JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34983

RESEARCH ARTICLE

Sinapis alba In Status Epilepticus: A Preclinical Study

Gayatri R. Nayak[1], Amrita Parida[1], Shreya Hegde[1*], Smita Shenoy[1], K. G. Mohandas Rao[2], K. Shivakumar Reddy[3] and V. Manju[4]

[1]Department of Pharmacology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India; shramikahegde@gmail.com

[2]Department of Basic Medical Sciences, Manipal, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India

[3]Centre for Molecular Neurosciences, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India

[4]Department of Paediatrics, Dr. T.M. A. Pai Rotary Hospital, Karkala, Manipal, Manipal Academy of Higher Education, Manipal - 576104, Karnataka, India

Abstract

In this study, the anti-convulsant effect of *Sinapis alba* seed oil and its combination with sodium valproate was evaluated in Wistar albino rats using the lithium-pilocarpine Status Epilepticus (SE) model. The experimental groups included: Group I - normal control; Group II - disease control; Group III - *Sinapis alba* seed oil; Group IV - sodium valproate; Group V - *Sinapis alba* seed oil + ½ dose sodium valproate. Antioxidant markers and Brain-Derived Neurotrophic Factor (BDNF) levels were measured from the brain samples. The pathological changes were also determined using Cresyl violet staining. *Sinapis alba* oil did not prevent rats from developing status epilepticus but reduced the intensity and frequency of occurrence. Oil administration increased antioxidant levels and decreased lipid peroxide levels as well. The combination of oil and sodium valproate showed a synergistic effect in the status epilepticus model. The study results show that *Sinapis alba* can be used as an adjuvant in status epilepticus along with other antiepileptic drugs.

Keywords: Epilepsy, Seizures, Traditional Medicine, Yellow Mustard

1. Introduction

Epilepsy is the second most commonly occurring complex neurodegenerative disorder and has plagued mankind for decades[1]. The World Health Organization (WHO) reported that around 50 million of the world's population is suffering from epilepsy. It is a condition that occurs due to spontaneous discharge of electrical impulses due to neuronal insult, which results in recurrent seizures. The excitatory neurotransmitter - "glutamate" and inhibitory transmitter- "γ-aminobutyric acid" (GABA) play a prominent part in epilepsy[2]. Pathophysiology of epilepsy includes the mechanism involved in initiating seizures (ictogenesis) as well as the process of epileptogenesis wherein the normal brain changes to a seizure-prone brain[3]. Epileptic seizure occurs through mechanisms involving reactive oxygen species due to the generation of free radicals[4]. Clinical features range from warnings like sensory or visual auras, and tingling fingers to severe symptoms like exhausting fits, mental health challenges and processing abnormalities leading to the syndrome of Status Epilepticus (SE). This can put life at risk and is caused due to intermittent episodes of excessive, aberrant cerebral activity[5,6]. Status epilepticus is defined as "A seizure with 5 minutes or more of continuous clinical and/or electrographic seizure activity or recurrent seizure activity without recovery between seizures". It is a medical emergency and should be treated immediately[7].

*Author for correspondence

Article Received on: 08.09.2023 Revised on: 29.11.2023 Accepted on: 01.12.2023

Neuronal damage occurs during the initial SE episode followed by spontaneous seizures, which precipitate a few weeks later. It is difficult to develop a Temporal Lobe Epilepsy (TLE) model since it is time-consuming. Chemo-convulsant like pilocarpine primed with lithium is an extensively used SE model because it reproduces certain human features, is comparatively easier to use and does not require complex setup[8]. Drugs that are currently available only prevent the seizure but do not completely stop the process of epileptogenesis. *In vivo*, testing with the choice of a suitable animal model to check the potential of the compound for its anti-convulsant property becomes an important step[9]. Though many Anti-Epileptic Drugs (AEDs) are available and despite making remarkable progress in understanding the mechanism, underlying cause, and pathophysiological changes, still around 30% of patients are intractable to the treatment (pharmaco-resistant)[10]. This is accompanied by dependent behaviour, social isolation, unemployment, low marital rates, psychological issues etc. More than 50% of patients on AEDs have cognitive impairment as the major side effect and the risk exponentially increases with the dose taken[11,12]. The adverse effects associated with AEDs have a greater effect on the normal quality of life of patients than the severity of seizure occurrence and the cost of the AED itself, which questions its usage[13].

The focus is increasing on finding Complementary and Alternative Medicines (CAMs). Herbal medicine is a type of CAM[14]. Herbal treatments are quite safe when formulated and prescribed correctly[15]. Traditional medicine systems are famous among developing countries; with up to about 80% of the country's population relying on herbal medicine for their prime healthcare needs[6]. Usually, medicinal plants have many properties, which help in the overall attainment of symptomatic relief or cure in many of the disease conditions prevailing, with minimal or no adverse effects associated. More than thirty herbal extracts and their derivatives from Indian, Chinese, and Japanese therapies have been evaluated in the Anticonvulsant Screening Project (ASP) by the Harvard program. Around 2/3rd of these compounds have shown good results in the *in vivo* and *in vitro* studies[16].

Sinapis alba, commonly known as yellow mustard, is a less pungent version of the black mustard. It is commonly used as a spice in different parts of the world. In traditional medicine, the seeds have been used for neurological disorders including epilepsy[17,18]. Its use in epilepsy is also mentioned in German Renaissance herbals[19]. There are no evidence-based studies on the effectiveness of *Sinapis alba* in epilepsy. There are few studies on the antiepileptic effect of black mustard (*B. nigra*) for generalized tonic-clonic seizures and positive results have been seen[4,20]. So, this research was performed to check for the usefulness of *Sinapis alba* seed oil in status epilepticus. We hypothesize that the possible neuroprotective action of *S. alba* would be due to the potent antioxidant property that helps in reducing neuronal oxidative damage during epileptogenesis.

2. Materials and Methods

2.1 Materials

Sodium Valproate (SV) (encorate 300mg tablet) was indented from Radha Medicals, Manipal. Cold-pressed yellow mustard seed oil – Brand Planton was obtained from Green Trade Company, New Delhi.

2.2 Experimental Animals

The study was conducted as per Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines (Institutional animal ethics clearance number -IAEC/KMC/65/2021). Forty albino Wistar rats of 200 - 300 grams weight were sanctioned for experiment and were housed at the central animal research facility, Manipal. Housing conditions were as follows: - 3 rats per cage with 12:12 hours of light: dark lighting conditions, the temperature maintained at 25±3 °C and humidity was 50%. Normal rodent diet pellet (VRK Nutritionals, Maharashtra, India) and water *ad libitum* were provided.

