Indian Journal of Experimental Biology
Vol. 62, June 2024, pp. 379-392
DOI: 10.56042/ijeb.v62i06.4177

Sinapic acid reduces pentylenetetrazol induced seizures in rats

Mehmet Ekici*[1], Handan Güneş[2] & Arzu Gezer[3]

[1]Department of Veterinary Physiology, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, 58140, Sivas, Türkiye
[2]Department of Physiology, School of Medicine, Sivas Cumhuriyet University, 58140, Sivas, Türkiye
[3]Health Care Services, School of Health Services, Atatürk University, 25240, Erzurum, Türkiye

Received 24 July 2023; *revised 17 January 2024*

Seizure is known to induce oxidative stress which may initiate neuronal death. Oxidant-antioxidant imbalance often leads to mitochondrial dysfunction, inflammation, and apoptosis in the brain which may further result in the development of seizure. Phenolic compounds such as curcumin and rosmarinic acid are reported to control convulsions and seizures in pentylenetetrazol induced seizures models by suppressing seizure time, oxidative stress and inflammation indirectly. Sinapic acid (SA), a polyphenolic product of hydroxycinnamic acid found in various plants, exhibits anti-inflammatory, antioxidant and anxiolytic effects. In this study, we investigated the effects of sinapic acid on pentylenetetrazol induced seizures in rats through oxidative stress, inflammation, apoptosis, and neurotrophic factor. A total of 28 male Wistar Albino rats weighing 200-220 g were divided into four equal groups (n=7/group). The treatment groups received 10 mg/kg and 20 mg/kg SA, respectively, by oral gavage for five consecutive days along with pentylenetetrazol (45 mg/kg, intraperitoneal) to induce seizures. The levels of Total oxidant status (TOS), Total antioxidant status (TAS), TNF-α, IL-1β, and Brain-derived neurotrophic factor (BDNF) were measured in the cortex and hippocampus. Additionally, caspase 3 and caspase 9 levels, as well as the immunoreactivity of Cleaved caspase 3, were determined in the hippocampus. The results showed that pretreatment with 20 mg/kg SA delayed the latency of generalized tonic-clonic seizures (GTCS) and first myoclonic jerk, reduced GTCS duration, and improved seizure score and cognitive function. Importantly, the 20 mg/kg SA pretreatment resulted in decreased levels of TOS, TNF-α, IL-1β, and BDNF in the cortex and hippocampus, while increasing TAS levels in these brain areas. Moreover, the 20 mg/kg SA reduced hippocampal caspase 3 and caspase 9 levels, as well as the immunoreactivity of Cleaved caspase 3 in rats with pentylenetetrazol-induced seizures. These findings suggest that the anti-seizure effects of SA are mediated by BDNF modulation, as well as its antioxidant, anti-inflammatory, and anti-apoptotic properties.

Keywords: Anticonvulsant activity, Antiseizure activity, Apoptosis, Brain-derived neurotrophic factor (BDNF), Epilepsy, Experimental acute seizure model, Generalized tonic-clonic seizures (GTCS), Inflammation, Oxidative stress, Racine convulsion scale (RCS)

A seizure is defined as the appearance of transient signs or symptoms in the brain due to abnormally excessive and asynchronous neuronal activity. The most prominent neurodegenerative changes include neuronal loss, increased astrocyte number and size, increased blood-brain barrier permeability, axonal damage, increased inflammation, and oxidative stress[1]. Neurodegeneration in the brain can alter the functions of dynamic synapses that store information, particularly in the hippocampus, possibly causing cognitive impairment after the seizure[2], which changes the quality of life leading to depression, anxiety and perceptual disorders[3]. Drugs such as antiepileptics that are used to treat seizures cannot cure the condition. Rather, they can only treat its symptoms. The long-term use of antiepileptic medications is restricted because of their negative side effects, withdrawal symptoms, harmful drug interactions, and financial load, particularly in developing countries. Additionally, some antiepileptic medications on the market have the potential to intensify types of seizures[4]. It has also been reported that the current treatment options may cause cognitive impairment[4].

Studies have reported that seizure induces oxidative stress and initiates neuronal death[5]. The generated reactive oxygen species because of imbalance oxidant-antioxidant lead to mitochondrial dysfunction, inflammation, and apoptosis in the brain which may further lead to the development of

*Correspondence:
Phone: +90 346 219 1010 (ext: 3625)
E-Mail: mehmetekici@cumhuriyet.edu.tr
ORCİD: https://orcid.org/0000-0002-2163-6214

seizure[6]. In addition, focal or systemic inflammatory processes cause abnormal neuronal plasticity and neural connectivity which may overstimulate the neuronal network that mediates the onset of seizure[7]. It has been known that the brain-derived neurotrophic factor (BDNF) plays an important role in many pathophysiological processes in the brain (e.g. epilepsy), as well as several physiological processes such as neuronal excitability, viability, development, and cell survival[8]. It has been suggested that a possible therapeutic strategy for seizure could be to suppress BDNF-TrkB signaling[9].

New treatment strategies for seizure have frequently been tried in experimental studies. For instance, phenolic compounds such as curcumin and rosmarinic acid have been recently reported to control convulsions and seizures in pentylenetetrazol induced seizures models by suppressing seizure time, oxidative stress, and inflammation indirectly[10,11]. Sinapic acid (SA), a polyphenolic product of hydroxycinnamic acid found in various plants, have been reported to show anti-inflammatory, antioxidant[12], and anxiolytic effects[13]. Moreover, SA has a neuroprotective effect in experimental rodent models such as kainic acid-induced hippocampal damage and β amyloid (1-42) protein-induced Alzheimer's disease in mice and in the 6-hydroxydopamine-induced hemi-parkinsonian rat[14-16]. SA also improves the impaired cognitive functions induced by scopolamine in rats[17].

In this study, we examined the effects of sinapic acid on oxidative damage, inflammation, apoptosis, and brain-derived neurotrophic factor levels in the cortex and hippocampus of rats with acute seizure induced by pentylenetetrazol.

Materials and Methods

Chemicals and pharmaceutics

Pentylenetetrazol and SA were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were of analytical quality. Commercial ELISA kits of TAS, TOS, BDNF, TNF-α, IL-1β, caspase 3, and caspase 9 were purchased from Bioassay Technology Laboratory (Shanghai Korain Biotech Co., Ltd, Shanghai, China. See below for more details).

Animals

In this study, a total of 28 healthy male, 8-10 weeks old, Wistar Albino rats weighing 200-220 g were used. The rats in each group housed in the macrolon type 4 cages and their daily health status were monitored. Each cage was numbered, and the chemicals applied were hidden from those who performed the experiment and analysis. However, the corresponding author was aware during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis. During the experiment, the rats were housed under standardized laboratory conditions at 21±2°C, 12/12 h light-dark period, in Sivas Cumhuriyet University Experimental Animals Application and Research Center. Food and tap water were given *adlibitum*. An adaptation period was applied for one week prior to the experiment. Experiments were carried out between 9 a.m. and 5 p.m. in an isolated laboratory room. The experimental protocols were approved by the Sivas Cumhuriyet University Animal Experiments Local Ethics Committee (Approval Code: 65202830-050.04.04-395). All protocols followed national guidelines for the care and use of laboratory animals and were compliant All protocols were followed national and international guidelines for the care and use of laboratory animals. Reporting follows ARRIVE 2.0 guidelines[18].

Experimental protocol

The study protocol is summarized in Fig. 1. At the beginning of the study, the rats were randomly divided into four groups (n=7/group); The rats in the control group (Gr. I) were given 2 mL of distilled water per rat (10 mL/kg) orally for 5 days. On day 5, sterile saline (1 mL/kg, i.p.) was administered to the same control rats one hour after the last distilled water administration. The rats in the Pentylenetetrazol group (Gr. II-IV) were administered distilled water through oral gavage for 5 days. On the 5th day, a single dose of pentylenetetrazol (45 mg/kg) in a volume of 1 mL/kg (0.2 mL/rat) was intraperitoneally administered to these (Gr. II) rats one hour after the last administration of distilled water to induce a seizure. The rats in the Pentylenetetrazol+SA10 (Gr. III) and Pentylenetetrazol+SA20 (Gr. IV) groups were given SA (10 and 20 mg/kg, respectively) by oral gavage for 5 days and on day 5, Pentylenetetrazol was administered (45 mg/kg, i.p.) one hour after the last SA administration. The doses of pentylenetetrazol and SA were determined based on previous studies[19,20]. In previous experimental studies on neurodegeneration, the effects of SA alone have been shown similar effects with those of the control groups[14,21]. Therefore,

