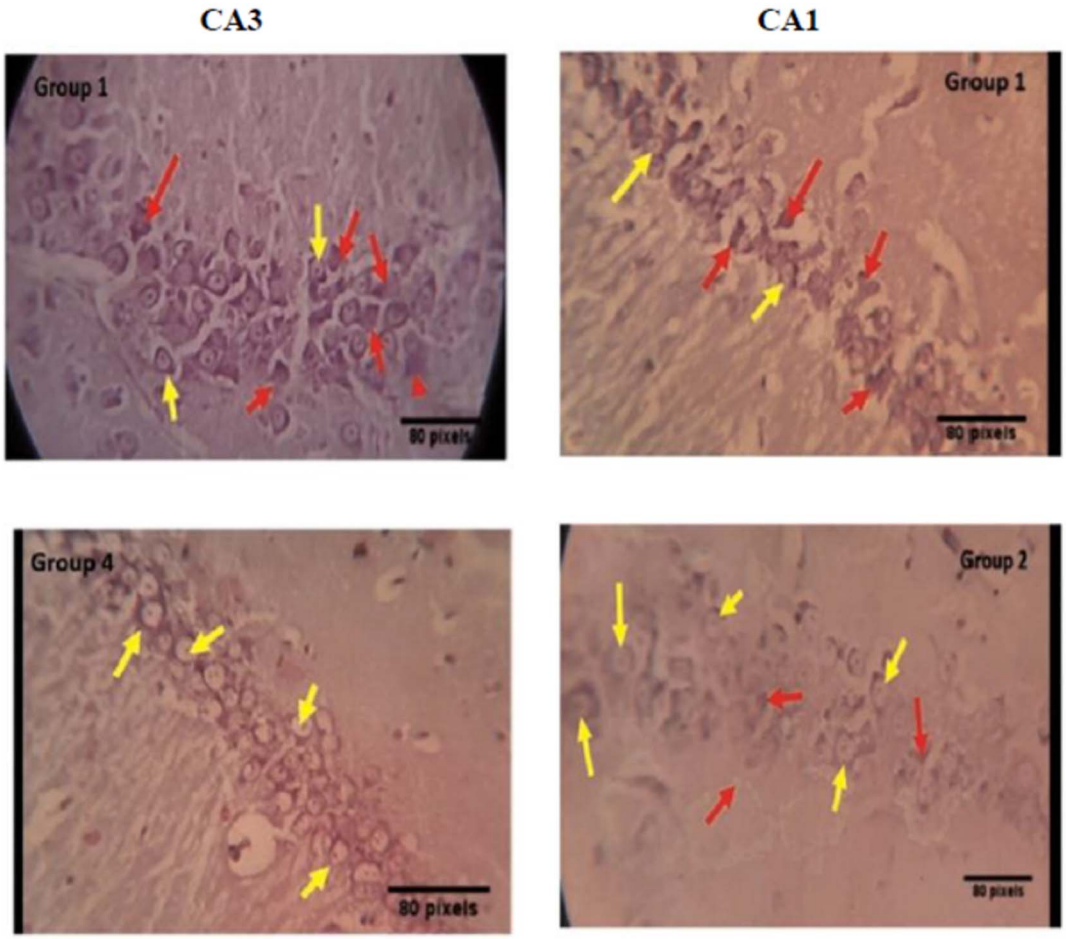
E-ISSN: 2149-7222 (Online)

Journal Cellular Neuroscience and Oxidative Stress



OPEN ACCESS and NO PUBLICATION FEE

http://dergipark.org.tr/jcnos

Former name; Cell Membranes and Free Radical Research

Volume 16, Number 2, 2024

Journal of Cellular Neuroscience and Oxidative Stress

http://dergipark.gov.tr/jcnos

BSN Health Analyses, Innovation, Consultancy, Organization, Industry and Trade Limited Company

http://www.bsnsaglik.com.tr/

info@bsnsaglik.com.tr

Formerly known as:

Cell Membranes and Free Radical Research (2008 - 2014)

Volume 16, Number 2, 2024

[CONTENTS]

Volume 16, Number 2, 2024
E-ISSN Number: 2149-7222 (Online)
Indexing: Scopus (Elsevier), CAS (Chemical Abstracts Service), Citation Index Database, EBSCOhost Research Database, Google Scholar

AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

J Cell Neurosci Oxid Stress 2024;16(2): 1195-1204

Empagliflozin Modulates Seizure Activity and Oxidative Stress in Rats with Epilepsy

Neha HOLLA[1], Shalini ADIGA[*1], Meena KUMARI[1], Mohandas Rao Kapettu GADAHAD[2], Prameetha NAIK[1]

[1]Department of Pharmacology, Kasturba Medical College Manipal, Manipal Academy of Higher Education Manipal, India
[2]Department of Basic Medical Sciences, Manipal Academy of Higher Education, Manipal, India

Received: 21 June 2024; **Accepted:** 7 August 2024

***Address for correspondence:**

Dr. Shalini Adiga,
Department of Pharmacology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal. Karnataka. India, 576104
e-mail: *Shalini.adiga@manipal.edu*

List of Abbreviations;

GSH, *reduced glutathione;* ***MES,*** *maximal electric shock;* ***MDA,*** *malondialdehyde,* ***NO,*** *nitric oxide;* ***PTZ,*** *pentylenetetrazol;* ***RSS,*** *Racine stages scoring;* ***SGLT,*** *sodium-glucose linked transporters;* ***THLE,*** *tonic hind limb extension;* ***TBA,*** *thiobarbituric acid;* ***T2DM,*** *type 2 diabetes mellitus*

