1.5 min. The solution was heated to 100 °C for 6 min and then allowed to cool down at room temperature to obtain absorbance at 330–375 nm in spectrofluorometer. The calculation of the dopamine level was carried out as per previous research reports(Schlumpf et al., 1974).

4.1.2. Serotonin level

For the assessment of serotonin level, 0.2 ml of O-phthaldialdehyde (OPT) reagent(20 mg in 100 ml conc. HCl) was added into the 0.2 ml of the aqueous phase of each rat brain extract and heated at 100 °C for 10 min to develop fluorophore. Once the solution gets cool down at room temperature, the absorbance was taken at 360–470 nm in the spectrofluorometer. Tissue blanks for Dopamine were prepared by adding the reagents of the oxidation step in reversed order and for serotonin tissue blank, 0.25 ml cont. HCI without OPT was added. Standard solution (500 μg/ml) of dopamine and serotonin was prepared using distilled water and HCl-butanol (1:2 ratio). The calculation of the serotonin level was carried out as per previous research reports (Schlumpf et al., 1974).

4.1.3. Blood collection for the assessment of serum biomarkers

For the blood collection, the rat from each group was restrained in a restrainer, the neck gently scuffed and the eye made to bulge. A capillary tube is inserted medially, laterally, or dorsally. Blood was allowed to flow by capillary action into the capillary tube/pipette, ensure proper aseptic condition. After collection of the whole blood, it was allowed to clot by leaving it undisturbed at room temperature for 30 min. Then it was centrifuged at 10,000 RPM for 10 min in a cooling refrigerated centrifuge. The resulting clear white supernatant was collected for various serum biochemical parameters.

4.2. Assessment of serum biochemical biomarkers

4.2.1. Estimation of serum glucose

One ml of the working solution was added to the test tube containing 10 µl of the serum sample. Similarly, standard and blank were prepared by using 10 µl of glucose standard (provided in the kit) and distilled water respectively. They were then mixed and incubated at room temperature for 30 min (endpoint reaction). The absorbance of test and standard was measured against blank at 505 nm using UV-Visible spectrophotometer (UV-1601 Shimadzu, Japan).

4.2.2. Estimation of CRP levels

Working reagent was prepared by adding 9 ml of diluent into 1 ml of latex reagent as per kit manual. Bring all the kit reagents (CRP Calibrator, Diluent (R1), Latex (R2)) to a room temperature (37 °C) before experiment. Assay conditions were: Wavelength 540 nm, Temperature 37 °C and 1 cm cuvette path length. Adjust the spectrophotometer to zero with distilled water. 10 µl serum samples were added to 1 ml of working reagent and absorbance were taken immediately after 4 min of incubation at 540 nm. The results for each group were calculated as CRP concentration in the sample by interpolation of its (A2- A1) in the calibration curve from given standard curve.

4.2.3. Estimation of serum CK-MB levels

One ml of the working solution was added to the test tube containing 50 µl of serum sample. Similarly standard and blank were prepared by using 50 µl of standard (provided in the kit) and distilled water respectively. The contents were mixed and incubated for 10 min at R.T. and change in absorbance was measured every minute for the next 5 min at 340 nm using UV-Visible spectrophotometer (UV-1601 Shimadzu, Japan).

4.2.4. Estimation of GGT levels

The estimation of GGT was done as per user manual given provided into the kit. One ml of substrate reagent and four ml of buffer regent were mixed to produce working reagent as per provided working manual kit. 100 µl of serum sample were added to the 1 ml of the prepared working reagent. The final mixture were gently mixed well and incubated for 60 s at normal room temperature (37 °C). The contents were mixed and absorbance of the sample (AT) and standard (AS) against reagent blank was measured at 405 nm for 1,2 and 3 min continuously using UV-Visible spectrophotometer (UV-1601 Shimadzu, Japan). Gamma-glutamyl is transfer red from gamma-glutamyl-p-nitroanilide to glycylglycine by Gamma-GT. The p-nitroaniline formed absorbs at 405 nm. The amount of p-nitroaniline formed is directly proportional to GGT activity. The estimated GGT is noted as U/l GGT.

Estimation of Serum LDH: One ml of the working solution was added to the test tube containing 25 µl of serum sample. Similarly standard and blank were prepared by using 25 µl of standard (provided in the kit) and distilled water respectively. The contents were mixed and incubated for 1 min at R.T. and measured the change in absorbance per min. (Δ A/min.) for the next 2 min at 340 nm using a UV-Visible spectrophotometer (UV-1601 Shimadzu, Japan).

Assessment of Oxidative stress: The Collected cool stored brain tissues were weighed and ipsilateral region were separated. 10 % w/v homogenate were prepared using 500 mg of ipsilateral part of each brain in 0.2 M phosphate buffer saline (PH 7.5). The tissue was homogenized with 25 strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. Half portion of homogenate was taken for estimation of MDA level before centrifugation at 10,000 rpm for 10 min at room temperature. Supernatant was used for the estimation of reduced glutathione,nitrite level and super oxide dismutase.

Estimation of MDA: Malondialdehyde formation was estimated by the method of Ohkawa. The method estimates MDA, a product of lipid peroxidation process. One molecule of MDA reacts with two molecules of thiobarbituric acid (TBA) under mildly acidic conditions to form a pink coloured chromogen, which intensity was measured colorimetrically at 535 nm (Ohkawa et al., 1979).

Estimation of GSH: Reduced of glutathione (GSH) was estimated by the method of Moran.Glutathione present in RBC consist of sulfhydryl groups. 5,5 dithiobis 2- nitro benzoic acid (DTNB), A disulphide compound, gets readily attacked by these sulfhydryl groups and forms a yellow coloured anion which measured colorimetrically at 412 nm (Moron et al., 1979).

Estimation of Nitrite: Determination of nitrite level in left ventricle was performed according to method described by Green. The measurement of nitrite content was done by the Griess reaction, by adding 100 µl of Griess reagent (1 % sulphanilamide, 0.1 % naphthyl ethylene diamine dihydrochloride in 2 % H3PO4) to 100 µl of samples. Griess reagent converts nitrite into a deep purple azo compound. Photometric measurement of the absorbance at 550 nm due to this azochromophore accurately determines NO^{2-} concentration. Nitrite concentration was calculated by comparison with absorbance at 550 nm of standard nitrite solution. Results are expressed as µmol/mg protein(Green et al., 1982).