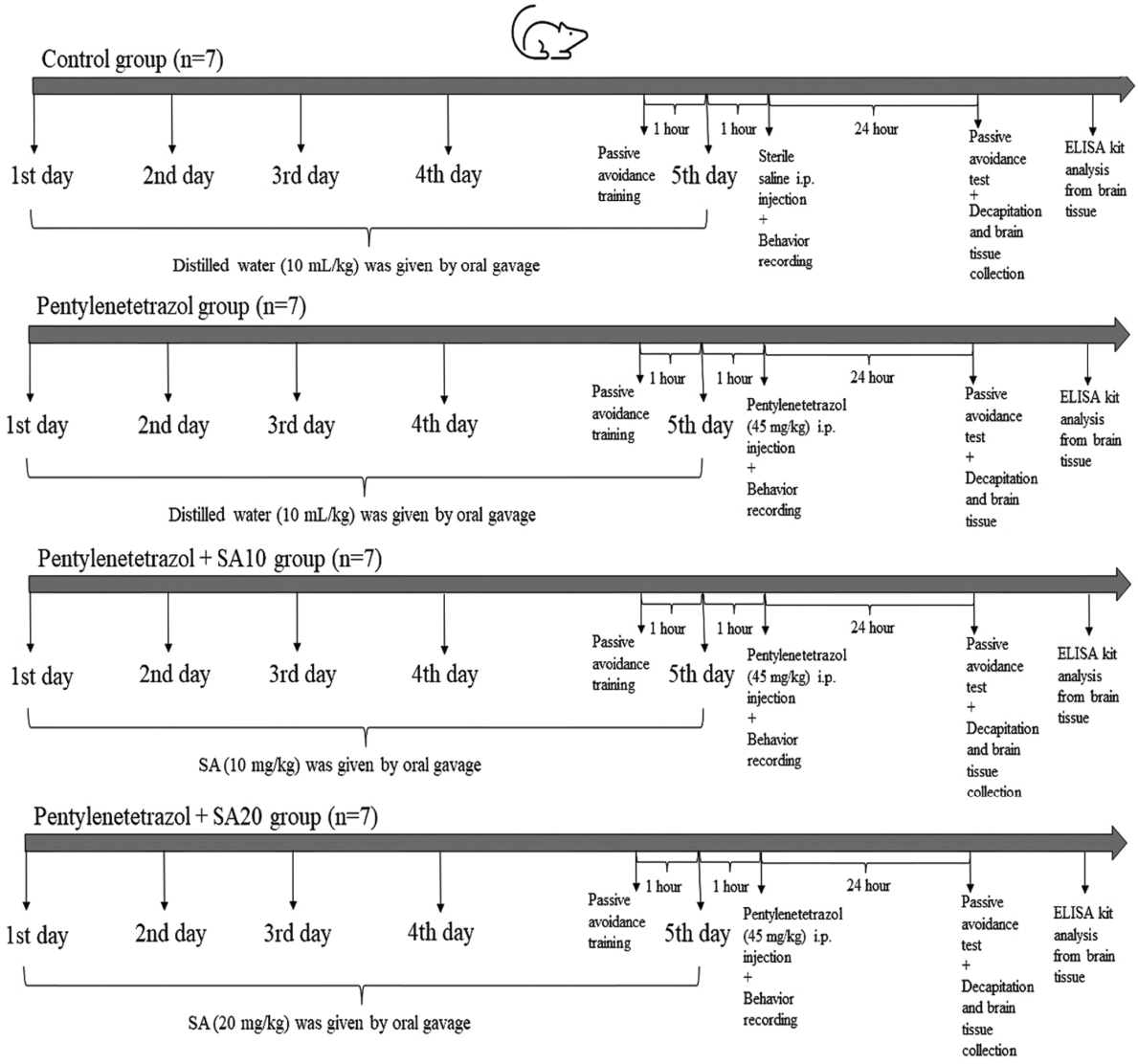

Fig. 1 — Experimental protocols of the study.

a SA group was not included in the study. Seizure severity was determined according to the modified racine convulsion scale (RCS): 0- no change in behaviour, 1- twitching of whisker and auricle, 2- motor arrest with more apparent twitching, 3- myoclonic body jerks, 4- tonic-clonic seizure while the animal remained on its feet, 5- tonic-clonic seizure with lacking of the righting reflex, 6- tonic-clonic seizure with wild climbing and jumping, and 7- lethal seizure[3]. The behaviour of the rats was recorded on a video camera for 30 min after pentylenetetrazol injection to assess seizure severity. The number of rats with seizures, seizure score, first myoclonic jerk, the latency and the duration of the generalized tonic-clonic seizure (GTCS) were analyzed from the video recordings. The passive avoidance test was performed 24 h after pentylenetetrazol injection. At the end of the experiment, the rats were euthanized by decapitation under ketamine (60 mg/kg)/xylazine (10 mg/kg) anesthesia. According to the guidelines and regulations set by the institute, the dead rats were disposed by buried at a licensed waste disposal site after euthanization and sample collection. No seizure-related deaths were observed during the study. The cortex and hippocampus samples were collected and kept at −80°C until analysis.

Passive avoidance test

The passive avoidance learning test was performed based on negative reinforcement as previously described[22]. Negative reinforcement is applied in passive avoidance experiments with rats, where they learn to avoid a particular area or stimulus linked to

an aversive electric shock. The removal of the shock acts as negative reinforcement, strengthening the avoidance behaviour. The principle of the test is that the rat acts against their natural tendency to prefer the dark area and avoids bright area. The shuttle box consisted of two parts, a light compartment (20×20×30 cm) and a dark compartment (20×20×30 cm). A guillotine door opening (6×6 cm) was formed on the floor of the partition between the two compartments. The stainless-steel grids, each approximately 5 mm in diameter, were spaced 1 cm apart on the compartment's floor to create foot shock. The animals spent at least 30 min getting used to the testing environment. The habituation experiment was conducted 30 min before the experiment. Each animal was placed in the light chamber, and after 5 s the guillotine door was opened. The door was immediately closed when the animal entered the dark room, and a foot shock (50 HZ, 5 s, 1 mA strength) was applied to the grid floor, and time latency to enter the dark compartment was recorded. After 20 s, the rat was taken out of the machine and placed back to its cage. The training ended after the rat spent 120 s in the light chamber. The training test was performed 2 h before the seizure. Then the seizure was carried out. A memory performance passive avoidance test was performed 24 h after the seizure. Each animal was placed in the light chamber and the experiment ended when they either moved into the dark chamber or were still there after 300 s. No electric shocks were administered to the animals throughout these sessions.

Preparing cortex and hippocampus homogenates

After passive avoidance testing, the hippocampus and cortex tissue samples that were collected from each rat were placed into 500 µL 1 × phosphate-buffered saline (PBS Tablets – Calbiochem, Merck Millipore, Germany; dissolving one tablet of PBS buffer in 1 liter of deionized H2O yields 140 mM NaCl, 10 mM phosphate buffer, and 3 mM KCl, pH 7.4 at 25°C) assay buffer containing Complete mini protease inhibitor cocktail (1 tablet per 3 mL PBS final concentration, 3.5× protease inhibitor, Roche Diagnostics) were mechanically homogenized with a bead beater (Bead Blaster 24, Edison, USA). The buffer solution was free of organic solvents and detergents. The homogenates were centrifuged for 10 min at 4°C at 4,000 rpm. The obtained supernatants were stored in dry ice until analysis. A total protein analysis was performed on an autoanalyzer (BS 200 Mindray, China).

Determination of hippocampal and cortical oxidative status, inflammation, apoptosis and BDNF levels

All ELISA assays mentioned below were purchased from Bioassay Technology Laboratory, Shanghai, China and performed according to the manufacturer's protocol. The effect of SA on oxidative stress of hippocampus and cortex in rats with seizures induced by pentylenetetrazol was determined by measuring TAS (Cat. No: 201-11-1187) and TOS (Cat. No: E1512Ra) levels with a commercial ELISA kit. To determine the inflammatory response to the seizures model, TNF-α (Cat. No: E0764Ra) and IL-1β (Cat. No: E0119Ra) levels in the hippocampus and cortex were determined with commercial ELISA kits. Hippocampal apoptosis was determined by analyzing caspase 3 (Cat. No: E1648Ra) and caspase 9 (Cat. No: E1898Ra) levels with commercial ELISA kits. The levels of BDNF (Cat. No: E0476Ra) from cortex and hippocampus supernatants were measured with ELISA kit.

Histopathological study

The brains of rats were examined after necropsy, and brain tissues were preserved in a 10% neutral formalin solution. The tissues underwent standard procedures of dehydration using alcohol and xylene, followed by embedding in paraffin blocks. Thin sections measuring 5 µm were prepared on slides coated with poly-lysine and stained with a combination of hematoxylin and eosin. The observed neuronal changes in the cornu ammonis (CA1, CA3) regions were evaluated semiquantitatively based on the presence or absence of pycnotic alterations, categorized as follows: absent (-), mild (+), moderate (++), or severe (+++).