Abstract

Drug-resistant epilepsy is a commonly devastating condition, affects more than 50 million people globally. Type 2 diabetes mellitus (T2DM) is associated with an increased risk of neurological disorders, and a potential association between epilepsy and subsequent T2DM has emerged. Inhibiting sodium-glucose linked transporters, which are differentially expressed in the brain, has been shown to reduce epileptic episode activity. This study aimed to evaluate the anticonvulsive effect of empagliflozin in rats with seizures induced by maximal electric shock (MES) and pentylenetetrazol (PTZ). Generalized tonic–clonic seizures were induced in the rats using an electroconvulsive meter, and pentylenetetrazol was injected to induce absence seizures. The duration of all the stages of seizure and Racine stage scoring (RSS) were performed. Malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH) levels, and histopathological analysis in the brain tissues were determined. A significant ($p < 0.01$) decrease in the duration of tonic hind limb extension, a significant decrease in the levels of pro-oxidants such as MDA and NO, and an increase in the levels of antioxidants such as GSH were observed in the low dose 10 mg/kg and high dose 20 mg/kg empagliflozin groups compared to the disease control group. Histopathological analysis revealed a greater number of healthy neurons with few dark-stained cells in the treatment groups, suggesting the neuroprotective effect of empagliflozin. In conclusion, results of the present study, showed that empagliflozin modulates epileptic activity. Empagliflozin has a potential role in the management of epilepsy in diabetic patients.

Keywords: Antioxidants, maximal electroshock, empagliflozin, SGLT2 inhibitor, epilepsy

Introduction

Epilepsy is a neurological disorder characterized by repeated spontaneous epileptic seizures that are connected to specific neurobiological and behavioral changes. Epilepsy is thought to affect 80–100 per 100000 people per year, with a higher incidence in people residing in low-income countries. The term "drug-resistant epilepsy" refers to epilepsy that is refractory to treatment and affects 20 to 30% of patients despite continuing advancements in the diagnosis and management of this condition (Thijs et al., 2019). Side effects are frequently experienced by patients who need a combination of antiepileptic agents for the treatment of epilepsy. These adverse reactions might range from mild, such as dizziness, headaches, and cognitive decline, to more serious, such as mood swings, arrhythmias, and interactions with the effects of different medications. Although there are numerous antiepileptic medications available for the treatment of epilepsy, there is still a need for better medications with target-based therapeutics and fewer side effects.

Due to temporary imbalances between the inhibitory neurotransmitter gamma-aminobutyric acid and the excitatory neurotransmitter glutamate, neuronal hyper synchronization occurs. Increased glutamate levels induce excitotoxicity, which allows calcium ions to enter cells more easily and activate enzymes such as proteases, phospholipases, and endonucleases, which produce reactive oxygen species (ROS). This ROS cause DNA fragmentation, lipid peroxidation and the alteration of nitrogenous bases (Dogan and Yildizhan 2021)

Sodium-glucose cotransporter 2 (SGLT2) inhibitors, a class of newly developed oral antidiabetic drugs, prevent the reabsorption of glucose by the kidneys. Canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin are SGLT2 inhibitors that have received approval from the United States Food and Drug Administration. SGLT2 inhibitors can normalize lipid profiles, lower blood pressure, and enhance visceral adiposity, in addition to reducing blood glucose. Due to their capacity to lower the oxidative stress caused by elevated glucose levels, SGLT2 inhibitors function as indirect antioxidants. SGLT2 inhibitors have also been shown to upregulate the levels of the antioxidants reduced glutathione (GSH) and superoxide dismutases (SODs), as well as to block pro-oxidants such as thiobarbituric acid-reactive substances (TBA) and NADPH oxidase 4. The blood brain barrier may be crossed by SGLT2 inhibitors, allowing brain cells to benefit from their antioxidative properties (Tsai et al. 2021). The stabilizing impact of sodium-glucose linked transporters (SGLTs) inhibitors on excitability and depolarization is made possible by the reduction in sodium transportation across neuronal membranes and the availability of glucose (Erdogan et al. 2018).

It has been proven that inhibiting SGLTs that are differentially expressed in the brain decreases episodic activity in epilepsy. The recent generation of SGLT2 inhibitors, such as empagliflozin, has shown the highest SGLT2 selectivity over SGLT1 compared to others. It was authorized by the Food and Drug Administration (FDA) in 2014 and is used to treat type 2 diabetes (Levine 2017). We were inspired to carry out this research to learn more about how empagliflozin affects seizure behavior in maximal electroshock (MES) and pentylenetetrazol (PTZ) models of epilepsy in rats.

Materials & methods

Animals

The study was carried out in accordance with the rules set forth by the Committee for Control and Supervision of Experimentation on Animals, and the protocol was authorized (IAEC/KMC/65/2021) by the Institutional Animal Ethics Committee. At the Central Animal Research Facility, fifty-six male albino Wistar rats weighing between 200 and 300 grams were kept in cages with three rats each under 12:12 light: dark lighting conditions at a temperature of 25 ± 30 °C and 50% humidity. Normal rodent diet pellets and water were provided ad libitum.

MES-induced generalized tonic–clonic seizure model

After acclimatization for 1 week, the Wistar albino rats were divided into 4 groups with 6 animals per group. Disease control rats were administered saline, group 2 received 300 mg/kg sodium valproate, group 3 received 10 mg/kg low-dose empagliflozin, and group 4 received 20 mg/kg high-dose empagliflozin for 14 days.

Generalized tonicclonic seizures were induced using an electroconvulsiometer (Alachkar et al. 2018). Electric stimulation at an intensity of 150 mA for 0.2 seconds (50-60 Hz frequency) via ear clip electrodes was used to induce seizures on the 14th day of the experiment after 1 hour of dosing with empagliflozin, valproate and saline.

The duration of all the seizure stages, which included flexion, tonic hind limb extension (THLE), clonic

convulsions and stupor, was noted. The Racine stage score (RSS) was determined. Death/recovery after twenty-four hours of induction was recorded. THLE was the endpoint (backward extension of the hind limb > 90° against the plane of the body axis). The percentage inhibition of convulsions and percentage protection were calculated.

