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The interplay between aging, epilepsy, and memory

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Abstract - *Age, epilepsy, and memory have complicated relationships that vary from person to person. Brain disorders such as epilepsy are characterized by frequent, spontaneously recurring seizures. Alternatively, aging is a normal and intricate process that leads to changes in the brain structure and function. Memory can be affected by seizures, and this effect may be more pronounced in people with epilepsy. The degree of memory impairment can vary depending on the type and frequency of seizures, and specific brain regions. Specific memory functions, such as working memory and episodic memory, may deteriorate with age and in epilepsy, which may be evident in electrophysiology and behavioral analysis. Neural connections and the volume of certain brain regions decrease with age, among other brain structural changes that can be detected in immunohistochemistry, histology, biochemical, and molecular studies. It is crucial in improving cognitive defects in aging and epilepsy, especially in old and epileptic animals. Thus, controlling seizure activity in older epileptic animals is essential in improving cognitive function. Cognitive improvement is possible through the use of bioactive compounds, which are often administered to treat seizures. Therefore, investigating the pharmacotherapeutic potential of Hippophae rhamnoides (Seabuckthorn/ Sbt) on cognitive functions, aging, and seizure activity is imperative.*

Keywords: *Aging, epilepsy, memory, seabuckthorn*

I. INTRODUCTION

In recent years, the spotlight has increasingly turned towards natural remedies in the pursuit of holistic health and wellness. Among these, *Hippophae rhamnoides* (Seabuckthorn/ Sbt) has emerged as a botanical marvel that offers several benefits such as antiseizure properties, anti-aging effects, and memory enhancement abilities.

Epilepsy, a neurological disorder characterized by recurrent seizures, represents a significant medical challenge worldwide [1]. Emerging research suggests that Sbt may provide a natural solution with its rich cocktail of bioactive compounds including flavonoids and antioxidants [2]. These compounds are thought to modulate neuronal activity and potentially reduce the frequency and severity of seizures [3]. Furthermore, the anti-inflammatory properties of Sbt may play a role in alleviating the neuroinflammation associated with epilepsy, providing hope for improved treatment of this condition [4].

Although aging is accompanied by a myriad of physiological changes, including oxidative stress, inflammation, and a decline in cognitive function [5]. Sbt, with its potent antioxidant content, has garnered attention for its ability to combat these age-related processes. By scavenging free radicals and strengthening the body's defense mechanisms [6], Sbt may help ward off the signs of aging and promote skin health, cardiovascular function, and

overall vitality [7]. Its rejuvenating effects extend beyond the surface, potentially enhancing cellular resilience and longevity from within [8].

As we navigate the complexities of modern life, cognitive function, and memory retention are of paramount importance. Sbt shows promise as a natural memory stimulant with its unique blend of nutrients including omega fatty acids, vitamins, and flavonoids [9]. These compounds are believed to support neuronal health and synaptic plasticity, facilitating efficient neurotransmission and cognitive performance [10]. Whether consumed as a dietary supplement or incorporated into culinary creations, Sbt may provide a flavorful path to sharper focus and improve memory [10].

Intriguingly, the multifaceted benefits of Sbt are deeply rooted in scientific research and centuries-old traditional practices [11]. As ongoing research sheds light on its mechanisms of action and therapeutic potential, Sbt stands poised to revolutionize our approach to seizure management, antiaging interventions, and cognitive enhancement. In our pursuit of optimal health and well-being, it is essential to harness nature's bounty and explore the intersection between tradition and innovation.

II. METHODOLOGY

Materials **-** All chemicals used were of analytical grade and provided by Sigma Aldrich (USA). Male Wistar rats aged 18-20 months were used for the experiment. The animals were provided by Central Laboratory Animal Resources (CLAR), Jawaharlal Nehru University (JNU), New Delhi, India. The JNU, Institutional Animal Ethical Committee (IAEC) approved the experimental protocols (with IAEC code 29/2014). Animal experiments were carried out following the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CPCSEA).