2.3 Procedure: Lithium-pilocarpine Induced Status Epilepticus Model[21-23]

A total of 40 albino Wistar rats (weight- 200-300 grams) were grouped into five groups with 8 rats per group by simple randomization technique. Motor activity was accessed for all animals using rotarod before the start of the study. All rats exhibited adequate neuromuscular coordination hence all animals were included. No animals are excluded. The rats were dosed for 14 days, doses as mentioned in the table 1 below. Sodium valproate was taken as the standard drug[24,25]. On the

13th day, the rats were injected with lithium chloride (3 mEq/kg) intraperitoneally (i.p.). Pilocarpine 30 mg/kg was given after 18-20 hours (i.e., on the 14th day) through the i.p. route to induce status epilepticus. Atropine sulphate (1 mg/kg, i.p.) was given 20 minutes before pilocarpine injection, to reduce muscarinic effects in the periphery. The rats were monitored separately keeping them single per cage. Delay in the onset of stage 4 (tonic-clonic convulsions with forelimb rearing) was noted and animals presenting with stage 4 were kept for an hour following the onset. One hour after stage 4/5 exhibition, diazepam (10 mg/kg, i.p.) was given to control status epilepticus. Extra doses were given until the seizure stopped to increase the rate of survival[26]. Recovery or death of the animals was also noted after 24 hours.

The seizures were assessed based on the Racine scale with slight modification and were scaled as following stages: 1. Facial autism 2. Head nod 3. Wet dog shakes, forelimb clonus 4. Tonic-clonic convulsions with forelimb rearing 5. Rearing with loss of balance with GTCS[27].

Table 1. Different groups along with the treatment doses

Groups	Treatment
Group 1 Normal control	Distilled water (10 mL/kg, p.o.)
Group 2 Disease control	Distilled water (10 mL/kg, p.o.)
Group 3 Test	Yellow mustard oil (200mg/kg) (p.o.)
Group 4 Standard	Sodium valproate (300mg.kg) (p.o.)
Group 5 Test + Standard	Yellow mustard oil (200mg/kg) (p.o.) + sodium valproate (150mg/kg) (p.o.)

2.4 Post-induction Care[26]

Rats were housed singly in a husk-free cage for 1-2 days until they stopped salivating and recovered. The rats were administered 2mL of normal saline, 2 hours after giving diazepam. Close monitoring is required for at least a week after induction since they do not consume food and are depressed. 0.9% saline was given orally twice daily to prevent dehydration and mortality. Animals exhibiting stage 4 and/or stage 5 seizures are prone to develop recurrent seizures after a few days.

2.5 Behavioural Assessment[28]

Preliminary behavioural tests were carried out and scoring was done based on already available protocols[28,29]. Usually, epileptic rats are startled more compared to normal. This exercise was done to evaluate differences in hyperexcitability between the different groups. The Post-Seizure-Behavioural Battery (PSBB) can be easily performed, without extra training for animals and does not require any special apparatus. Behaviour was assessed based on four experiments:

2.5.1 Approach Response

In this test, an object like a pen was slowly moved near the face of the animal, holding it vertically. The scoring of responses was as follows: 1. No reaction to approach with the pen; 2. Sniffing of pen; 3. Moving away from pen; 4. Freezing; 5. Jumping away; 6. Attacking the pen.

2.5.2 Touch Response

Animals were prodded in the rump area using the blunt end of the pen. The response is then scored as follows: 1. No reaction to poking; 2. Turn towards the touched area; 3. Moving away from touch in a forward direction; 4. Freezing; 5. Jerking around towards touch; 6. Turn away from touch; 7. Jumping with or without vocalization.

2.5.3 Loud Noise Response[30]

In this test, a click noise was made to be heard several centimetres above the animal's head. Responses were scored: 1. No reaction; 2. Flinching or flicking of the ears; 3. Abrupt jumping.

2.5.4 Pick-up Test

Rats were grasped around its body and picked up. Scoring was done as: 1. Easy pick up; 2. Easy but with vocalization; 3. Little difficult, trying to rear and turn towards the experimenter's hand; 4. Freezing 5. Difficult to catch, avoid by moving away.

After 30 days of induction, the rats are euthanized by thiopentone sodium (120 mg/kg i.p.). Animals were dissected for brain samples and cleaned with ice-cold normal saline.

2.6 Assessment of Biochemical Parameters

Tissue homogenate preparation[31] - The euthanized animals were cardially perfused with cold saline. The

dissected brain was kept in phosphate buffer saline (0.1M, pH - 7.4, ice cold) for biochemical estimations. Then it was homogenized by using a Teflon homogenizer. For supernatant, the homogenate thus obtained was centrifuged at 10,000rpm (4°C) for 10 minutes.

After the tissue homogenate was prepared, superoxidase dismutase, Malonaldehyde Assay (MDA), nitrite levels, glutathione, and Brain-Derived Nuclear Factor (BDNF) were assessed. BDNF levels were measured using an ELISA kit.

2.7 Histopathological Analysis[32]

The brain tissues were collected and fixed in 10% formalin. The cresyl violet staining technique was used to assess the extent of neuronal degeneration. Serial coronal brain sections of 30-50 µm thickness were taken using a cryostat at frozen condition (−18°C). Sections were hydrated with xylene, 100%, 90% and 70% alcohol. Following this, the sections were stained with cresyl violet at 37-50°C. This stain is helpful since it stains neuronal cell bodies and degenerating or pyknotic neurons can easily be identified under the light microscope. Dehydration of sections was carried out using 70%, 95% and 100% alcohol grades and later washed with xylene. Mounting was done using mountant - DPX. Cresyl violet stained sections were then observed under a light microscope for the qualitative analysis of pyramidal neurons of the hippocampal CA3 region.

2.8 Statistical Analysis

Statistics were carried out using GraphPad Prism 8.0.1. For biochemical and behavioural parameters, analysis was done using one-way ANOVA followed by Post Hoc Tukey's multiple comparison test. For body weight, two-way ANOVA followed by post hoc Bonferroni's test was used. All the data were expressed as mean ± Standard Error of the Mean (SEM).

