Topamax® and Ginger Oil Potential Neurotoxicity And Their Interaction in Mice Thru Oxidative Stress and Inflammatory Markers, Neurotransmitters Expression and Immunohistochemical Measurements

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Research Article

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Abstract

Background: Topamax® has multiple pharmacological mechanisms that are efficient to treat epilepsy and migraine. Ginger has been demonstrated to have gingerols and shogaols compounds that proven to cross the blood-brain barrier causing migraine relief, implying that it is useful in the treatment of migraines. Moreover, Topamax has many off-label uses. So it was necessary to explore the possible neurotoxicity of Topamax®, Ginger oil and their interaction in the mice brain.

Methods and Results: Male mice were orally gavage with Topamax®, ginger oil (400mg/kg), and Topamax® plus ginger oil with the same pattern for one month. Oxidative stress markers, acetylcholinesterase (AChE) and gamma aminobutyric acid (GABA) and tumor necrosis factor-alpha (TNF- α), were analyzed in brain tissue. Histopathological examination by hematoxylin and eosin, immunohistochemical glial fibrillary acidic protein (GFAP), and Bax expression analysis were done. The mRNA levels of GABAAR subunits, Gabra1, Gabra3, and Gabra5 were evaluated by RT qPCR. The analysis of data revealed that Topamax® elevated the levels of oxidative stress markers, neurotransmitters, TNF- α , and diminished the level of glutathione reduced (GSH). Topamax® exhibited various neuropathological alterations, strong Bax expression, and GFAP immune-reactivity in the cerebral cortex. The interaction effect of Topamax® plus ginger oil attenuated the changes induced by Topamax® in the abovementioned parameters. Both Topamax® and ginger oil upregulated the mRNA expression of gabra1 and gabra3 while their interaction markedly downregulated them.

Conclusion: We can conclude that the Topamax® overdose could possibly cause neurotoxicity, but the interaction with ginger oil can reduce Topamax® -induced neurotoxicity and should be taken in parallel.

Introduction

Topamax® is the brand name of topiramate, a sulfamate-substituted monosaccharide with multiple pharmacologic mechanisms of action that is efficiently used to treat epilepsy and migraine. It has been illegally marketed or prescribed for other symptoms including alcohol dependence, cocaine addiction, bipolar disorder, binge eating disorder, post-traumatic stress disorder, and obesity [1-3]. It operates as an anticonvulsant by inhibiting voltage-dependent sodium channels, increasing the activity of the neurotransmitter gamma-aminobutyric acid, and antagonizing the glutamate receptor subtypes A-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainite. It's also a mild inhibitor of carbonic anhydrase (CA, types II and IV) isoenzymes, which may control pH-dependent activation of voltage- and receptor-gated ion channels [4]. Topamax® using is often discontinued due to its adverse effects. Slow titration and low initial doses may often, but not always, minimize side effects [5]. Tiredness, paresthesia, drowsiness, loss of appetite, nausea, diarrhea, and a sour taste in the mouth are the most prevalent side effects associated with Topamax® use. Blurred vision [6], anxiety, or suicidal thoughts [7], hyperchloremic acidosis, hepatic-renal-toxicity, and negative consequences on cognition and memory have also been recorded (5, 8- 10).

Traditional medicine is still an important source of alternative medicine around the world. The negative side effects of currently available medications have renewed interest in the development of novel drug candidates derived from natural sources [11]. Ginger, the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae family), is very rich in therapeutic properties. It has been used in the cure of numerous ailments including pain, cold, vomiting, arthritis, rheumatism constipation, hypertension, and infectious diseases [12]. Ginger anticonvulsant activity has been confirmed implying that it plays an essential role in the treatment of migraines [13]. The effectiveness of ginger in the common migraine treatment is comparable to antimigraine drugs [14]. Moreover, the clinical adverse effects of ginger were less than the anti-migraine drugs [15]. In addition to its anti-inflammatory, antioxidant, and anticancer properties [16,17], recent research suggested that ginger has neuroprotective properties [18 -20].

Oxidative stress is outcome of an imbalance between the creation and detoxification of reactive oxygen species (ROS). The brain is very susceptible to oxidative damage owing to its high energy demand, oxygen consumption and plenty of peroxidizable fatty acids [21]. ROS generation is the primary mechanism of neurotoxicity for numerous prominent toxins [22-24]. Neurodegenerative disorders are linked to oxidative stress and neuroinflammation in the central nervous system [25]. Consequently, generated free radicals may oxidize neurotransmitters and impairing their function. In addition, lipid peroxidation targets polyunsaturated fatty acid-rich neuronal membranes, resulting in decreased membrane fluidity and altered membrane transport [26].

