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Protective effect of huperzine A on phenytoin induced cognition impairment: Behavioral and biochemical study

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Phenytoin, a drug of choice for Epilepsy is also known for its adverse effects. The most common adverse effect due to phenytoin is cognition impairment. Cognition impairment is a serious problem in society as it debars the person's social life. Thus to overcome such a problem demand for a solution arises. Huperzine, sesquiterpene alkaloids having immense neuroprotective properties. Thus in this study, it was aimed to evaluate the effectiveness of huperzine on Phenytoin- induced Cognition Impairment. The protective effect of huperzine on phenytoin-induced cognition impairment was evaluated in rats. The effect of Huperzine on phenytoin-induced cognitive impairment was evaluated by behavioral, biochemical, and histopathological studies. The co-administration of huperzine with phenytoin showed significant results. The treatment of Huperzine with phenytoin resulted in significant improvement in learning and memory. The oxidative stress induced by Phenytoin was reversed by huperzine. A significant decrease in cholinesterase activity was also observed. The histopathology showed damaged neuronal cells in periventricular regions and cortex due to phenytoin which was altered by Huperzine. Thus, the present study demonstrates the protective effect of huperzine on phenytoin-induced cognition impairment.

Keywords: Acetylcholinesterase (AChE), Butyrylcholinesterase (BChE), Cerebrum, Neurprotection, Nootropic

Phenytoin is the most commonly used antiepileptic drug for decades. It is found effective against almost all types of epilepsies. Apart from the promising effect of Phenytoin on epilepsy, it produces some serious side effects including Cognition impairment, Impaired memory, Nystagmus, Dysarthria, Pruritis, Paresthesia, *etc*[1]. It is noteworthy that oxidative stress is mainly responsible for the development and pathogenesis of epilepsy, which further leads to cognitive impairment in Patients on treatment with Phenytoin[2,3]. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) are the chemicals that are involved in oxidative processes and are important for the functioning of the hippocampus and Cortex entorhinalis. It has been reported that Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) play a key role in cognitive functions and the brain cholinergic system[4]. The learning neurotransmitter *i.e*. Acetylcholine (Ach) and butyrylcholine(Bch) are involved in a variety of functions including muscle contractions *etc*. The inhibitors of cholinesterase are involved in increasing the level of Acetylcholine (Ach) and butyrylcholine (Bch) by inhibiting the hydrolysis of their enzymes at their neuronal Synaptic Cleft. This results in improved neuronal transmission[5]. The established Nootropic agents like physostigmine, galantamine, donepezil, and tacrine have shown a similar potential of cholinesterase inhibition[6]. One such drug that has been found to be active in nootropic activities is huperzine. Huperzine is a plant based alkaloid obtained from the herbs of *Huperzia serrata* of *Huperziceae* family[7]. It is an established Nootropic agent which has been used for the treatment of HupA has been used for centuries to treat fever, inflammation, blood disorders, and schizophrenia, *etc*. It has also been reported for its highly potent and specific inhibitory activity of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) across blood–brain barrier[8,9]. This results in increased neuronal activity across the synaptic cleft. In order to explore the effectiveness of huperzine in Thus in this study, the possibility of huperzine to combat the Present study aims for the assessment of Protective Effect Huperzine on Phenytoin induced Cognition Impairment.

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Materials & Methods

Drugs and chemicals

The Drugs used *i.e*. Galantamine and Huperzine were purchased from Sigma Aldrich Chemical Company USA. The chemicals used *i.e*. (DTNB), and reduced glutathione (GSH) were purchased from Sigma Chemical Company USA. The other reagents used were of analytical grade and were obtained from CDH, India.

Animals

Adult albino rats of either sex weighing 100-200 g were maintained at RT (25 ± 3°C) and were fed with a rodent lab diet and tap water *ad libitum*. The protocol for the study was approved by the Institutional Animal Ethical Committee of College of Veterinary Science and Animal Husbandry, Jabalpur Ref. No.2071/Re/S/19/CPCSEA

Study protocol

The rats were divided into four groups having six animals in each group. The first group was controlled and was treated with Distilled water for 21 days (orally). The Second group was Toxic Control and was Phenytoin Treated (dose) 20 mg/Kg phenytoin dissolved in 0.2% CMC (*p.o*) for 21 days (*i.v*). The third group was Nootropic Control and was treated with (dose) Galantamine 20 mg/Kg phenytoin dissolved in 0.2% CMC for 21 days (*i.v*). Phenytoin + Galantamine Treated. The next group was the test group *i.e.* Phenytoin with Huperzine Treated (*i.v*) (Table 1).