PTZ-induced absence seizure model

Animals were assigned to four groups of eight animals in each group at random. Before beginning the experiment, they were acclimatized for 7 days in the animal facility. Animals in Group I received saline water, Group II received 300 mg/kg sodium valproate intraperitoneally, and Groups III and IV were pretreated with empagliflozin at doses of 10 mg/kg and 20 mg/kg, respectively, for 2 weeks. The freshly prepared pentylenetetrazol was injected at a dose of 60 mg/kg, i.p., following 30 minutes of drug administration on the 14th day. Rats were monitored separately for 60 minutes. (Sarangi et al., 2017)

According to the Racine classification with slight modifications (Lüttjohann et al. 2009; Alachkar et al. 2018) seizures were scored as follows:

Stage 0 - no change in behavior
Stage 1 - stereotype mouthing, eye blinking, and/or mild facial clonus
Stage 2 - head nodding and/or severe facial clonus
Stage 3 - Myoclonic jerk in forelimbs
Stage 4 - clonic convulsions in the forelimbs with rearing
Stage 5 - generalized clonic convulsions associated with loss of balance

On the 15th day, the rats in both models were euthanized by an overdose of thiopentone sodium (120 mg/kg i.p.). Rat brains were quickly dissected and stored in phosphate-buffered saline (0.1 M, pH 7.4) for biochemical estimations and in 10% formalin for histopathological analysis.

MDA Assay

The brain tissue homogenate that was produced was centrifuged for 10 minutes at 10,000 rpm (4°C) to obtain the supernatant. By using the TBA reaction, the MDA levels were calculated. Lipoproteins were precipitated with trichloroacetic acid (pH 2-3). It is boiled with thiobarbituric acid, which reacts with malondialdehyde to generate MDA-TBA2 and provides a pink color. The complex that turned pink was cooled to room temperature and measured at 532 nm with a spectrophotometer (Eppendorf AG 22331 Hamburg, Germany). The concentrations of MDA were presented as μM/g tissue (Senthilkumar. 2021).

NO - Griess Reagent Assay

Nitrite is transformed into nitrous acid in acidic solution, and this acid diozolizes sulfonamides. After reacting with N-(1-naphthyl)-ethylenediamine, this sulphanilamide-dizonium salt creates a chromophore that is detected at 540 nm. (Lee et al., 2003)

GSH Assay

This method is based on the redox reaction between GSH and DTNB, which results in the formation of a yellow pigment when the Ellman's reagent DTNB is introduced to sulfhydryl compounds. The pigment which forms is stable for approximately 10 minutes, and temperature changes have a small impact on the process. At 412 nm, the reaction was recorded. (Beutler et al., 1963)

Histopathological analysis

The degree of neuronal degeneration was evaluated via the Nissl staining technique. A cryostat operating under frozen conditions (-18°C) was used to cut serial coronal sections with a thickness of 30–50 μm. To improve penetration and staining, cresyl violet was used for staining at 37–50°C. This stain is useful since it may be used to identify neuronal cell death and stain both glial and neuronal cells. Sections were dehydrated using 70%, 95%, and 100% alcohol before being washed with xylene. Mounting was performed using mountant-DPX. (Smiałowska et al. 2003)

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.1. The analysis was performed using one-way ANOVA followed by post hoc Tukey's multiple comparison test for the biochemical and behavioral parameters. All the data are expressed as the mean ± standard deviation (SD).

Results

Table 1. Effect of empagliflozin on MES-induced epilepsy in rats, *All values are expressed as the mean ± SD. Statistical analysis was performed by one-way ANOVA, followed by post hoc Tukey's multiple comparison test.* $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{****}p<0.0001$ *vs disease control*

Group No	Group Name	Flexion (sec)	THLE (sec)	Clonic Convulsions (sec)	Stupor (sec)	Inhibition of THLE (%)
1	Disease control	12.00 ± 1.41	18.33 ± 5.88	22.83 ± 2.31	134.3 ± 32.65	0%
2	Sodium valproate	6.83 ± 1.941***	0.00 ± 0.00****	6.50 ± 1.87****	66.00 ± 66.00***	100%
3	Empagliflozin 10 mg/kg	9.16 ± 1.72*	5.66 ± 6.53**	14.50 ± 6.77**	96.00 ± 18.30*	66.6%
4	Empagliflozin 20 mg/kg	7.50 ± 1.37***	1.66 ± 4.08****	8.00 ± 3.34****	80.50 ± 7.66**	83.3%