Neurotransmitters serve a crucial part in neurochemical synaptic transmission, which is an important function of the central nervous system. In the mammalian central nervous system, γ -aminobutyric acid (GABA) is the most important inhibitory neurotransmitter. Furthermore, astrocytes play a critical role in the response to a range of CNS lesions. Upregulation of glial fibrillary acidic protein (GFAP), an astrocytic marker, has been documented in all forms of central nervous system injury. Increased GFAP-immunoreactive cells and branches, hyperplasia, and enhanced GFAP expression are all signs of reactive astrogliosis [27]. GABA receptors (GABAR) are channel receptors that responds to the neurotransmitter GABA, and they are subdivided into two classes: GABAA receptors (GABAAR) (ionotropic receptors) are ligand-gated ion channels; GABAB receptors (GABABR) (metabotropic receptors) are G protein-coupled receptors. In addition, activation of GABA receptors lead to the so-called shunting inhibition, which reduces the excitability of the cell independent of the changes in membrane potential [28]. GABAARs modulation is one of several possible anti-seizure mechanism of Topamax® [29].

The objective of the current study was to explore the possible neurotoxicity of Topamax®, ginger oil and their interaction in the brain of mice. The studies were performed by estimation of the oxidative marker, neurotransmitters, neuropathological, Immunohistochemical, and genetical deviations.

Materials And Methods

Chemicals

Topamax® tablet (100mg) was purchased from Kahira pharmaceutical company (Cairo, Egypt). Ginger oil was purchased from EL-Captain Company, Egypt. ELISA kits for Reactive Oxygen Species (ROS), α -tumor necrosis factor were purchased from Sunlong Biotech, Co (Zhejiang, China). Gamma Aminobutyric Acid (GABA) and acetylcholinesterase kits were purchased from MyBioSource (USA). Lipid peroxide (MDA) and glutathione reduced (GSH) kits were purchased from Bio diagnostic (Egypt). All chemicals and reagents used were of analytical grade.

Animals

Adult male Swiss albino mice, aged 7–8 weeks and 25–30g weight used in this investigation, were obtained from the animal breeding house of the National Research Centre, Egypt. The animals were maintained at a constant temperature ($22 \pm 2^{\circ}\text{C}$) on a 12h light/dark cycle with free access to food and water. The National Research Centre's Animal Care and Use Committee and the National Institutes of Health's (NIH publication 86-23 revised 1985) requirements were followed for all animals. The study was authorized by the National Research Centre's Ethics Committee (Approval No. 18038).

Experimental Design

Thirty five male albino mice were distributed into five groups, each with seven mice. Animals in control group were administered saline solution (0.25mg/mice/day) via oral gavages, vehicle group were given corn oil (0.25mg/mice/day). Animals in the Topamax® group were orally gavage with four multiplied the therapeutic dose of Topamax® (400 mg/kg/mice/day). In the ginger oil group animals were orally gavage with ginger oil at the same dose according to Rashidian et al. [30]. The animals of the interaction group were given orally ginger oil and Topamax® (400mg/kg/mice/day). The period of administration in all experimental groups was one month.

Biochemical Analysis

Brain homogenate preparation

Brain tissue pieces was weighed and homogenized in PBS with a glass homogenizer on ice. The cells suspension were sonicated with an ultrasonic cell disrupter or subjected to freeze-thaw cycles to further break down. The homogenates were centrifuged for 5 min at $5000\times g$ to get the supernatant.

Estimation of Oxidative Stress Markers and antioxidant enzyme in Brain Tissue

Lipid peroxidation

Lipid peroxidation is frequently investigated using the assays for thiobarbituric acid-reactive substances (TBARSs) according the method of Ohkawa et al. [31] by (Cat. No. MD 25 29, Biodignostic. Co, Egypt). Malondialdehyde (MDA) formed a colored complex in the presence of thiobarbituric acid, which was detectable by measurement of absorbance at 534nm. Absorbance was measured with Shimadzu UV-160 spectrophotometer. Levels were calculated as mmol/g.