Immunohistochemical study

The brains of rats were examined after necropsy, and brain tissues were fixed in a 10% neutral formalin solution. The tissues were processed through standard procedures of dehydration using alcohol and xylene, followed by embedding in paraffin blocks. Thin sections measuring 5 µm were prepared on slides coated with poly-lysine. The sections were deparaffinized using xylene and alcohol series, followed by washing with phosphate-buffered saline (PBS). Endogenous peroxidase activity was inhibited by treating the tissues with 3% H_2O_2 for 10 min. Antigen retrieval was performed using an antigen retrieval solution (10 mM sodium citrate buffer, pH 6.0, Santa cruz, Catalog No: sc-294091), applying 2 rounds of 5 min at 500 watts. After washing with PBS, the tissues were incubated with

primary antibodies against Cleaved caspase 3 (ElabScience, Catalog No. E-AB-30004, diluted at a ratio of 1/1200) at 25 °C for 45 min. For detection, the Large Volume Detection System: anti-Polyvalent, HRP (Thermofisher, Catalog No: TP-125-HL) was used following the manufacturer's instructions. The chromogen used was AEC (3-Amino-9-Ethyl-carbazole). Counterstaining was performed using Mayer's Hematoxylin, and the slides were examined under a light microscope. Immunopositivity in the cornu ammonis (CA1, CA3) regions of the brain was semiquantitatively assessed and categorized as absent (-), mild (+), moderate (++) or severe (+++).

Statistical analyses

The sample size (n=7 per group) was calculated with the G*Power Version 3.1.9.6 (Germany) program according to the previously report[23]. The effect size, α error probability, and the power (1 - β error probability) were selected as 0.72, 0.05, and 0.80, respectively. Data were analyzed using IBM SPSS Statistics for Windows (version 26). The normal distribution analysis of the data obtained from the study was performed with the Shapiro–Wilk test, and the homogeneity of the data was performed with the Levene test. Data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test. The obtained histo-pathological and immunohistochemical data were analyzed using nonparametric tests, specifically the Kruskal-Wallis test to identify the group(s) responsible for the differences. If a significant difference was found, the Mann-Whitney U test was performed to determine the specific group(s) that contributed to the difference. Results were expressed as mean ± standard error of mean (S.E.M.). The P values less than 0.05 were considered statistically significant.

Results

Effects of SA on seizure score, the latency of first myoclonic jerk, the latency and duration of GTCS

The seizures behaviour was evaluated by the video camera recordings following pentylenetetrazol injection to the rats. Fig. 2 shows that the seizure score point according to the modified racine scale and the duration of GTCS were significantly higher in the penty-lenetetrazol group than that of the penty-lenetetrazol+SA20 group (P = 0.005, F (3, 24) = 53.792; P = 0.001, F (3, 24) = 15.749, respectively). The latency of first myoclonic jerk and GTCS of the penty-lenetetrazol+SA20 group (Gr. IV) was statistically higher than that of the pentylenetetrazol group (Gr. II) (P = 0.000, F (3, 24) = 365.98; P = 0.001, F (3, 24) = 29.032, respectively). However, there were no significant differences between the pentylenetetrazol group (Gr. II) and the pentylenetetrazol+SA10 group (Gr. III) in any behaviour parameters (P >0.05).

Effects of SA on passive avoidance test

This test was conducted to determine memory dysfunction and fear after seizures caused by pentylenetetrazol. In the passive training stage, there was no statistical difference between the groups (P >0.05, F (3, 24) = 0.073) (Fig. 2E). On the other hand, the pentylenetetrazol group (Gr. II) had a lower test time than those of the control group (P = 0.000, F (3, 24) = 45.685). The latency of test time of the pentylenetetrazol+SA20 group (Gr. IV) was notably higher than the pentylenetetrazol group (Gr. II) (P = 0.000, F (3, 24) = 45.685). However, there were no significant differences between the penty-lenetetrazol group and the pentylenetetrazol+SA10 (Gr. III) group in passive avoidance test latency (P >0.05).

Effects of SA on brain oxidative status

The total antioxidant status (TAS) and total oxidant status (TOS) levels were analyzed to determine

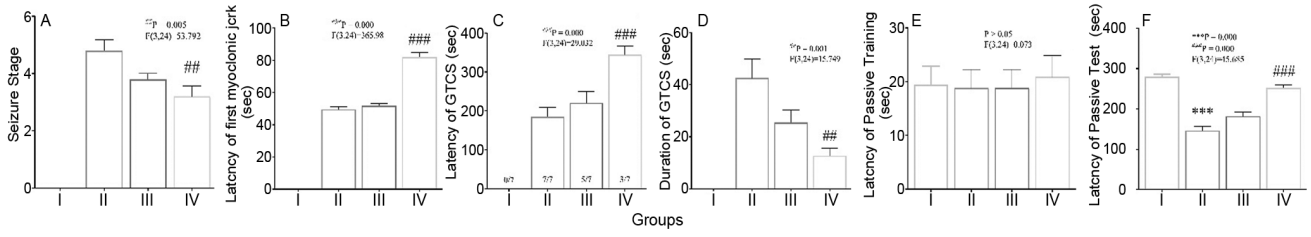

Fig. 2 — (A) Seizure score (RCS); (B) latency of first myoclonic jerk; (C & D) generalized tonic-clonic seizure (GTCS) latency and duration; (E & F) Passive avoidance test in pentylenetetrazol induced behavioural seizures. [Gr. I: Control; Gr. II: Pentylenetetrazol; Gr. III & IV: Pentylenetetrazol + SA10 and SA20, respectively. Data were expressed as mean ± S.E.M. and analyzed with one-way analysis of variance (ANOVA) followed by Tukey HSD multiple comparison test. ***P <0.001 compared to the control group; $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 compared to the Pentylenetetrazol group]

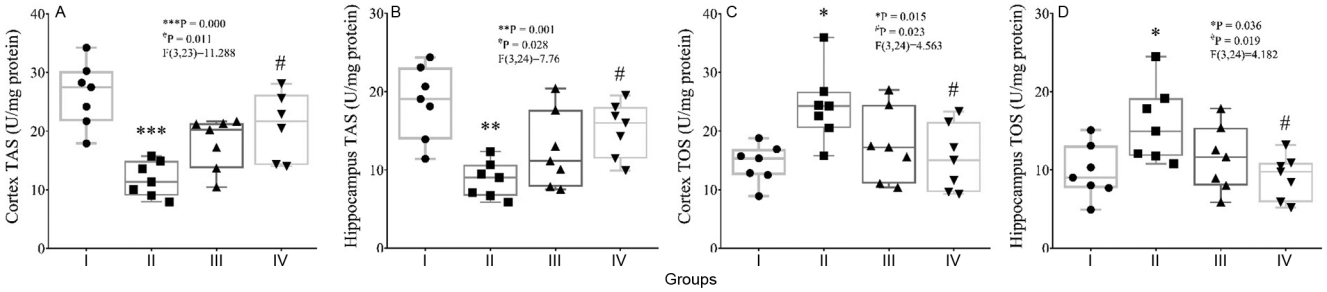

Fig. 3 — (A & B) Total antioxidant status (TAS) levels; and (C & D) Total oxidant status (TOS) levels in the cortex and hippocampus of rats. [Gr. I: Control; Gr. II: Pentylenetetrazol; Gr. III & IV: Pentylenetetrazol + SA10 and SA20, respectively. Statistical difference between groups is indicated as follows: **P* <0.05, ***P* <0.01, ****P* <0.001 compared with the control group; $^{\#}P$ <0.05 compared with the Pentylenetetrazol group. One-way ANOVA followed by Tukey post hoc test was used to determine the differences between the independent variables]

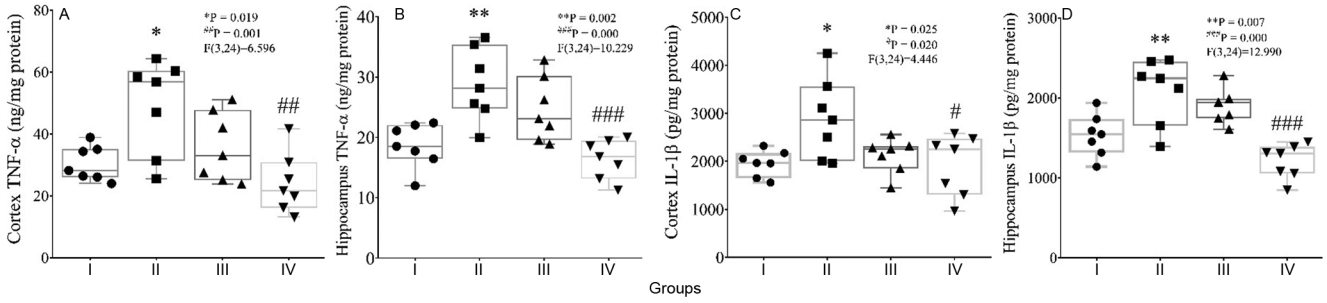

Fig. 4 — (A & B) TNF-α levels; and (C& D) IL-1β levels in the cortex and hippocampus of rats. [Gr. I: Control; Gr. II: Pentylenetetrazol; Gr. III & IV: Pentylenetetrazol + SA10 and SA20, respectively. Statistical difference between groups is indicated as follows: **P* <0.05, ***P* <0.01 compared to the control group; $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001 coompared to the Pentylenetetrazol group. One-way ANOVA followed by Tukey post hoc test was used to determine the differences between the independent variables]

the oxidative status in the cortex and hippocampus. Fig. 3A shows the TAS results in the cortex. The cortex TAS levels were significantly lower in the pentylenetetrazol group compared to the control group (P = 0.000, F = 11.288). The administration of 20 mg/kg SA significantly increased the reduction in TAS levels in the cortex caused by pentylenetetrazol (P = 0.011, F (3, 23) = 11.288). However, there was no difference between the pentylenetetrazol group and the pentylenetetrazol+SA10 group (*P* >0.05). Fig. 3B shows the TAS results in the hippocampus. The TAS levels in the pentylenetetrazol group (Gr. II) were significantly lower in the hippocampus than the control group (P = 0.001, F (3, 24) = 7.761). On the other hand, the 20 mg/kg SA administration significantly increased the hippocampal TAS levels compared to those of the pentylenetetrazol group (P = 0.028, F (3, 24) = 7.761). There was not statistically significance between the pentylenetetrazol (Gr. II) and the pentylenetetrazol+SA10 groups (Gr. III) (*P* >0.05).

