The Protection by Vitamin E Against Tramadol-Induced Proconvulsant Effects and Brain Damage in Pentylenetetrazole-Induced Status Epilepticus in Rats

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Abstract: We investigated the effect of the opioid analgesic tramadol on the development of epileptic seizures and neuronal injury and the possible modulatory effect of vitamin E (Vit E) in rats with pentylenetetrazole (PTZ)-induced status epilepticus. Rats received repeated intraperitoneal (i.p.) injections of PTZ till the development of status epilepticus and were pretreated once with tramadol (30, 60 or 90 mg/kg), vitamin E (Vit E, 70 mg/kg) or both tramadol (90 mg/kg) and Vit E (70 mg/kg) prior to starting PTZ injections. Seizure scores, the latency time and the PTZ dose for each group required to reach status epilepticus were determined and histopathological examination of the brain tissue was done. Results indicated that tramadol produced both anticonvulsant and proconvulsant effects. The anticonvulsant effects of tramadol were observed for facial twitching (stage 1), convulsive body waves (stage 2), and myoclonic jerks and rearing (stage 3) and turn over onto one side position (stage 4) that were significantly inhibited by tramadol. In contrast, tonic-clonic convulsions (stage 5) were significantly increased by 60 or 90 mg/kg of tramadol as compared to PTZ control group. The mean latency and PTZ threshold dose for status epilepticus were markedly decreased after tramadol. The administration of Vit E exerted beneficial effects in decreasing epilepsy scores and increasing both the latency time and threshold dose of PTZ for reaching status epilepticus. Meanwhile, rats treated with both tramadol and Vit E exhibited significant increase in tonic-clonic convulsions and markedly shortened latency time to reach status epilepticus compared to those treated with only Vit E. In cerebral cortex and hippocampus, PTZ resulted in apoptotic cells, darkly stained degenerated and vacuolated neurons and gliosis. These pathological changes increased after tramadol but were markedly reduced by Vit E treatment. Collectively, these results suggest that: (i) tramadol exerts both anticonvulsant and proconvulsant effects; (ii) tramadol shortened the latency time and decreased the threshold dose of PTZ for evoking status epilepticus; (iii) PTZ-induced seizures and brain damage can be inhibited by Vit E; (iv) tramadol at high doses interferes with the effect of Vit E in inhibiting tonic-clonic convulsions and in reducing brain damage.

Keywords: Tramadol, Vitamin E, Epilepsy, Anticonvulsant, Pentylenetetrazole.

INTRODUCTION

Epileptogensis summarizes interactive changes in cellular processes that culminate in the development of epilepsy [1]. Tramadol (1*RS*; 2*RS*)-2-(dimethylaminomethyl)-1-(3-methoxyphenyl)-cyclohexanol-hydrochloride is a synthetic analog of codeine and an effective analgesic drug in use for the treatment of pain of severe and moderate intensity such as that occurring in cancer patients, after surgery, trauma, low back pain and rheumatoid arthritis [2-5]. The mechanism of action involves weak agonist activity at μ-opioid receptors besides an inhibitory action on the reuptake of serotonin and noradrenaline [6, 7]. The drug is a racemic 1:1 mixture of two enantiomers, (+)-tramadol and (-)-tramadol. These two enantiomers differ as regards their potencies at opioid receptors and monoamine uptake sites [7]. In addition, tramadol is metabolized by cytochrome P450 (CYP) enzymes CYP2D6 and CYP3A4 to the more potent opioid analgesic metabolite o-desmethyltramadol (M1) [8]. The drug also increases the release of dopamine in several brain regions and thus may mediate reinforcing effects [9, 10]. In this respect, studies have shown that compared with short acting opioids, tramadol alone administered after surgery carries the risk of prolonged opioid use [11]. Tramadol can increase a patient's likelihood for developing seizures through lowering of the seizure threshold [12]. Occurrence of generalized tonic-clonic seizures has been reported with high doses of tramadol [13] but may also develop after such low doses as 37.5 mg twice daily [14]. Seizures may occur within the first 24 hours of tramadol administration. Those with history of traumatic brain injury and seizure activity or on antipsychotic drugs are more susceptible to development of seizures by tramadol [15].