4.3. Estimation of SOD

SOD was estimated by the method of Kono.The O^{2-}, substrate for SOD is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine.The assessment of superoxide dismutase was carried out as per Soni, 2014. 0.2 ml of brain homogenate's supernatant sample from each group were well mixed with 0.1 ml of EDTA, 0.5 ml carbonate buffer, and 1 ml of epinephrine. The absorbance was read against the blank at an interval of 30 s for 3 min at 480 nm. The SOD level was calculated using the standard curve which was plotted using standard SOD (MP Bio, USA) (Kono, 1978).

4.4. Statistical analysis

The data were expressed as mean ± SEM of 6 animals, as per animal protocol and groups. Comparisons among groups were performed by one-way ANOVA followed by Tukey's multiple comparisons post-hoc-test. The significant differences were established at $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. All statistics were done using GraphPad Prism® software (version 5.01, California, USA).

5. Results

5.1. Effects of anti-diabetic agents on learning, memory and mobility in Morris water maze after anaesthesia exposure

5.1.1. Mean escape latency

We reported a significant delay in spatial memory and learning and increased mean escape latency in diabetic MCAo control as compared to diabetic control, MCAo control. We provide evidence that, the acute exposure of anaesthesia increased dementia and cognitive decline associated with diabetic and MCAo insult. We also observed a quick increase in escape latency in diabetic MCAo control group. Voglibose and saxagliptin treatment improved the escape latency time in diabetic MCAo operated groups compared to diabetic MCAo control group. Treatments with repaglinide and dapagliflozin were found to be not much effective in improving mean escape time in diabetic MCAo operated treated groups (Fig. 1a).

5.1.2. Mean immobility time

Immobility time during the Morris water maze is also considered as the impact of cognitive decline and depression. The chronic anaesthesia impacted on learning and memory and exposure over brain insult fasten the neurodegenerative process. In our study, we observed increased mean immobile time in diabetic control, MCAo control and diabetic MCAo control group compared to normal control. The increased immobility was found to be in this order: Diabetic MCAo control>MCAo

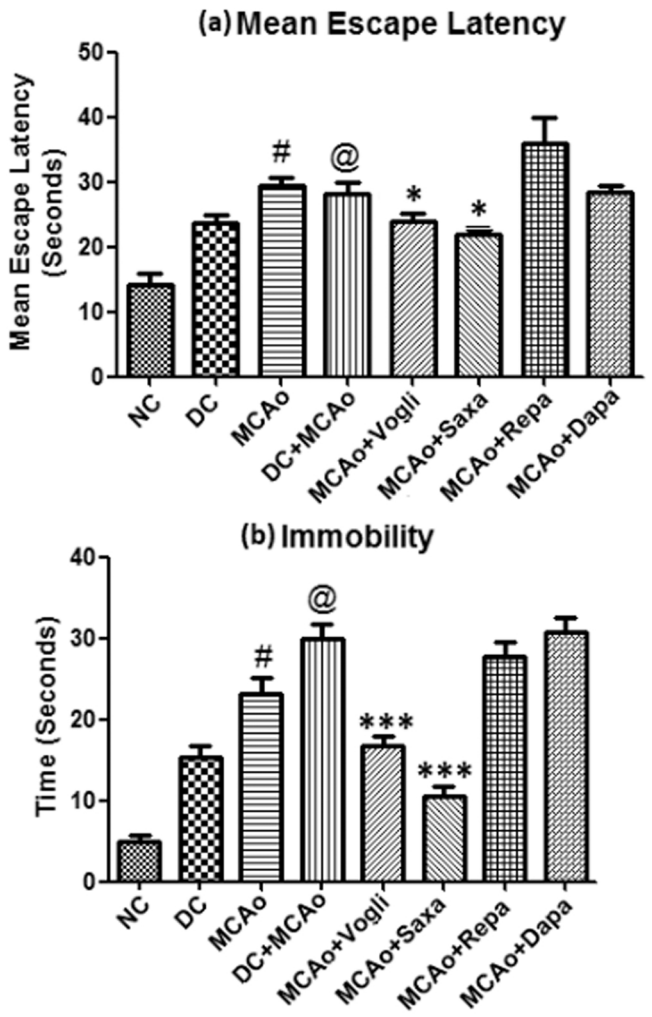


Fig. 1. Effects of anti-diabetic agents on learning, memory and mobility in Morris water maze after anaesthesia exposure: (a) Mean escape latency; (b) Immobility. # significantly different from normal control, @ significantly different from normal control, diabetic control and MCAo control. Values of significance are expressed as: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM of 6 animals. NC: Normal Control; DC: Diabetic Control; MCAo: Middle cerebral artery occlusion operated; DC+MCAo: Diabetic MCAo operated; MCAo+Vogli: Diabetic MCAo operated Voglibose treated (10 mg/kg oral); MCAo+Saxa: Diabetic MCAo operated Saxagliptin treated (10 mg/kg oral); MCAo+Repa: Diabetic MCAo operated Repaglinide treated (1.0 mg/kg oral); MCAo+Dapa: Diabetic MCAo operated Dapagliflozin treated (0.5 mg/kg oral).

control> Diabetic control. Treatment with anti-diabetic agents significantly improved mobility and reduced mean immobility time in voglibose and saxagliptin treated group compared to diabetic MCAo control. The anti-diabetic treatments with repaglinide and dapagliflozin were found to be less or not effective in improving mobile time compared to diabetic MCAo control (Fig. 1b).