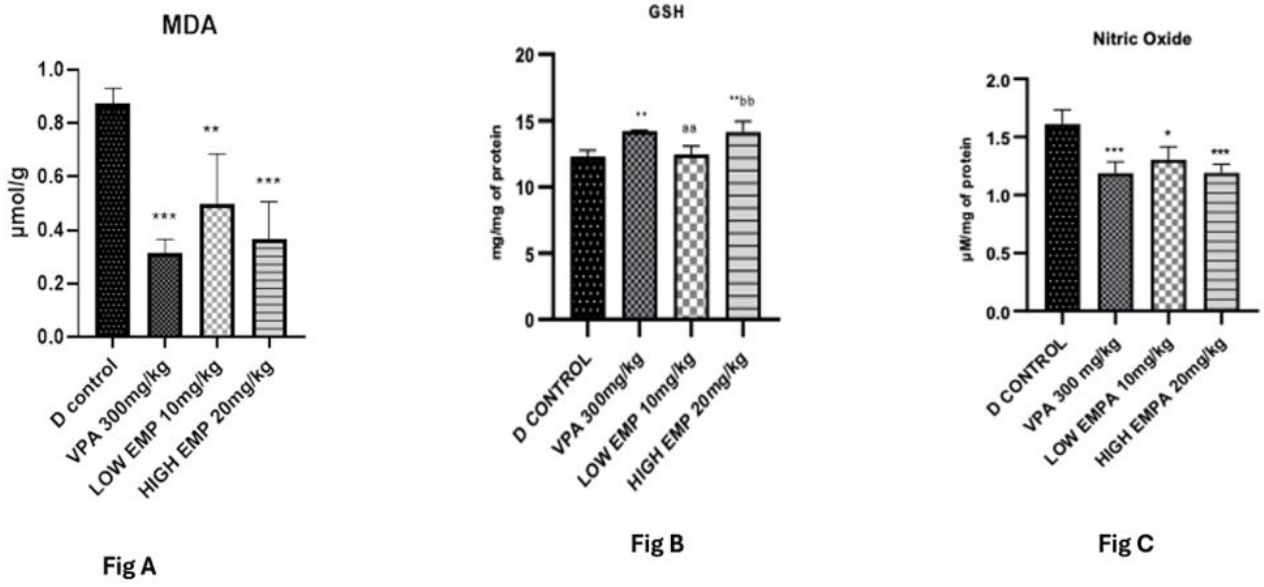


Fig A Fig B Fig C

Figure 2. Effect of empagliflozin on the brain oxidative markers MDA (fig A), GSH (fig B) and Nitric oxide (fig C) in MES-model, *All values are expressed as the mean ± SD. Statistical analysis was performed by one-way ANOVA, followed by post hoc Tukey's multiple comparison test. (MDA: F=57.08; GSH: F=25.78; NO: F=39.43)* $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ *vs disease control* $^{bb}p<0.01$ *vs low dose empagliflozin* $^{aa}p<0.01$ *vs sodium valproate*

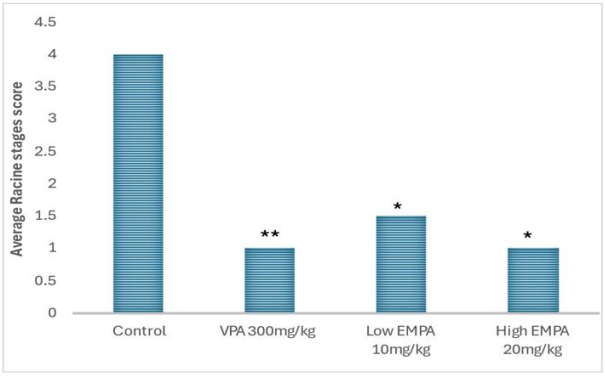


Figure 1. Effect of sodium valproate and empagliflozin on average Racine stage score- MES, *All values are expressed as the median (IQR). Statistical analysis was performed by Kruskal-Wallis test followed by post hoc DSCF pairwise comparison test. *p<0.05, **p<0.01; vs disease control.*

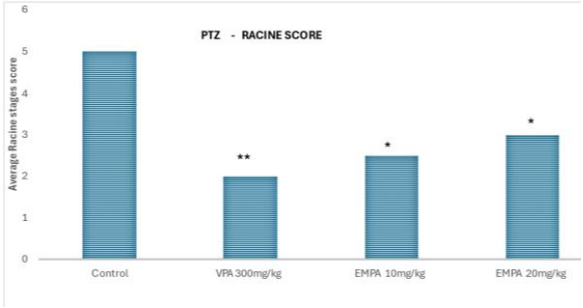


Figure 4: Effect of sodium valproate and empagliflozin on average Racine stage score- PTZ model, *All values are expressed as the median (IQR). Statistical analysis was performed by Kruskal-Wallis test followed by post hoc DSCF pairwise comparison test. *p<0.05, **p<0.01; vs disease control*

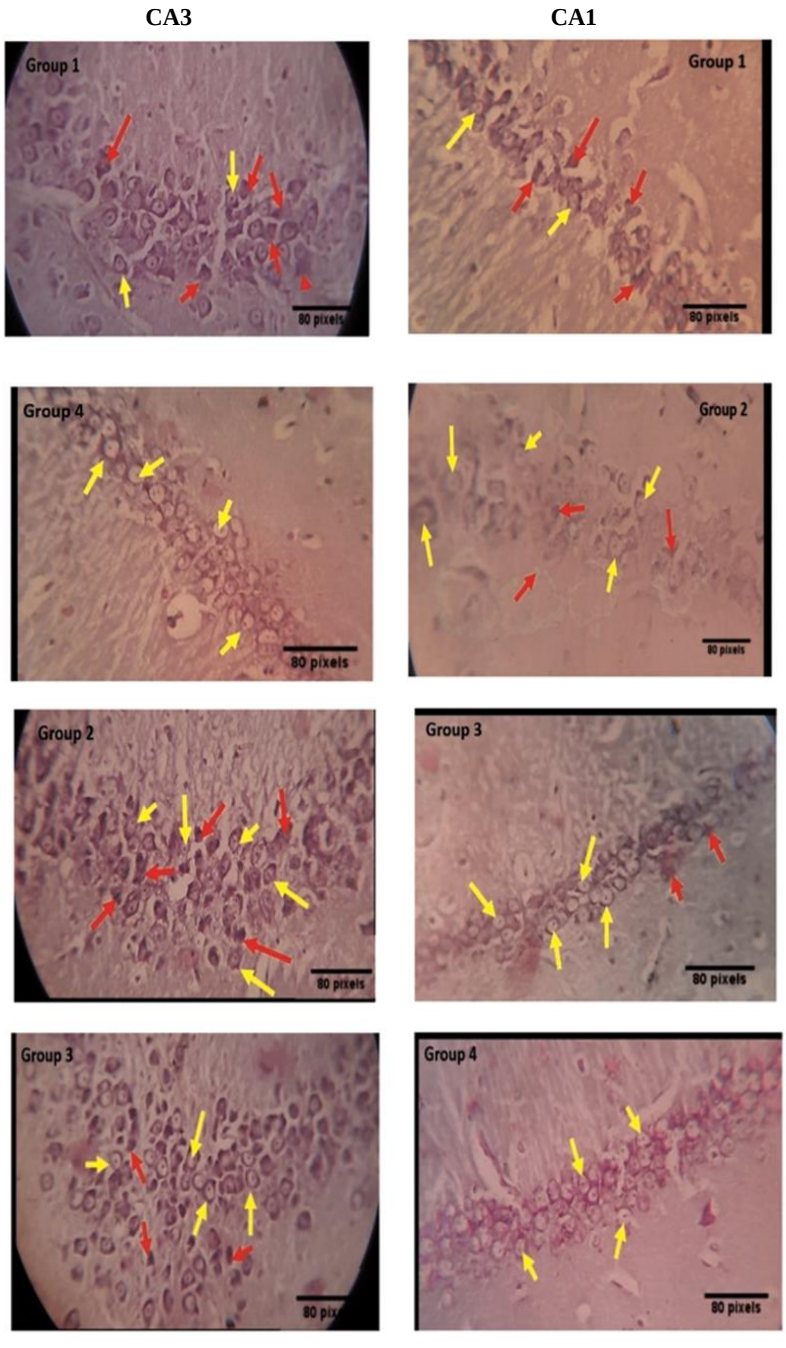


Figure 3: Representative photomicrographs of Cresyl hippocampal CA3 and CA1 regions of group 1 (disease control), group 2 (standard drug), group 3 (low-dose test drug) and group 4 (high-dose test drug) rats showing the cell bodies of the pyramidal neurons. [Magnification: 40x10 X] – MES model