Figure 3C shows the TOS results in the cortex. The cortex TOS levels in the pentylenetetrazol group (Gr. II) were statistically higher compared to the control group (P = 0.015, F (3, 24) = 4.563). However, the 20 mg/kg SA administration decreased TOS levels compared to the pentylenetetrazol group (P = 0.023, F (3, 24) = 4.563). No statistical difference was found between the pentylenetetrazol+SA10 and pentylenetetrazol groups (*P* >0.05). Fig. 3D shows the TOS results in the hippocampus. The hippocampal TOS levels, which were elevated by pentylenetetrazol significantly decreased in pentylenetetrazol+SA20 group (Gr. IV) (P = 0.019, F (3, 24) = 4.182) while no change was found between the pentylenetetrazol and pentylenetetrazol+SA10 groups (Gr. III) (*P* >0.05).

Effects of SA on brain inflammation

The levels of TNF-α and IL-β were analyzed to determine inflammation in the cortex and hippocampus. Figure 4A shows the TNF-α results in the cortex. TNF-α levels in the cortex were significantly higher in the pentylenetetrazol group compared to the control group (P = 0.019, F (3, 24) = 6.596). The increased cortex TNF-α levels by pentylenetetrazol significantly decreased by the 20 mg/kg SA administration (P = 0.001, F (3, 24) =

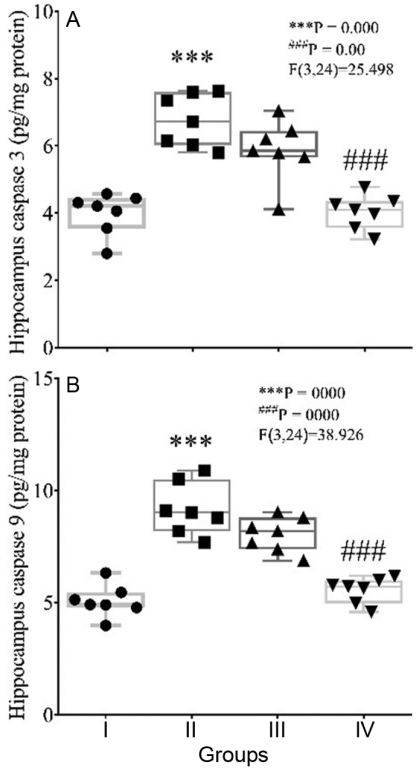

Fig. 5 — (A) Caspase 3; and (B) caspase 9 levels in the hippocampus of rats. [Gr. I: Control; Gr. II: Pentylenetetrazol; Gr. III & IV: Pentylenetetrazol + SA10 and SA20, respectively. The statistical difference between groups is indicated as follows: ***P <0.001 compared to the control group; $^{###}P$ <0.001 compared to the Pentylenetetrazol group. One-way ANOVA followed by Tukey post hoc test was used to determine the differences between the independent variables]

6.596). However, there was no statistically significant difference in the cortex TNF-α levels between the pentylenetetrazol (Gr. II) and pentylenetetrazol+SA10 groups (Gr. III) (P >0.05). Fig. 4B shows the TNF-α results in the hippocampus which were significantly higher in the pentylenetetrazol group (Gr. II) compared to the control group (P = 0.002, F (3, 24) = 10.229). The 20 mg/kg SA significantly reduced the increased levels of TNF-α caused by pentylenetetrazol (P = 0.000, F (3, 24) = 10.229) whereas there was not statistically difference between the pentylenetetrazol (Gr. II) and pentylenetetrazol+SA10 groups (Gr. III) (P >0.05).

Figure 4C shows the IL-β results in the cortex. Compared to the control group, cortex IL-β levels in the pentylenetetrazol group statistically increased (P = 0.025, F (3, 24) = 4.446). The increased cortex IL-1β levels by pentylenetetrazol significantly decreased by the 20 mg/kg SA administration (P = 0.020, F (3, 24) = 4.446). However, there was no change in cortex IL-1β levels between pentylenetetrazol and pentylenetetrazol+SA10 groups (P >0.05). Fig. 4D shows the IL-β results in hippocampus. Compared to the control group, the hippocampal IL-1β level was significantly higher in the pentylenetetrazol group (P = 0.007, F (3, 24) = 12.990). The increased IL-1β levels by pentylenetetrazol significantly reduced in pentylenetetrazol+SA20 groups (P = 0.000, F (3, 24) = 12.990). However, there were no notable changes in the hippocampus IL-1β levels between pentylenetetrazol and Pentylenetetrazol+SA10 groups (P >0.05).

Effects of SA on hippocampal caspase 3 and caspase 9 levels

Hippocampal apoptosis levels were determined by analyzing caspase 3 and caspase 9 levels. Figure 5A presents the caspase 3 results. Caspase-3 levels in the hippocampus were statistically significant and higher in the pentylenetetrazol group (Gr. II) than in the control group (P = 0.000, F (3, 24) = 25.498). The increased caspase 3 levels by pentylenetetrazol decreased in the pentylenetetrazol+SA20 group (Gr. IV) (P = 0.000, F (3, 24) = 25.498). Fig. 5B presents the caspase 9 results. Caspase 9 levels in the hippocampus were significantly higher in the pentylenetetrazol group (Gr. II) compared to the control group (P = 0.000, F (3, 24) = 38.926). The increased caspase 9 levels by Pentylenetetrazol decreased in the Pentylenetetrazol+SA20 group (Gr. IV) (P = 0.000, F (3, 24) = 38.926). However, there were no significant changes in the hippocampal caspase 3 and caspase 9 levels between penty-lenetetrazol (Gr. II) and pentylenetetrazol+SA10 groups (Gr. III) (P >0.05).

Effects of SA on the brain BDNF levels

The levels of the cortex and hippocampal BDNF were determined by ELISA kits. Figure 6A shows BDNF results in the cortex. BDNF levels in the cortex were statistically higher in the pentylenetetrazol group (Gr. II) compared to the control group (P = 0.000, F (3, 24) = 20.963). However, the increased cortex BDNF levels decreased in the Penty-lenetetrazol +SA20 group (Gr. IV) (P = 0.000, F (3, 24) = 20.963). Fig. 6B shows BDNF results in the hippocampus. BDNF levels in the hippocampus were statistically higher in the penty-lenetetrazol group (Gr. II) compared to the control group (P = 0.048, F (3, 24) = 4.051). The 20 mg/kg SA administration significantly decreased the elevated hippocampal BDNF levels (P = 0.019, F (3, 24) = 4.051). However, there was change in the cortex and hippocampal BDNF levels between the penty-lenetetrazol (Gr. II) and pentylenetetrazol+SA10 groups (Gr. III) (P >0.05).

Effects of SA on histopathological changes

Significant statistical differences were observed among the groups in the histopathological evaluation (Table 1, P <0.05). The cornu ammonis (CA1, CA3) sections of rats in the control group exhibited normal histological appearance. However, histopathological examination revealed pyknotic changes in the CA1 and CA3 regions of the cornu ammonis. The pentylenetetrazol group (Gr. II) showed moderate-level changes in the CA1 region, while the pentylenetetrazol+SA10 group (Gr. III) exhibited mild-level changes in the CA1 region. In contrast, no significant changes were observed in the CA1 region of the pentylenetetrazol+SA20 group (Gr. IV). Similar correlations were observed in the CA3 region. The pentylenetetrazol group (Gr. II) displayed severe pyknotic changes in the neurons of the CA3 region, whereas these changes were reduced to a moderate level in the pentylenetetrazol+SA10 group (Gr. III) and to a mild level in the pentylenetetrazol+SA20 group (Gr. IV). The nuclei of pyknotic neurons appeared dark and shrunken (Fig. 7).