Evaluation of behavioral parameters

The behavioral parameters were studied after 2 hrs of drug administration. Memory, passive avoidance, motor coordination, locomotor activity, and exploratory behavior were assessed on 0 days, 14th, and 21st day. On 21th day the behavioral studies were carried out 3 h after drug administration.

Elevated plus maze test

Elevated plus maze test is used to determine the acquisition and retention of memory. This is an apparatus that consists of 4 arms 2 open and 2 closed having a quadrangular point. The rat is placed at an open arm away from the central platform. The time taken by the animal to explore and move from the open to the closed arms is noted. This is the initial transfer latency. Transfer latency is defined as the time taken by the animal to move from the open arm to the closed arm. If the animal did not show a response to move towards the closed arm it is gently taken back and the transfer latency is noted as 60 sec[10].

Table 1 — Study protocol

Group	Treatment
Group I	Normal saline (2 mL/kg)
Group II	Phenytoin (20 mg/kg)
Group III	Galantamine (4mg/kg)
Group IV	Phenytoin (20 mg/kg) and Galantamine (4mg/kg)
Group V	Phenytoin (20 mg/kg) and Huperzine A (5 mg/kg)

One trail passive avoidance

Memory retention is evaluated with a step-through passive avoidance apparatus (Ugo Basile, Italy). It consisted of two separate chambers connected by a guillotine door. One chamber is lit using a bulb, and the other was dark. The floor in both chambers consisted of steel grids used to deliver electric shocks. On the acquisition trial, each rat is placed in the lighted chamber. After 30 sec of habituation, the guillotine door separating the light and dark chambers is opened, and the initial latency to enter the dark chamber is recorded. Immediately after the rat entered the dark chamber, the guillotine door is closed and an electric foot shock (75 V, 0.2 mA, 50 Hz) is delivered to the floor grids for 0.2 sec. The rat was removed from the dark chamber 5 sec later and returned to its home cage. Twenty-four hours after seizure induction, retention latency is measured in the same way as in the acquisition trial, but the foot shock is not delivered, and the latency is recorded up to a maximum of 600 sec[11].

Test for alertness (exploratory behavior)

0.5 m3 wooden board with 16 holes (3 cm in diameter) is employed for the study. Each rat is placed individually on the board for 6 min. In the first 2 min, the animal is allowed for acclimatization and then the number of head dipping performed in the next 4 min is noted for each animal[12].

Motor coordination test

Motor coordination test is conducted in rats using a Rota- Rod apparatus (Inco-Ambala, India). The animals are screened for motor co-ordination and the animals which stayed on the rotating rod without falling for 120 sec are chosen for the study. Each animal is placed on the Rotarod and the time taken by the animal to fall is noted[13].

Test for locomotor activity

Spontaneous motor activity is monitored using Actophotometer. Each animal is subjected to an adaptation period of 2-5 min after which their

locomotor activity is assessed for 5 min. An increase in the count is regarded as CNS stimulant activity. A decrease in the count is considered CNS depressant activity[14].

Biochemical Analysis

Biochemical study

Tissue preparation

The whole-brain of each rat is dissected out and divided into two cerebral hemispheres for biochemical estimations. From one-half, 10% (w/v) homogenate is prepared with ice-cold 0.1M phosphate buffer (pH 7.4), and lipid peroxidation product and reduced glutathione are assessed. With the other half, 10% (*w/v*) homogenate was prepared with 0.1 M Tris-HCl buffer containing 1% Triton-X and was used to assess cholinesterase activity[15].

Brain lipid peroxidation

The extent of lipid peroxidation in tissues is assessed by measuring the level of malondialdehyde (MDA) according to the method of Ohkawa, et al. Briefly, 1 mL (10%) tissue homogenate was added to the reaction mixture containing 1 mL of trichloroacetic acid (15%) and 2 mL of thiobarbituric acid (0.38%). The reaction mixture is heated for 60 min at 90°C, cooled, and centrifuged at 6900 rpm for 15 min. The absorbance of the supernatant is measured at 532 nm against the blank, which contained all reagents except homogenate. MDA is quantified and expressed as μmol of MDA per mg of wet tissue[16].

Brain reduced glutathione

Glutathione (GSH) is measured according to the method of Ellman. The homogenate is mixed with an equal quantity of 10% trichloroacetic acid and centrifuged to separate the proteins. Two milliliters of 0.3 M phosphate buffer (pH 8.4), 0.5 mL of DTNB, and 0.4 mL of double-distilled water are added to 100 μL of supernatant thus obtained. A parallel standard GSH was run to determine the concentration of GSH in test samples. Absorbance was read in a spectrophotometer (Analytik Jena, Germany) at 412 nm within 15 min. The concentration of reduced glutathione is expressed as micrograms per gram of wet tissue[17].