The duration of flexion, duration of clonic convulsion and duration of stupor were significantly shorter in the low-dose and high-dose empagliflozin and sodium valproate groups than in the disease control group. There was a significant decrease in the duration of THLE in the sodium valproate group ($p<0.0001$), low-dose empagliflozin group ($p<0.01$) and high-dose empagliflozin group ($p <0.0001$). The sodium valproate group exhibited 100% protection from THLE, while the high-dose empagliflozin group and low-dose empagliflozin group exhibited 83.3% and 66.6% protection, respectively. There was no mortality recorded after 24 hours of induction in any of the groups.

There was a significant reduction in the MDA levels in the groups treated with sodium valproate ($p <0.001$), low-dose empagliflozin ($p <0.01$) and high-dose empagliflozin ($p <0.001$) in comparison to the disease control group.

Compared with that in the disease control group, there was a significant increase in GSH in the sodium valproate ($p < 0.01$) and high dose empagliflozin ($p < 0.01$) groups. Additionally, GSH levels in the high-dose empagliflozin group were significantly greater than those in the low-dose empagliflozin group.

There was a significant decrease in nitric oxide levels in the groups treated with sodium valproate ($p < 0.001$), low-dose empagliflozin ($p < 0.05$) and high-dose empagliflozin ($p < 0.001$) in comparison to the disease control group.

Qualitative analysis of cresyl violet-stained pyramidal neurons in the hippocampal CA3 and CA1 regions: Fig 3&6

The rats in group 1 had pyramidal neuronal cell bodies in the hippocampal CA3 region that displayed many flame-shaped, pyknotic, degenerating pyramidal neurons (shown by the red arrow in the figure). The presence of profoundly basophilic, flame-shaped cells is a sign of karyopyknosis in hippocampal neurons. Compared with the rats in group 1, the rats in group 3 and group 2 groups showed a slight decrease in flame-shaped, degenerating pyramidal neuronal cell bodies and a slight increase in healthy neurons in the hippocampal CA3 region.

Nonetheless, the high dose empagliflozin group demonstrated a notable increase in the quantity of healthy neurons (shown by the yellow arrow).

The average Racine stage score (RSS) significantly decreased in the sodium valproate group ($p < 0.01$), low dose& high dose empagliflozin group ($p < 0.05$)

In PTZ model; MDA levels were significantly lower in sodium valproate group ($p < 0.0001$), low-dose empagliflozin group ($p < 0.01$) and high-dose empagliflozin group ($p < 0.0001$) than in the disease control group.

Compared with those in the disease control group, there were significant increases in GSH (reduced form) in the sodium valproate group ($p < 0.01$), low-dose empagliflozin group ($p < 0.05$) and mg/kg high-dose empagliflozin group ($p < 0.01$).

There was a significant decrease in nitric oxide levels in the groups treated with sodium valproate ($p < 0.001$), low dose empagliflozin ($p < 0.01$) and high dose empagliflozin ($p < 0.001$) in comparison with the disease control group.

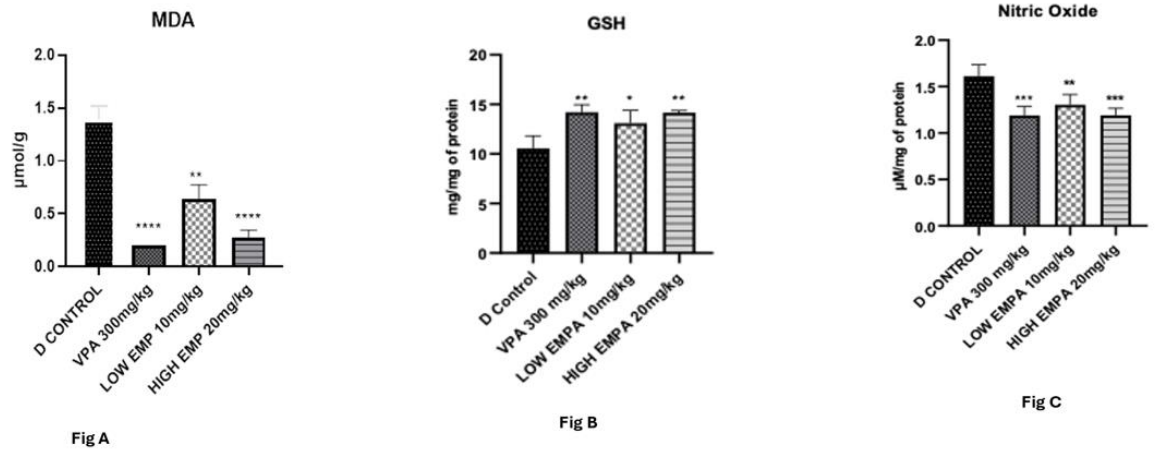


Figure 5: Effect of empagliflozin on the brain oxidative markers MDA (fig A), GSH (fig B) and Nitric oxide (fig C) in PTZmodel, *All values are expressed as the mean ± SD. Statistical analysis was performed by one-way ANOVA, followed by post hoc Tukey's multiple comparison test. (MDA F=77.90; GSH F=9.09; NO F=10.62)* $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{****}p<0.0001$ *vs disease control*