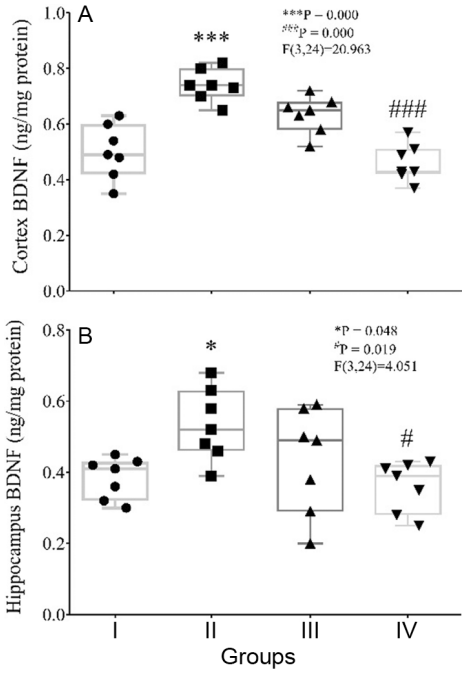

Fig. 6 — BDNF levels in (A) the cortex; and (B) hippocampus of rats. [Gr. I: Control; Gr. II: Pentylenetetrazol; Gr. III & IV: Pentylenetetrazol + SA10 and SA20, respectively. The statistical difference between groups is indicated as follows: *P <0.05, ***P <0.001 compared to the control group; $^{\#}P$ <0.05, $^{\#\#\#}P$ <0.001 compared to the Pentylenetetrazol group. One-way ANOVA followed by Tukey post hoc test was used to determine the differences between the independent variables]

Table 1 — Pyknotic changes observed in neurons

Groups	CA1 region	CA3 region
Control	0.00 ± 0.00^{aA}	0.16 ± 0.51^{aA}
Pentylenetetrazol	1.66 ± 0.51^{bA}	3.00 ± 0.00^{bB}
Pentylenetetrazol+SA10	0.83 ± 0.40^{cA}	1.83 ± 0.40^{cB}
Pentylenetetrazol+SA20	0.16 ± 0.40^{dA}	1.00 ± 0.00^{dA}

[^{a,b,c,d}Different letters in the same column show the difference between groups (P <0.05); and ^{A,B}Different letters on the same line show the difference between groups (P <0.05)]

Table 2 — Immunohistochemical staining with Cleaved caspase 3

Groups	CA1 region	CA3 region
Control	$0,16\pm0,40^{aA}$	$0,33\pm0,51^{aA}$
Pentylenetetrazol	$1,83\pm0,00^{bA}$	$2,83\pm0,40^{bB}$
Pentylenetetrazol+SA10	$1,66\pm0,51^{bA}$	$1,83\pm0,40^{cA}$
Pentylenetetrazol+SA20	$0,83\pm0,00^{cA}$	$1,00\pm0,00^{dA}$

[^{a,b,c,d}Different letters in the same column show the difference between groups (P <0.05); and ^{A,B}Different letters on the same line show the difference between groups (P <0.05)]

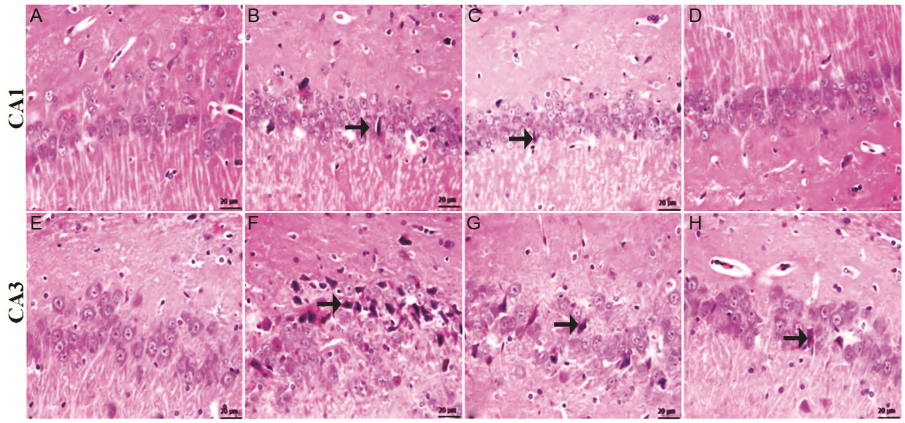

Fig. 7 — (A-D) CA1 sections: (A) Control group displaying normal histological appearance, (B) Pentylenetetrazol group showing neurons with intermediate pyknotic changes (arrow), (C) Pentylenetetrazol+SA10 group exhibiting neurons with mild pyknotic changes (arrow), and (D) Pentylenetetrazol+SA20 group with normal histological appearance; and (E-H) CA3 sections: (E) Control group demonstrating normal histological appearance (F) Pentylenetetrazol group presenting neurons with severe pyknotic changes (arrow), (G) Pentylenetetrazol+SA10 group displaying neurons with intermediate pyknotic changes (arrow), and (H) Pentylenetetrazol+SA20 group showing neurons with mild pyknotic changes (arrow). [H-E: Hematoxylin-Eosin Staining]

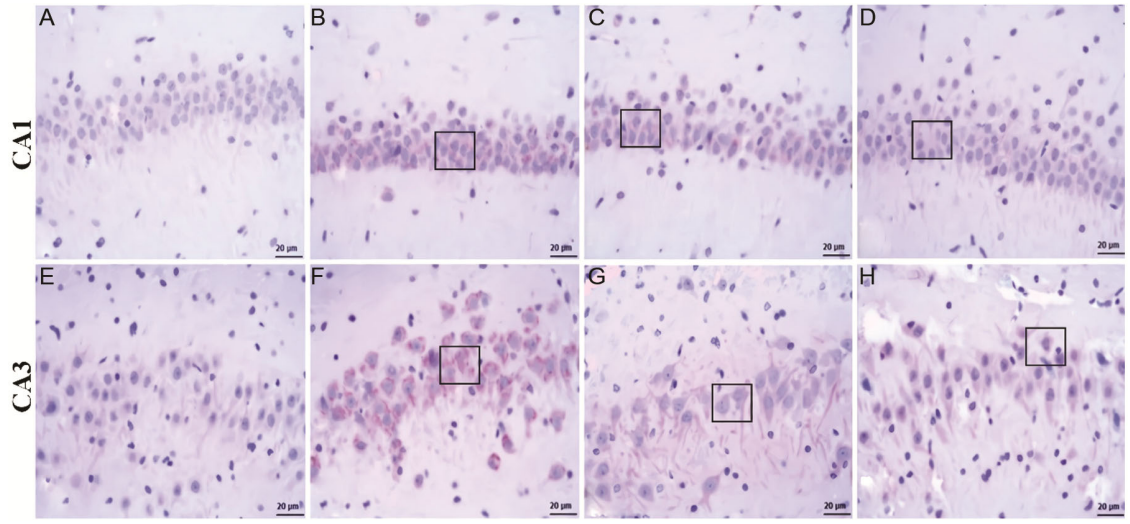

Fig. 8 — (A-D) CA1 sections: (A) Control group showing immune negativity, (B) Pentylenetetrazol group displaying moderate immunopositivity (□), (C) Pentylenetetrazol+SA10 group exhibiting moderate immunopositivity (□), and (D) Pentylenetetrazol+SA20 group demonstrating mild immunopositivity (□); and (E-H) CA3 sections: (E) Control group indicating immune negativity, (F) Pentylenetetrazol group presenting severe immunopositivity (□), (G) Pentylenetetrazol+SA10 group displaying moderate immunopositivity (□), and (H) Pentylenetetrazol+SA20 group showing mild immunopositivity (□). [IHC: Immunohistochemistry]

Effects of SA on cleaved caspase3 immunohistochemical expression

Statistically significant differences were found between the groups in immunohistochemical staining for cleaved caspase 3 (Table 2, P <0.05). The control group rats did not show significant immunopositivity in the cornu ammonis. In the treatment groups, moderate immunopositivity was observed in CA1 of both the pentylenetetrazol group (Gr. II) and pentylenetetrazol +SA10 group (Gr. III), while mild immunopositivity was observed in CA1 of the pentylenetetrazol+SA20 group (Gr. IV). Similar patterns of immunopositivity were observed in CA3. The pentylenetetrazol group (Gr. II) exhibited severe immunopositivity in CA3, the pentylenetetrazol +SA10 (Gr. III) group displayed moderate immuno-positivity, and the pentylenetetrazol +SA20 group (Gr. IV) showed mild immunopositivity (Fig. 8).