Reactive Oxygen Species (ROS)

Reactive oxygen species levels were measured using 2, 7-dichlorofluorescein diacetate (DCFH-DA) (SI 1189Ra, Sunlong Biotech Co., Ltd, China), which measures intracellular generation of hydrogen peroxide, a procedure widely used for estimation of ROS [32].

Estimation of Glutathione Reduced concentration

Reduced glutathione was estimated by the method of Khan et al. [33] using kit (Cat. No. GR 25 11, Biodiagnostic. Co, Egypt). The method based on the reduction of 5, 5'-dithiobis (2 - nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 412 nm and expressed as nmol GSH/g tissue.

Measurement of Tumor Necrosis Factor-Alpha (TNF- α)

TNF- α was measured in brain tissue by using ELISA kit (SL0722Ra) supplied by (Sunlong Biotech Co., Ltd, China) according to the manufacturer's instructions [34].

Estimation of Gamma Aminobutyric Acid (GABA)

GABA level was measured in brain cells by using ELISA kit (Cat No. MBS269152) supplied by MyBioSource (USA) according to the manufacturer's instructions [35].

Acetylcholine esterase estimation

AChE activity was estimated in the whole brain homogenates using ELISA kit Cat No: E-EL-R0355) supplied by MyBioSource (USA) according to the manufacturer's instructions [36].

Histopathological Studies

Brain specimens were harvested from all mice/ group, immediately fixed in 10% neutral buffered formalin and routinely processed according to Suvarna et al. [37]. Sections of 5 μ m thickness were prepared and stained with Hematoxylin and Eosin (H&E) for subsequent histopathological examination by a light microscope (Olympus BX50, Tokyo, Japan). Neuropathological damage in the cerebral cortex was graded from (0-4) as follow: (0) indicated no changes; (1) indicated percentage area affected (<10%); (2) indicated percentage area affected (20-30%); (3) indicated percentage area affected (40-60%) and (4) indicated percentage area affected (>60%) [38].

Immunohistochemical Analysis

Glial Fibrillary Acidic Protein (GFAP)

The deparaffinized and rehydrated sections were incubated with GFAP, mouse monoclonal antibody (1:500) (Dako, N-series Ready- to use primary antibody). The immune-staining was amplified and

completed by Horseradish Peroxidase complex (Dako, REALTM Envision TM / HRP, Mouse ENV). Sections were developed and visualized using 3, 3-diaminobenzidine (Dako, REALTM DAB + Chromogen). The substrate system produced a crisp brown end product at the site of the target antigen. Sections were counterstained with haematoxylin, then dehydrated in alcohol, cleared in xylene and cover slipped for microscopically examination [39]. Quantification of GFAP was estimated by measuring the area % expression from 5 randomly chosen non overlapping fields in each section and averaged using image analysis software (Image J, version 1.46a, NIH, Bethesda, MD, USA).

Bax Expression

Bax expression level in the brain sections was examined according to El makawy et al. [40] Sections were incubated with primary antibodies against Bax (1:100 dilution) (Santa Cruz Biotechnology Inc., Dallas, TX, USA). The immune reaction was visualized using diaminobenzidine tetrachloride (DAB, Sigma, St. Louis, MO, USA).The positive immune reactive cells showed brown-stained cytoplasm. Quantification of Bax apoptotic protein was estimated by measuring the area % expression from 5 randomly chosen fields in each section and averaged using image analysis software (Image J, version 1.46a, NIH, Bethesda, MD, USA).

Real-Time Quantitative Reverse Transcription PCR (RT-qPCR)

Brain tissue was grinded using liquid nitrogen then homogenized in Easy red total RNA extraction kit (Intronbio, Korea) to extract total RNA according to the manufacturer’s instructions. RNA quality and concentration were measured using NanoDrop[™] 1000 Spectrophotometer (Thermo Fisher Scientific, USA). One µg RNA was treated with 1U DNase I, RNase-free (Thermo Scientific), to remove genomic DNA then HiSenScript TM cDNA kit (Intronbio, Korea) was used for cDNA synthesis. RT-qPCR was carried out on Stratagene Mx3005P Real-Time PCR System (Agilent Technologies) in a 20 µL reaction volume using, 1µl cDNA, 10 µM of forward and reverse primers, 10µl TOPrealTM qPCR 2X PreMIX (SYBR Green with low ROX) (Enzynomics) and DNase-free water. The reaction program consisted of 12 min at 95°C, followed by 40 cycles at 95°C for 15 sec, at 55-60°C for 30sec (table 1) and 72°C for 30 sec. Gyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control. Raw data obtained were exported to Microsoft Excel and analyzed using the 2-ΔΔ Ct method [41].