Surgery and electrophysiology **-** In the current study. two brain regions were examined, namely the cortex and hippocampus. To measure the local field potential of the brain from the cerebral cortex and hippocampus of all the animal groups, stereotaxic surgery was performed to implant brain electrodes. The surgical, perioperative care and electrophysiology protocols were followed according to Ladol and Sharma, 2021b [12]. Sbt was administered to rats at a dose of 1 ml/kg body weight after iron injection.

Na^+K^+ATPase activity **-** The crude synaptosomal fraction was used to estimate the Na^+K^+ATPase activity. Activity was determined following the protocol of Beltowski et al., (2003) [13]. A reaction mixture comprising of NaCl (100mM), KCl (20mM), ATP (3mM), $MgCl_2$ (5mM), and Tris (50mM, pH 7.4) to which protein (50µg) was added and then kept for 30 min at 37℃ in a water bath. Later, the reaction was stopped with 1 ml of chilled TCA solution (10%). 1 ml of supernatant was collected after centrifugation (4000 rpm, 5 min). The supernatant was added to a reaction mixture comprising 8.1ml dH_2O and 0.5ml of acid ammonium molybdate (0.5%) in H_2SO_4 (5N) and then incubated at 25℃ for 10 minutes. A reducing agent (0.4ml) containing sodium bisulfite (15%), aminonaphtholsulfonic acid (0.5g), and sodium sulfite (20%) was then added. A specific ouabain blocker (1mM) was used to prevent the Na^+K^+ATPase activity. The absorbance at 660nm was then measured using a spectrophotometer. ATP was used as substrate and the released inorganic phosphate was determined in a spectrophotometer. Na^+K^+ATPase activity (ouabain-sensitive) was measured and the activity was presented in nm of inorganic phosphate released/min/mg protein [14].

Spatial learning and memory test **-** The MWM test was conducted to assess cue-based spatial learning and memory in animals. It was carried out following Morris's (1984) protocol [15] with minor modifications [16]. The test was performed in a round tank half filled with water and marked into four equal quadrants with distal cues of different sizes, shapes, and colors. A submerged escape platform was placed in one of the four quadrants, obscuring the background. Each rat was placed in one of the quadrants of the tank facing the wall and allowed to explore for 60 seconds. The latency to find the hidden platform was recorded. Five trials per rat per day were carried out on five consecutive days. The test was videotaped using a web camera. The behavioral experiment was conducted between 10:30 a.m. and 1:30 p.m. to eliminate performance fluctuations due to animals' circadian rhythms [12].

Tissue preparation for histopathology and immunohistochemistry (IHC) **-** Rats were anesthetized and perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde overnight. The brains were then equilibrated in a 10% to 30% sucrose gradient and then stored in a 30% sucrose solution. The coronal sections of the brain tissue (15µm) were cut using a cryostat (Leica CM 1860, Germany). The brain sections from level 4 were collected and 35 parallel tissue sections were

obtained per animal. Sections were then mounted on gelatin-coated slides for further analysis.

For histopathological analysis, cresyl violet (CV) staining was performed in the brain tissue sections. Sections were air-dried for 30 minutes and rehydrated through a series of alcohol gradients. The sections were then kept in CV stain. It is then dehydrated through a series of alcohol gradients and cleared by immersion in xylene for 5 minutes. The sections were then mounted with DPX, analyzed, and photographed under an optical microscope (Motic Instruments Co. Ltd., Chengdu, China).

For IHC, tissue sections were air-dried for 1 hour at room temperature, and then washed with phosphate buffer saline (PBS). The sections were then treated with Triton X-100 (1%) and immersed in H_2O_2 (1%) for 10 minutes. Sections were then incubated with NGS (3%) for 35 minutes and incubated in rabbit polyclonal primary antibodies of GFAP (1:800) overnight at 4℃. The sections were then washed three times in PBS for 5 minutes each. The tissue sections were then incubated with HRP-labelled secondary antibody (1:200) for 90 minutes at 25℃. Then rinsed three times in PBS for 5 minutes each and immersed in DAB and H_2O_2 solution (0.25%) in PBS for 10 minutes. DPX medium was used to mount glass slides with a coverslip, and images were captured with an optical microscope (Nikon Eclipse Ti, Tokyo, Japan).

