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Research Article

Pharmacotherapeutic Effects of *Hippophae rhamnoides* in Rat Model of Post-traumatic Epilepsy in View of Oxidative Stress, Na+,K+ATPase Activity and Sodium Ion Channel Expression

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Abstract

Background: Post-traumatic epilepsy (PTE) is a brain disorder characterized by an occurrence of spontaneous and recurrent seizures post brain insults. Initiation and progression of epilepsy is a complex process involving numerous cellular, molecular, and signalling mechanisms. Therefore, understanding the mechanism involved and finding safer treatments is of paramount importance.

Objectives and Methods: In this study, the antiepileptic effect of *Hippophae rhamnoides* (sea buckthorn/sbt) in post-traumatic epilepsy model was assessed. Post-traumatic epilepsy was induced by intracortical iron (5µl of 100mM $FeCl_3$) injection. *Hippophae rhamnoides* berry extract was administered orally at a dose of 1ml/kg b.wt. for 1 month. Then, the effect of sbt on the oxidative stress, Na^+,K^+ATPase activity and sodium ion channel expression was evaluated.

Results and Discussions: In cortex and hippocampus of epileptic rats, the results demonstrate altered electrophysiology, elevated oxidative stress, and reduced antioxidant defense. Additionally, reduced Na^+,K^+ATPase activity and elevated sodium channel $Na_v1.1$ and $Na_v1.6$ expression were also observed. Sbt administration has attenuated epileptiform activity, counteracted oxidative stress, elevated Na^+,K^+ATPase activity, and decreased sodium channel expression in the cortex and hippocampus of epileptic rats. In summary, our results demonstrate the antiepileptic effect of sbt that have possibly exerted by its antioxidative and ion channel regulatory properties in post-traumatic epilepsy model.

Keywords: Post-traumatic Epilepsy; Iron-induced Epilepsy; *Hippophae rhamnoides;* Sea Buckthorn; Seizure

Abbreviations

BSA: Bovine Serum Albumin; CAT: Catalase; DNPH: 2,4-dinitrophenylhydrazine; DTNB: 5,5-dithiobis-2-Nitrobenzoic Acid; EEG: Electroencephalographic; LP: Lipid Peroxidation, MUA: Multiple Unit Activity; PTE: Post-Traumatic Epilepsy; TBI: Traumatic Brain Injury; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; VGSC: Voltage Gated Sodium Channels; DAB: 3,3′-Diaminobenzidine; DEHA: Dehydeoepiandrosterone; CA: Cornu Ammonis; MDA: Malondialdehyde; PBS: Phosphate Buffer Saline; Sbt: Sea Buckthorn

Introduction

Traumatic brain injury (TBI) often causes seizure and is a major contributor to the induction and progression of post-traumatic epilepsy [1]. TBI trigger mechanism including oxidative stress and neuroinflammation that sporadically contribute to epileptogenesis

[2,3]. The complex mechanism of epileptogenesis results in causing a wide spectrum of symptoms and seizure types, leading to unresolved treatment issues. Broadly, post-traumatic epilepsy (PTE) accounts for 10–20% of epilepsy globally [4].

In case of PTE, the mechanism of epileptogenesis is mainly initiates by free iron, release from cortical infraction and extravasations of haemoglobin after a brain insult. The free iron initiates generation of reactive oxygen species (ROS) via fenton reaction and results in elevated oxidative stress. In this study, an iron-induced rodent model of post-traumatic epilepsy was used [5]. The iron injection in brain initiates fenton reaction and results in elevated oxidative stress that adequately recapitulates human post-traumatic epilepsy [5].

Oxidative stress at a cellular level disrupts membrane physiology and biomolecules via the formation and release of ROS [6,7]. Additionally, it alters neuronal electrophysiology and biochemistry [8]. Moreover, brain is highly vulnerable to free radicals damage due to its high metabolic demand and the presence of polyunsaturated fatty acids [9].