3. Results

The results of the study are as follows:

3.1 Anti-epileptic Effect of *Sinapis alba* Seed Oil (Yellow Mustard Oil) In Status Epilepticus

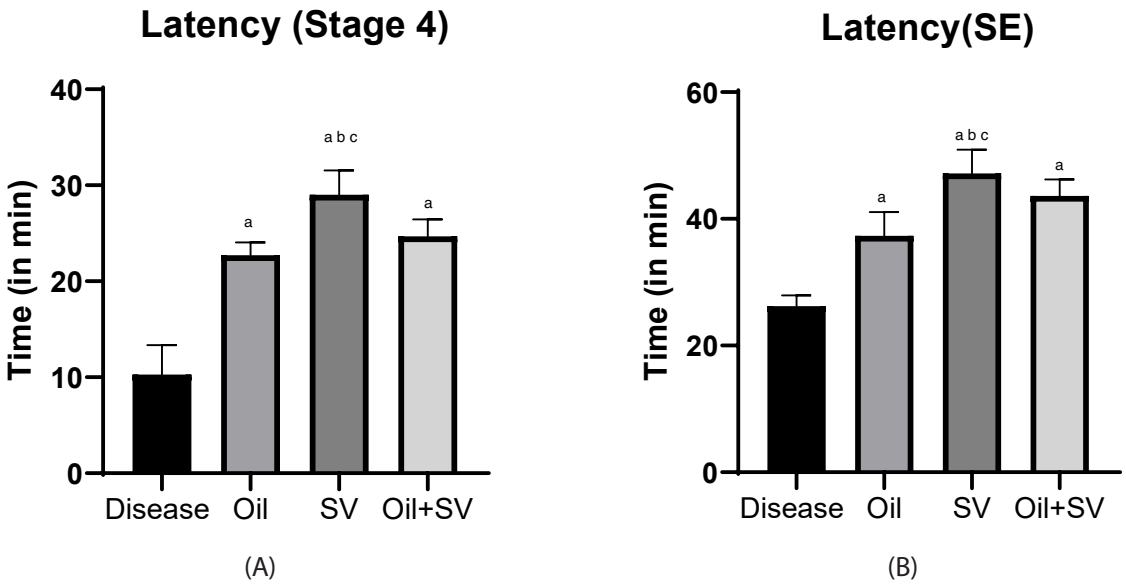
After lithium-pilocarpine injections, the animals showed alterations in their behaviour including stereotyped movements like paw licking, sniffing, and rearing. After around an hour they also exhibited cholinergic symptoms (peripheral) like diarrhoea, piloerection, salivation, and scratching. The scoring was done as mentioned above. The percentage of animals showing different stages of seizures is depicted in Table 2. Latencies to develop seizure and status epilepticus were recorded and presented in Figure 1.

In the disease group (Group 2) all the rats (n=8) showed stages 4 and 5. All the animals (n=8) in the disease control exhibited stage 4/5. All the treatment groups increased the latency to onset of 1st - stage 4 seizure ($p<0.05$) compared to lithium pilocarpine control characterized by forelimb clonus along with rearing. However, there was a significant difference between Group 3 (oil 200 mg/kg) when compared to Groups 4, and 5 in onset latency to stage 4. The latencies of onset of stage 4 were more in Group 4 dosed with SV 300 mg/kg. The latencies of Groups 4 and 5 were comparable.

We consider the condition as status epilepticus if the animals exhibit 2 or more - stage 4/5 seizures

Table 2. Evaluation of the anti-epileptic effect of *Sinapis alba* seed oil in status epilepticus

Sl. No.	Groups	% of Animals showing stage 4	% of Animals showing stage 5	% of Animals showing SE	% Mortality after 24 hours (n=8)
1	Normal	-	-	-	-
2	Disease	100	100	100	12.5
3	Oil 200mg/kg	87.5	87.5	87.5	0
4	SV 300 mg/kg	87.5	75	75	0
5	Oil 200mg/kg + SV 150 mg/kg	87.5	87.5	87.5	0



(Data was analyzed using one-way ANOVA, followed by post hoc Tukey's multiple comparisons test. Data represented as Meantime ± SEM. Means were compared with the disease control group.
a - p <0.0001 vs disease control; b - p <0.001 vs SV 300 mg/kg; c - p <0.05 vs Oil 200mg/kg + Sodium valproate (SV) 150 mg/kg)

Figure 1. **(A).** Latency to the onset of the first generalized seizure (stage 4/5); **(B).** Latency to status epilepticus after lithium-pilocarpine injection (in minutes).

continuously without gain of consciousness in between 5-10 minutes or recurrent seizures occurring over about 30 minutes. In our study, we could observe a significant increase in latency to SE among test groups ($p<0.05$) in comparison to disease control. SV 300 mg/kg showed more delay to the onset of SE when compared to other groups. The delay in onset of SE was comparable between Groups 4 and 5, but not with oil alone.

There was the death of 1 rat in Group 2 (disease control) within 24 hours of induction. We also witnessed mortality of rats one week after induction, 1 rat from Group 2 (disease control) and 1 rat from Group 5 (oil 200 mg/kg + SV 300 mg/kg) this could be due to a severe reduction in weight and dehydration.

3.2. Post-seizure Behavioural Battery (PSBB) of Rats in Lithium-pilocarpine Model

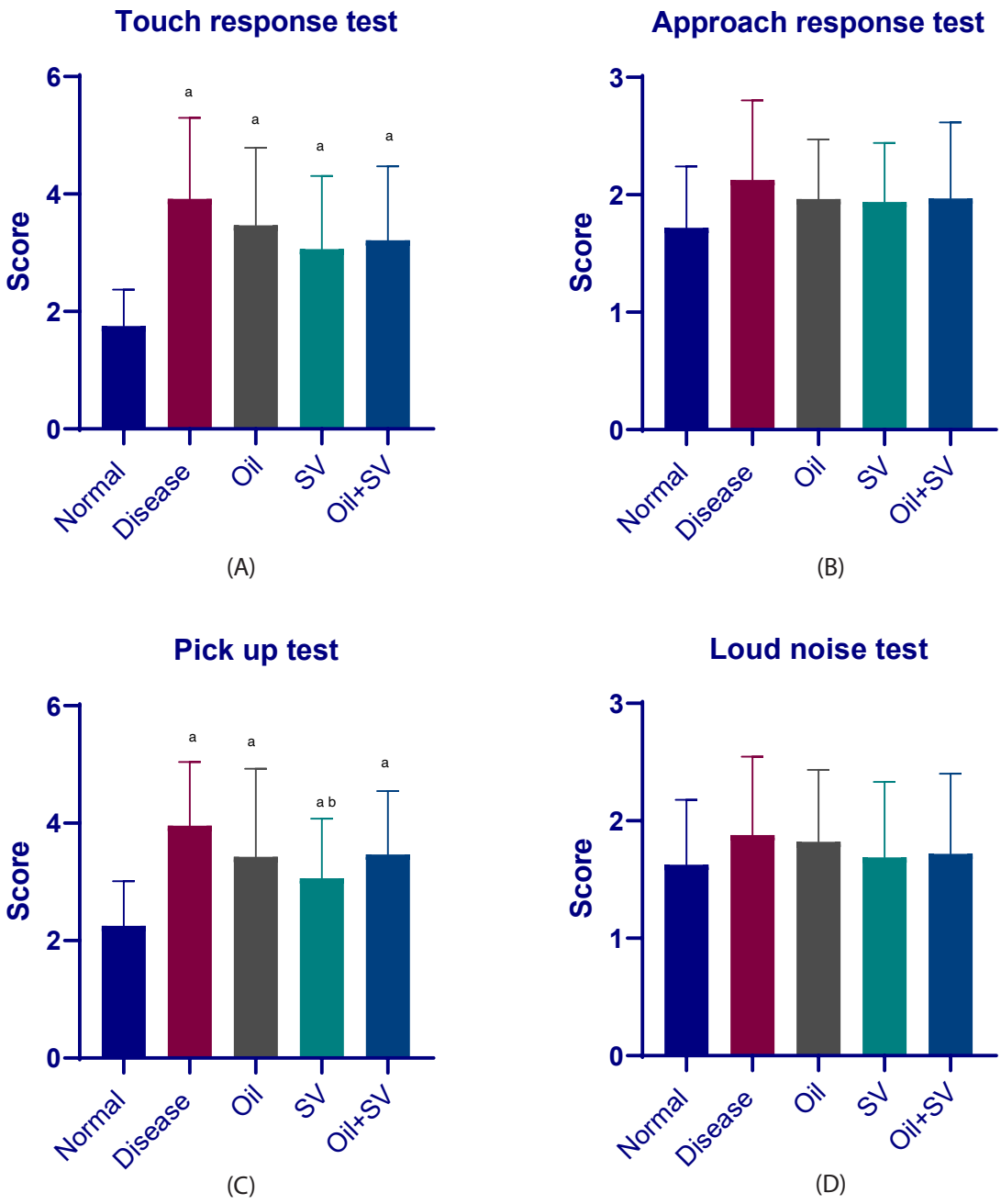
Four preliminary hyperexcitability tests were carried out: a) touch response test; b) approach test; c) pick up test, and d) loud noise test. The results are depicted in Figure 2.