Table1. Sequence of primer

Gene	Accession No.	Nucleotide sequence 5'–3'	Size(bp)
Gabra1	NM_010250.5	CCAAGTCTCCTTCTGGCTCAACA GGGAGGGAATTTCTGGCACTGAT	111
Gabra3	NM_008067.4	CAAGAACCTGGGGACTTTGTGAA AGCCGATCCAAGATTCTAGTGAA	119
Gabra5	NM_176942.4	CCCTCCTTGTCTTCTGTATTTCC TGATGTTGTCATTGGTCTCGTCT	99
GAPDH	NM_008084.3	AACCTTTGGCATTGTGGAAGG ACACATTGGGGGTAGGAACA	223

Statistical Analysis

All data are presented as the mean $\pm$ standard error (SE) of at least three determinations. Statistical analysis of the experimental data was performed by one way analysis of variance (ANOVA), followed by Duncan's multiple comparison test using the Statistical Package for Social Sciences (SPSS version 16.0, SPSS Inc., Chicago, IL, USA). All differences were statistically significant at $P \leq 0.05$.

Results

Oxidative stress markers and GSH reduced concentration

Data in the Figures 1-3 illustrate the brain tissue levels of Malondialdehyde (MDA), ROS and glutathione reduced (GSH) in control and treated mice groups. The results revealed that there was no difference between the levels of Malondialdehyde (MDA), and ROS in brain tissue of controls and ginger oil-treated groups. While the brain tissue MDA and ROS levels in Topamax® -treated animals was significantly higher ($P \leq 0.01$) than all other groups. At the same time, the interaction between Topamax® and Ginger oil, significantly lower ($P \leq 0.01$) the brain MDA and ROS levels than those in Topamax® treated animals. The level of brain glutathione reduced concentration (GSH) in ginger oil-treated mice was significantly higher ($P \leq 0.01$) than in all groups. Whereas, the GSH level of Topamax® -treated mice was significantly lower ($P \leq 0.01$) than all groups. In addition, the interaction elevated the level of GSH than those of Topamax® treated mice brain.

Effect of Interaction between Topamax® and Ginger Oil on Neurotransmitters

The results indicated that the Topamax® exhibited significant elevation ($P \leq 0.01$) in AChE and GABA in mice brain tissue as compared to control and ginger treated animals as seen in Figures 4 & 5. Ginger oil interaction with Topamax® significantly ($P \leq 0.01$) ameliorates their levels but did not reach the normal control levels.

Effect of Topamax® and Ginger Oil on Tumor Necrosis Factor-Alpha

To evaluate the potential inflammatory response resulting from the exposure to Topamax® the cytokines TNF- α was measured in the brain tissue. In comparison to the control group, Topamax® elevated significantly ($P \leq 0.01$) the TNF- α level, while ginger significantly declined ($P \leq 0.01$) its level as show in Figure 6. While, the interaction between Ginger and Topamax® significantly diminished ($P \leq 0.01$) the raise in TNF- α level induced by Topamax®.

Effect of Topamax® and Ginger Oil on Histopathology of the Cerebral Cortex

Microscopically, cerebral cortex of normal control mice as well as mice treated with corn oil and ginger oil reveled the normal histological architecture. On contrary, cerebral cortex of Topamax® treated mice exhibited various neuropathological alterations. The most pronounced changes observed were congestion of cerebra blood vessel, neuronal pyknosis and necrosis, neuronophagia, proliferation of glia cells, necrosis of granular cells and intercellular edema. Meanwhile, interaction of Topamax® and Ginger oil significantly regressed the neuropathic lesions. Examined cerebral cortex revealed necrosis, pyknosis of some neurons and neuronophagia (Figure 7). Table 2 summarizes the histopathological lesion score in the cerebral cortex of mice in different experimental groups.

Glial Fibrillary Acidic Protein (GFAP)

Microscopic examination of normal control as well as corn oil and Ginger oil treated mice revealed normal small-sized astrocytes with lightly stained GFAP positive short processes. On contrary, strong immune-reactivity of hypertrophied astrocytes with deeply brown stained GFAP positive processes was recorded in cerebral cortex of mice treated with Topamax® 400mg/kg.. On the other hand, moderate immune-reactive astrocytes were demonstrated in sections from mice treated with combined Topamax® and Ginger oil. Furthermore, the percentage area of staining GFAP % was significantly elevated in Topamax® 400mg/kg treated group, as compared to negative control mice. Co-treatment of Topamax® and Ginger oil resulted in a significant reduction of GFAP area %, as compared to Topamax® 400mg/kg treated mice. Additionally, corn oil alone as well as Ginger oil alone exhibited insignificant change in GFAP area%, as compared to negative control group (Figure 8).