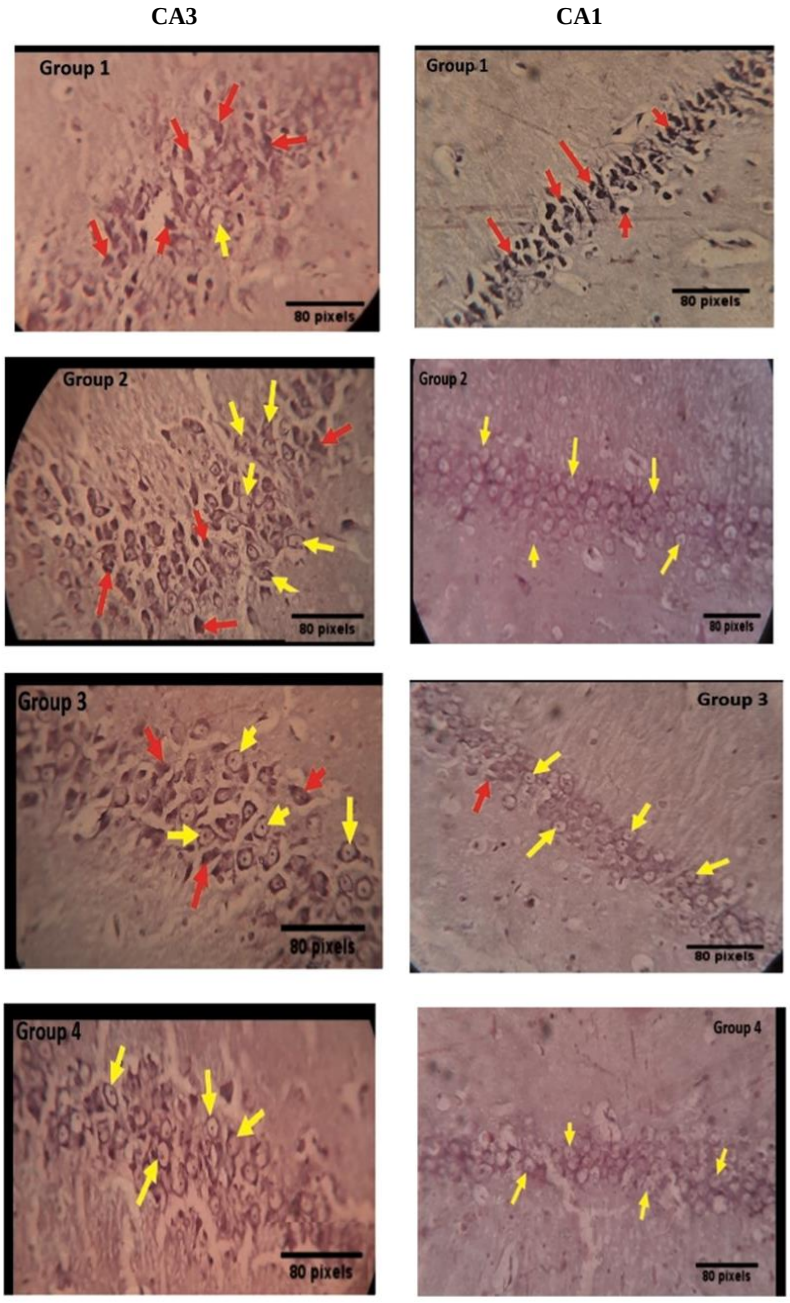


Figure 6: Representative photomicrographs of Cresyl violet-stained hippocampal CA3 and CA1 regions of group 1 (disease control), group 2 (standard drug), group 3 (low-dose test drug) and group 4 (high-dose test drug) rats showing the cell bodies of the pyramidal neurons. [Magnification: 40x10 X] - PTZ model

Discussion

In the present study, empagliflozin was tested at doses of 10 mg/kg and 20 mg/kg in rats that had been subjected to MES and PTZ-induced seizures.

Sodium valproate showed 100% protection, while low dose empagliflozin showed 66.6% protection, and high dose empagliflozin showed 83.3% protection against THLE and as shown by the Racine stage scores. Dapagliflozin, an SGLT2 inhibitor at both high and low doses, significantly improved the RSS in a study by Erdogan MA et al. Likewise, both high and low doses of empagliflozin reduced the RSS compared to disease control and the high dose empagliflozin group exhibited a substantial increase in the number of healthy neurons in our study. This finding suggested that high-dose empagliflozin exhibited potential neuroprotective effects against seizures in a dose-dependent manner.

Numerous disorders, including epilepsy, involve free radicals as part of their pathophysiology. The main effects of free radicals are tissue damage and membrane lipid peroxidation, which lead to cell membrane malfunction and apoptosis. Normally, many antioxidants regulate the biological impacts of free radicals, such as GSH and SOD. Currently, research is being conducted to identify antiepileptic medications with antioxidant and neuroprotective benefits because free radicals are thought to be involved in augmenting convulsions (Sudha et al. 2001). Seizures can encourage lipid peroxidation, and antioxidants have been found to prevent cell death in the hippocampus and lipid peroxidation in both hemispheres (Frantseva et al. 2000).

SGLT2 inhibitors such as canagliflozin and dapagliflozin increased GSH levels while decreasing MDA and nitric oxide levels, as reported in earlier reports (Hassanein et al. 2023; Abd et al. 2022). Our study revealed reduced MDA and nitric oxide levels and elevated GSH levels, suggesting that empagliflozin has antioxidative effects.

A cohort examination of national health insurance claims in Taiwan revealed that individuals with type 2 diabetes had a greater likelihood of seizures than controls, irrespective of severe hypoglycemia (Lu et al. 2018). The relationship between type 2 diabetes and epilepsy as a comorbid condition is well established (Sander et al. 2016; Nadeem et al. 2023).

Improved glycemic management is associated with a reduction in seizure activity in people with diabetes mellitus and seizure disorders, even after antiepileptic medications are stopped. Several epileptic disorders, including treatment-resistant epilepsy in children, have been shown to be controlled by increasing the metabolism of glucose, often by lowering its availability and pushing the brain to rely on alternate energy sources such as ketones (Neal et al. 2008).