5.2. Effects of anti-diabetic agents on neuromodulators levels on anaesthesia exposed rats

5.2.1. Serotonin

From our study, it was observed that chronic exposure of anaesthesia to diabetic rats, MCAo operated rats and diabetic MCAo operated rats reduced the brain dopamine levels compared to normal control. The diabetic MCAo operated anaesthesia exposed rats showed significant decrease in brain serotonin levels among all groups. Post treatment with all anti-diabetic agents showed improved brain serotonin levels compared to diabetic MCAo operated anaesthesia exposed rats. Among them, dapagliflozin was found to be very significant ($p < 0.001$) in improving the brain serotonin levels compared to diabetic MCAo operated anaesthesia exposed rats. Voglibose and saxagliptin treated group also improved ($p < 0.01$) brain serotonin levels compared to disease control. Anti-diabetic agent, repaglinide was found to be least effective in improving the brain serotonin levels (Fig. 2a).

5.3. Dopamine

Chronic anaesthesia exposure to rats induced significant decrease in brain dopamine levels in all groups. Diabetic control, MCAo control and diabetic MCAo control groups showed significant reduction ($p < 0.01$) in brain dopamine levels compared to normal control groups. Post treatment with anti-diabetic agents showed improved brain dopamine levels in chronic anaesthesia exposed rats. Among all, saxagliptin and dapagliflozin were found to be very significant ($p < 0.001$) in improving brain dopamine levels compared to disease control (chronic anaesthesia exposed diabetic MCAo rats). Voglibose and repaglinide treated groups were found to be least significant ($p < 0.05$) among all anti-diabetic drug treatment compared to disease control (Fig. 2b).

5.4. Effects of anti-diabetic agents on serum biomarkers upon chronic exposure of anaesthesia in rats

5.4.1. Serum glucose

From our experimental data, we observed a significant elevation in serum glucose levels in all groups upon chronic exposure of anaesthesia. We found significant elevation ($p < 0.001$) in serum glucose levels in diabetic MCAo operated chronic anaesthesia treated groups compared to normal control groups. Post treatment with voglibose and saxagliptin showed reduced ($p < 0.01$) serum glucose levels compared to disease control (diabetic MCAo operated chronic anaesthesia treated group). Post treatment with repaglinide and dapagliflozin treated groups showed very poor glucose control ($p < 0.05$) compared to disease control. So, overall we can conclude that, chronic exposure of anaesthetic agents reduced the anti-diabetic potential of anti-diabetic drugs in diabetic rats and diabetic MCAo operated rats (Fig. 3a).

5.5. Serum CRP levels

The chronic exposure of anaesthesia induced significant elevation in serum CRP levels in all diabetic and diabetic MCAo groups. We found significantly elevated serum CRP levels in diabetic MCAo operated group compared to normal control and among all groups. Post treatment with voglibose and saxagliptin significantly reduced ($p < 0.001$) serum CRP levels compared to disease control group. Post treatment with serum dapagliflozin and repaglinide also reduced ($p < 0.01$) serum CRP levels compared to disease control (Fig. 3b).

5.6. Serum CK-MB levels

In our study, we found significantly elevated levels of serum CK-MB in MCAo operated and diabetic MCAo operated chronic anaesthesia treated groups. Post treatment with all anti-diabetic agents induced significant reduction in serum CK-MB levels compared to disease control group. Post treatment with dapagliflozin showed significant reduction in serum CK-MB levels ($p < 0.001$) compared to all anti-diabetic agents and disease control group (Fig. 3c).

5.7. Serum GGT levels

From our experimental data, we observed significant elevation ($p < 0.001$) in serum GGT levels in MCAo control and diabetic MCAo operated chronic anaesthesia treated groups compared to normal

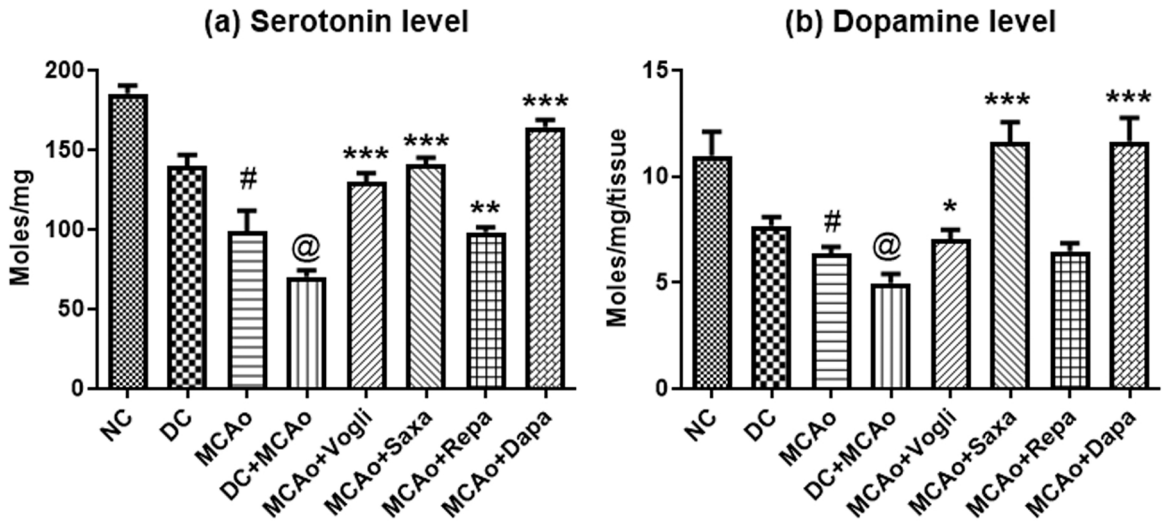


Fig. 2. Effects of anti-diabetic agents on neuromodulators on anesthesia exposed rats. (a) Serotonin levels and (b) Dopamine levels. # significantly different from normal control, @ significantly different from normal control, diabetic control and MCAo control. Values of significance are expressed as: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM of 6 animals. NC: Normal Control; DC: Diabetic Control; MCAo: Middle cerebral artery occlusion operated; DC+MCAo: Diabetic MCAo operated; MCAo+Vogli: Diabetic MCAo operated Voglibose treated (10 mg/kg oral); MCAo+Saxa: Diabetic MCAo operated Saxagliptin treated (10 mg/kg oral); MCAo+Repa: Diabetic MCAo operated Repaglinide treated (1.0 mg/kg oral); MCAo+Dapa: Diabetic MCAo operated Dapagliflozin treated (0.5 mg/kg oral).

control. Post treatment with all anti-diabetic agents showed significant reduction in serum GGT levels compared to normal control. Voglibose and dapagliflozin treated groups showed similar reduced levels in all anti-diabetic agents compared to diabetic MCAo operated treated group (Fig. 3d).