Discussion

Pentylenetetrazol-induced seizure in the rat is a related model of human absence epilepsy and generalized tonic-clonic epilepsy. In this experimental study, the effects of SA on a single dose of pentylenetetrazol (45 mg/kg) induced seizures were determined in rats. The results show that the 20 mg/kg SA reduces the seizure score and the duration of GTCS but increases the latency of first myoclonic jerk and GTCS. In addition, the SA administration improves the memory impairment caused by pentylenetetrazol-induced seizures. Furthermore, SA decreases TOS, TNF-α, IL-1β, BDNF levels in the cortex and hippocampus, and hippocampal caspase 3 and caspase 9 levels. In addition, SA elevates the cortical and hippocampal TAS levels after the induction of the seizures in rats. However, the 10 mg/kg SA administration does not have any significant improving effect on the selected parameters which is consistent with a previous study[21].

Neurodegeneration in brain locations is directly related to seizure severity[24]. Clinical efficacy of anti-epilepsy medications, including preventing and reducing their is essential to prevent seizures and reduce frequency and severity of seizure. Recently, studies have demonstrated the anticonvulsant effectiveness of antioxidants in experimental epilepsy models[25-27]. In a previous study, it has been shown that SA improves passive avoidance test memory by suppressing oxidative-nitrosative stress in the hippocampus in kainic acid induced seizure and it shows an anticonvulsant effect of SA[14]. Similarly, in our study, SA decreased the seizure score and the duration of GTCS depending on the dose, but it increased the latency of first myoclonic jerk and GTCS. Moreover, it improved the cognitive impairment caused by pentylenetetrazol. It can be suggested that the reduction in the severity of pentylenetetrazol induced seizures with SA pretreatment is due to the antioxidant, anti-inflammatory, and neuroprotective effects of SA.

Oxidative stress, caused by mitochondrial dysfunction, influences the pathogenesis of seizures which can cause neuronal damage in epilepsy. High oxidative status is also linked to seizure intensity and recurrence[28]. Clinical and experimental studies have suggested that antioxidant supplementation prevents subjects from the progressive deterioration of seizures[29,30]. A previous study showed that pentylenetetrazol administration did not affect the level of brain TAS while it increased TOS levels significantly in rats[20]. On the other hand, pentylenetetrazol-induced neurotoxicity leads to an increase in TOS levels and a reduction in TAS levels in SH-SY5Y cells[22]. In a previous study of rats with hepatotoxicity induced by arsenic, SA significantly reduced lipid peroxidation and hydroperoxides and protein carbonyl levels. In addition, SA increased the levels of antioxidant parameters such as hepatic SOD, CAT, GPx, and GSH[31]. In another study, SA increased renal antioxidant levels and decreased lipid peroxidation in a cisplatin-induced nephrotoxicity model[32]. In this study, TAS levels decreased in the cortex and hippocampus in pentylenetetrazol-induced rats whereas TOS levels elevated in both cortex and hippocampus. Importantly, SA increased cortical and hippocampal TAS levels and decreased TOS levels in both cortex and hippocampus, suggesting that SA administration causes a crucial antioxidative effect which is consistent with previous studies[31]. Thus, oxidative stress can play an essential role in the etiopathology of seizures induced by pentylenetetrazol in rats.

Seizures can contribute an inflammatory reaction, which could further lead to a progression in seizure. Imbalances between pro-inflammatory and anti-inflammatory responses are found to be related to epilepsy[33]. Microglia and astrocytes, activated in damaged brain tissue, generate proinflammatory cytokines, including TNF-α, IL-1β, interferon-gamma, and chemokines[34]. These important proinflammatory factors can influence neuronal excitability and glial function. As a result, communication between glial cells and neurons may be impaired as evidenced by the fact that the changes in cytokine productions lower the seizure threshold and decrease neuronal survival[35]. It has been reported that proinflammatory cytokine levels increase in the cerebral cortex and hippocampus of rats that were administered pentylenetetrazol which this effect can be suppressed by anti-inflammatory agents[36]. In previous studies on the rat seizure model induced by pentylenetetrazol, they reported an increase in the levels of MDA, IL-1β, TNF-α, and PGE2 in the brain tissue, along with a decrease in SOD activity. Thus, they emphasized the significant role of oxidative stress and inflammation in the pathophysiology of seizures induced by pentylenetetrazol[26]. It has been reported that SA administration significantly reduces cortical and hippocampal TNF-α and IL-1β levels in an Alzheimer's disease rat model[21]. In addition, SA significantly reduces TNF levels in neuronal degeneration induced by intracerebroventricular streptozotocin injection in rats[19]. Together, previous studies and the findings from the current study suggest that inflammation is an important factor for the progression of epilepsy and SA, as a potent anti-inflammatory agent, has beneficial effects on at least some of proinflammatory cytokines in the cortex and hippocampus of rats with seizure induced by pentylenetetrazol.

Apoptosis activated by a series of caspase cascades such as caspase 3, which can be activated in seizures. The hippocampus is vulnerable to acute seizures, and seizures induced by pentylenetetrazol can lead to the loss of neuronal cells in this region of the brain[37,38]. A study reported that seizures cause high apoptosis with neuronal damage in the cortex and hippocampus[39]. Pentylenetetrazol has been found to induce histopathological damage specifically in cornu ammonis CA1 and CA3 neurons, leading to significant structural alterations in these brain regions[20]. Furthermore, research findings have indicated that pentylenetetrazol possesses an augmenting impact on the manifestation of Cleaved caspase 3 within the CA1 and CA3 areas of the cornu ammonis[40,41]. Consistent with the literature findings in our study, we observed increased pyknotic changes and enhanced immunohistochemical expression of Cleaved caspase 3 in CA1 and CA3 neurons following acute seizures. It was determined that a dose of 20 mg/kg of SA significantly reversed these alterations. The decreased immune expression of Cleaved caspase 3 suggests that SA may interfere with the programmed cell death pathways in neurons, potentially contributing to the development and progression of seizure-related pathology. Activation of apoptotic pathways in the pentylenetetrazol-induced seizure may be a result of high level of reactive oxygen species that may activate the mitochondrial pathway[42]. Inflammation also triggers the apoptosis pathway. Stimulation of tumor necrosis factor receptor 1 (TNFR-1) can lead to strong activation of the apoptotic pathway. Moreover, NF-

κB and the apoptotic pathway are reported to be tightly linked[43]. It has been reported that any lesion in the brain regions, including the hippocampus and cerebral cortex, may cause impairment in learning and memory function[44]. As a learning processing center of the brain, the hippocampus mediates memory and cognition, and neuronal injury of this area induces memory and cognitive impairment[45]. In a passive avoidance test of rats with seizures induced by pentylenetetrazol, a delay in entering the dark zone and impaired passive avoidance memory have been reported which is similar to our results. Moreover, some studies have shown that seizures caused by pentylenetetrazol lead to memory dysfunction, cognitive disturbances, and deterioration passive avoidance memory in experimental rat models[10]. It has been reported that SA suppressed oxidative stress and prevented rats from losing their memory in the Alzheimer's disease rat model[19]. In another Alzheimer's disease model, it has been shown that SA alleviates neuronal cell death and cognitive dysfunction with its antioxidant and anti-inflammatory activity in rats[15]. In the current study, pentylenetetrazol increased hippocampal caspase 3 and caspase 9 levels while SA decreased them in the hippocampus. Furthermore, SA significantly improved memory impairment caused by pentylenetetrazol administration. These findings, along with others, indicate a potential role of apoptosis in epilepsy and possible antiapoptotic effects of SA in seizure rat models.

The brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that includes numerous brain neuronal traffic such as neuronal survival, differentiation and development, synaptogenesis, synaptic transmission, plasticity and excitability of the neurons, and improves cognitive functions[46]. BDNF potentiates glutamatergic neurotransmission and inhibits GABAergic neuro-transmission[47]. Excessive activity of CNS caused by pentylenetetrazol increases neuronal excitability and may increase BDNF secretion, leading to epilepsy[48]. In the hippocampus and cortex of animal epilepsy models and in human brain tissue, mRNA and protein levels of BDNF are upregulated and elevated in seizure activities[9]. Interventions in the expression of BDNF mRNA and protein as well as BDNF/TrkB pathway, which increase seizure activity have been reported to inhibit the development of the seizure state *in vivo*[49]. On the other hand, seizures cause depolarization-induced activation of the synaptic NMDA subtype of the excitatory amino acid receptor, which results in an influx of Ca^{2+} and the rapid release of BDNF, which binds to and activates TrkB-FL[50]. Furthermore, the high-frequency activity found in seizures causes tissue plasminogen activator release, which causes pro-BDNF to mature BDNF conversion[50]. A previous study reported that pre-treatment of myricetin, a potent antioxidant and anti-inflammatory agent, reduced the increased hippocampal BDNF-TrkB protein expression caused by pentylenetetrazol and alleviated the severity of pentylenetetrazol induced epilepsy seizure[25]. Similarly, Garcinol reduced the increased hippocampal expression of BDNF and TrkB caused by penty-lenetetrazol[51]. Consistent with these findings, BDNF levels increased both cortex and hippocampus following pentylenetetrazol-induced seizures in the current study. In the cortex and hippocampus, the administration of SA decreased the increased BDNF levels induced by pentylenetetrazol induced seizures. Therefore, the SA-mediated reduction in BDNF levels in the cortex and hippocampus may have suppressed seizures and supported the increased memory function.