In PTE, an alteration in Na^+,K^+ATPase activity and sodium ion channel ($Na_v1.1$ and $Na_v1.6$) expression was observed [10,11]. Na^+,K^+ATPase is an ion transporters that maintains Na^+/K^+ equilibrium, regulates neuronal excitability, electrochemical gradient, resting membrane potential, neurotransmitters release and uptake. Decrease in activity or expression of Na^+,K^+ATPase leads to neuronal dysfunction and cause seizure activity [12] furthermore, alteration of $Na_v1.1$ and $Na_v1.6$ expression produces neuronal burst and hyperexcitability [13]. $Na_v1.1$ channel are confines to cell bodies [14], $Na_v1.6$ channel in myelinated axons and dendrites [15] and contribute in initiation of action potentials [16]. In epilepsy, upregulation in the activity or expression of sodium channel were reported [17].

In last few decades, many antiepileptic drugs being introduced but epileptic patients were soon reported to develop resistant towards the drugs [18]. Moreover, terrible side effects of antiepileptic drugs deteriorate the quality of life in epileptic patients and results in treatment complications. Several conventional antiepileptic drugs, such as phenobarbital, valproic acid, carbamazepine were reported to increase lipid peroxidation in blood cells [19]. Therefore, finding an alternative and effective treatment strategy is important.

At present, focus on plant research is increasing globally because of its pharmacotherapeutic properties and lesser side effects. Emphasis is on the use of plant crude extracts or their isolated fractions containing polyphenols. Polyphenols are secondary metabolites widely present in plants that have medicinal properties [6]. Structure of flavonoids is ideal for radical scavenging, that chelates free radicals and transition metal ions and contributes towards antioxidative potential [20].

Hippophae rhamnoides is commonly known as sea buckthorn (sbt). Nutritional and therapeutic values of sbt are well known in China and Russia since ancient times [21]. Almost every bioactive compound of sbt has been reported to prevent different diseases [21]. A unique feature of sbt berry is its high oil content [22], that may provide an advantage towards absorption and bioavailability. It is rare to find almost all the major vitamins and minerals in a single fruit type. Sbt has reported to possess antioxidative, antiageing and neuroprotective properties [21], but its effect on iron-induced epilepsy is still unknown. Thus, to evaluate the role of sbt in counteracting or healing the effects of post-traumatic epilepsy is of special interest.

It was reported that there may be interactions and co-operations between different antioxidant compounds [23], that may provides a synergistic effect and produces an antioxidative capacity that have greater potential than the use of a single bioactive compound [24]. Therefore, in this study we used whole berry extract rather than using a single bioactive compound.

Objective of the Study

The main objectives of this study were to determine whether sbt administration attenuates epileptiform activity and oxidative stress. And to evaluate whether it can regulate Na^+,K^+ATPase activity and sodium ion channel ($Na_v1.1$ and $Na_v1.6$) expression in the rat model of post-traumatic epilepsy.

Materials and Methods

Materials

Chemicals including ferric chloride ($FeCl_3$), ouabain, 2,4-dinitrophenylhydrazine (DNPH), bovine serum albumin (BSA), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), 3, 3′-diaminobenzidine (DAB), used in the experiments were procured from Sigma-Aldrich, (USA). Surgical and electrophysiological recording equipment were of

high-grade stainless steel, tissue friendly, and procured from Plastic One, Roanoke-VA, (USA).

Extract preparation

Hippophae rhamnoides (sbt) berries were collected from Ladakh during the month of September and always from the same region to avoid phytochemical variations. Fresh berries were cleaned and weighed in different batches of 500 gm each. A single batch of berries was ground using a blender. Crude extract was then filtered using a muslin cloth and the filtrate was stored in a dark bottle at -20°C in a refrigerator. Extract from same batch was used for all the experiments. Oral administration of sbt extract was given at a dose of 1 ml/kg body weight for one month [25]. Control rats received an equal volume of RO water.

Animals and treatment plan

Thirty-two male adult Wistar rats of 4 months age, weighing around 250-350 gm were used for this study. Standard laboratory cages of 12″×9″×6″ size made up of polypropylene with stainless-steel covers were used to house rats. Rats were provided free access to food and water. Animal room was maintained at 23-25°C, and a 12 hour light and dark cycle. Prior approval was taken from the Institutional Animal Ethical Committee of Jawaharlal Nehru University, New Delhi, India for performing all the experiments. A guideline of Committee for Control and Supervision of Experimental Animals was followed.