5.8. Serum LDH levels

From our data, it was reported that chronic exposure of anaesthesia in diabetic rats and diabetic MCAo operated rats induced significant elevation ($p < 0.001$) in serum LDH levels compared to normal control.

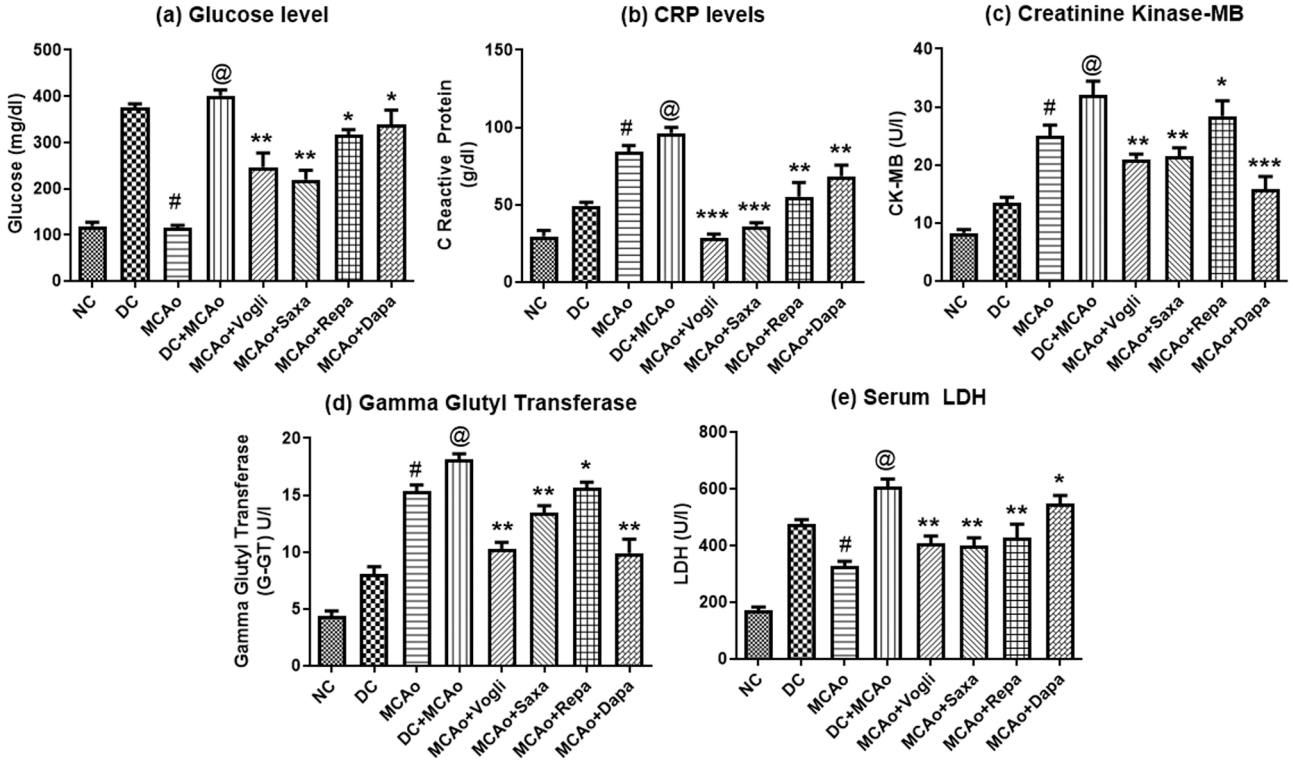


Fig. 3. Effects of anti-diabetic agents on serum biochemical biomarkers upon chronic exposure of anesthesia in rats. (a) Glucose level; (b) CRP levels; (c) CK-MB levels; (d) GGT levels; (e) LDH levels. # significantly different from normal control, @ significantly different from normal control, diabetic control and MCAo control. Values of significance are expressed as: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM of 6 animals. NC: Normal Control; DC: Diabetic Control; MCAo: Middle cerebral artery occlusion operated; DC+MCAo: Diabetic MCAo operated; MCAo+Vogli: Diabetic MCAo operated Voglibose treated (10 mg/kg oral); MCAo+Saxa: Diabetic MCAo operated Saxagliptin treated (10 mg/kg oral); MCAo+Repa: Diabetic MCAo operated Repaglinide treated (1.0 mg/kg oral); MCAo+Dapa: Diabetic MCAo operated Dapagliflozin treated (0.5 mg/kg oral).