Antiepileptic drugs are ineffective against seizures caused by hyperglycemia. Neuronal hyperexcitability, neuropropagation, and seizures have all been connected to elevated extracellular glucose levels. Low levels of GABA and adenosine triphosphate-sensitive potassium channels (KATP) have been linked to these outcomes (Huang et al. 2007). One of the hypothesized causes is the decrease in GABA levels caused by depression of glucose uptake and the Krebs cycle, which increases the activity of alternative glucose metabolism pathways. Due to these metabolic alterations, the succinic-semialdehyde pathway synthesizes succinic acid from GABA, meeting up to 40% of the energy requirements of brain tissue (Guisado and Arieff 1975). In contrast, diabetic ketoacidosis has an antiepileptic effect because it meets most of the energy needs of brain tissue through ketone bodies. In a case with refractory status epilepticus dapagliflozin was initiated to achieve persistent ketosis along with infusions of propofol and midazolam (Blunck et al. 2018). The patient was able to be weaned off the propofol injection within a week of starting SGLT2i because a consistent state of ketosis was met. This report supported the hypothesis that SGLT2i can enhance ketogenesis, which has been confirmed in past research studies to be beneficial in the treatment of refractory status epilepticus (D'Andrea et al. 2019).

The connection between glucose availability and seizures is complicated since low glucose availability sometimes causes seizures rather than preventing them (Rovet and Ehrlich 1999). In some patients with intolerance to metformin or renal disease, metformin may not be useful as the standard drug for treating diabetes. The alternative choices include sulfonylureas (SUs), pioglitazone, acarbose, meglitinides and dipeptidyl peptidase-4 (DPP-4) inhibitors. These medications may result in an increase in weight and hypoglycemia. The use of SGLT2 inhibitors such as dapagliflozin, empagliflozin, and canagliflozin for the treatment of type 2 diabetes does not increase the frequency of hypoglycemic incidents.

SGLT activity is elevated in seizure-prone brain regions that are experiencing seizures, as indicated by the tracking of the radioactive emissions produced by methyl alpha-D-[U-14C] glucopyranoside, an isotope-labeled nonmetabolizable SGLT substrate that tends to accumulate in seizure-prone neurons in earlier report (Poppe et al. 1997). SGLTs physiologically create inward currents to transport sodium and glucose through cellular membranes and into the cytoplasm. Reduced neuronal excitability and a reduction in the available metabolic resources for cellular respiration resulting in an increase in the seizure threshold were hypothesized for the control of seizures observed with the SGL2i dapagliflozin (Erdogan et al. 2018). The SGLT inhibitor phlorizin was shown to exacerbate the severity of pilocarpine-induced limbic seizures during status epilepticus (SE) in mice, as well as the extent to which neurodegeneration occurred in the hippocampus 24 hours after SE (Melo et al. 2016). The different effects of phlorizin and dapagliflozin reported were attributed to phlorizin inhibiting both SGLT1 and SGLT2, whereas dapagliflozin inhibiting only SGLT2 (Tharmaraja et al. 2022). Empagliflozin has been shown to have greater selectivity for SGLT2 (2500-fold) than dapagliflozin (1200-fold) and canagliflozin (250-fold) (Wiciński et al. 2020). Due to these implicit processes, SGLT2-selective inhibition may prevent seizure activity and offers a plausible explanation for the findings of this investigation. Further studies are necessary to explore the exact molecular mechanism of empagliflozin in epilepsy.

Acknowledgments

The authors thank the department of pharmacology for providing the necessary support for conducting the study.

Funding

There is no financial disclosure for the current study.

Conflict of interest

None

Authors' contributions

NH: methodology, analysis, original draft preparation. SA: conceptualization, resource supervision, analysis, review, and editing. M.K : conceptualization, analysis, review, and editing; MRKG: histopathology analysis; P: methodology. The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The corresponding author can provide access to the data used to support the findings of the current inquiry upon request.

Consent for publication

The final manuscript as submitted was approved by the authors.

ORCID

Meena KUMARI; 0000-0002-1506-1442
Mohandas RAO; 0000-0001-5832-0718
Neha HOLLA; 0009-0008-2264-5703
Prameetha NAIK; 0009-0009-8564-5943
Shalini ADIGA; 0000-0002-3369-194X

References

Abd El-Fattah EE, Saber S, Mourad AAE, et al. (2022). The dynamic interplay between AMPK/NFκB signaling and NLRP3 is a new therapeutic target in inflammation: Emerging role of dapagliflozin in overcoming lipopolysaccharide-mediated lung injury. Biomed Pharmacother. 147:112628. doi: 10.1016/j.biopha.2022.112628.

Alachkar A, Łażewska D, Latacz G, et al. (2018). Studies on Anticonvulsant effects of Novel Histamine H3R Antagonists in Electrically and Chemically Induced Seizures in Rats. Int J Mol Sci.19(11):3386. doi:10.3390/ijms19113386

Beutler E, Duron O, Kelly BM. (1963). Improved method for the determination of blood glutathione. J Lab Clin Med. 61:882-888.

Blunck JR, Newman JW, Fields RK, Croom JE. (2018) Therapeutic augmentation of ketogenic diet with a sodium-glucose cotransporter 2 inhibitor in a super refractory status epilepticus patient. Epilepsy Behav Case Rep. 10:61-64. doi: 10.1016/j.ebcr.2018.05.002.

D'Andrea Meira I, Romão TT, Pires do Prado HJ, Krüger LT, Pires MEP, da Conceição PO. (2019). Ketogenic Diet and Epilepsy: What We Know So Far. Front Neurosci. 13:5. doi:10.3389/fnins.2019.00005.

Doğan M & Yildizhan K. (2021). Investigation of the effect of paracetamol against on glutamate-induced cytotoxicity in C6 glia cells. Cumhuriyet Sci. J.. 42(4); 789-794

Erdogan MA, Yusuf D, Christy J, et al. (2018). Highly selective SGLT2 inhibitor dapagliflozin reduces seizure activity in pentylenetetrazol-induced murine model of epilepsy. BMC Neurol. 18(1):81. doi:10.1186/s12883-018-1086-4.