The aim of the present study is to examine the effect of tramadol on the development of epileptic seizures and neuronal injury in an experimental model of status epilepticus induced by pentylenetetrazole in the rat. Pentylenetetrazole (PTZ) is an antagonist at γ-aminobutyric acid (GABA) (A) receptor which is widely

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employed in elucidation of the pathogenetic mechanisms involved in epilepsy and in the screening of new compounds with potential antiepileptic activity [16]. We also aimed to examine the possible modulation by the antioxidant vitamin E of seizures caused by PTZ alone and in presence of tramadol.

MATERIALS AND METHODS

Animals

This study was conducted on male Sprague-Dawley rats weighing 180-200 g. Rats were group-housed under temperature- and light-controlled conditions and allowed standard laboratory rodent chow and water *ad libitum*. Animal experiments were done at 9 O'clock in order to avoid changes in the circadian rhythm. The study was done in accordance to the regulations of the Ethics Committee of the National Research Centre and the Guide for Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Publication No. 85-23, revised 1996).

Drugs and Chemicals

Pentylenetetrazole (PTZ) was purchased from Sigma (St. Louis, USA). Tramadol was a gift from the Ministry of Justice (Egypt). The drug was dissolved in saline and i.p. administered. Vitamin E (alpha-tocopherol) was purchased from Pharco Co. (Egypt). The rest of chemicals and reagents were purchased from Sigma (St. Louis, USA). The doses of tramadol and Vit E used in the study was based on that used for humans after conversion to that of rat using Paget and Barnes conversion tables [17].

Pentylenetetrazol-Induced Seizures in Rats

Pentylenetetrazole (PTZ) was i.p. injected at an initial starting dose of 30 mg/kg, followed every 10 min by repeated doses of 10 mg/kg each, until the occurrence of status epilepticus. Seizure behaviors during the study were classified into 5 stages and scored as follows: 0: no response; 1: ear and facial twitching; 2: convulsive waves through the body; 3: myoclonic jerks, rearing; 4: turn over onto one side position; 5: turn over onto back position, generalized tonic-clonic seizures [18]. The average score of each stage was determined and compared between the PTZ control and different treated groups. In addition, the average latency time and dose of PTZ required by each treated group of animals to reach status epilepticus were determined.

Experimental Groups

Rats were randomly divided into six different groups (7-8 rats/group) and treated as follows:

Group 1: received saline prior to the start of PTZ injections and served as PTZ control.

Groups 2, 3 and 4: received tramadol at doses of 30, 60 or 90 mg/kg, respectively, before PTZ injections.

Group 5: was treated with Vit E at the dose of 70 mg/kg before PTZ injections.

Group 6: was treated with Vit E (70 mg/kg) + tramadol (90 mg/kg) before PTZ injections.

Seizures scores were recorded and rats were quickly euthanized by decapitation carried out under light ether anaethesia, 2 hr after the last dose of PTZ. The brain of each rat was then quickly removed on an ice cold glass plate. One half of each brain was stored at -80 ^{o}C until the biochemical assays while the other half was kept in 10% formol saline for histopathological processing.

Histopathological Studies

Representative specimens of brain were fixed in 10% neutral buffered formalin for 48 h, dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Sections were cut at 5 µm using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (Hx & E) for the histopathological study.

Statistical Analysis

Results are expressed as mean ± SEM. Data of the behavioral study were analyzed by Kruskal-Wallis test followed by uncorrected Dunn's test. Graphpad Prism software, version 5 (GraphPad Prism Software Inc., San Diego, USA) was used for the statistical analysis. A probability value < 0.05 was considered as statistically significant.