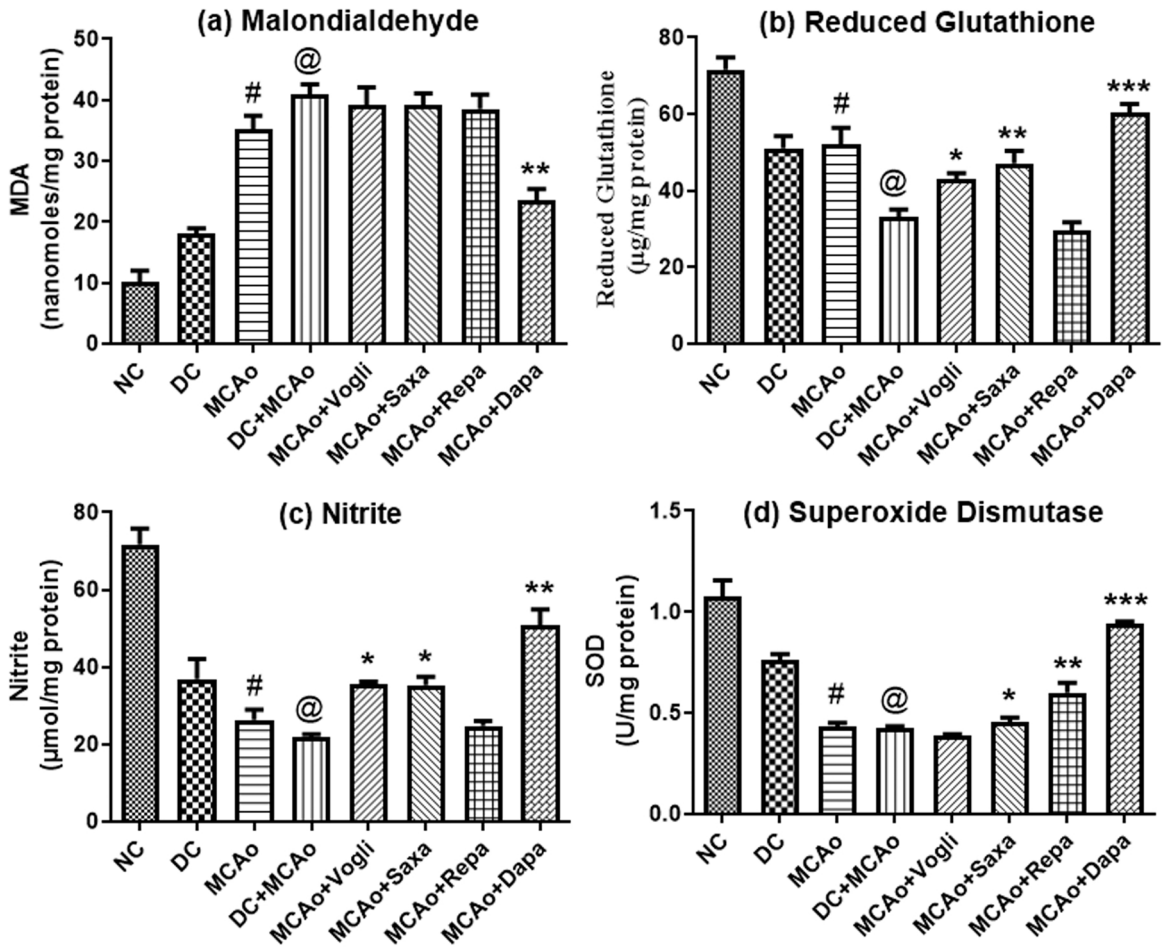


Fig. 4. Effects of anti-diabetic agents on oxidative stress markers upon chronic exposure of anesthesia in rats. (a) Malondialdehyde levels; (b) Reduced glutathione levels; (c) Nitrite levels; and (d) Superoxide dismutase levels. # significantly different from normal control, @ significantly different from normal control, diabetic control and MCAo control. Values of significance are expressed as: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. Values are expressed as Mean ± SEM of 6 animals. NC: Normal Control; DC: Diabetic Control; MCAo: Middle cerebral artery occlusion operated; DC+MCAo: Diabetic MCAo operated; MCAo+Vogli: Diabetic MCAo operated Voglibose treated (10 mg/kg oral); MCAo+Saxa: Diabetic MCAo operated Saxagliptin treated (10 mg/kg oral); MCAo+Repa: Diabetic MCAo operated Repaglinide treated (1.0 mg/kg oral); MCAo+Dapa: Diabetic MCAo operated Dapagliflozin treated (0.5 mg/kg oral).

The diabetic MCAo operated chronic anaesthesia treated groups showed significantly elevated levels of serum LDH compared to normal control. Post treatment with all anti-diabetic agents reduced the levels of serum Dapagliflozin compared to disease control group (Fig. 3e).

5.9. Effects of anti-diabetic agents on oxidative stress markers upon chronic exposure of anaesthesia in rats

5.9.1. MDA levels

As per our experimental data, we found significant elevated levels of brain MDA levels in all diabetic, MCAo operated and diabetic MCAo operated chronic anaesthesia operated groups compared to normal control. Post treatment with anti-diabetic agents did not show any significant effects in reducing brain MDA levels except dapagliflozin ($p < 0.01$) (Fig. 4a).

5.9.2. GSH levels

In our study, upon chronic anaesthesia exposure brain glutathione levels get significantly reduced in diabetic control, MCAo control and diabetic MCAo control group compared to normal control group. Post treatment with all anti-diabetic agents except repaglinide showed significant improvement in brain glutathione levels compared to disease control group (Fig. 4b).

5.10. Nitrite levels

From our data, it was observed that chronic exposure of anaesthesia significantly reduces brain nitrite levels compared to normal control in diabetic MCAo and MCAo control groups. Voglibose and saxagliptin post treated group showed increased ($p < 0.01$) brain nitrite levels compared to diabetic MCAo operated group. Dapagliflozin treated group showed significantly improved ($p < 0.001$) nitrite levels compared to disease control group and also among all anti-diabetic drug treatments (Fig. 4c).

5.11. SOD levels

Chronic exposure of anaesthesia in diabetic, diabetic MCAo and MCAo operated rats induced significant decrease in brain SOD activity compared to normal control group. Post treatment with all anti-diabetic agents except voglibose and saxagliptin significantly improved brain SOD levels (repaglinide $p < 0.01$; dapagliflozin $p < 0.001$). Post treatment with voglibose and saxagliptin was found to be very less effective or no effective in improving brain SOD levels (Fig. 4d).

6. Discussion

Diabetics are routinely subjected to procedures and are exposed to a variety of anaesthetic drugs, some of which are neurodegenerative.

Furthermore, persistent anaesthetic exposure has been extensively confirmed in earlier study publications as causing progressive neurotoxicity following repeated exposures. As a result of metabolic imbalance, diabetes creates altered brain pathways and decreased neuronal signalling; persistent anaesthetic exposure in diabetics may aggravate diabetes and associated risk factors for stroke and cognitive decline (Belrose and Noppens, 2019). The goal of this study was to see how anti-diabetes medicines affected diabetic MCAo rats' prolonged anaesthetic exposure. In diabetic MCAo rats, the Morris Water Maze was utilized to examine cognitive impairment linked with anaesthesia and hyperglycemia. We discovered that diabetic rats under persistent anaesthesia had a longer mean escape delay and immobility period in our investigation. After treatment with voglibose and saxagliptin, the mean escape latency and immobility time were substantially reduced. Furthermore, as compared to the disease control group, the average mean path length walked by rats with voglibose and saxagliptin was shown to be shorter. The therapy with repaglinide or dapagliflozin had no effect on mean escape latency or immobility time. Voglibose and saxagliptin modify depressed behaviour and improve cognition in anaesthesia-exposed MCAo mice, according to the findings of this study.