Rats were randomly divided into four groups to minimize selection bias. Each group consists of 8 rats (n = 8):

- **Group 1:** In this group, rats were injected with 5µl saline (0.9% NaCl) in the somatosensory cortex.
- **Group 2:** In this group, rats were injected with 5µl iron (100 mM $FeCl_3$) in the somatosensory cortex to induce post-traumatic epilepsy.
- **Group 3:** In this group, rats were injected with 5µl iron (100 mM $FeCl_3$) in the somatosensory cortex to induce post-traumatic epilepsy. Post 2 day surgery, oral administration of sbt was given at a dose of 1ml/kg body weight for one month.
- **Group 4:** In this group, rats were injected with 5 µl saline (0.9% NaCl) in the somatosensory cortex. Post 2 day surgery, oral administration of sbt was given at a dose of 1 ml/kg body weight for one month.

Surgical procedure

Rats were anesthetized using isoflurane (4%) and were placed in a stereotaxic surgical apparatus for administration of saline or iron and implantation of epidural electrodes. Head was shaved and an incision in the scalp was made and then muscles were cleaned to expose the underlying skull surface. Brain coordinates were stereotaxically marked and burr holes of 0.5 mm diameter were drilled into the skull [26]. In the saline and iron-injected rats, 5µl of 0.9% NaCl and 100 mM $FeCl_3$ were injected respectively over 5 minutes from hole into somatosensory cortex (coordinates: anteroposterior-1.0 mm, mid-lateral-1.0 mm from bregma and ventral-2.0 mm to dura) using a motorized injector. The burr hole was sealed using bone wax after injection. On the frontal sinus, one screw electrode was placed that serves as an animal ground. Four epidural screw electrodes were sited (coordinates: anteroposterior-2mm and mid-lateral-2mm from bregma) for cortical electrophysiological recording. Intra-cerebral bipolar electrodes were placed in the CA1 subregion (coordinates: anteroposterior -2.8 mm and mid-lateral-2.5 mm from bregma and ventral-2.71 mm to dura) for hippocampus recording. Nine-pin connector was used and soldered to screw electrodes, which was then attached to head using dental acrylic cement. All rats were given perioperative care and kept under observation until recovery.

Electrophysiological recording

Electroencephalography (EEG) and multiple-unit activity (MUA) recording

Before recording, animals were habituated for a week in a recording room. Recording was done post surgical recovery from cortex and CA1 subregion of hippocampus of rat brain using a Grass Technologies polyVIEW 16 Data Acquisition System with high-performance AC preamplifiers (Model P511). The recordings were restricted to the alert stationary state to reduce noise. Extracellular signals were passed through Grass HIP 511 with FET, signals were amplified and filtered at 1Hz to 100Hz and 300Hz to 10kHz for EEG and MUA recordings respectively using Grass Polygraph P511 AC preamplifiers to reject undesired frequencies. A window discriminator (WPI, Florida, USA) electronically discriminated MUA and displayed signals on an oscilloscope. Quantification of the epileptic activity was performed by counting corresponding MUA to that of epileptiform waveforms.

Preparation of tissue homogenate

Once the electrophysiological recordings were completed, rats were sacrificed and brains were isolated instantly for further analysis. Each brain sample was weighed and then washed in normal saline. Brain was dissected out over an iced petri dish and both hemispheres (left and right) cortex and hippocampi are pooled together. The brain samples were homogenized (diluted 1:10) in the 50 mM Tris buffer (pH 7.4) using a homogenizer. Thereafter, homogenization samples were centrifuged at 6000 rpm for 10 min using a refrigerated centrifuge (Sorvall RC5C). Resultant supernatant was again centrifuged at 14,000 rpm for 25 min to separate synaptosomes from the cytosol. The synaptosomal fraction was used to measure the Na^+,K^+ATPase activity whereas cytosolic fraction was used to performed lipid peroxidation, SOD and CAT assay. Protein concentration of each sample was determined by the method of Lowry., *et al*. (1951) using BSA as a standard [27].