Statistical analysis - Data are expressed as mean ± SD. The mean value of Na^+K^+ATPase activity was determined in different groups. The MWM data were averaged across five trials/day/animal in all groups. A daily average was treated and evaluated as an individual measure. Blind analysis was used to examine the CV and GFAP-stained photomicrograph data. The one-way analysis of variance (ANOVA) and the Holm-Sidak post hoc test compare the results statistically. Assessments were performed between control and epileptic rats and between the epileptic and epi+Sbt-treated rats. Sigma Plot software version 12.0 was used for statistical analysis and probabilities of < 0.05 were set as significant.

III. RESULTS

The complete experimental design and timeline of the study are explained in Fig. 1.

The effect of Sbt on seizure activity - In the present study, to diagnose epileptiform activity, synchronized video EEG was recorded from the rat's brain to identify the seizure and correlate it with behavioral manifestations. The epileptiform activity was identified based on the waveform morphology, seizure discharge rate, and behavioral changes. Observation of electrobehavioral indices helps confirm epileptiform activity. Normal brain waves consisting of synchronized low-voltage patterns were generally observed in the control and saline-injected control group, as shown in Fig. 2. This recording shows no epileptiform spike activity. Epileptiform activity was observed in the EEG stretches of the cortex and hippocampus of the iron-injected rat group (Fig. 2). A relationship between electrographic alterations and behavioral changes validated that the experimental technique induced epilepsy.

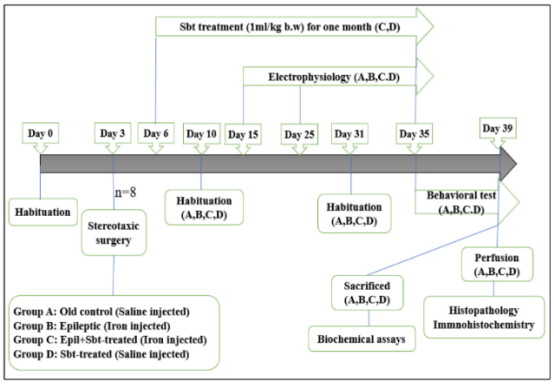

Fig. 1: Experimental design and timeline.

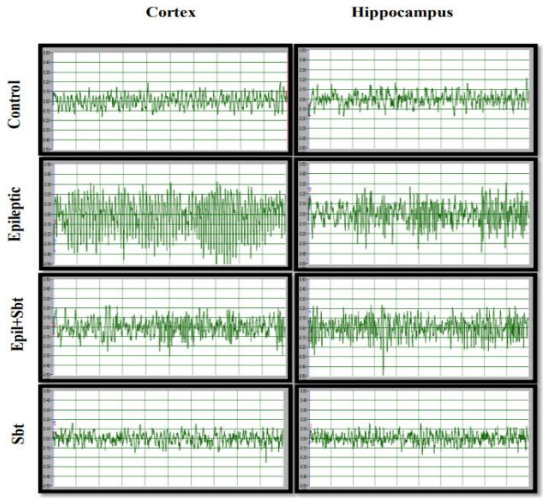

Fig. 2: Representative 20 seconds of EEG stretch from the cortex and hippocampus of a rat's brain. A) Old control B) Epileptic C) Epil+Sbt-treated and D) Sbt-treated groups (on their 30th day of Sbt treatment). Spike wave complex and sharp waves represent seizure activity.

Sbt treatment reduced the seizure frequency in the epileptic rats. Nonetheless, there were no significant differences between the Sbt-treated control and the control. Sbt treatment reduced the occurrence of seizure activity in epileptic rats. When Sbt was administered to the epileptic rats, there was a significant reduction in the seizure severity,

suggesting the possible seizure attenuation property of Sbt.

The effect of Sbt on Na^+K^+ATPase activity - Na^+K^+ATPase activity in the cortex and hippocampus was reduced by 57.8% and 50.5% respectively in the epileptic rats compared to the control rats. When Sbt was administered to epileptic rats, Na^+K^+ATPase activity increased by 76.4% in the cortex and 40.5% in the hippocampus compared to epileptic rats (Fig. 3), indicating a beneficial effect of Sbt on seizure activity.