RESULTS

Effect of Tramadol and/or Vitamin E on PTZ-Induced Status Epilepticus

Effect of Tramadol

Figure **1** shows the changes in the mean seizure scores for the stages 1-3 during the study. Compared with the PTZ control group, treatment with tramadol at

doses of 60 or 90 mg/kg significantly decreased the development of stage 1 (ear and facial twitching) by 83% and 79.3%, respectively (1.25 ± 0.16, 1.5 ± 0.19 *vs.* 7.25 ± 0.31). The occurrence of convulsive waves through the body (stage 2) was inhibited by 93.0%, 93.0%, and 85.0% (0.22 ± 0.04, 0.22 ± 0.04, 0.50 ± 0.18 *vs.* 3.250 ± 0.31) and myoclonic jerks and rearing (stage 3) by 85%, 85% and 50% (0.67 ± 0.12, 0.67 ± 0.12, 2.25 ± 0.49 *vs.* 4.50 ± 0.57) after tramadol doses of 30, 60 or 90 mg/kg, respectively. The scores for stage 4 (turn over onto one side position) were inhibited by 91.6%, 91.6% by the drug at 30 or 60 mg/kg, respectively. The higher dose of 90 mg/kg tramadol elicited only 53.6% reduction in the stage 4 scores. In contrast to the above, the scores for generalized tonic-clonic seizures (stage 5) were increased by 114.0% by 60 or 90 mg/kg of tramadol compared with the PTZ control value (Figure **2**).

The changes in the threshold dose of PTZ and latencies to develop status epilepticus are shown in Figure **3**. The administration of tramadol at doses of 30, 60 or 90 mg/kg was associated with 36.4%, 51.8% and 100% decrements in the threshold dose required for developing status epilepticus (from a control value of 55.0 ± 3.2 to 35.0 ± 1.9, 32.5 ± 1.6 and 0.23 ± 0.04). The latency for status epilepticus decreased by 55.0%, 78.9% and 99.0%, respectively, by the above doses compared to the PTZ control value (20.0 ± 0.53, 9.5 ± 0.62, 0.45 ± 0.08 *vs.* 45.0 ± 1.9) (Figure **3**).