Lipid peroxidation (LP)

Lipid peroxidation assay was performed following the method of Ohkawa., *et al*. (1979) with slight changes [28]. To a tissue homogenate (250 µl), 4% sodium dodecyl sulfate (10 µl) was added. After that, a reaction mixture was prepared in a centrifuge tube using 2 M HCl (750 µl) in 0.8% TBA and 1% CH_3COOH. Then the reaction mixtures were incubated in a water bath at 95°C for 1 hour. Later, solutions were kept at 25°C for cooling and then centrifuged at 2000 rpm for 20 min. OD was recorded at 532 nm using a Shimadzu UV-1800 spectrophotometer. Total MDA formed was estimated and expressed as nmol MDA/mg protein.

Superoxide dismutase (SOD) assay

The method described by Marklund., *et al*. (1974) was followed to measure SOD activity [29]. To 50 µl of tissues homogenate, a reaction mixture of 1.0 ml of 20 mM pyrogallol solution and 1.0 ml of tris-buffer (50 mM) was added. Pyrogallol auto-oxidation was estimated at 420 nm using a spectrophotometer to assay SOD activity. SOD activity was expressed as U/mg protein, the amount of SOD requires to inhibit 50% of pyrogallol auto-oxidation is considered as a unit.

Catalase (CAT) assay

The method described by Claiborne (1986) was followed with slight modifications to assay CAT activity [30]. To 100 ul tissues homogenate, 1 ml of a reaction mixture was added. Reaction mixture consisted of 50 mM potassium phosphate (pH 7.0) and 19 mM H_2O_2. Reaction was initiated by adding H_2O_2 and changes in absorbance were measured at 240 nm for 30 s using a spectrophotometer. Activity was calculated and expressed as a number of H_2O_2 consumed per min/mg protein.

Na^+,K^+ATPase assay

Crude synaptosomal fraction was used to estimate Na^+,K^+ATPase activity. The method described by Beltowski., *et al*. (2003) was followed [31]. To a reaction mixture containing 20 mM KCl, 100 mM NaCl, 5 mM $MgCl_2$, 3 mM ATP, and 50 mM Tris (pH 7.4), 50 ug protein was added and incubated at 37°C for 30 min. To stop the reaction, 1 ml chilled 10% TCA solution was added. The reaction mixture was centrifuged at 4000 rpm for 5 min. After that 1 ml supernatant was collected and added to a reaction mixture containing 8.1 ml distilled water, and 0.5 ml of (0.5%) acid ammonium molybdate in 5 N H_2SO_4 and then incubated for 10 min at room temperature. After incubation 0.4 ml of reducing agent that contained 0.5 g aminonaphtholsulfonic acid, 15% sodium bisulfite, and 20% sodium sulfite was added. Later, absorbance was read at 660 nm using a spectrophotometer. ATP was used as a substrate and liberated inorganic phosphate was estimated using spectrophotometer. Na^+,K^+ATPase activity was blocked using specific blocker ouabain (1 mM). Ouabain sensitive Na^+,K^+ATPase activity was calculated and expressed as nanomoles of inorganic phosphate released/min/mg protein.

Immunohistochemistry (IHC)

After anesthetization, 4% paraformaldehyde was used to perfuse rats. Brain was removed and kept in 4% paraformaldehyde for 24 h for tissue fixation. Brain sections (15 µm) were cut using cryostat and mounted on gelatine-coated clean glass slides. For IHC, the sections were air-dried for 2 h at room temperature and then washed with phosphate buffer saline (PBS). Then the sections were treated with 1% Triton X-100 and were immersed in 1% H_2O_2 in PBS for 10 min to quench the endogenous peroxidase. For blocking nonspecific antigen binding, sections were incubated with 3% NGS for 30 min. Then the sections were incubated overnight at 4°C in rabbit polyclonal primary antibodies of $Na_v1.1$ and $Na_v1.6$ (1:1000). Thereafter, the sections were washed 3 times in PBS for 5min each. After that, the sections were incubated with HRP-labelled secondary antibody (1:200) at room temperature for 90 min. The sections were then washed 3 times with PBS and treated with DAB and 0.25% H_2O_2 solution in PBS for 10 min. Slides were cover slipped using DPX mounting medium and photographed using a light microscope (Nikon Eclipse Ti, Tokyo, Japan). Protein level of $Na_v1.1$ and $Na_v1.6$ was determined using Image J software by comparing optical intensity of $Na_v1.1$ or $Na_v1.6$.