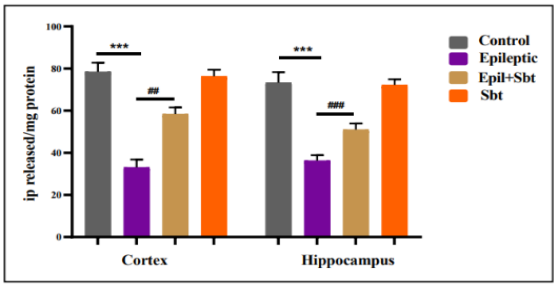

Fig. 3: The bar diagram represents Na^+K^+ATPase activity in the cortex and hippocampus of old control, epileptic, epil+Sbt-treated, and Sbt-treated rats groups. Data expressed as mean ± SD. ***p ≤ 0.001represents a significant difference in old control vs. epileptic rats; ###p ≤ 0.001, ##p ≤ 0.01 represent a significant difference in epileptic vs. epil+Sbt-treated rats.

The effect of Sbt on spatial learning and memory - Impairment in learning and memory was observed in the epileptic rats, as evidenced by a longer escape latency of 170% (on day 5) compared to the control. Old epileptic rats treated with Sbt showed a 40.7% reduced escape latency (at day 5) as compared to the epileptic control group (Fig. 4), suggesting a beneficial effect of Sbt on learning and memory. No significant changes were observed between old Sbt-treated control and control rats. Daily spatial learning and memory assessments were also assessed for percent change in escape latency.

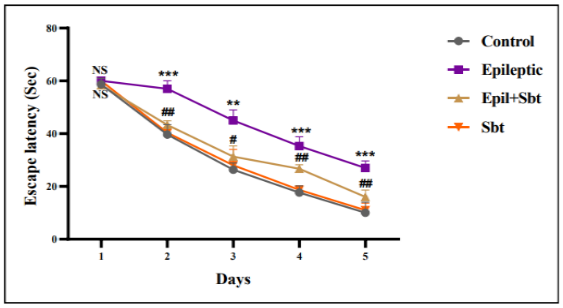

Fig. 4: Line plot showing the escape latency in old control, epileptic, epil+Sbt-treated, and Sbt-treated rats groups. Data expressed as mean ± SD. ***p ≤ 0.001, **p ≤ 0.01 represent a significant difference in old control vs. epileptic rats; ##p ≤ 0.01, # p ≤ 0.05 represent a significant difference in epileptic vs. epil+Sbt-treated rats.

The effect of Sbt on histopathology - Impaired neuronal morphology was observed in the brain tissue of CV-stained epileptic rats. The irregular shape and size of the neurons confirm the neuronal damage in the epileptic brain tissue sections. Improved neural structure was observed in Sbt-treated old epileptic rats, as shown by the improved neuronal morphology (Fig. 5) with fewer pyknotic neurons. The present results suggest the potential neuroprotective ability of Sbt. Nevertheless, no significant differences were observed between the Sbt-treated and the control rats.

The effect of Sbt on protein expression of GFAP - GFAP protein expression in the coronal sections of the cortex and hippocampus of control, epileptic, Sbt-treated epileptic, and Sbt-treated control rats was examined. Cortex and hippocampal sections showed characteristic changes in GFAP labeling in reactive astrocytes. The results showed that GFAP was increased in the epileptic rats compared to the control rats (Fig. 6). Administration of Sbt to epileptic rats decreased GFAP protein expression in both the cortex and the hippocampus suggesting a possible anti-inflammatory effect of Sbt.

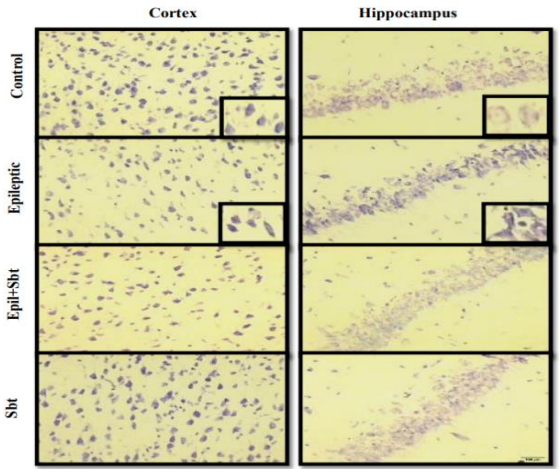

Fig. 5: Photomicrographs representing the coronal sections (CV-stained) from the cortex and hippocampus regions of old control, epileptic, epil+Sbt-treated, and Sbt-treated rats. Inset showing normal neurons in old control and shrunken neurons

in epileptic brain tissue sections (Scale bar: 10 µm).