We intended to explore how anti-diabetic medications affected altered neuromodulation after evaluating the neurobehavioral benefits of anti-diabetic medicines in the Morris Water Maze paradigm. Chronic anaesthetic exposure produces neuronal damage and neurodegeneration, which is a well-known mechanism (Belrose and Noppens, 2019). Diabetes affects metabolic brain function, impairing neuromodulation in a variety of ways and ultimately to cognitive deterioration (Sun et al., 2020). We wanted to see how anti-diabetic drugs affected anaesthetic exposure since behavioural alterations are inversely related to brain neuromodulation. Cognitive and reward functions are linked to dopamine and serotonin. A well-balanced serotonin and dopamine system function in parallel to facilitate the brain's memory and learning processes (Bang et al., 2020). We found a significant drop in serotonin and dopamine levels in diabetic MCAo operated rats exposed to protracted anaesthesia in our research. After treatment, all anti-diabetic drugs improved serotonin and dopamine levels significantly. It is undeniable that all anti-diabetic drugs have a positive effect on neuromodulation. Thus, the investigation of serotonin and dopamine levels corroborate the behavioural findings because both the modulators plays crucial for more effective interventions of neuropsychiatric conditions.

We investigated into biochemical indicators linked with persistent anaesthetic drug exposure in diabetic stroke patients after screening anti-diabetic medications. Stroke symptoms are exacerbated by diabetes and hyperglycaemia. Cognitive decline and neurodegeneration are further exacerbated by anaesthesia and high blood sugar levels (Akoudad et al., 2016). In our investigation, diabetic MCAo rats exposed to continuous anaesthesia had significantly raised serum glucose levels, which were effectively lowered by post-treatment with all anti-diabetic medications. As a result, we can conclude that reducing serum glucose levels provides neuroprotection. CRP is an important indicator of inflammation and possible tissue damage in the blood. Inflammation and inflammatory marker activation are influenced by both hyperglycaemia and persistent anaesthetic exposure (Chavda et al., 2021; Belrose and Noppens, 2019). In our research, we discovered that diabetic MCAo rats exposed to continuous anaesthesia had considerably higher serum CRP levels, which were significantly lowered by all anti-diabetic drugs after therapy. As a result, we can extrapolate that anti-diabetes drugs have substantial anti-inflammatory properties, resulting in neuroprotective behavior in diabetic MCAo rats exposed to anaesthesia. Cardiovascular problems are caused by hyperglycemia and long-term anaesthetic exposure, which lead to cerebrovascular events (Roizen, 2004). In this study, we looked at serum CK-MB levels for cardiovascular research and discovered that diabetic MCAo operated groups had significantly higher levels, which were significantly lowered by post-treatment with all anti-diabetic drugs. Anti-diabetic medicines give cardiovascular protection during protracted anaesthetic exposure in diabetic MCAo operated rats, according to the findings. GGT, or Gamma Glutamyl Transferase, has been associated to catastrophic cardiovascular risk factors in diabetics in previous studies. In diabetics, it has also been associated to cerebrovascular events and cognitive deterioration (Komuroglu et al., 2018). In our study, we reported that diabetic MCAo rats exposed to continuous anaesthesia had significantly increased GGT levels, which were significantly lowered by all anti-diabetes therapy. LDH induces cardiovascular and cerebrovascular damage in hyperglycemic patients and worsens stroke pre and post stroke conditioning, according to earlier study (Shah et al., 2020). LDH levels in the diabetic MCAo group were four times greater than normal controls, according to our findings. Chronic anaesthesia appears to accelerate not just the neurodegenerative process but also increases cardiac and cerebrovascular risk factors markedly. After treatment, all anti-diabetic drugs significantly lower serum LDH levels. All anti-diabetic drugs, according to the findings of the previous study, exhibit cardioprotective and cerebroprotective effects. Furthermore, we could hypothesize that all anti-diabetic agents improve cardiac serum indicators, contributing in neuroprotection.

After neuromodulation and behavioural investigation, we aim to explore into oxidative stress in diabetic MCAo operated anaesthetic treated groups. Diabetics who are exposed to anesthetics experience higher oxidative stress and cognitive impairment (Ghoneim and Block, 2012). In diabetic MCAo rats exposed to continuous anaesthesia, we studied the effects of anti-diabetes medications on malondialdehyde, reduced glutathione, nitrite, and superoxide dismutase levels. Higher MDA levels, lower glutathione levels, lower nitrite levels, and lower superoxide dismutase levels were detected in disease control. All anti-diabetic drugs, with the exception of dapagliflozin, did not reduce MDA levels following treatment. Furthermore, post-treatment with voglibose, saxagliptin, and dapagliflozin significantly improved reduced glutathione levels, whereas repaglinide treatment was ineffective, suggesting that voglibose, saxagliptin, and repaglinide may have an anti-oxidant role in anaesthesia-induced stress and neurodegeneration. Cellular damage and degeneration are caused by a nitrite deficiency. Diabetes causes persistent neuronal death by impairing metabolic cell-cell function. Voglibose, saxagliptin, and dapagliflozin significantly reduced nitrite levels in the disease control group and significantly increased nitrite levels in the anaesthesia-exposed diabetic groups, which were significantly improved by voglibose, saxagliptin, and dapagliflozin, according to our findings. In a diabetic group that had been treated to persistent anaesthesia, repaglinide had no effect on nitrite levels. Voglibose, saxagliptin, and dapagliflozin all appear to have anti-oxidant characteristics through raising nitrite levels. As a consequence of mitochondrion respiration, Increased Reverse Electron Transport (RET) SOD levels and activity are key contributors to oxidative stress and can be exceedingly harmful to proper cell-cell function (Clausen et al., 2010) (Chavda et al., 2022). In our research, we observed significant decrease in SOD activity in diabetic MCAo group which had been subjected to persistent anaesthesia, that was greatly alleviated by dapagliflozin, repaglinide, and saxagliptin. Voglibose, on the other hand, was found to be ineffective in improving brain SOD activity in diabetic MCAo group. In our study, We found that dapagliflozin, repaglinide, and saxagliptin have anti-oxidant activity by increasing SOD activity.