Brain cholinergic status

The cholinergic markers AChE and BuChE are estimated in the rat brain according to the method of Ellman. The assay is a spectrophotometric method that involves two linked reactions to produce a colored compound. Briefly, 10 mM DTNB in 0.1 M Tris–HCl buffer (pH 8.0), 100 μL of supernatant, and 30 mMacetylthiocholine iodide as substrate were added for AChE estimation. For BChE estimation, 10 mM DTNB in 0.1 M Tris-HCl buffer (pH 8.0), 300 μL of supernatant, and 7.5 mMbutyrylthiocholine iodide as substrate were added. Absorbance was measured at 412 nm for 3 min at 30 sec intervals using a spectrophotometer. AChE activity is expressed as micromoles of acetylthiocholine iodide hydrolyzed per milligram of protein per minute, and BChE activity is expressed as micromoles of butyrylthiocholine iodide hydrolyzed per milligram of protein per minute. One unit is defined as 1 mole of acetylthiocholine hydrolyzed per minute per milligram of protein[18].

Assessment of oxidative stress

The brain samples are quickly removed, cleaned with chilled saline, dissected into the cortex, midbrain, medulla, pons, and cerebellum were stored at −40°C[19].

Estimation of acetylcholine (ACH) content

The content of acetylcholine is estimated by the method of Metcalf (1951) as given by Augustins on (1957). The different regions of the brain are quickly frozen in liquid nitrogen, weighed accurately, and placed in pyrex glass tubes. These tubes are placed in boiling water for 5 min to terminate the AChE enzyme activity, and also to release the bound ACh. The tissues are then homogenized in 1 mL of distilled water. To the homogenate, 1 mL of alkaline hydroxylamine hydrochloride followed by 1 mL of 50% hydrochloric acid solution (l:l:HCl: H, O) are added. The contents are mixed thoroughly and centrifuged. To the supernatant 0.5 mL of 0.37 M, ferric chloride solution is added and the brown color developed is read at 540 nm against a reagent blank in a spectrophotometer. The acetylcholine content is expressed as p moles of AChIg wet wt. of tissue[20].

Histopathological Study

Histopathological Investigation on brain tissue: Brain tissues are dissected out carefully and were kept in 10% formalin solution prepared with normal saline. Tissues were stained using Hematoxylin and Eosin stain[21].

Results

Behavioral analysis

Elevated plus maze test

There was a nominal significant change in transfer latency among the groups whereas, one-way ANOVA

revealed a significant difference in retention transfer latency. The retention transfer latency increased (P> 0.0001) in phenytoin treated group. The co-administration of phenytoin with huperzine decreased the retention transfer latency. The values decreased in phenytoin-treated groups when compared with phenytoin and huperzine-treated groups on day 14. The change in retention transfer latency at day 21 was noticeable in phenytoin and huperzine treated groups. The transfer latency in Nootropic control *i.e*. galantamine treated was close to the control group in all treatments. Promising results could be witnessed while comparing the Nootropic control galantamine with phenytoin treated, phenytoin and huperzine treated group. It could be seen that huperzine produced an effective change in the phenytoin induced, memory impairment which was in a dose-dependent manner. The values did not reach the normal control but were close to control (Fig 1).

One trail passive avoidance

There was a nominal significant change in transfer latency among the groups at 0 days of the study. Further with the increasing study protocol till day 21 no such significant change in the Initial transfer latency was witnessed in comparison to the control groups (Fig 2). The retention transfer latency decreased from 3.16 ± 0.31 to 2.5 ± 0.2 (21 day) (P> 0.0001) in phenytoin treated group. The co-administration of phenytoin with huperzine decreased the retention transfer latency. The values decreased in phenytoin treated, phenytoin and huperzine treated groups, respectively, at day 14. The change in retention transfer latency at day 21 was noticeable in all groups. The transfer latency in nootropic control *i.e*. galantamine treated was close to the control group in all treatments. It could be seen that Huperzine produced an effective change in the Phenytoin induced, memory impairment which was in a dose-dependent manner. The values did not reach the normal control but were close to control.