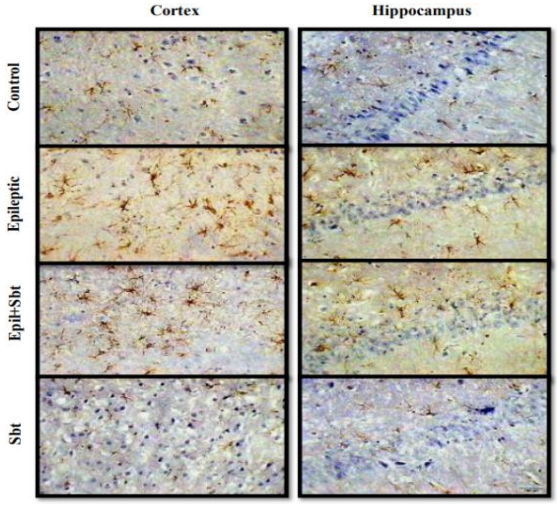

Fig. 6: Photomicrographs representing protein expression of GFAP in the coronal sections of the cortex and hippocampus of old control, epileptic, epil+Sbt-treated, and Sbt-treated rats. Cortex and hippocampus sections showing characteristic changes of GFAP labeling in reactive astrocytes (Scale bar: 10 µm)

IV. DISCUSSION

To investigate the effect of Sbt on epilepsy, local field potential from the cerebral cortex and hippocampus of the rat brain were examined. Additionally, the timed video for behavioral correlates was recorded. Since, an exclusive EEG recording often leads to false-positive results when movement artifacts such as eating, drinking, and grooming often result in epileptiform-like activities [17]. The brain waves in control animals have a standard pattern, and there are no spikes discharges. Nonetheless, the brain waves in epileptic rats consist of transient sharp waves, poly-spikes, and bursts of spike-wave, which differ from standard brainwave patterns [18],[19]. The distinct epileptic discharges in rats generally last a few seconds and are often accompanied by a postictal inhibition lasting several minutes, after which a regular electrographic pattern resumes. These results are consistent with previous studies from our laboratory in which seizure activity was observed in iron-induced epilepsy [17]. The electrobehavioral indices are an essential tool for studying seizure-related disorders. Various behavioral characteristics can also be recorded during an epileptic seizure, such as frequent rearing with backward falling, face twitching, nodding of the head, and teeth chattering [17]. Administration of Sbt to epileptic rats is shown to reduce the epileptiform activity in the cortex and hippocampus. In addition, we also examined Na^+K^+ATPase activity in rats. Na^+K^+ATPase is a transmembrane protein and an intact membrane is required for its normal functioning. Na^+K^+ATPase therefore plays a crucial role in restoring membrane potential, its dysfunction is mainly related to epileptiform activities [20]. Decreased Na^+K^+ATPase activity was observed in epileptic rats compared to control rats. The results show the normal activity of Na^+K^+ATPase in the cortex and hippocampus of epileptic rats treated with Sbt, which is due to the ameliorative nature of Sbt against seizure activity. Furthermore, Na^+K^+ATPase activity in the brain has been reported to be reduced by low concentrations of free radicals [21]. The ROS-facilitated oxidation of sulfhydryl groups inhibits the Na^+K^+ATPase activity [22]. A change in membrane fluidity and neuronal excitability is reported in epilepsy [23]. Chronic stress and depression reduce Na^+K^+ATPase activity in the brain [24], followed by impairments in memory, learning, and exploration behavior [25]. Studies have shown that structural integrity and lipid composition of the membrane are crucial for enzyme activity [22]. In addition, reduced Na^+K^+ATPase activity has been reported to be related to learning and memory impairments, neuronal hyperexcitability, epileptogenesis, and even cell death [20]. In our study, we observed lower Na^+K^+ATPase activity in epileptic rats, while improved activity was observed in the cortical region and hippocampus of Sbt-treated epileptic rats.