7. Conclusion

Results of our findings revealed that persistent anaesthetic exposure deteriorates cognitive profile and increases the risk of stroke biomarkers which can be improved by treatment with voglibose and saxagliptin. In addition, behavioural studies demonstrated that both medications were effective in reducing neurodegeneration and depressive-like behaviour. In the post-operative anaesthetic exposed neurodegenerative brain microenvironment, voglibose and saxagliptin, also showed a strong

neuroprotective property. Thus, we may conclude that anti diabetic drugs can reduce post-operative mortality, morbidity, and cognitive dysfunction caused by post-operative stress and persistent anaesthesia-induced cognitive dysfunction considerably and could be considered for clinical translation as a promising treatment.

CRediT authorship contribution statement

Vishal Chavda: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Snehal Patel:** Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Data Availability

The data will be available on reasonable request.

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Author contributions

All authors contributed in execution of the work and preparation of this manuscript.

Consent to participate

All authors participated in this work.

Consent for publication

All authors consent to publish this work.

Conflict of interest

All authors of this manuscript have no any conflict of interest to declare.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2022.10.009.

References

Akoudad, S., Wolters, F.J., Viswanathan, A., De Bruijn, R.F., Van Der Lugt, A., Hofman, A., Vernooij, M.W., 2016. Association of cerebral microbleeds with cognitive decline and dementia. JAMA Neurol. 73 (8), 934–943. https://doi.org/10.1001/jamaneurol.2016.1017.

Bang, D., Kishida, K.T., Lohrenz, T., White, J.P., Laxton, A.W., Tatter, S.B., Montague, P. R., 2020. Sub-second dopamine and serotonin signaling in human striatum during perceptual decision-making. Neuron 108 (5), 999–1010. https://doi.org/10.1016/j.neuron.2020.09.015 e6.

Bederson, J.B., Pitts, L.H., Tsuji, M., Nishimura, M.C., Davis, R.L., Bartkowski, H., 1986. Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. Stroke 17 (3), 472–476. https://doi.org/10.1161/01.STR.17.3.472.

Belrose, J.C., Noppens, R.R., 2019. Anesthesiology and cognitive impairment: a narrative review of current clinical literature. BMC Anesthesiol. BioMed. Cent. Ltd. https://doi.org/10.1186/s12871-019-0903-7.

Bilotta, F., Qeva, E., Matot, I., 2016. Anesthesia and cognitive disorders: a systematic review of the clinical evidence. Expert Review of Neurotherapeutics. Taylor and Francis Ltd. https://doi.org/10.1080/14737175.2016.1203256.

Chavda, V., Shah, P., Patel, S.S., Bhadada, S., 2021. Pre-exposure of voglibose exerts cerebroprotective effects through attenuating activation of the polyol pathway and inflammation. Eur. J. Neurosci. 53 (8), 2541–2552. https://doi.org/10.1111/ejn.15151.

Chavda, V., Chaurasia, B., Garg, K., Deora, H., Umana, G.E., Palmisciano, P., Scalia, G., Lu, B., 2022. Molecular mechanisms of oxidative stress in stroke and cancer. Molecular mechanisms of oxidative stress in stroke and cancer 5, 100029. https://doi.org/10.1016/j.dscb.2021.100029.

Clausen, A., Doctrow, S., Baudry, M., 2010. Prevention of cognitive deficits and brain oxidative stress with superoxide dismutase/catalase mimetics in aged mice. Neurobiol. Aging 31 (3), 425–433. https://doi.org/10.1016/j.neurobiolaging.2008.05.009.

Davis, K.E., Burnett, K., Gigg, J., 2017. Water and T-maze protocols are equally efficient methods to assess spatial memory in 3xTg Alzheimer's disease mice. Behav. Brain Res. 331, 54–66. https://doi.org/10.1016/j.bbr.2017.05.005.

Dhillon, S., 2015. Saxagliptin: A Review in Type 2 Diabetes. Drugs. Springer International Publishing. https://doi.org/10.1007/s40265-015-0473-z.

Finnerty, C.C., Mabvuure, N.T., Kozar, R.A., Herndon, D.N., 2013. The surgically induced stress response. J. Parenter. Enter. Nutr. 37, 21S–29S. https://doi.org/10.1177/0148607113496117.

Garg, R., Schuman, B., Bader, A., Hurwitz, S., Turchin, A., Underwood, P., Lortie, M., 2018. Effect of preoperative diabetes management on glycemic control and clinical outcomes after elective surgery. Ann. Surg. 267 (5), 858–862. https://doi.org/10.1097/SLA.0000000000002323.

Ghoneim, M.M., Block, R.I., 2012. Clinical, methodological and theoretical issues in the assessment of cognition after anaesthesia and surgery: a review. Eur. J. Anaesthesiol. https://doi.org/10.1097/EJA.0b013e328356bd6e.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R. , 1982. Analysis of Nitrate, Nitrite, and [15N] Nitrate in Biological Fluids Automated NO; and NO ? Analysis. *Analysis*, 126(1), 131–138. Retrieved from ⟨https://www.researchgate.net/publication/313426110⟩.

Hudetz, J.A., Iqbal, Z., Gandhi, S.D., Patterson, K.M., Byrne, A.J., Hudetz, A.G., Warltier, D.C., 2009. Ketamine attenuates post-operative cognitive dysfunction after cardiac surgery. Acta Anaesthesiol. Scand. 53 (7), 864–872. https://doi.org/10.1111/j.1399-6576.2009.01978.x.