3.3. Effect of *Sinapis alba* Seed Oil on Oxidative Stress Markers and BDNF Levels

The effect of *Sinapis alba* on various oxidative stress markers and BDNF is depicted in Figure 3.

3.4. Qualitative Analysis of Cresyl Violet Stained Pyramidal Neurons of Hippocampal CA3 Region

Cresyl violet stained pyramidal neuronal cell bodies of hippocampal CA3 region of rats of normal control (Group 1) showed normal healthy neurons (indicated by the yellow arrow in Figure 4) with healthy-looking cell membrane, clear cytoplasm, and prominent nucleus. However, the cresyl violet stained hippocampal CA3 region of the lithium-pilocarpine model of chronic epilepsy disease control (Group 2) showed many degenerating, flame-shaped deeply basophilic, pyknotic cell bodies of pyramidal neurons (indicated by the red arrow in Figure 4) which are indicative of karyopyknosis of the neurons of the hippocampus. It is noted that there is a substantial increase in the healthy

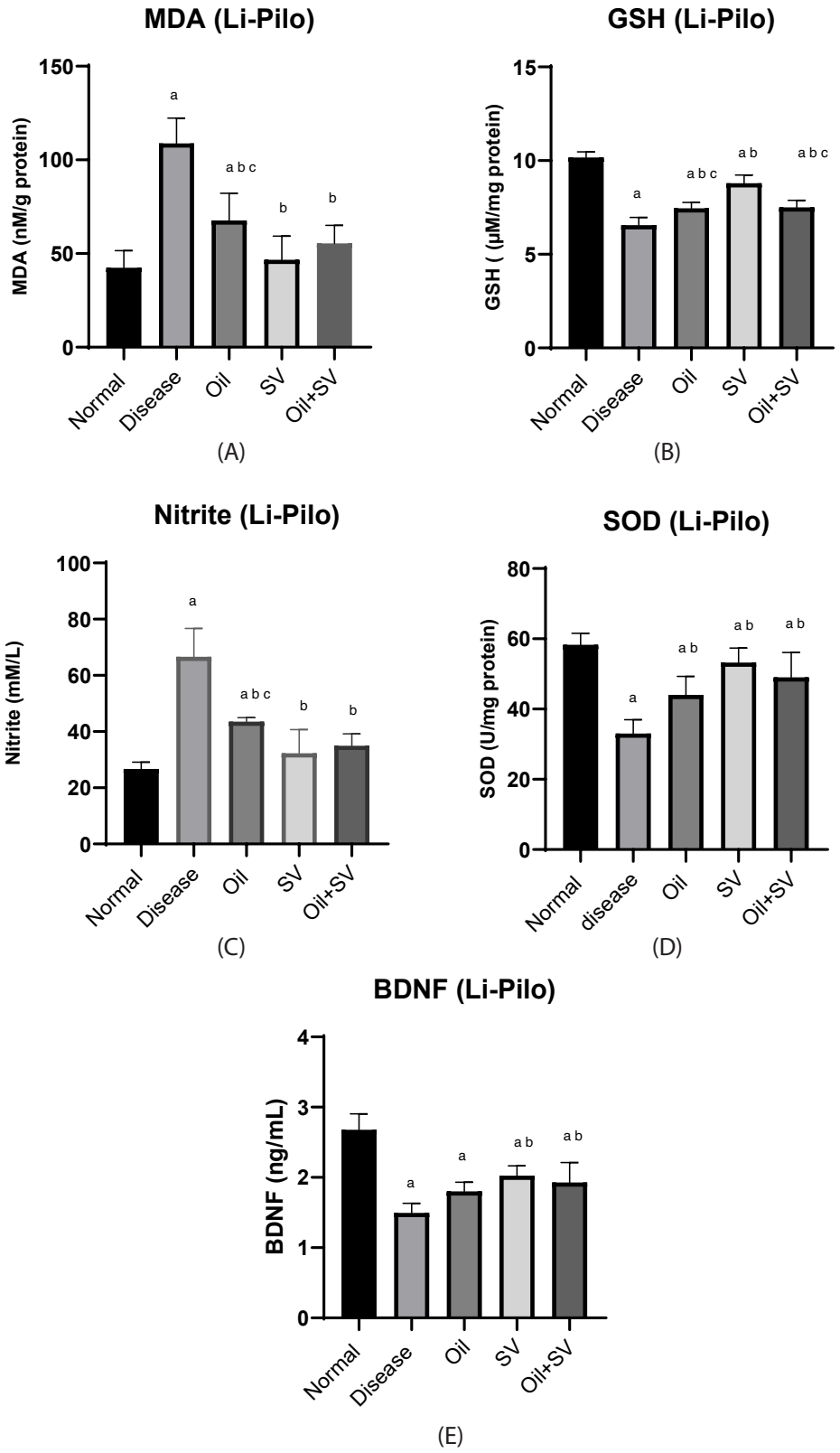


(One way ANOVA then post hoc Tukey's multiple comparison test. Data is denoted as Mean score ± SEM. a - p <0.05; vs normal control; b - p <0.05 vs disease control

Figure 2. Observation of behavioural battery (scores) of lithium-pilocarpine-induced rats to test for behavioural hyperexcitability. The data is presented as: **(A).** Approach response test; **(B).** Touch–response test; **(C).** Pick up test; and **(D).** Loud noise test.

neurons and very few or negligible numbers of flame-shaped, degenerating pyramidal neuronal cell bodies in the hippocampal CA3 region of rats belonging to Groups 3, 4 and 5 when compared to those of Group 2.