Statistical analysis

All data obtained were expressed as mean ± SD. One-way analysis of variance (ANOVA) and Holm-Sidak post hoc tests were performed to analyze statistical calculations using Sigma Plot software version 12.0. Statistical significance was set at $p < 0.05$.

Results

Effect of sbt on epileptiform activity

EEG and MUA recordings from all groups were analyzed for epileptiform activity. EEG stretches of 20 sec from cortex and hippocampus were compared among the groups. Elevated seizure activity was observed in both cortex and hippocampus of epileptic rats. Epileptic seizures can be easily distinguished from normal brain activity because of its unique spike and polyspike brain wave patterns that are represent by an increased in amplitude and frequency as clearly depicted in figure 1A. Administration of sbt significantly attenuated epileptiform activity. Changes are also quantified at a level of MUA counts. Results showed MUA counts in both cortex (70.8%) and hippocampus (73.4%) were elevated in epileptic rats as compared to control. Reduced MUA counts in cortex (8.9%) and hippocampus (9.3%) were observed in sbt administered epileptic rats as compared to epileptic control (Figure 1B).

Figure 1: EEG and MUA recordings from the cortex and hippocampus of rats' brain. (A) Represents the 20 sec stretches of EEG of control, epileptic, Sbt treated epileptic and Sbt treated rats. (B) MUA counts of 1 min duration of control, epileptic, epileptic + Sbt treated and Sbt treated rats. Data are expressed as mean ± SD, ***p ≤ 0.001 significantly different from control; ###p ≤ 0.001 significantly different from epileptic group.

Effect of sbt on oxidative stress

MDA formed in tissue is a marker for oxidative stress. Results showed significantly increased MDA in both cortex (122%) and hippocampus (81%) of epileptic rats as compared to control rats. Significantly decreased MDA was observed in cortex (36.5%) and hippocampus (29.1%) of sbt administered epileptic rats as compared to epileptic rats. This suggests antioxidative potential of sbt against iron-induced epilepsy (Figure 2).

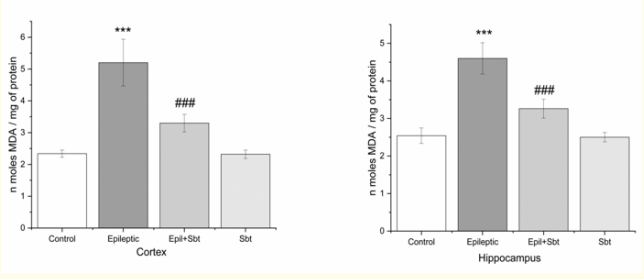

Figure 2: Effect of sbt administration on LP in cortex and hippocampus of epileptic rats. Data are expressed as mean ± SD, ***p ≤ 0.001 significantly different from control; ###p ≤ 0.001 significantly different from epileptic group.

Significant decreases in SOD and CAT activity were also observed in cortex (35.3%), (39.2%) and hippocampus (43%), (37.5%) of epileptic rats due to an increase in ROS (because of iron injection and the cascading reaction). Contrarily, there was a significant increased in SOD and CAT activity in both cortex (25.9%), (28.3%) and hippocampus (37.7%), (21.8%) on sbt administration in epileptic rats that suggests chelating effect of a polyphenol compounds present in sbt (Table 1).

Effect of sbt on Na^+,K^+ATPase activity

Na^+,K^+ATPase activity was lower in cortex (49.3%) and hippocampus (49.6%) of epileptic rats as compared to control rats. On sbt administration in epileptic rats, activity of Na^+,K^+ATPase in cortex (56.6%) and hippocampus (47.8%) was observed higher as compared to epileptic rats (Figure 3).

SOD (U/mg protein)				
	Control	Epileptic	Epil+Sbt	Sbt
Cortex	3.34 ± 0.24	2.16 ± 0.17***	2.72 ± 0.23##	3.38 ± 0.30
Hippocampus	4.28 ± 0.19	2.44 ± 0.18***	3.36 ± 0.25##	4.26 ± 0.30
CAT (µmol H_2O_2 decomposed/min/mg protein)				
	Control	Epileptic	Epil+Sbt	Sbt
Cortex	54.14 ± 2.1	32.95 ± 1.9***	42.24 ± 1.9##	56.14 ± 2.1
Hippocampus	57.86 ± 2.9	35.76 ± 2.9***	43.54 ± 1.5##	66.94 ± 2.6

Table 1: Effect of sbt administration on SOD and CAT activities in cortex and hippocampus of epileptic rats.