Kessler, R.C., Crum, R.M., Warner, L.A., Nelson, C.B., Schulenberg, J., Anthony, J.C., 1997. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the national comorbidity survey. Arch. Gen. Psychiatry 54 (4), 313–321. https://doi.org/10.1001/archpsyc.1997.01830160031005.

Komuroglu, A.U., Yur, F., Ekin, I.H. , 2018. Influence of lycopene administration on neopterin, myeloperoxidase and gamma glutamyl transferase in diabetic rats. *Fresenius Environmental Bulletin*, 27(6), 4285–4292. Retrieved from ⟨https://www.researchgate.net/publication/329209094⟩.

Kono, Y., 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 186 (1), 189–195. https://doi.org/10.1016/0003-9861(78)90479-4.

Kotagal, M., Symons, R.G., Hirsch, I.B., Umpierrez, G.E., Dellinger, E.P., Farrokhi, E.T., Flum, D.R., 2015. Perioperative hyperglycemia and risk of adverse events among patients with and without diabetes. Ann. Surg. 261 (1), 97–103. https://doi.org/10.1097/SLA.0000000000000688.

Momen, N.C., Plana-Ripoll, O., Agerbo, E., Benros, M.E., Børglum, A.D., Christensen, M. K., McGrath, J.J., 2020. Association between mental disorders and subsequent medical conditions. N. Engl. J. Med. 382 (18), 1721–1731. https://doi.org/10.1056/nejmoa1915784.

Moron, S.M., Depierre, W.J., Mannervik, B. , 1979. Levels of glutathione-S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, 582(April), 67–78. Retrieved from ⟨https://www.researchgate.net/publication/281266409⟩.

Munukutla, S., Pan, G., Deshpande, M., Thandavarayan, R.A., Krishnamurthy, P., Palaniyandi, S.S., 2016. Alcohol toxicity in diabetes and its complications: a double trouble. Alcoholism: Clinical and Experimental Research. Blackwell Publishing Ltd. https://doi.org/10.1111/acer.13008.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95 (2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Palermo, N.E., Gianchandani, R.Y., McDonnell, M.E., Alexanian, S.M., 2016. Stress hyperglycemia during surgery and anesthesia: pathogenesis and clinical implications. Curr. Diabetes Rep. https://doi.org/10.1007/s11892-016-0721-y.

Pishdad, R., Pishdad, P., Pishdad, G.R., 2020. Acarbose versus repaglinide in diabetes treatment: a new appraisal of two old rivals. Am. J. Med. Sci. 359 (4), 212–217. https://doi.org/10.1016/j.amjms.2020.01.011.

Roizen, M.F., 2004. Anesthetic implications of diabetes. Rev. Mex. De. Anestesiol. Vol. 27, 9–18.

Sanders, R.D., Pandharipande, P.P., Davidson, A.J., Daqing, M., Maze, M., 2011. Anticipating and managing postoperative delirium and cognitive decline in adults. BMJ. https://doi.org/10.1136/bmj.d4331.

Schlumpf, M., Lichtensteiger, W., Langemann, H., Waser, P.G., Hefti, F., 1974. A fluorometric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochem. Pharmacol. 23 (17), 2437–2446. https://doi.org/10.1016/0006-2952(74)90235-4.

Semenkovich, K., Brown, M.E., Svrakic, D.M., Lustman, P.J., 2015. Depression in type 2 diabetes mellitus: Prevalence, impact, and treatment. Drugs 75 (6), 577–587. https://doi.org/10.1007/s40265-015-0347-4.

Shah, P., Chavda, V., Patel, S., Bhadada, S., Ashraf, G.M., 2020. Promising Anti-stroke Signature of Voglibose: Investigation through In- Silico Molecular Docking and Virtual Screening in In-Vivo Animal Studies. Curr. Gene Ther. 20 (3), 223–235. https://doi.org/10.2174/1566523220999200726225457.

Simha, V., Shah, P., 2019. Perioperative glucose control in patients with diabetes undergoing elective surgery. JAMA - J. Am. Med. Assoc. https://doi.org/10.1001/jama.2018.20922.

Steinmetz, J., Rasmussen, L.S., 2016. Peri-operative cognitive dysfunction and protection. Anaesthesia. Blackwell Publishing Ltd. https://doi.org/10.1111/anae.13308.

Sun, Y., Ma, C., Sun, H., Wang, H., Peng, W., Zhou, Z., He, X., 2020. Metabolism: a novel shared link between diabetes mellitus and Alzheimer's disease. J. Diabetes Res. *2020*. https://doi.org/10.1155/2020/4981814.

Vashi, R., Chavda, V., Patel, S.S., 2020. Cerebrovascular complications of diabetes: SGLT-2 inhibitors as a promising future therapeutics. Curr. Drug Targets *21*. https://doi.org/10.2174/1389450121666201020163454.

Vorhees, C.V., Williams, M.T., 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 1 (2), 848–858. https://doi.org/10.1038/nprot.2006.116.

Wang, L., Gao, P., Zhang, M., Huang, Z., Zhang, D., Deng, Q., Wang, L., 2017. Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. JAMA - J. Am. Med. Assoc. 317 (24), 2515–2523. https://doi.org/10.1001/jama.2017.7596.

Wei, L.A., Fearing, M.A., Sternberg, E.J., Inouye, S.K., 2008. The confusion assessment method: a systematic review of current usage. J. Am. Geriatr. Soc. https://doi.org/10.1111/j.1532-5415.2008.01674.x.

Woo, M.H., Lee, H.S., Kim, J., 2019. Effect of pioglitazone in acute ischemic stroke patients with diabetes mellitus: a nested case-control study. Cardiovasc. Diabetol. 18 (1) https://doi.org/10.1186/s12933-019-0874-5.

Xiong, X.-H., Chen, C., Chen, H., Gao, R., Deng, Q.-Y., Cai, X.-W., Zhu, T., 2020. Effects of intravenous and inhalation anesthesia on blood glucose and complications in patients with type 2 diabetes mellitus: study protocol for a randomized controlled trial. Ann. Transl. Med. 8 (13), 825-825. https://doi.org/10.21037/atm-20-2045a.