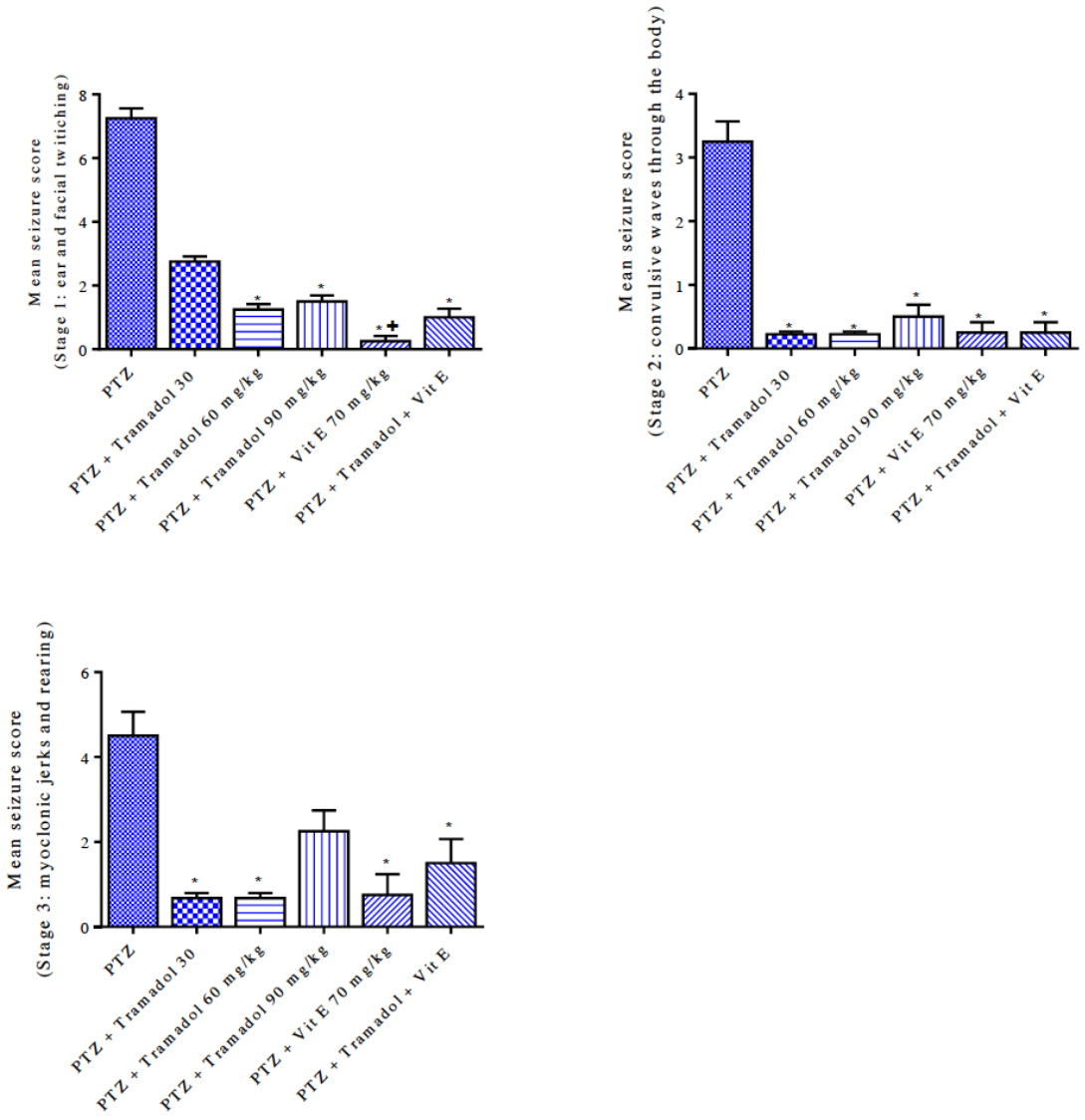

Figure 1: Mean scores of individual epilepsy stages 1-3 in PTZ only, PTZ + tramadol, PTZ + Vit E and PTZ + tramadol + Vit E treated groups. Each bar represents mean ± S.E. of 7-8 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 *vs.* PTZ control. +: p<0.05 *vs.* PTZ + tramadol 30 mg/kg.

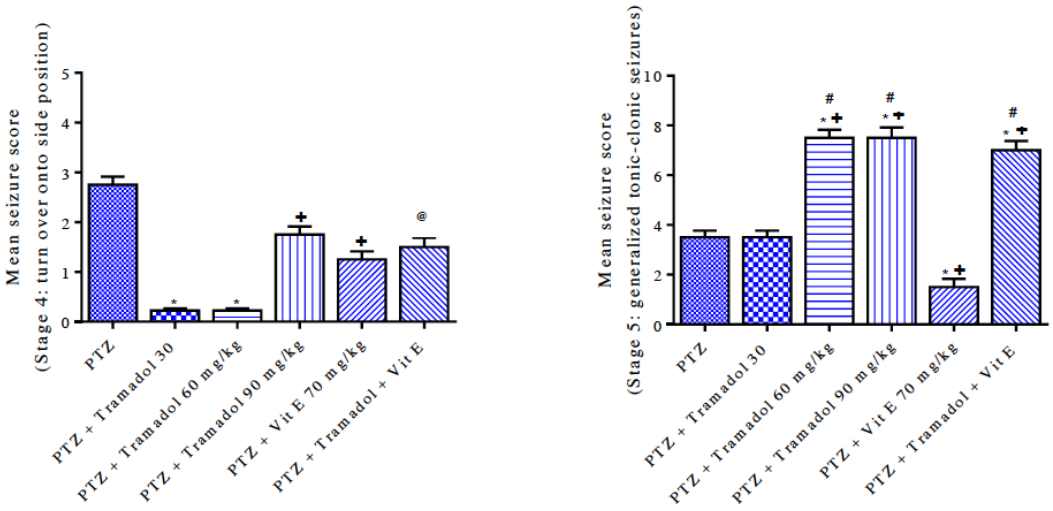

Figure 2: Mean scores of individual epilepsy stages 4 & 5 in PTZ only, PTZ + tramadol, PTZ + Vit E and PTZ + tramadol + Vit E treated groups. Each bar represents mean ± S.E. of 7-8 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 *vs.* PTZ control and between different groups as shown in the graph. +: p<0.05 *vs.* PTZ + tramadol 30 mg/kg. @: p<0.05 *vs.* PTZ + tramadol 90 mg/kg. #: p<0.05 *vs.* PTZ + Vit E.