Bax protein immune expression

Immune histochemical expressions of Bax in the cerebral cortex of mice were analyzed (Figure 9). Briefly, Microscopic examination of normal control, corn oil and Ginger oil (400mg/kg) treated mice exhibited no immune expression. Meanwhile, strong Bax expression was demonstrated in mice treated with Topamax® 400 mg/kg.. The interaction between Topamax® and Ginger downregulated the Bax expression in cerebral cortex of treated mice. Co-treatment of Topamax® and ginger oil resulted in a significant reduction of Bax area %, as compared to Topamax® 400mg/kg treated mice. Additionally, corn oil alone as well as Ginger oil alone exhibited insignificant change in Bax area%, as compared to negative control group.

Table 2
Histopathological lesion score in the cerebral cortex of all experimental groups

Histopathological lesion	Control	Corn oil	Ginger oil	Topamax®	Topamax® + Ginger oil
Congestion of cerebral blood vessel	0	0	0	2	0
Necrosis and pyknosis of neurons	0	0	0	4	2
Neuronophagia	0	0	0	3	2
Necrosis of granular cells	0	0	0	3	1
proliferation of glia cells (gliosis)	0	0	0	3	0
Intracellular edema	0	0	0	2	0

Real Time- Quantitative Reverse Transcription PCR (RT-qPCR)

The mRNA levels of three GABA_AR subunits, namely Gabra1, Gabra3 and Gabra5 were evaluated by RT-qPCR. As shown in figure 10, Topamax® treatment up-regulated the relative mRNA expression of Gabra1 (1.5 fold). Similarly, significant up-regulation was found in animals treated with Ginger oil (1.7 fold). In the same way, the mRNA levels of Gabra3 were significantly up-regulated in Topamax® (1.4 fold, $P \leq 0.05$) and Ginger oil (1.5 fold, $P \leq 0.05$) treated animals compared to control. Conversely, interaction of Topamax® and Ginger oil markedly down-regulated mRNA expression level of Gabra1 and Gabra3 ($P \leq 0.05$). Regarding Gabra5, there was no significant change in Topamax® and Ginger oil treated animals compared to control. Also, no significant change was found in animals treated with Topamax® plus Ginger oil.

Discussion

Topamax® is a medication used in the treatment of epilepsy and migraine. It also has a variety of off-label uses, such as neuropathic pain, psychotropic drug-induced weight gain, alcohol use disorders, cluster headache prophylaxis, and more [42]. The objective of this research was to estimate the potential neurotoxicity of Topamax® and ginger oil high doses in the brain of mice and the ginger oil attenuating role against the toxicity of Topamax®. The current study showed that Topamax® at a dose of 400mg/kg induced various biochemical and histological alterations in the brain cells, supported by a strong immune expression of Bax protein. Our results showed an increase in the brain oxidative stress markers (MDA and ROS) and a decrease in the GSH levels. Oxidative stress is caused by an imbalance between the overproduction of ROS and the detoxification of these highly reactive species. The brain is highly susceptible to oxidative stress due to its high oxygen consumption rate, modest antioxidant defence, and the presence of redox-active metals [43]. In addition, neuronal membranes are rich in polyunsaturated fatty acids that become a target for lipid peroxidation [44]. It is known that excessive ROS generation can

cause damage to neuronal cells inducing cell death via either an apoptotic or a necrotic pathway [26, 45]. GSH plays a crucial role in the central nervous system defense against ROS overproduction. During excessive ROS production, the reduced GSH is consumed and the oxidized form prevails [47]. Malonaldehyde (MDA) is a product of the peroxidative degradation of polyunsaturated fatty acids and serves as a marker for assessing the extent of lipid peroxidation that considered as an imperative cause of neuronal damage [48]. The current study indicated that Topamax® increased the MDA levels in the brain tissue of mice. This result is consistent with study reported that Topamax® elevated the level of MDA and declined the glutathione activity in brain tissue [49].