Values are expressed as mean ± SD, ***p ≤ 0.001 significantly different from control; ##p ≤ 0.01 significantly different from epileptic group.

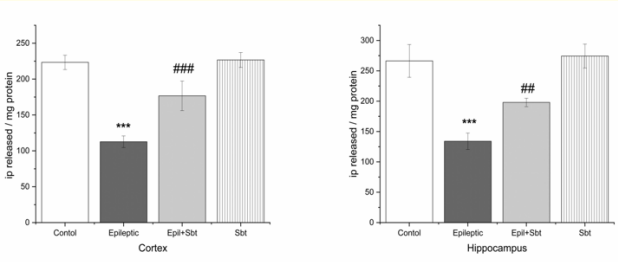

Figure 3: Effect of sbt administration on Na^+,K^+-ATPase activity in cortex and hippocampus of epileptic rats. Data are expressed as mean ± SD, ***p ≤ 0.001 significantly different from control; ###p ≤ 0.001, ##p ≤ 0.01 significantly different from epileptic group.

Effect of sbt on protein expression of $Na_v1.1$ and $Na_v1.6$

Changes in expression of voltage-gated sodium ion channel subtypes $Na_v1.1$ and $Na_v1.6$ in response to epilepsy and sbt administration were determined. Protein expression of $Na_v1.1$ in both brain sections of cortex and hippocampus was observed. The channel expression was significantly higher in cortex (37.2%) and hippocampus (42.1%) of epileptic rats as compared to control rats. Sbt administration reduced epileptic associated increase in the expression of $Na_v1.1$ in cortex (14.8%) and hippocampus (13.3%) of epileptic rats (Figure 4A and 4B).

Protein expression of $Na_v1.6$ in both brain sections of cortex and hippocampus was also observed. Significant differences between groups were reported. The channel expression was significantly higher in cortex (45.2%) and hippocampus (42.7%) of epileptic rats as compared to control rats. Sbt administration reduced epileptic associated increase in the expression of $Na_v1.6$ in both cortex (18.3%) and hippocampus (15.2%) of epileptic rats (Figure 5A and 5B).

Figure 4: Protein expression of $Na_v1.1$ in the coronal sections of cortex and hippocampus of control, epileptic, Sbt treated epileptic and Sbt treated rats. (A) Cortex and hippocampus sections showing immuno-reactivity of $Na_v1.1$. (B) Optical intensity data are expressed as mean ± SD, ***p ≤ 0.001 significantly different from control; ##p ≤ 0.01 significantly different from epileptic group.

Figure 5: Protein expression of $Na_v1.6$ in the coronal sections of cortex and hippocampus of control, epileptic, Sbt treated epileptic and Sbt treated rats. (A) Cortex and hippocampus sections showing immuno-reactivity of $Na_v1.6$. (B) Optical intensity data are expressed as mean ± SD, ***p ≤ 0.001 significantly different from control; ##p ≤ 0.01 significantly different from epileptic group.

Discussion and Conclusion

Post-traumatic epilepsy results in spike-wave discharges that can be measure through EEG. Seizure waveform generation is associated with many factors that converge to produce a complex phenotype [32]. Cortex and hippocampus are highly susceptible to structural and functional damages, such as neuronal loss and neuoinflammation [33]. It has been reported that the onset of spontaneous seizure discharge occurs at an injection site and later spreads to entire cerebral cortex of brain and subcortical structures like hippocampus [34]. This study demonstrates changes in EEG and MUA in cortex and hippocampus of epileptic rats and sbt administered epileptic rats.

EEG of epileptic rats shows interictal spikes that are brief (20-70 ms) conspicuous sharp waveforms and an indicator of epileptic seizures. MUA is electrophysiological marker that demonstrates activity of a neuronal population. The change in MUA counts indicates underlying alteration in electrophysiological, biochemical and behavioral parameters of neurons [35]. Sbt administration attenuated spikes and polyspikes waveform associated with epileptiform activity. Additionally, a corresponding decreased in MUA counts further confirmed reduced epileptiform discharges by sbt administration.