It can be noted that several degenerating pyknotic neurons (indicated by red arrow) which look characteristically flame-shaped are relatively more in Group 2 when compared to those of other groups. Groups 3, 4 and 5 showed predominantly healthy neurons (indicated by yellow arrow) when compared to Group 2. The neurons of Groups 4 and 5 looked almost at par with the normal control group.



One-way ANOVA, followed by post hoc Tukey's multiple comparisons test. Data represented as Mean ± SEM.
a - p <0.05 vs normal control; b - p <0.05 vs disease control; c - p <0.05 vs SV 300mg/kg

Figure 3. Effect of *Sinapis alba* seed oil and sodium valproate on brain oxidative markers MDA; **(A).** GSH; **(B).** Nitrite; **(C).** SOD**; (D).** BDNF; **(E).** Levels in lithium pilocarpine-induced status epilepticus model.

3.5. Qualitative Histopathological Evaluation of Cresyl Violet Stained Neurons of the Amygdala

Cresyl violet stained amygdaloid neurons of rats belonging to the normal control group showed normal healthy cell bodies (indicated by the yellow arrow in Figure 5). Their plasma membrane looked healthy with distinct edges. The cytoplasm was clear, and the nucleus was easily recognizable. However, the cresyl violet stained amygdala region of the Lithium-Pilocarpine model of chronic epilepsy-disease control (Group 2) showed several degenerating, flame-shaped, pyknotic cell bodies of neurons (indicated by the red arrow in Figure 5). Deeply basophilic flame-shaped cells indicate the degenerating nature of these neurons which could be due to karyopyknosis. It is noted that there is a marked increase in the healthy neurons and very few or negligible numbers of flame-shaped, degenerating neuronal cell bodies in the amygdala of rats belonging to Groups 3, 4, and 5 when compared to those of Group 2.

It can be noted that several degenerating pyknotic neurons (indicated by the red arrow) are relatively more in Group 2 when compared to those of other groups. Groups 3, 4, and 5 showed predominantly healthy neurons (indicated by yellow arrow) when compared to Group 2. The neurons of Groups 4 and 5 looked almost at par with the normal control group.

4. Discussion

In the present study, we evaluated the effect of *Sinapis alba* seed oil (yellow mustard) in lithium-pilocarpine-induced epileptic rat models. Several studies have been carried out to check the efficacy of AED sub-therapeutic dose combinations with products of plants with antioxidant properties in different epileptic models[33]. Therefore, we wanted to check if the combination of *S. alba* oil with a sub-therapeutic dose of sodium valproate had some synergistic effect.

Lithium-pilocarpine model is the commonly used model in the development of SE that mimics temporal lobe epilepsy of humans. Lithium when given alone does not have any pro-convulsant action in rats[34]. However, lithium treatment helps sensitize rats and produces limbic seizures at low (sub-convulsant) doses35. This combination produces several changes in the body like increased inositol monophosphate accumulation,

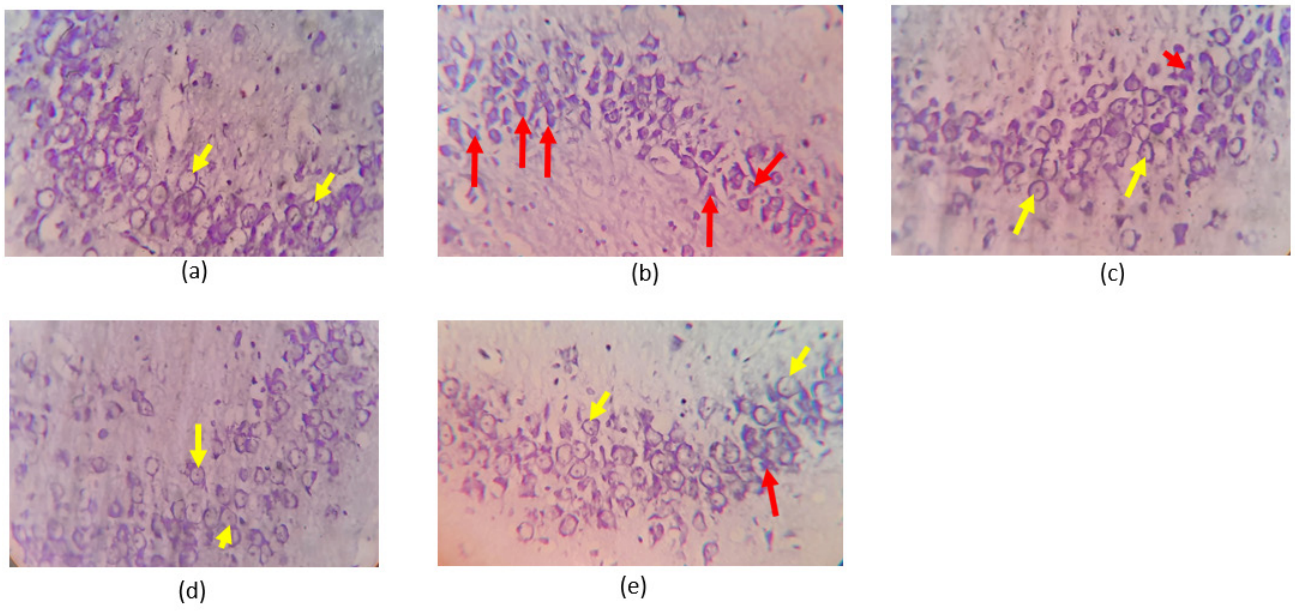


Figure 4. Representative photomicrographs of cresyl violet stained hippocampal CA3 region of: **(a).** Group 1 (normal control); **(b).** Group 2 (disease control); **(c).** Group 3 (treated with 200 mg/kg yellow mustard oil); **(d).** Group 4 (treated with 300 mg/kg Sodium valproate); **(e).** Group 5 (treated with 150 mg/kg sodium valproate + 200 mg/kg yellow mustard oil) [magnification: 40x10 X].