Astrocytes are unique glial cells that have many essential tasks in the brain and it is critical in neuroinflammation. They respond to diverse sorts of brain traumas by a process called reactive astrogliosis [50]. Additionally, astrocyte activation can be triggered and amplified by multiple inflammatory factors, including TNF- α , IFN- γ , IL-6, and IL-1 β [51]. Activated astrocytes highly express the glial fibrillary acidic protein (GFAP) levels as early markers for apoptosis and astrogliosis [52]. These findings are in agreement with our results, where the study revealed that Topamax® increased the level of pro-inflammatory cytokine TNF- α and showed strong immune-reactivity of hypertrophied astrocytes with deeply brown stained GFAP positive processes as indicative of astrogliosis. Secretion of large amounts of TNF- α by the activated glial cells is mostly implicated in neuroinflammatory disorders [53]. GFAP is the chief intermediate filament in astrocytes that responsible for the astrocytes cytoarchitecture and mechanical strength, moreover, it plays a role in the interaction between astrocytes and neurons [54]. Dag et al. [55] found that Topamax® upregulated the expression of GFAP in the brain of rat offspring. Brenner and Messing [56] reported that the GFAP upregulation is a marker for reactive astrogliosis (gliosis) that considered an early pathological sign to utmost neurodegenerative diseases.

The proapoptotic protein Bax is activated by signaling actions in cells undergoing apoptotic cell death. In neurons, Bax is thought to be the main executioner and plays an essential role in neuronal cell death in processes as diverse as developmental apoptosis, hypoxia, ischemia, oxidative stress, nerve growth factor withdrawal, and nerve injury ([57]. It was postulated that neuronal apoptosis represents one of the mechanisms causing side effects of antiepileptic drugs [19]. In the present study, the brain of Topamax® treated animals showed strong Bax protein expression. Elevation in levels of Bax protein and mRNA were detected in traumatic Brain injury in rats and considered as an indicator of neuronal cell death [58].

Taken together, our results led us to hypothesize that apoptosis, neuroinflammation, oxidative stress, and astrogliosis were implicated in the Topamax® apparent neurotoxicity. Oxidative stress with neuroinflammation, neural death, and abnormal connections between neurons are possible pathophysiological features [59]. *Zingiber officinale* (Zingiberaceae) is used in traditional medicine as an antiepileptic alternative medication that has antioxidant activity and neuroprotective effects [60]. Also, the effectiveness of ginger in the therapy of migraine attacks and its similarity to the antiepileptic drug was confirmed [15]. The results showed that a dose of 400mg/kg ginger oil had no neurotoxicity. Also, in the present study the interaction of ginger oil and Topamax® revealed its neuroprotective effect evidenced by overall enhancement in the investigated brain tissues with regressed the neuropathic

lesions, decreased expression of GFAP and Bax protein, in addition, to improve the histopathological lesion score. Moreover, the biochemical investigation showed improvement in the levels of oxidative stress markers and inflammatory marker (TNF- α) levels. This is consistent with Badawy et al. [19] study that confirmed the protection of ginger against the neurochemical and histopathological damage of brain and CNS tissues induced by the antiepileptic drug, gabapentin. Furthermore, El-Akabawy and El-Kholy [61] showed that ginger reduced the inducible levels of TNF- α , GFAP, and AChE in diabetic rats. In addition, the ginger active compounds 6-gingerol and 6-shogaol were shown to have neuroprotective properties by attenuating MDA, TNF- α , and GSH levels in brain tissue [18]. This led us to hypothesize that the neuroprotective effect of ginger oil may be mediated by its free radical scavenging and antioxidant activity and oxidative stress inhibition. Besides, our previous study on fatty acid profile and tocopherols composition of cold pressed ginger oil revealed that it is rich in oleic and stearic acids and contains high levels of α -, γ - and δ -tocopherols and α -, β -, γ - and δ -tocotrienols [40]. Song et al. [64] revealed the neuroprotective effects of Oleic acid against transient and permanent global cerebral ischaemia, as well as focal cerebral ischaemia. Similarly, stearic acid was found to protect cortical neurons against oxidative stress by boosting the internal antioxidant enzymes. It is known that Oleic and stearic acid neuroprotective effects may be mostly mediated by the activation of the proliferator-activated receptor (PPAR γ) and recovers the neurological functions [65]. In addition, tocopherols and tocotrienols are considered as neuroprotective and antioxidant agents in astrocytes, chronic cerebral hypoperfusion-induced neurodegeneration [66]. In addition, Azar et al. [67] displayed that α -tocopherol reduced ischemic edema formation through increasing antioxidant activity (GSH) and reducing MDA levels. From the mentioned above findings, we can confirm that the ginger oil neuroprotective property was attributed to their high content of fatty acids and tocopherols.