In PTE, it was reported that oxidative stress plays an important role in the onset and progression of seizures [36]. Oxidative stress triggered neuroinflammation and causes membrane lipid peroxidation (LP) that contributes to seizure generation [37]. In this study, we evaluated membrane lipid peroxidation in response to epilepsy and sbt administration. Our results demonstrate a significant increased in LP in the cortex and hippocampus of epileptic rats. Administration of sbt significantly decreased LP in cortex and hippocampus of epileptic rats.

Antioxidant enzyme like SOD and CAT function as detoxifying enzymes in a cellular system. In Haber-Weiss reaction, superoxide reacts with H_2O_2 and produces toxic hydroxyl radicals [38]. SOD is crucial to remove O^{2-} and CAT breaks down H_2O_2. Their main role is to protect cellular system from oxidative stress.

In present study, we observed disrupt enzymatic activity of SOD and CAT in cortex and hippocampus of epileptic rats. Administration of sbt significantly enhanced SOD and CAT in cortex and hippocampus of epileptic rats. These results suggest that bioactive compounds of sbt probably have a summation effect and contribute largely towards its antioxidative potential. Our results are consistent with previous study from our laboratory, where administration of dehydroepiandrosterone (DEHA) significantly increases SOD and CAT in epileptic rats [39]. It was reported that polyphenol rich foods provide antioxidative effects and neuroprotection against neuronal disorders [40].

Further, it was reported that Na^+,K^+ATPase activity in brain is inhibit by free radicals even at low concentrations [41,42]. ROS mediated oxidation of sulfhydryl groups inhibit activity of Na^+,K^+ATPase [43]. Studies showed that membrane structural integrity and lipid composition are vital for enzyme activity. An alteration in membrane fluidity and neuronal excitability is observed in PTE [6]. Fenproporex was also reported to decrease Na^+,K^+ATPase activity [44]. Furthermore, a decrease in Na^+,K^+ATPase activity was reported to be associated with hyperexcitability and epileptogenesis [45]. Malfunctioning of Na^+,K^+ATPase activity is associated with neuronal hyperexcitability [43]. In this study, decrease in Na^+,K^+ATPase activity was observed in epileptic rats as compared to control rats.

Our results demonstrate normal activity of Na^+,K^+ATPase in cortex and hippocampus of sbt administered epileptic rats that is because of the protective nature of sbt against oxidative stress.

Voltage-gated sodium channel (VGSCs) are expressed ubiquitously in central nervous system and are crucially important for regulating neuronal excitability. Epilepsy-associated mutations are identified in genes that encode for VGSCs [46]. Mutations affect channel biophysics and contribute to network excitability. Loss of function mutation in $Na_v1.1$ was correlates to hyperexcitability and epilepsy [47]. Increase expression of $Na_v1.6$ modulates glial-neuronal communication to release excessive glutamate and contribute towards generation of epileptic seizures [48]. Our results reported differential expression of $Na_v1.1$ and $Na_v1.6$ in cortex and hippocampus of epileptic rats and sbt administered epileptic rats. Further, it confirms that high expression of $Na_v1.1$ and $Na_v1.6$ was correlated with hyperexcitability and epileptiform activity. These results are consistent with previous study from our laboratory where the expression of $Na_v1.1$ and $Na_v1.6$ was reported to be higher in epileptic rats [11]. Administration of sbt restores the normal expression of both $Na_v1.1$ and $Na_v1.6$ in cortex and hippocampus that correlate with attenuate epileptiform activity in epileptic rats.

In conclusion, our results suggest that sbt administration improve electrophysiological alterations, reduce oxidative stress, and enhance antioxidant defense in cortex and hippocampus of epileptic rats there by restoring the brain biochemical parameters. Moreover, sbt also restore Na^+,K^+ATPase activity, and regulate the expression of sodium channel $Na_v1.1$ and $Na_v1.6$. Therefore, we propose pharmacotherapeutic potential of sbt in healing post-traumatic epilepsy.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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