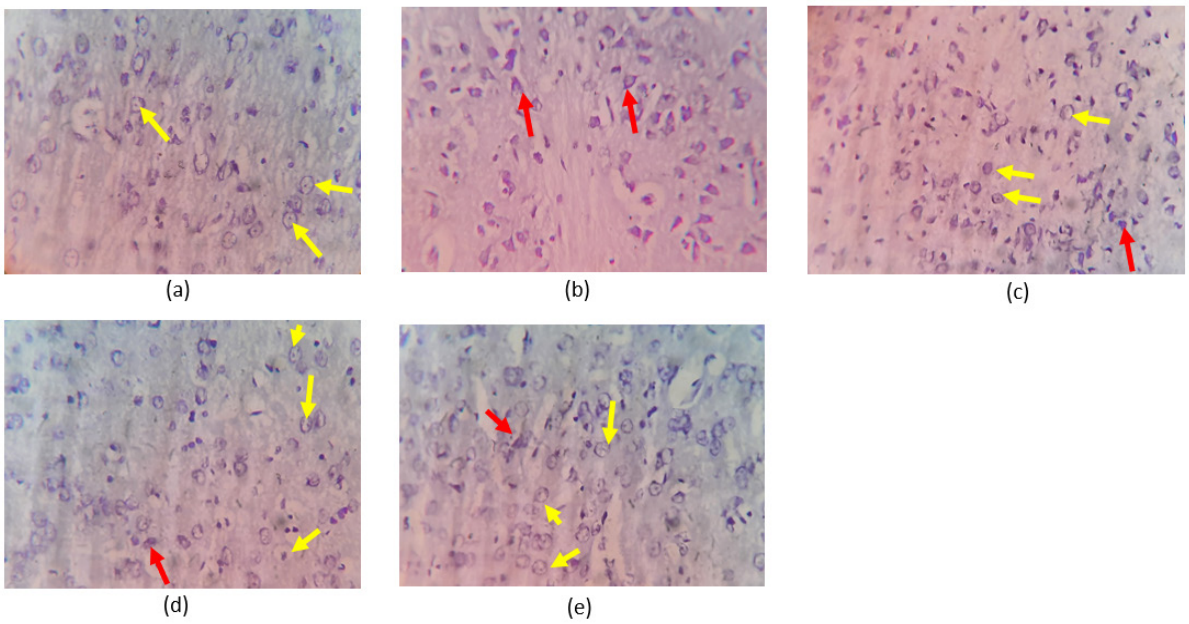


Figure 5. Representative photomicrographs of cresyl violet stained cell bodies of amygdaloid neurons of: **(a).** Group 1 (normal control); **(b).** Group 2 (disease control); **(c).** Group 3 (Treated with 200 mg/kg yellow mustard oil); **(d).** Group 4 (Treated with 300 mg/kg Sodium valproate); and **(e).** Group 5 (Treated with 150 mg/kg sodium valproate + 200 mg/kg yellow mustard oil) [Magnification: 40x10 X].

decreased inositol in cortical region, and elevated levels of acetylcholine which may be responsible for seizure precipitation and lethality associated[35].

In this study, status epilepticus induction was carried out using systemic lithium-pilocarpine injection and the effect of yellow mustard oil 200 mg/kg, sodium valproate 300 mg/kg and a combination of yellow mustard oil 200 mg/kg + sodium valproate 150 mg/kg was investigated. Yellow mustard oil helped in the prevention of the deleterious effect of SE by decreasing the mortality rate, and mean latency to onset of stage 4 and SE. The results also have shown that a combination of yellow mustard oil with half a dose of sodium valproate has an additive effect on status epilepticus. The intensity and frequency of seizures were decreased, and latency for the onset of seizures was increased with the combination. When oxidative markers were studied, oil, SV and the combination were able to lower the levels of nitrite and MDA and increase GSH, SOD. This correlated with similar studies conducted previously[36]. However, the treatment could not significantly normalize the levels of these markers. The effect of SV alone and combination groups lower the pro-oxidants level to a significant extent. Mustard oil alone was not that effective in the li-pilocarpine model. BDNF plays a role in neurogenesis. Skupien *et al.,* and Simato *et al*., also mention the potential of using BDNF in developing novel AED therapies[37,38]. In correlation with these studies, we observed treatment group elevated BDNF levels and lower levels were seen in diseased conditions.

The cresyl violet stained sections were observed for morphological changes at the level of the CA3 region of the hippocampus as well as the amygdaloid region. It was observed that neuronal cells of li-pilocarpine control were severely affected showing dark stained pyknotic cells, while the treatment group showed the presence of normal cells with little count of dead cells. Though the oil group showed healthy neurons, cell survival was higher in SV and combination groups. This shows that the treatment with SV alone and in combination increased the neuronal survival to some extent showing neuroprotective action at the level of hippocampus and amygdaloid region.

The data obtained from body weight indicated that 2 weeks of dosing with yellow mustard oil accelerated growth in rats. This shows its appetite-stimulating action, which was mentioned in a few studies[39]. In

the SE model, li-pilocarpine control lost weight while oil-dosed animals recovered better within a week post-induction. Current antiepileptic drugs act by i) inhibition of voltage-gated Na^+ channel-valproate, phenytoin, lamotrigine, felbamate ii) NMDA receptor blockers – felbamate[40-42]. So, we can predict that the oil might have action through one of these mechanisms. In one of the studies conducted to check the anti-convulsant effect of black mustard in rabbits, plasma calcium level decreased with a substantial increase in muscle Ca^{2+} ions after induction, this is due to utilization of plasma Ca^{2+} to reduce muscle spasms. Ca^{2+} is required to close the sodium channels. During epilepsy, this channel remains open, and continuous firing will be there, which is presented as muscle spasms. The rise in plasma Ca^{2+} levels after treatment showed that mustard oil corrected the abnormalities[20].

Preliminary phytochemical analysis confirmed the presence of terpenoids, flavonoids, phenols, and fatty acids in *S. alba* seeds[43]. Phenolic compounds of various plants were neuroprotective in earlier studies[44]. This is due to their extensive anti-oxidative property. Phenolic compounds are abundant in *S. alba* which are already determined, characterized, and are studied[45]. Mustard seeds are also rich in n-3 Polyunsaturated Fatty Acids (PUFAs). It consists of about 6-11 % omega 3-PUFAs[46]. There are several attempts made to understand the action of n-3-PUFAs in multiple neuronal disorders. It is found that chronic treatment with n-3-PUFAs has a neuroprotective effect, brings out plastic changes in the epileptic brain and reduces neuronal death in hippocampal CA1 and CA3 areas[47,48]. There are studies indicating the anti-convulsant action of PUFA[49]. Limitations of the study - for ethical reasons, we could not use a greater number of animals and had to restrict them to a smaller sample size. Using a larger sample would have strengthened the findings.

5. Conclusion

Sinapis alba oil did not prevent rats from developing status epilepticus but reduced its intensity and frequency. Oil administration increased antioxidant levels and decreased lipid peroxide levels as well. The combination showed a synergistic effect in the status epilepticus model.