Pentylenetetrazol, a GABA competitive inhibitor, blocks GABA from binding to GABAA receptors found on the surface of muscles. The excitatory-inhibitory signal ratio increases in the absence of GABA binding, leading to a convulsive phenotype[52]. SA is a natural phenolic acid found in plants, particularly in spices like cinnamon, cloves, and black pepper. In cells and the brain, SA has been shown to have antioxidant and anti-inflammatory properties. It can also act as a neuroprotective agent by protecting neurons from oxidative stress and apoptosis[53]. One of the main targets of SA in cells is the Nrf2 (nuclear factor erythroid 2-related factor 2) pathway. Nrf2 is a transcription factor that regulates the expression of antioxidant and detoxifying enzymes, and SA has been shown to activate this pathway. By activating Nrf2, SA can increase the levels of antioxidant enzymes like catalase, superoxide dismutase, and glutathione peroxidase, which help to reduce oxidative stress in cells[54]. In the brain, SA has been found to modulate several neurotransmitters and their receptors, including acetylcholine, dopamine, and GABA[13,14,16,21]. SA has also been shown to enhance the activity of BDNF, a protein that promotes the growth and survival of neurons[55]. Overall, SA appears to have multiple targets and mechanisms of action in

cells and the brain, which may contribute to its beneficial effects on health and well-being. Protein kinases such as protein kinase A (PKA) and protein kinase C (PKC) have been shown to phosphorylate the GABAA-Cl-channel complex[56]. The potentiation of the GABA response caused by opioid receptor agonists is modulated through the long-latency regulation of protein kinase A (PKA) and protein kinase C (PKC)[56]. Flavonoids have an antiepileptic effect by modifying the GABAA-Cl-channel complex, as they are structurally like benzodiazepines[57]. It was reported that SA ameliorated kainic acid-induced excitotoxic cell death and seizure-like behavior with GABAA receptor agonistic activity *in vivo* and *in vitro*[13]. In a previous study in mice, it has been shown that SA has neuroprotective and anticonvulsive effects through its radical scavenging property and GABA receptor activation[14]. Our study revealed that SA, with its free radical scavenging, anti-inflammatory, and apoptosis-inhibiting characteristics, may repair pentylenetetrazol induced convulsions and memory impairment, particularly via BDNF and GABAA receptors activation.

There are several limitations of this study: (i) the effects of SA were not investigated in the pentylenetetrazol-induced seizure model with GABA agonists and antagonists; (ii) it might have been better to confirm behavioural seizure symptoms using the electroencephalography (EEG) method; and (iii) the Western blot methods could have applied.

Conclusion

The overall findings of this study show that sinapic acid (SA) has antiseizure activity and prevents rats from memory impairment caused by seizures. These potent beneficial effects may be due to the inhibition of oxidative stress, inflammation, and suppression of increased brain-derived neurotrophic factor (BDNF) levels, suggesting that SA may be used as an antiseizure medicine in the future.

Conflict of interest

Authors declare no competing interests.

References

1 Casillas-Espinosa PM, Ali I & O'Brien TJ, Neuro degenerative pathways as targets for acquired epilepsy therapy development. *Epilepsia Open*, 5 (2020) 138.

2 Kanner AM, Helmstaedter C, Sadat-Hossieny Z & Meador K, Cognitive disorders in epilepsy I: Clinical experience, real-world evidence and recommendations. *Seizure*, 83 (2020) 216.

3 Taskiran AS & Tastemur Y, The role of nitric oxide in anticonvulsant effects of lycopene supplementation on pentylenetetrazol-induced epileptic seizures in rats. *Exp Brain Res*, 239 (2021) 591.

4 Laue-Gizzi H, Discontinuation of antiepileptic drugs in adults with epilepsy. *Aust Prescr*, 44 (2021) 53.

5 Lin TK, Chen SD, Lin KJ & Chuang YC, Seizure-induced oxidative stress in status epilepticus: is antioxidant beneficial? *Antioxidants* (Basel), 9 (2020) 1029.

6 Alrashdi BM, Fehaid A, Kassab RB, Rizk S, Habotta OA & Abdel Moneim AE, Biosynthesized selenium nanoparticles using epigallocatechin gallate protect against pentylenetetrazole-induced acute epileptic seizures in mice via antioxidative, anti-inflammatory, and anti-apoptotic activities. *Biomedicines*, 11 (2023) 1955.

7 Rana A & Musto AE, The role of inflammation in the development of epilepsy. *J Neuroinflammation*, 15 (2018) 144.

8 Chen NC, Chuang YC, Huang CW, Lui CC, Lee CC, Hsu SW, Lin PH, Lu YT, Chang YT, Hsu CW & Chang CC, Interictal serum brain-derived neurotrophic factor level reflects white matter integrity, epilepsy severity, and cognitive dysfunction in chronic temporal lobe epilepsy. *Epilepsy Behav*, 59 (2016) 147.

9 Iughetti L, Lucaccioni L, Fugetto F, Predieri B, Berardi A & Ferrari F, Brain-derived neurotrophic factor and epilepsy: a systematic review. *Neuropeptides*, 72 (2018) 23.

10 Hamdy M, Antar A, El-Mesery M, El-Shafey M, Ali AN, Abbas KM, Abulseoud OA & Hussein AM, Curcumin offsets PTZ-induced epilepsy: involving inhibition of apoptosis, Wnt/β-catenin, and autophagy pathways. *Egypt J Basic Appl Sci*, 7 (2020) 240.

11 Grigoletto J, Oliveira CV, Grauncke AC, Souza TL, Souto NS, Freitas ML, Furian AF, Santos AR & Oliveira MS, Rosmarinic acid is anticonvulsant against seizures induced by pentylenetetrazol and pilocarpine in mice. *Epilepsy Behav*, 62 (2016) 27.

12 Pandi A & Kalappan VM, Pharmacological and therapeutic applications of sinapic acid-an updated review. *Mol Biol Rep*, 48 (2021) 3733.

13 Yoon BH, Jung JW, Lee JJ, Cho YW, Jang CG, Jin C, Oh TH & Ryu JH, Anxiolytic-like effects of sinapic acid in mice. *Life Sci*, 81 (2007) 234.

14 Kim DH, Yoon BH, Jung WY, Kim JM, Park SJ, Park DH, Huh Y, Park C, Cheong JH, Lee KT, Shin CY & Ryu JH, Sinapic acid attenuates kainic acid-induced hippocampal neuronal damage in mice. *Neuropharmacology*, 59 (2010) 20.

15 Lee HE, Kim DH, Park SJ, Kim JM, Lee YW, Jung JM, Lee CH, Hong JG, Liu X, Cai M, Park KJ, Jang DS & Ryu JH, Neuroprotective effect of sinapic acid in a mouse model of amyloid β(1-42) protein-induced Alzheimer's disease. *Pharmacol Biochem Behav*, 103 (2012) 260.

16 Zare K, Eidi A, Roghani M & Rohani AH, The neuroprotective potential of sinapic acid in the 6-hydroxy-dopamine-induced hemi-parkinsonian rat. *Metab Brain Dis*, 30 (2015) 205.