Furthermore, the behavioral changes associated with epilepsy and the effect of Sbt treatment on epileptic rats were tested using Morri's water maze. In our study, an increased escape latency suggests reduced spatiotemporal learning and memory in epileptic rats. This is in line with previous studies showing learning and memory impairments in epilepsy [26]. However, reduced escape latency was observed in Sbt-treated rats, suggesting enhanced spatial learning and memory compared to epileptic rats. In learning and memory deficits, neuronal death has been observed in the CA1 region of the hippocampus [27]. These results imply the beneficial effect of Sbt, which may gradually contribute to memory retention. Kaempferol, a bioactive compound of Sbt, has been reported to be effective in preventing depression, mood swings, and cognitive disorders [28].

Many studies have shown that seizures trigger neurodegeneration and hippocampal sclerosis [29], as well as neuropathology [30]. Studies reported that epileptic seizures cause neuronal damage in the

cortex, amygdala, hippocampus, and thalamus [31]. In particular, the cerebral cortex and hippocampus are vulnerable to anatomical and physiological damage, such as neurodegeneration and neuroinflammation [32]. Therefore, the histological parameters were evaluated in the current study. In CV-stained brain sections, we observed deformed neurons which were illustrated by morphological aberrations in epileptic rats. While Sbt-treated epileptic rats showed slightly improved neuronal morphology in the tissue sections. CV selectively stained the brain tissue sections, providing further evidence of histological impairments, showing greater staining of Nissl granules in the cortical and the hippocampal CA1 regions of the control rats compared to the epileptic rats' brain sections [33]. Increased neuronal abnormalities were confirmed with histopathological changes in old epileptic rats. Moreover, quercetin a bioactive compound of Sbt has shown neuroprotective effects against colchicine-induced neuronal damage [34]. Therefore, Sbt may have protected the brain from neuronal damage caused by seizure activity.

In addition, the GFAP is an important marker of reactive astrocyte activity and is mainly distributed in the central nervous system [35]. Astrocytes serve as a second line of defense in the brain and therefore play a crucial role in the normal function of neurons. Moreover, astrocyte activation is closely linked to cell progression and inflammation [35]. In the present study, higher expression of GFAP was observed in epileptic rats as compared to Sbt-treated rats. The increasing levels of expression of GFAP indicate neuroinflammation in epileptic rats. Sbt treatment in epileptic rats decreased the GFAP expression in both the cortex and hippocampus of epileptic rats. Previous studies also reported an increase in GFAP expression associated with plaque formation and inflammatory reactions [36]. Moreover, studies have reported anti-inflammatory and immunomodulatory effects of Sbt [37]. Therefore, the attenuation of reactive astrogliosis by Sbt might have been attributed to the repair processes of epilepsy-induced neuronal death.

In summary, we evaluated the effects of Sbt on seizure activity in old epileptic rats in terms of all parameters confirming epileptiform activity. These results suggest that Sbt administration attenuated seizure activity in the cerebral cortex and hippocampus of epileptic rats, thereby restoring brain biochemical parameters. Sbt treatment helps restore membrane potential by regulating Na^+K^+ATPase activity, which is essential for normal brain function. Furthermore, the treatment counteracted neuronal impairments and neuroinflammation in epileptic rats, as evidenced by improved neuronal morphology in tissue sections. This further leads to improvement in learning and memory in old epileptic rats. The study suggested that Sbt modulates age-associated defects and regulates epileptic seizures, as evident from the various parameters studied. Sbt treatment improved neuroprotection and showed antiseizure effects. Moreover, the study indicated that the antiseizure effect of Sbt was also escorted by its Na^+K^+ATPase activity regulation. Therefore, Sbt may have a pharmacotherapeutic effect on both epilepsy and aging. However, further interventions are required to clearly understand the exact mechanism of action and metabolic pathways responsible for these responses.

DISCLOSURE

The authors declare no conflicts of interest.

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