6. References

1. Shin EJ, Jeong JH, Chung YH, Kim WK, Ko KH, Bach JH, Hong JS, Yoneda Y, Kim HC. Role of oxidative stress in epileptic seizures. Neurochem Int. 2011; 59(2):122-37. https://doi.org/10.1016/j.neuint.2011.03.025 PMid:21672578 PMCid: PMC3606551
2. Chen T-S, Huang T-H, Lai M-C, Huang C-W. The role of glutamate receptors in epilepsy. Biomedicines. 2023; 11(3):783. https://doi.org/10.3390/biomedicines11030783 PMid:36979762 PMCid: PMC10045847
3. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J Jr. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia. 2005; 46(4):470-2. https://doi.org/10.1111/j.0013-9580.2005.66104.x PMid:15816939
4. Kiasalari Z, Khalili M, Roghani M, Sadeghian A. Antiepileptic and antioxidant effect of *Brassica nigra* on pentylenetetrazol-induced kindling in mice. Iran J Pharm Res. 2012; 11(4):1209-17.
5. Al-Rafiah AR, Mehdar KM. Histopathological and biochemical assessment of neuroprotective deffects of sodium valproate and lutein on the pilocarpine Albino rat model of epilepsy. Behav Neurol. 2021; 2021:5549638. https://doi.org/10.1155/2021/5549638 PMid:34149964 PMCid: PMC8195670
6. Shashi PKr , Jangra MK, Yadav AK. Herbal and synthetic approaches for the treatment of epilepsy. Int J Nutr Pharmacol Neurol Dis. 2014; 4:43-52. https://doi.org/10.4103/2231-0738.124613
7. Trinka E, Cock H, Hesdorffer D, Rossetti AO, Scheffer IE, Shinnar S, Shorvon S, Lowenstein DH. A definition and classification of status epilepticus: Report of the ILAE Task Force on Classification of Status Epilepticus. Epilepsia. 2015; 56(10):1515-23. https://doi.org/10.1111/epi.13121 PMid:26336950
8. Ahmed Juvale II, Che Has AT. The evolution of the pilocarpine animal model of status epilepticus. Heliyon. 2020; 6(7):e04557. https://doi.org/10.1016/j.heliyon.2020.e04557 PMid:32775726 PMCid: PMC7393986
9. Castel-Branco MM, Alves GL, Figueiredo IV, Falcão AC, Caramona MM. The Maximal Electroshock Seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol. 2009; 31(2):101-6. https://doi.org/10.1358/mf.2009.31.2.1338414 PMid:19455265
10. Luszczki JJ, Wu JZ, Raszewski G, Czuczwar SJ. Isobolographic characterization of interactions of retigabine with carbamazepine, lamotrigine, and valproate in the mouse maximal electroshock-induced seizure model. Naunyn Schmiedebergs Arch Pharmacol. 2009; 379(2):163-79. https://doi.org/10.1007/s00210-008-0349-9 PMid:18773196

11. Jokeit H, Krämer G, Ebner A. Do antiepileptic drugs accelerate forgetting? Epilepsy Behav. 2005; 6(3):430-2. https://doi.org/10.1016/j.yebeh.2004.12.012 PMid:15820354
12. Naidech AM, Kreiter KT, Janjua N, Ostapkovich N, Parra A, Commichau C, Connolly ES, Mayer SA, Fitzsimmons BF. Phenytoin exposure is associated with a functional and cognitive disability after subarachnoid haemorrhage. Stroke. 2005; 36(3):583-7. https://doi.org/10.1161/01.STR.0000141936.36596.1e PMid:15662039
13. Wahab A. Difficulties in treatment and management of epilepsy and challenges in new drug development. Pharmaceuticals (Basel). 2010; 3(7):2090-110. https://doi.org/10.3390/ph3072090 PMid:27713344 PMCid: PMC4036655
14. Spinella M. Herbal medicines and epilepsy: The potential for benefit and adverse effects. Epilepsy Behav. 2001; 2(6):524-32. https://doi.org/10.1006/ebeh.2001.0281 PMid:12609386
15. Elvin-Lewis M. Should we be concerned about herbal remedies? J Ethnopharmacol. 2001; 75(2-3):141-64. https://doi.org/10.1016/S0378-8741(00)00394-9 PMid:11297844
16. Schachter SC. Botanicals and herbs: a traditional approach to treating epilepsy. Neurotherapeutics. 2009; 6(2):415-20. https://doi.org/10.1016/j.nurt.2008.12.004 PMid:19332338 PMCid: PMC5084222
17. Gokhale S, Joshi P, Tatiya A, Bakliwal S, Fursule R. Some neglected spices in India. Indian J Nat Prod Resour. 2004; 3(5):343–6.
18. Nadkarni KM, Nadkarni AK. *Dr K. M.* Nadkarni's Indian Materia Medica: With Ayurvedic, Unani-tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies, Appendices and Indexes. Popular Prakashan; 1976.
19. Adams M, Schneider SV, Kluge M, Kessler M, Hamburger M. Epilepsy in the Renaissance: A survey of remedies from 16th and 17th-century German herbals. J Ethnopharmacol. 2012; 143(1):1-13. https://doi.org/10.1016/j.jep.2012.06.010 PMid:22710294
20. Obeagu EI, Aloh GS, Emeka OC, Kanu SN, Okpara KE, Chibunna OM, *et al.* The anti-epileptic/anti-convulsive effects of mustard seed extracts. World Journal of Pharmaceutical Research. 2015; 4(3):219-31.
21. Faure JB, Akimana G, Carneiro JE, Cosquer B, Ferrandon A, Geiger K, Koning E, Penazzi L, Cassel JC, Nehlig A. A comprehensive behavioural evaluation in the lithium-pilocarpine model in rats: effects of carisbamate administration during status epilepticus. Epilepsia. 2013; 54(7):1203-13. https://doi.org/10.1111/epi.12219 PMid:23663139
22. Kim JE, Choi HC, Song HK, Kang TC. Perampanel Affects Up-stream regulatory signaling pathways of GluA1 phosphorylation in normal and epileptic rats. Front Cell Neurosci. 2019; 13:80. https://doi.org/10.3389/fncel.2019.00080
23. Tan X, Tu Z, Han W, Song X, Cheng L, Chen H, Tu S, Li P, Liu W, Jiang L. Anticonvulsant and neuroprotective effects of dexmedetomidine on pilocarpine-induced status epilepticus in rats using a metabolomics approach. Med Sci Monit. 2019; 25:2066-78. https://doi.org/10.12659/MSM.912283 PMid:30892279 PMCid: PMC6437718
24. Amiri-Nikpour MR, Nazarbaghi S, Eftekhari P, Mohammadi S, Dindarian S, Bagheri M, Mohammadi H. Sodium valproate compared to phenytoin in the treatment of status epilepticus. Brain Behav. 2018; 8(5):e00951. https://doi.org/10.1002/brb3.951 PMid:29761006 PMCid: PMC5943732
25. Perucca P, Scheffer IE, Kiley M. The management of epilepsy in children and adults. Med J Aust. 2018; 208(5):226-233. https://doi.org/10.5694/mja17.00951 PMid:29540143
26. Glien M, Brandt C, Potschka H, Voigt H, Ebert U, Löscher W. Repeated low-dose treatment of rats with pilocarpine: low mortality but a high proportion of rats developing epilepsy. Epilepsy Res. 2001; 46(2):111-9. https://doi.org/10.1016/S0920-1211(01)00272-8 PMid:11463512
27. Joushi S, Salmani ME. Effect of eugenol on the lithium-pilocarpine model of epilepsy: behavioural, histological, and molecular changes. Iran J Basic Med Sci. 2017; 20(7):745-52.
28. Rice AC, Floyd CL, Lyeth BG, Hamm RJ, DeLorenzo RJ. Status epilepticus causes long-term NMDA receptor-dependent behavioral changes and cognitive deficits. Epilepsia. 1998; 39(11):1148-57. https://doi.org/10.1111/j.1528-1157.1998.tb01305.x PMid:9821978
29. Polascheck N, Bankstahl M, Löscher W. The COX-2 inhibitor parecoxib is neuroprotective but not antiepileptogenic in the pilocarpine model of temporal lobe epilepsy. Exp Neurol. 2010; 224(1):219-33. https://doi.org/10.1016/j.expneurol.2010.03.014 PMid:20353773
30. Nizinska K, Szydlowska K, Vouros A, Kiryk A, Stepniak A, Vasilaki E, Lukasiuk K. Behavioral characteristics as potential biomarkers of the development and phenotype of epilepsy in a rat model of temporal lobe epilepsy. Sci Rep. 2021; 11(1):8665. https://doi.org/10.1038/s41598-021-88088-9 PMid:33883658 PMCid: PMC8060252
31. Asuntha G, Raju YP, Sundaresan CR, Rasheed A, Chowdary VH, Vandana KR, Babu KS, Prasad KV. Effect of *Argemone mexicana* (L.) against lithium-pilocarpine induced status epilepticus and oxidative stress in Wistar rats. Indian J Exp Biol. 2015; 53(1):31-5.
32. Smiałowska M, Wierońska JM, Szewczyk B. Neuroprotective effect of NPY on kainate neurotoxicity in the hippocampus. Pol J Pharmacol. 2003; 55(6):979-86.
33. Kumar R, Arora R, Sarangi SC, Ganeshan N S, Agarwal A, Kaleekal T, Gupta YK. Pharmacodynamic and pharmacokinetic interactions of hydroalcoholic leaf extract of *Centella asiatica* with valproate and phenytoin in experimental models of epilepsy in rats. J Ethnopharmacol. 2021; 270:113784. https://doi.org/10.1016/j.jep.2021.113784 PMid:33429032
34. Ormandy GC, Song L, Jope RS. Analysis of the convulsant-potentiating effects of lithium in rats. Exp Neurol.