17 Lee IS, Choi GY, Sreelatha I, Yoon JW, Youn SH, Maeng S & Park JH, Effect of sinapic acid on scopolamine-induced

learning and memory impairment in SD rats. *Brain Sci*, 13 (2023) 427.
18 Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T & Würbel H, The arrive guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol*, 18 (2020) e3000410.
19 Shahmohamady P, Eidi A, Mortazavi P, Panahi N & Minai-Tehrani D, Effect of sinapic acid on memory deficits and neuronal degeneration induced by intracerebroventricular administration of streptozotocin in rats. *Pol J Pathol*, 69 (2018) 266.
20 Akkaya R, Gümüş E, Akkaya B, Karabulut S, Gülmez K, Karademir M, Taştemur Y & Taşkıran AŞ, Wi-Fi decreases melatonin protective effect and increases hippocampal neuronal damage in pentylenetetrazole induced model seizures in rats. *Pathophysiology*, 26 (2019) 375.
21 Verma V, Singh D & Kh R, Sinapic acid alleviates oxidative stress and neuro-inflammatory changes in sporadic model of Alzheimer's disease in rats. *Brain Sci*, 10 (2020) 923.
22 Taskiran AS, Ergul M, Gunes H, Ozturk A, Sahin B & Ozdemir E, The effects of proton pump inhibitors (Pantoprazole) on pentylenetetrazole-induced epileptic seizures in rats and neurotoxicity in the SH-SY5Y Human Neuroblastoma Cell Line. *Cell Mol Neurobiol*, 41 (2021) 173.
23 Serdar CC, Cihan M, Yücel D & Serdar MA, Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. *Biochem Med* (Zagreb), 15 (2021) 010502.
24 Ono T & Galanopoulou AS, Epilepsy and epileptic syndrome. *Adv Exp Med Biol*, 724 (2012) 99.
25 Sun ZQ, Meng FH, Tu LX & Sun L, Myricetin attenuates the severity of seizures and neuroapoptosis in pentylenetetrazole kindled mice by regulating the of BDNF-TrkB signaling pathway and modulating matrix metalloproteinase-9 and GABA (A). *Exp Ther Med*, 17 (2019) 3083.
26 Erdogan A, Erdogan MA, Gurgul S & Erbas O, Effects of diclofenac sodium on seizure activity in rats with pentylenetetrazole-induced convulsions. *Neurochem Res*, 48 (2023) 1412.
27 Sahin H & Erbaş O, Beneficial effects of sennoside B on pentylenetetrazole-induced seizures in rats. *Hum Exp Toxicol*, 42 (2023) 9603271231168764.
28 Chang SJ & Yu BC, Mitochondrial matters of the brain: mitochondrial dysfunction and oxidative status in epilepsy. *J Bioenerg Biomembr*, 42 (2010) 457.
29 Borowicz-Reutt KK & Czuczwar SJ, Role of oxidative stress in epileptogenesis and potential implications for therapy. *Pharmacol Rep*, 72 (2020) 1218.
30 Madireddy S & Madireddy S, Therapeutic strategies to ameliorate neuronal damage in epilepsy by regulating oxidative stress, mitochondrial dysfunction, and neuroinflammation. *Brain Sci*, 13 (2023) 784.
31 Pari L & Mohamed Jalaludeen A, Protective role of sinapic acid against arsenic: induced toxicity in rats. *Chem Biol Interact*, 194 (2011) 40.
32 Ansari MA, Sinapic acid modulates Nrf2/HO-1 signaling pathway in cisplatin-induced nephrotoxicity in rats. *Biomed Pharmacother*, 93 (2017) 646.
33 Legido A & Katsetos CD, Experimental studies in epilepsy: immunologic and inflammatory mechanisms. *Semin Pediatr Neurol*, 21 (2014) 197.
34 Villasana-Salazar B & Vezzani A, Neuroinflammation microenvironment sharpens seizure circuit. *Neurobiol Dis*, 178 (2023) 106027.
35 Li W, Wu J, Zeng Y & Zheng W, Neuroinflammation in epileptogenesis: from pathophysiology to therapeutic strategies. *Front Immunol*, 14 (2023) 1269241.
36 Soltani Khaboushan A, Yazdanpanah N & Rezaei N, Neuroinflammation and proinflammatory cytokines in epileptogenesis. *Mol Neurobiol*, 59 (2022) 1724.
37 Flores-Soto M, Romero-Guerrero C, Vázquez-Hernández N, Tejeda-Martínez A, Martín-Amaya-Barajas FL, Orozco-Suárez S & González-Burgos I, Pentylenetetrazol-induced seizures in adult rats are associated with plastic changes to the dendritic spines on hippocampal CA1 pyramidal neurons. *Behav Brain Res*, 406 (2021) 113198.
38 Al Omairi NE, Albrakati A, Alsharif KF, Almalki AS, Alsanie W, Abd Elmageed ZY, Zaafar D, Lokman MS, Bauomy AA, Belal SK, Abdel-Daim MM, Abdel Moneim AE, Alyami H & Kassab RB, Selenium nanoparticles with prodigiosin rescue hippocampal damage associated with epileptic seizures induced by pentylenetetrazole in rats. *Biology* (Basel), 11 (2022) 354.
39 Branco Cdos S, Scola G, Rodrigues AD, Cesio V, Laprovitera M, Heinzen H, Dos Santos MT, Fank B, de Freitas SC, Coitinho AS & Salvador M, Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate (Ilex paraguariensis St. Hil.) on pentylenetetrazol-induced seizures in Wistar rats. *Brain Res Bull*, 92 (2013) 60.
40 Cavalcante TMB, De Melo JMA Júnior, Lopes LB, Bessa MC, Santos JG, Vasconcelos LC, Vieira Neto AE, Borges LTN, Fonteles MMF, Chaves Filho AJM, Macêdo D, Campos AR, Aguiar CCT & Vasconcelos SMM, Ivabradine possesses anticonvulsant and neuroprotective action in mice. *Biomed Pharmacother*, 109 (2019) 2499.
41 Shalaby AM, Sharaf Eldin HEM, Abdelsameea AA, Abdelnour HM, Alabiad MA, Elkholy MR & Aboregela AM, Betahistine attenuates seizures, neurodegeneration, apoptosis, and gliosis in the cerebral cortex and hippocampus in a mouse model of epilepsy: a histological, immune-histochemical, and biochemical study. *Microsc Microanal*, 10 (2022) 1.
42 Méndez-Armenta M, Nava-Ruíz C, Juárez-Rebollar D, Rodríguez-Martínez E & Gómez PY, Oxidative stress associated with neuronal apoptosis in experimental models of epilepsy. *Oxid Med Cell Longev*, 2014 (2014) 293689.
43 Webster JD & Vucic D, The balance of TNF mediated pathways regulates inflammatory cell death signaling in healthy and diseased tissues. *Front Cell Dev Biol*, 8 (2020) 365.
44 Mahmoudi T, Lorigooini Z, Rafieian-Kopaei M, Arabi M, Rabiei Z, Bijad E & Kazemi S, Effect of Curcuma zedoaria hydro-alcoholic extract on learning, memory deficits and oxidative damage of brain tissue following

seizures induced by pentylenetetrazole in rat. *Behav Brain Funct*, 16 (2020) 7.

45 Zielinski MC, Tang W & Jadhav SP, The role of replay and theta sequences in mediating hippocampal-prefrontal interactions for memory and cognition. *Hippocampus*, 30 (2020) 60.

46 Kowiański P, Lietzau G, Czuba E, Waśkow M, Steliga A & Moryś J, BDNF: A key factor with multipotent impact on brain signaling and synaptic plasticity. *Cell Mol Neurobiol*, 38 (2018) 579.

47 Naderipoor P, Amani M, Abedi A, Sakhaie N, Sadegzadeh F & Saadati H, Alterations in the behavior, cognitive function, and BDNF level in adult male rats following neonatal blockade of GABA-A receptors. *Brain Res Bull*, 169 (2021) 35.

48 Liu X, Liu J, Liu J, Liu XL, Jin LY, Fan W, Ding J, Peng LC, Wang Y & Wang X, BDNF-TrkB signaling pathway is involved in pentylenetetrazole-evoked progression of epileptiform activity in hippocampal neurons in anesthetized rats. *Neurosci Bull*, 29 (2013) 565.

49 Lin TW, Harward SC, Huang YZ & McNamara JO, Targeting BDNF/TrkB pathways for preventing or suppressing epilepsy. *Neuropharmacology*, 167 (2020) 107734.

50 Marini AM, Jiang X, Wu X, Pan H, Guo Z, Mattson MP, Blondeau N, Novelli A & Lipsky RH, Preconditioning and neurotrophins: a model for brain adaptation to seizures, ischemia and other stressful stimuli. *Amino Acids*, 32 (2007) 299.

51 Hao F, Jia LH, Li XW, Zhang YR & Liu XW, Garcinol upregulates GABAA and GAD65 expression, modulates BDNF-TrkB pathway to reduce seizures in pentylenetetrazole (PTZ)-induced epilepsy. *Med Sci Monit*, 22 (2016) 4415.

52 Thapliyal S & Babu K, Pentylenetetrazole (PTZ)-induced convulsion assay to determine GABAergic defects in caenorhabditis elegans. *Bio Protoc*, 8 (2018) e2989.

53 Pandi A & Kalappan VM, Pharmacological and therapeutic applications of sinapic acid-an updated review. *Mol Biol Rep*, 48 (2021) 3733.

54 Raish M, Shahid M, Bin Jardan YA, Ansari MA, Alkharfy KM, Ahad A, Abdelrahman IA, Ahmad A & Al-Jenoobi FI, Gastroprotective effect of sinapic acid on ethanol-induced gastric ulcers in rats: involvement of Nrf2/HO-1 and NF-κB signaling and antiapoptotic role. *Front Pharmacol*, 12 (2021) 622815.

55 Lee IS, Choi GY, Sreelatha I, Yoon JW, Youn SH, Maeng S & Park JH, Effect of sinapic acid on scopolamine-induced learning and memory impairment in SD rats. *Brain Sci*, 13 (2023) 427.

56 Lee JJ, Hahm ET, Min BI, Han SH, Cho JJ & Cho YW, Roles of protein kinase A and C in the opioid potentiation of the GABAA response in rat periaqueductal gray neuron. *Neuropharmacology*, 44 (2003) 573.

57 Choudhary N, Bijjem KR & Kalia AN, Antiepileptic potential of flavonoids fraction from the leaves of Anisomeles malabarica. *J Ethnopharmacol*, 135 (2011) 238