Acetylcholine (ACh) is a neurotransmitter that has a crucial role in the brain. It alters neuronal excitability, influences synaptic transmission and plasticity, and coordinates firing of neurons. Disrupted levels of acetylcholine was shown to be implicated in neurodegenerative disorders [68]. Although AChE is not an apoptosis initiator, it has been discovered that cells with AChE overexpression are more susceptible to apoptosis [65]. Data of the present study revealed that Topamax® elevated the brain AChE activity, this is consistent with the study of Al-Kawaz et al. [69] they elucidated that the Topamax® diminished the ACh levels in pregnant rats. In line with the above findings, it appears that AChE activity is implicated in the neurotoxicity of Topamax®. Where, AChE's main function is to stop neuronal transmission and signalling between synapses, preventing ACh from spreading and activating neighbouring receptors [70]. Oppositely, the results demonstrated that interaction of Topamax® and ginger oil decreased the Topamax® induced AChE levels. This is attributed to the AChE inhibitory activity of ginger that may be due to its high content of oleic acid [61]. Velasco and Hijazi [71] suggested that oleic acid may have a role in the cholinergic system through induction of choline acetyltransferase expression, a transferase enzyme responsible for the synthesis of ACh.

Topamax® is an antiepileptic drug that works by inhibiting voltage-dependent Na(+)- and/or Ca(2+)-fluxes into neurons, increasing GABA-mediated Cl⁻-fluxes into neurons, increasing potassium currents, inhibiting kainate-mediated conductance at glutamate receptors of the AMPA/kainate type, and inhibiting

several carbonic anhydrase isozymes. The elevation of GABA levels in the brain potentiates the GABA-mediated inhibition by increasing the frequency of its interactions with its receptors [5]. Our results showed that Topamax® induced the GABA protein levels in brain tissue. On the other hand, we found an increase in GABA levels in ginger oil-treated animals. However, GABA levels induced by Topamax® highly exceeded that induced by ginger oil. In Consistent with our results, Farag et al. [20] revealed that ginger supplementation has been associated with an increased GABA concentration in the hippocampus as well as cerebral cortex of senile female rats. GABA levels elicited in the brain of mice treated with Topamax® and ginger oil were found to be lower than in Topamax® treated animals in the current investigation. This could be due to the theory that drug interaction with medicinal plant therapy may lead to lower pharmacological activity and efficacy of the drug [72].

Topamax® promotes GABA responses at the GABA_ARs and it may specially modulate a subset of GABA_ARs [73]. There are numerous subunit isoforms for the GABA_AR, α 1-6, β 1-3, γ 1-3, δ , ϵ , π , θ and ρ (1–3) which determine several properties of the receptor as its conductance, agonist affinity and opening chance [74]. Above all, α subunits are vital determinants of receptor function and localization [75]. Reyes-Nava *et al.* [76] indicated that knockdown of *gabra1* results in hypo-activity and defects in the expression of other GABAARs subunits. Also, many studies reported that the alteration of α 5 GABAAR levels have been observed in neurodevelopmental disorders [77, 78]. Additionally, down-regulation of *Gabra1*, 3 and 5 mRNA levels was reported in Alzheimer's disease (AD) patients [79]. In the present study, the expression level of three α subunit-encoding genes, *Gabra1*, *Gabra3* and *Gabra5*, were evaluated in response to Topamax and ginger oil and their interaction. The mRNA expression level for brain *Gabra1* and *Gabra3* was up-regulated in both Topamax® and ginger oil treated animals, while, it was markedly down-regulated in their interaction treated animals. This result agrees with Xiaoyuan et al. [80] who studied the therapeutic effect of Topamax® on global ischemia/reperfusion injury and they reported increased protein expressions of *Gabra1* in gerbil's hippocampus. Taherianfard *et al.* [81] revealed that ginger the anti-nociceptive effects prevents the suppression of the function of GABA_ARs by inhibiting eicosanoids. Guillen-Ruiz *et al.* [82] found that oleic acid in ginger oil reduced locomotor activity and motor incoordination apparently through actions at the GABA_AR. In the current study, the *Gabra5* mRNA expression was not changed in response to neither Topamax® or ginger oil nor their interaction. Our findings led us to hypothesize that Topamax® and ginger oil have enhanced, at least partly, *Gabra1* and *Gabra3*-mediated inhibition currents.