Test for alertness (exploratory behavior)

No noticeable change in the exploratory behavior of the animals was seen in control phenytoin treated, galantamine treated, phenytoin and galantamine treated, phenytoin and huperzine treated groups at day 0 of the study. The exploratory behavior was determined by the number of head dippings in the hole apparatus (Fig. 3). The number of head dipping in the phenytoin-treated groups decreased from 4.1 ± 0.41 at the day to 1.16 ± 0.1 at day 21 (P> 0.0001). The co-administration of the Nootropic agent huperzine with phenytoin increased the number of head dipping 2.5 ± 0.1 in phenytoin treated to 4.83 ± 0.4 and phenytoin and huperzine treated groups at day14 of the study. The increase in the

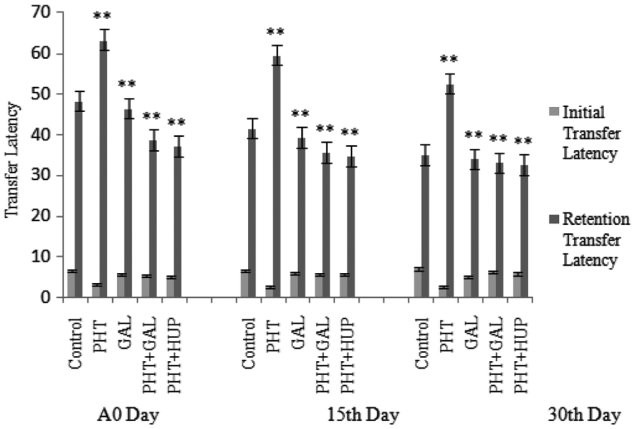

Fig. 2 — Effect of Huperzine on phenytoin passive avoidance (Initial and Retention Transfer Latency). Values are expressed as mean± SEM of 6 animals. ** (P< 0.01) *vs* Phenytoin group

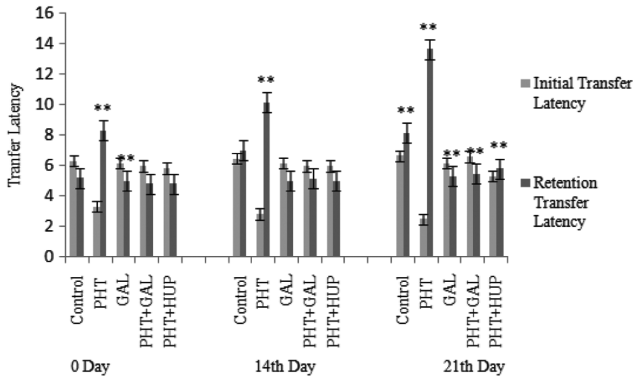

Fig. 1 — Effect of Huperzine on phenytoin induced memory impairment (Transfer Latency). Values are expressed as mean ± SEM of 6 animals. ** (P< 0.01) *vs* Phenytoin group

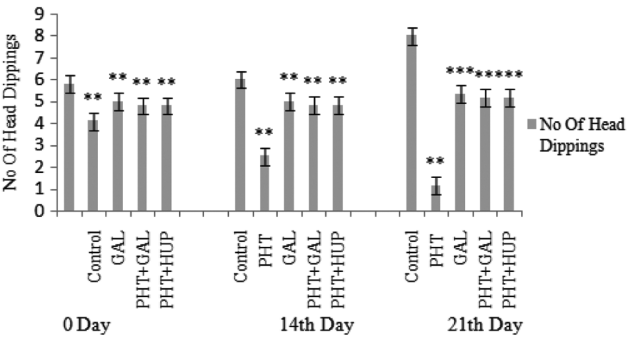

Fig. 3 Effect of Huperzine on phenytoin impaired exploratory behavior. Values are expressed as mean± SEM of 6 animals. ** (P< 0.01), *** (P< 0.001) *vs* Phenytoin group.

number of head dippings at day 21 was from 1.16 ± 0.1 to 5.16 ± 0.5 in phenytoin treated, phenytoin and huperzine treated groups, respectively. The number of head dippings in Nootropic control *i.e.* galantamine treated was close to the control group in all treatments. Effective results could be seen with comparison to galantamine with phenytoin treated, phenytoin and huperzine treated groups.

Motor coordination test

There was no effective change in the retention time of the animals at day 0 of the study. The Retention time in Nootropic control *i.e.* galantamine treated was close to the control group in all treatments. Striking results could be seen with comparison to galantamine with phenytoin treated, phenytoin and huperzine treated groups. It was noteworthy that phenytoin decreased the retention time at the Rotarod from 16.66 ± 1.1 at day 0 to 6.6 ± 0.6 at day 21. The co-administration of Huperzine with phenytoin increased the retention time from 10 ± 1.0 in Phenytoin treated to 13.16 ± 1.3 in Phenytoin and Huperzine treated groups at day 14 (P< 0.001). On day 21 there were a remarkable increase from 6.6 ± 0.6 in Phenytoin treated to 13.33 ± 1.3 in phenytoin and huperzine treated, Phenytoin and Huperzine groups, respectively, at day 21 (P< 0.001) (Fig. 4).