Frantseva MV, Perez Velazquez JL, Tsoraklidis G, et al. (2000) Oxidative stress is involved in seizure-induced neurodegeneration in the kindling model of epilepsy. Neuroscience. 97(3):431-435. doi:10.1016/s0306-4522(00)00041-5.

Guisado R, Arieff AI. (1975). Neurologic manifestations of diabetic comas: correlation with biochemical alterations in the brain. Metabolism. 24(5):665-679. doi:10.1016/0026-0495(75)90146-8.

Hassanein EHM, Saleh FM, Ali FEM, Rashwan EK, Atwa AM, Abd El-Ghafar OAM. (2023). Neuroprotective effect of canagliflozin against cisplatin-induced cerebral cortex injury is mediated by regulation of HO-1/PPAR-γ, SIRT1/FOXO-3, JNK/AP-1, TLR4/iNOS, and Ang II/Ang 1-7 signals. Immunopharmacol Immunotoxicol. 45(3):304-316. doi:10.1080/08923973.2022.2143371.

Huang CW, Huang CC, Cheng JT, Tsai JJ, Wu SN. (2007). Glucose and hippocampal neuronal excitability: role of ATP-sensitive potassium channels. J Neurosci Res. 85(7):1468-1477. doi:10.1002/jnr.21284.

Lee DU, Kang YJ, Park MK, et al. (2003). Effects of 13-alkyl-substituted berberine alkaloids on the expression of COX-II, TNF-alpha, iNOS, and IL-12 production in LPS-stimulated macrophages. Life Sci. 73(11):1401-1412. doi:10.1016/s0024-3205(03)00435-1.

Levine MJ. (2017). Empagliflozin for Type 2 Diabetes Mellitus: An Overview of Phase 3 Clinical Trials. Curr Diabetes Rev. 13(4):405-423. doi:10.2174/1573399812666160613113556.

Lu CL, Chang YH, Sun Y, Li CY. (2018). A population-based study of epilepsy incidence in association with type 2 diabetes and severe hypoglycaemia. Diabetes Res Clin Pract. 140:97-106. doi: 10.1016/j.diabres.2018.03.020.

Lüttjohann A, Fabene PF, van Luijtelaar G. (2009). A revised Racine's scale for PTZ-induced seizures in rats. Physiol Behav. 98(5):579-586. doi: 10.1016/j.physbeh.2009.09.005.

Melo IS, Santos YMO, Costa MA, et al. (2016). Inhibition of sodium glucose cotransporters following status epilepticus induced by intrahippocampal pilocarpine affects neurodegeneration process in hippocampus. Epilepsy Behav. 61:258-268. doi: 10.1016/j.yebeh.2016.05.026.

Nadeem M, Memon S, Qureshi K, et al. (2023) Seizing the Connection: Exploring the Interplay Between Epilepsy and Glycemic Control in Diabetes Management. Cureus 15(9): e45606. doi:10.7759/cureus.45606

Neal EG, Chaffe H, Schwartz RH, et al. (2008). The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. Lancet Neurol. 7(6):500-506. doi:10.1016/S1474-4422(08)70092-9.

Poppe R, Karbach U, Gambaryan S, et al. (1997). Expression of the Na+-D-glucose cotransporter SGLT1 in neurons. J

Neurochem.69(1):84-94. doi:10.1046/j.1471-4159.1997.69010084. x.

Rovet JF, Ehrlich RM. (1999). The effect of hypoglycemic seizures on cognitive function in children with diabetes: a 7-year prospective study. J Pediatr. 134(4):503-506. doi:10.1016/s0022-3476(99)70211-8.

Sander JW, Novy J, Keezer MR. (2016). The intriguing relationship between epilepsy and type 1 diabetes mellitus. Diabetologia. 59(7):1569-1570. doi:10.1007/s00125-016-3982-8

Sarangi SC, Joshi D, Kumar R, Kaleekal T, Gupta YK. (2017). Pharmacokinetic and pharmacodynamic interaction of hydroalcoholic extract of Ocimum sanctum with valproate. Epilepsy Behav.75:203-209. doi: 10.1016/j.yebeh.2017.08.018.

Senthilkumar M, Amaresan N, Sankaranarayanan. (2021). Plant-Microbe Interactions: Laboratory Techniques, Springer Protocols Handbooks, https://doi.org/10.1007/978-1-0716-1080-0_25, © Springer Science+Business Media, LLC, part of Springer Nature

Smiałowska M, Wierońska JM, Szewczyk B. (2003). Neuroprotective effect of NPY on kainate neurotoxicity in the hippocampus. Pol J Pharmacol. 55(6):979-986. PMID: 14730092.

Sudha K, Rao AV, Rao A.(2001). Oxidative stress and antioxidants in epilepsy. Clin Chim Acta.303(1-2):19-24. doi:10.1016/s0009-8981(00)00337-5.

Tharmaraja T, Ho JSY, Sia CH, et al. (2022). Sodium-glucose cotransporter 2 inhibitors and neurological disorders: a scoping review. Ther Adv Chronic Dis. 13:20406223221086996. doi:10.1177/20406223221086996.

Thijs RD, Surges R, O'Brien TJ, Sander JW.(2019). Epilepsy in adults. Lancet. 393(10172):689-701. doi: 10.1016/S0140-6736(18)32596-0.. PMID: 30686584.3.

Tsai KF, Chen YL, Chiou TT, et al. (2021). Emergence of SGLT2 Inhibitors as Powerful Antioxidants in Human Diseases. Antioxidants (Basel). 10(8):1166. doi:10.3390/antiox10081166.

Wiciński M, Wódkiewicz E, Górski K, Walczak M, Malinowski B. (2020). Perspective of SGLT2 Inhibition in Treatment of Conditions Connected to Neuronal Loss: Focus on Alzheimer's Disease and Ischemia-Related Brain Injury. Pharmaceuticals (Basel). 13(11):379. doi:10.3390/ph13110379