1991; 111(3):356-61. https://doi.org/10.1016/0014-4886(91)90103-J PMid:1847879
35. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of *Albizzia lebbeck*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. J Ethnopharmacol. 2000; 71(1-2):65-75. https://doi.org/10.1016/S0378-8741(99)00192-0 PMid:10904147
36. Ahmad P. Oxidative damage and antioxidants in plants: Antioxidant networks and signalling. San Diego: Academic Press. 2014; 345-67. https://doi.org/10.1016/B978-0-12-799963-0.00011-3 PMCid: PMC5121342
37. Simonato M. Gene therapy for epilepsy. Epilepsy Behav. 2014; 38:125-30. https://doi.org/10.1016/j.yebeh.2013.09.013 PMid:24100249
38. Skupien-Jaroszek A, Walczak A, Czaban I, Pels KK, Szczepankiewicz AA, Krawczyk K, Ruszczycki B, Wilczynski GM, Dzwonek J, Magalska A. The interplay of seizures-induced axonal sprouting and transcription-dependent Bdnf repositioning in the model of temporal lobe epilepsy. PLoS One. 2021; 16(6):e0239111. https://doi.org/10.1371/journal.pone.0239111 PMid:34086671 PMCid: PMC8177504
39. Ram MP, Pushpan R, Rohini S. Mustard and its uses in Ayurveda. Indian Journal of Traditional Knowledge. 2009; 8(3):400–4.
40. Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. Epilepsia. 1995; 36(Suppl 2):S2-12. https://doi.org/10.1111/j.1528-1157.1995.tb05996.x PMid:8784210
41. White HS, Harmsworth WL, Sofia RD, Wolf HH. Felbamate modulates the strychnine-insensitive glycine receptor. Epilepsy Res. 1995; 20(1):41-8. https://doi.org/10.1016/0920-1211(94)00066-6 PMid:7713059
42. Subramaniam S, Rho JM, Penix L, Donevan SD, Fielding RP, Rogawski MA. Felbamate block of the N-methyl-D-aspartate receptor. J Pharmacol Exp Ther. 1995; 273(2):878-86.
43. Sujatha R, Mariajancyrani J, Chandramohan G. Preliminary phytochemical investigation and antimicrobial activity of *Sinapis alba*. Sch J App Med Sci. 2013; 1:138-41.
44. Aldawsari HM, Eid BG, Neamatallah T, Zaitone SA, Badr JM. Anticonvulsant and neuroprotective activities of *Phragmanthera austroarabica* extract in pentylenetetrazole-kindled mice. Evid Based Complement Alternat Med. 2017; 2017:5148219. https://doi.org/10.1155/2017/5148219 PMid:28465705 PMCid: PMC5390588
45. Nicácio AE, Rodrigues CA, Visentainer JV, Maldaner L. Evaluation of the QuEChERS method for the determination of phenolic compounds in yellow (*Brassica alba*), brown (*Brassica juncea*), and black (*Brassica nigra*) mustard seeds. Food Chem. 2021; 340:128162. https://doi.org/10.1016/j.foodchem.2020.128162 PMid:33027718
46. El-Shenawy NS, Zaid AA, Al Harbi MS, Al-Thomali AW. Effects of mustard oil on oxidative stress parameters of male mice. Advances in Food Sciences. 2014; 36(2):78-85.
47. Cysneiros RM, Ferrari D, Arida RM, Terra VC, de Almeida AC, Cavalheiro EA, Scorza FA. Qualitative analysis of hippocampal plastic changes in rats with epilepsy supplemented with oral omega-3 fatty acids. Epilepsy Behav. 2010; 17(1):33-8. https://doi.org/10.1016/j.yebeh.2009.11.006 PMid:19969506
48. Ferrari D, Cysneiros RM, Scorza CA, Arida RM, Cavalheiro EA, de Almeida AC, Scorza FA. Neuroprotective activity of omega-3 fatty acids against epilepsy-induced hippocampal damage: Quantification with immunohistochemical for calcium-binding proteins. Epilepsy Behav. 2008; 13(1):36-42. https://doi.org/10.1016/j.yebeh.2008.01.001 PMid:18295545
49. Taha AY, Burnham WM, Auvin S. Polyunsaturated fatty acids and epilepsy. Epilepsia. 2010; 51(8):1348-58. https://doi.org/10.1111/j.1528-1167.2010.02654.x PMid:20608961