Test for locomotor activity

The impaired locomotor activity was assessed using Actophotometer. There was no such change in the locomotor activity in the groups at day 0 of the study. The Spontaneous Motor Activity in the phenytoin-treated group was decreased from 46.66 ± 4.6 at day 0 to 19.66 ±1.9 at day 21 (P< 0.001). The co-administration of huperzine with phenytoin increased the number of counts from 33.33 ± 3.3 in phenytoin treated to 47.5 ± 4.7 in phenytoin and huperzine treated at day 14 (P< 0.01). There was a noticeable increase On day 21 from 19.66 ±1.9 in phenytoin treated to 40.66 ± 4.0 in phenytoin and huperzine treated groups, respectively, at day 21 (P< 0.001) (Fig:5).

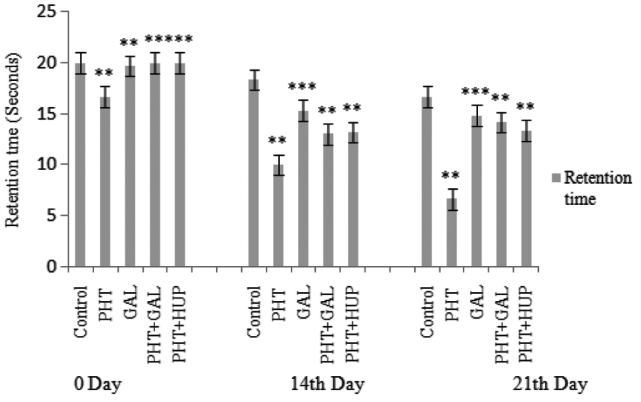

Fig. 4 — Effect of Huperzine on phenytoin-induced motor in-coordination. Values are expressed as mean± SEM of 6 animals. **(P< 0.01), ***(P< 0.001) *vs* Phenytoin group

Biochemical analysis

Brain lipid peroxidation

The level of lipid peroxidation was assessed by the level of Malondialdehyde (MDA) (mol/g wet tissue). The level of MDA was noticeably increased by phenytoin in the brain *i.e.* in Cortex Cerebellum, Medulla, Pons, and Midbrain. In the Cortex lipid peroxidation was increased by 16.66±1.6 mol/g wet tissue by phenytoin which was decreased up to 8.5 ± 0.8 mol/g wet tissue by co-administration of Phenytoin and Huperzine. In the Cerebellum lipid peroxidation was increased by 29.16 ± 1.7 mol/g wet tissues by phenytoin which was decreased up to 14.83 ± 1.4 mol/g wet tissue wet tissue by co-administration of Phenytoin and Huperzine. Similarly, in Medulla it was 30.1± 3.0 mol/g wet tissue in phenytoin treated groups which was decreased to 10.1 ± 1.1mol/g wet tissue in Huperzine co-administration with Phenytoin. In Pons, it was decreased to 12.66 ±1.2 mol/g wet tissue in Phenytoin and Huperzine. In Midbrain the level of MDA was decreased noticeably from 35± 3.2 mol/g wet tissue in phenytoin treatment to 11± 1.1 mol/g wet tissue in Phenytoin and Huperzine, Phenytoin (Fig. 6).

Brain reduced glutathione

Phenytoin drastically increased the level of Brain reduced glutathione in the Cortex, Cerebellum, Medulla, Pons, and Midbrain. Co-administration of

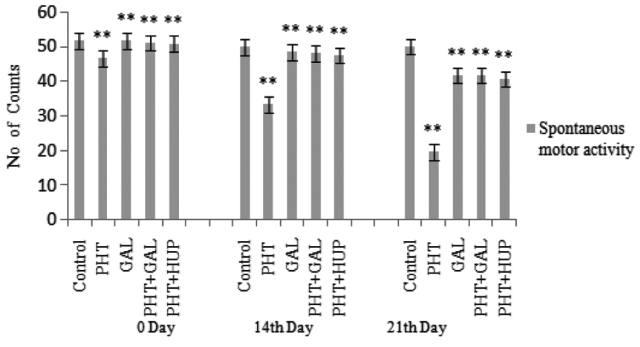

Fig. 5 — Effect of Huperzine on phenytoin induced impaired locomotor activity. Values are expressed as mean± SEM of 6 animals. **(P< 0.01) *vs* Phenytoin group