Declarations

- **Ethics approval**

The study was authorized by the National Research Centre's Ethics Committee (Approval No. 18038).

- **Consent for publication**

Not applicable

- **Availability of data and material**

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare no conflicts of interest.

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This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

- **Authors contributions**

AIE designed the study. DMM; FMI and KAA carried out the biological experiments and analyzed the data under the supervision of AIE. DMM; FMI, KAA and AIE co-wrote the manuscript. All approved the final version of the manuscript for publication.

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Figures

Figure 1

MDA levels in the brain tissue of mice treated with Topamax® and/or Ginger oil high doses

Data shown as mean $\pm$ SE; one way analysis of variance was used for data analysis (n=6), groups with unlike superscript letters were significantly different ($P \leq 0.01$)

Figure 2

ROS levels in the brain tissue of mice treated with Topamax® and/or ginger oil high doses.

Data shown as mean $\pm$ SE; one way analysis of variance was used for data analysis (n=6), groups unlike superscript letters were significantly different ($P \leq 0.01$)

Figure 3

GSH levels in the brain tissue of mice treated with Topamax® and/or Ginger oil high doses. Data shown as mean $\pm$ SE; one way analysis of variance was used for data analysis (n=6), groups with unlike superscript letters were significantly different ($P \leq 0.01$)

Figure 4

AChE activity in the brain tissue of mice treated with Topamax® and/or Ginger oil high doses. Data shown as mean $\pm$ SE; one way analysis of variance was used for data analysis (n=6), groups with unlike superscript letters were significantly different ($P \leq 0.01$)

Figure 5

GABA activity in the brain tissue of mice treated with Topamax® and/or Ginger oil high doses. Data shown as mean ± SE; one way analysis of variance was used for data analysis (n=6), groups with unlike superscript letters were significantly different ($P \leq 0.01$)

Figure 6

TNF-α in the brain tissue of mice treated with Topamax® and/or Ginger oil high doses. Data shown as mean ± SE; one way analysis of variance was used for data analysis (n=6), groups with unlike superscript letters were significantly different ($P \leq 0.01$)

Figure 7

Photomicrographs representing histological H&E stained sections of the cerebral cortex of mice (scale bar 25µm): Normal control, Corn oil, Ginger treated groups showing the normal histological architecture with normal neurons. Topamax® (400mg/kg) treated group, showing congestion of cerebral blood vessel (CO), neuronal pyknosis and necrosis (NP), neuronophagia (NH), proliferation of glia cells (GL), necrosis of granular cells (NG) and intracellular edema (CE). Combined Topamax® and Ginger oil treated group, showing pyknosis of some neurons (NP) and neuronophagia (NH).

Figure 8

Photomicrographs representing immunocytochemistry of GFAP expression in the cerebral cortex (scale bar 25μm) of mice: normal control, corn oil & Ginger oil (400mg/kg) showing normal small sized astrocytes with lightly stained GFAP positive short processes (arrow). Topamax® 400mg/kg; showing strong positive expression of hypertrophied astrocytes with deeply stained GFAP positive brown processes (arrow). Topamax® and Ginger oil, showing moderate immune-reactive astrocytes (arrow). Immune staining area (%) of GFAP expression in the cerebral cortex of mice from different experimental groups. Data shown as mean $\pm$ SE (n=6); groups with unlike superscript letters were significantly different ($P \leq 0.05$).

Figure 9

Photomicrographs representing immunocytochemistry of Bax expression in the cerebral cortex of mice (scale bar 25µm): Normal control; Corn oil and Ginger oil (400mg/kg) showing no immune expression. Topamax® 400mg/kg; showing strong positive expression (arrow). Topamax® and Ginger oil, showing weak immune reaction (arrow). Immune staining area (%) of Bax expression in the cerebral cortex of mice from different experimental groups. Data shown as mean ± SE (n=6); groups with unlike superscript letters were significantly different ($P \leq 0.05$).

Figure 10

The mRNA levels of GABAAR subunits, A) Gabra1, B) Gabra3 and C) Gabra5 in mice brain tissue of experimental groups. Data is presented as mean $\pm$ SE, one way analysis of variance was used for data analysis (n=6), groups with unlike superscript letters were significantly different ($p \leq 0.05$)