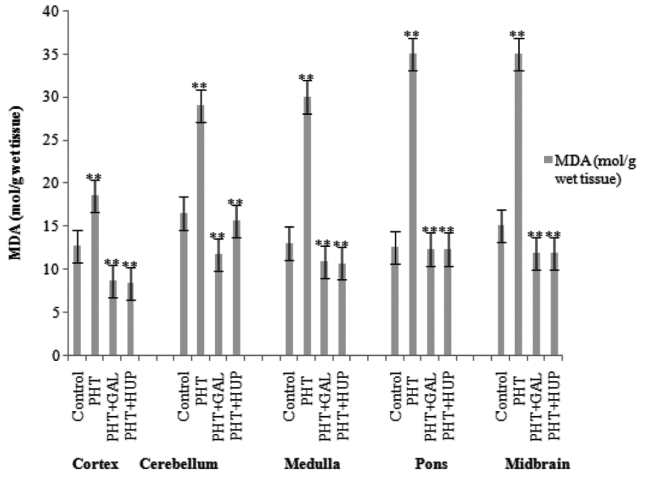

Fig. 6 — Effect of Huperzine on phenytoin-induced alterations in regional brain lipid peroxidation. Values are expressed as mean± SEM of 6 animals **(P< 0.001)

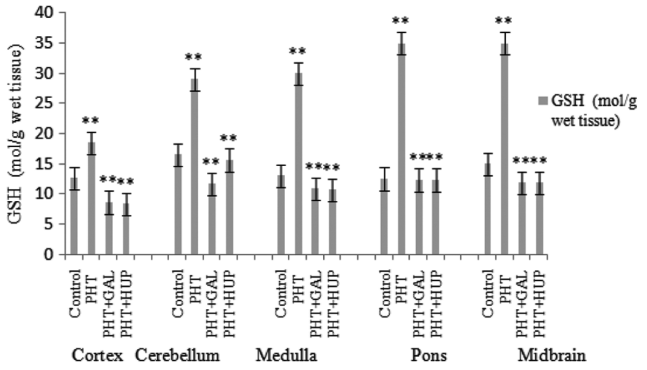

Fig. 7 — Effect of Huperzine on phenytoin- induced alterations in regional brain reduced glutathione. Values are expressed as mean± SEM of 6 animals ** (P< 0.01)

Huperzine with Phenytoin decreased the level of GSH in a dose-dependent manner. The values were somewhere close to the control group but did not reach the normal control (Fig 7).

Brain cholinergic status

Brain acetyl choline Activity was remarkably increased by Phenytoin to 1.121± 0.1, 1.129 ± 0.1, 1.112 ± 0.1, 1.109 ± 0.1, 1.114 ± 0.1 µmol/min/mg protein in Cortex, Cerebellum, Medulla, Pons and Midbrain, respectively. The Co administration of Huperzine with Phenytoin decreased the level of brain acetyl cholinesterase to 0.94 ± 0.09, 0.91 ± 0.09, 0.72 ± 0.06, 0.79 ±0.06, 0.49 ± 0.04 µmol/min/mg protein in Cortex, Cerebellum, Medulla, Pons and Midbrain, respectively, (Fig. 8).

Histopathological Study

The histopathology of brain cells showed damaged neuronal cells in periventricular regions and cortex.

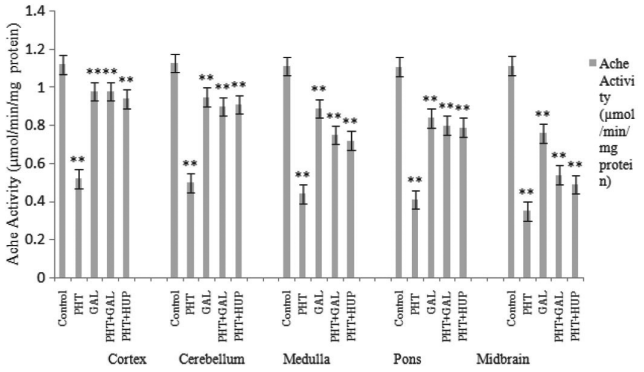

Fig. 8 — Effect of Huperzine on phenytoin-induced alterations in regional brain acetylcholinesterase activity. Values are expressed as mean ± SEM n= 6 animals. ** (P<0.001)

Further phenytoin-induced apoptosis and necrosis were seen in periventricular regions and cortex. Under the influence of Huperzine co-administration, the neuronal damage was minimized. The apoptosis and necrosis due to phenytoin were also reduced. Figure 9 illustrates the effect of phenytoin on the brain. The Control group showed normal brain architecture (Fig. 9A). Phenytoin treated group revealed severe necrosis in the cortex (Fig. 9B). The treatment of Nootropic agents with Phenytoin showed changes in the brain and a decrease in necrosis was seen. The decrease was most prominent in Phenytoin and Galantamine treated Group which was the Nootropic control (Fig. 9C). Phenytoin and Huperzine treated (Fig. 9D) had lower necrosis and some features of normal brain cortex were seen.

Discussion

In the present study, it was observed that Phenytoin demonstrated an adverse effect on the neurological status of animals. It was evident by alterations in motor coordination, cognition, exploratory behavior, spontaneous motor activity, and histopathological studies. Cognitive impairment is one of the serious and major side effects due to AEDs. Various Studies have shown impairment and memory loss due to phenytoin[22]. Our findings showed impaired memory and learning due to phenytoin. Phenytoin prolonged the transfer latency of animals in the elevated plus-maze and passive avoidance test which showed the risk of cognition impairment in healthy individuals[23]. The Co-administration of Phenytoin and Huperzine showed improved responses on learning and memory. The transfer latency of the animals was decreased in comparison to the phenytoin treated group. Phenytoin is known to affect exploration and cause sedation[24].

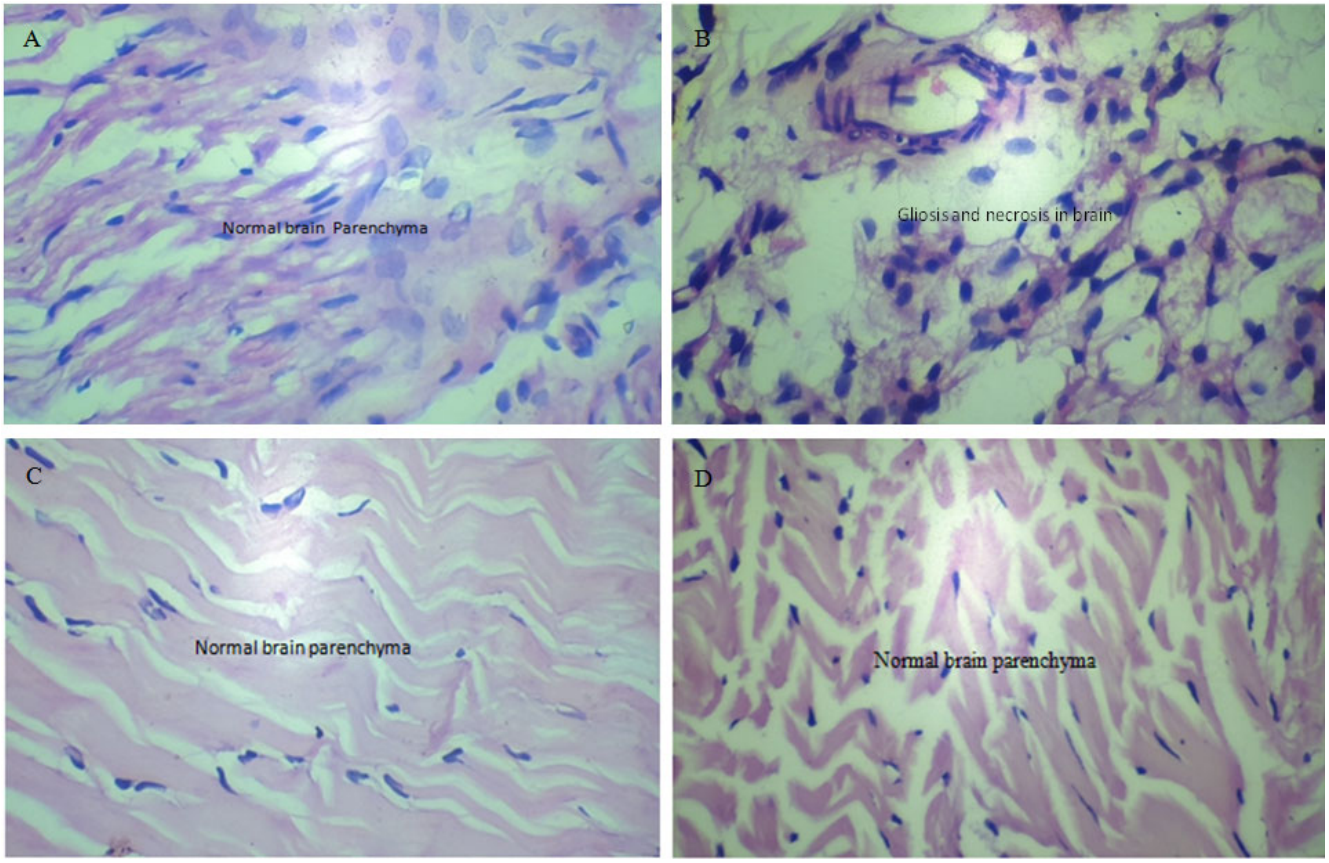

Figure 9 — (A) Normal Brain Cell; (B) Necrosis in Brain; (C) Brain cell phenytoin and galantamine treated group with less necrosis; and (D) Phenytoin and Huperzine treated with less necrosis

The number of head dippings was decreased which showed a decrease in exploratory behavior. Phenytoin when administered with huperzine showed a change in exploratory behavior and the animal showed alertness near to the control group. The impairment of performance when subject to the rotarod test showed weak muscular and motor coordination while the performance was significant in the huperzine with phenytoin treated group. There was a noticeable change in the spontaneous motor activity due to phenytoin which reflected its CNS depressant effect. Under the influence of huperzine, spontaneous motor activity was altered. The role of the cerebral cortex in long-term memory storage is well defined. It is majorly involved in high-level task control such as memory, consciousness, language, *etc*. under the influence of phenytoin the lipid peroxidation in brain regions increased in comparison to the huperzine with phenytoin treated groups. The increased lipid peroxidation in the brain causes injuries in the neuronal membranes which alters motor and neuronal functioning. The phenytoin-induced neuronal damage in the brain is responsible for various deficits such as memory impairment, sedation, ataxia, loss of exploratory behavior[25]. The brain cholinergic system is responsible for learning and memory which is regulated by Acetylcholine esterase (Ache). At the synaptic cleft of cholinergic synapse and neuromuscular junction's acetylcholinesterase (Ache) hydrolysis the neurotransmitter acetylcholine (Ach). The inhibitors of Ache enhance the availability of acetylcholine in synaptic cleft[26]. Various studies have provided evidence for the possible relationship between cognitive deficit and the brain cholinergic system. Although there are some other neurotransmitters involved in learning and memory the role of acetylcholine is vital[27]. Studies have shown the patients on phenytoin therapy have poor memory performance. It is also established that phenytoin decreases the brain acetylcholine level. Phenytoin-induced memory impairment is due to the enhanced acetylcholinesterase activity[28]. The role of huperzine in cognitive impairment has been studied[29] owing this fact it's the role in phenytoin induced cognition impairment was explored with promising results. In the present study, the effect of Huperzine on

Ache activity was promisable. The increase in the level of Ache activity due to the drug was decreased under the influence of Huperzine. The co-administration of phenytoin with Huperzine showed promising results and the reading were close to the normal control. Previous studies have established the fact that phenytoin at the therapeutic doses increased the Ache activity in brain regions in rats. It was believed that phenytoin *via* oxidative stress enhanced the AChE activity and thereby depleted the levels of ACh in brain regions resulting in subsequent memory impairment. Similarly, in the case of brain reduced glutathione, the levels were increased due to the influence of phenytoin which was decreased by Huperzine co-administration. The histopathological studies showed damaged neuronal cells in periventricular regions and cortex. Further, phenytoin-induced apoptosis and necrosis were seen in periventricular regions and cortex. Under the influence of Huperzine-A co-administration, the neuronal damage was minimized. The apoptosis and necrosis due to phenytoin were also reduced. Thus, in the present study, it could be concluded that the co-administration of Huperzine with phenytoin reduces the phenytoin induced cognition impairment.

Conclusion

The study concludes the protective effect of huperzine on phenytoin induced cognition impairment. The behavioural and biochemical parameters were analysed. The effectiveness of you huperzine on phenytoin induced cognition impairment was promising. Behaviourally, responses were altered. Significant responses were seen in learning and memory the stress induced due to oxidation was apparently affected by huperzine. Influential decrease in the cholinesterase activity was also witnessed. The histopathological evidences showed that the damaged neuronal cells in brain specifically periventricular regions and cortex altered and repaired to much extent. Thus the study concludes that huperzine when co administered with phenytoin encounters its adverse effect of cognition impairment.

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Conflict of interest

All authors declare no conflict of interest.

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