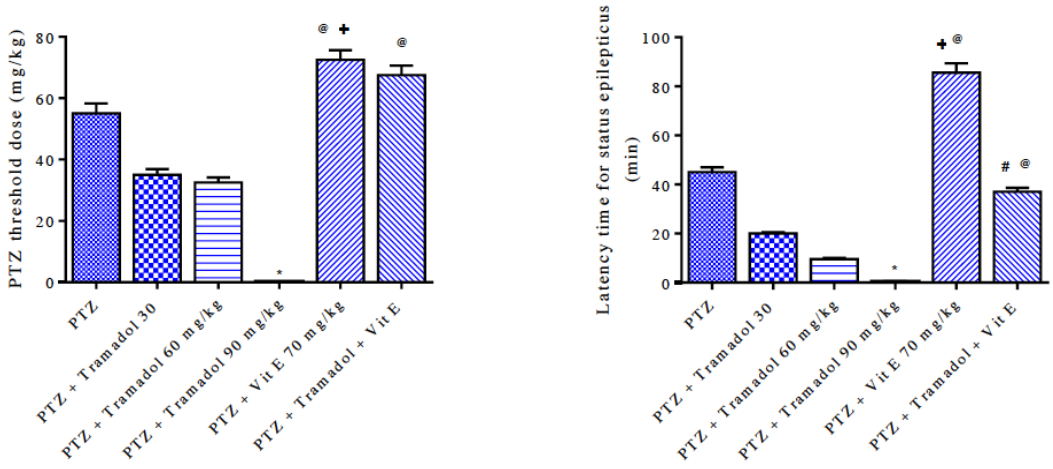

Figure 3: PTZ threshold dose and the mean latency time for status epilepticus. Each bar represents mean ± S.E. of 7-8 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 *vs.* PTZ control. +: p<0.05 *vs.* PTZ + tramadol 30 mg/kg. #: p<0.05 *vs.* PTZ + tramadol 90 mg/kg. @: p<0.05 *vs.* PTZ + tramadol 60 or 90 mg/kg.

Effect of Vitamin E

Vitamin E treatment significantly inhibited ear and facial twitching (stage 1) by 96.6% (0.25 ± 0.16 *vs.* 7.25 ± 0.31), convulsive waves through the body (stage 2) by 92% (0.25 ± 0.16 *vs.* 3.25 ± 0.31), myoclonic jerks and rearing (stage 3) by 83.3% (0.75± 0.49 *vs.* 4.5 ± 0.57). Stage 4 (turn over onto one side position) and stage 5 (generalized tonic-clonic seizures) were inhibited by 55.4% (1.25 ± 0.16 *vs.* 2.8 ± 0.16) and 57.1% (1.5 ± 0.32 *vs.* 3.5 ± 0.26), respectively (Figures **1** & **2**). Additionally, Vit E significantly increased the threshold dose to develop status epilepticus by 37.3% from a control value of 55.0 ± 3.2 to 75.5 ± 3.1 and increased the latency for status epilepticus by 90% compared to the PTZ control value (85.5 ± 3.9 *vs.* 45.0 ± 1.9) (Figure **3**).

Effect of Vitamin E in Combination With Tramadol

Compared with Vit E treatment the combination of tramadol/Vit E exhibited significantly higher scores for stage 5 (generalized tonic-clonic seizures) (Figure **2**) and markedly shortened latency time to reach status epilepticus (Figure **3**).

Effect of Tramadol and/or Vitamin E on Histopathological Damage in Brain of PTZ-Treated Rats

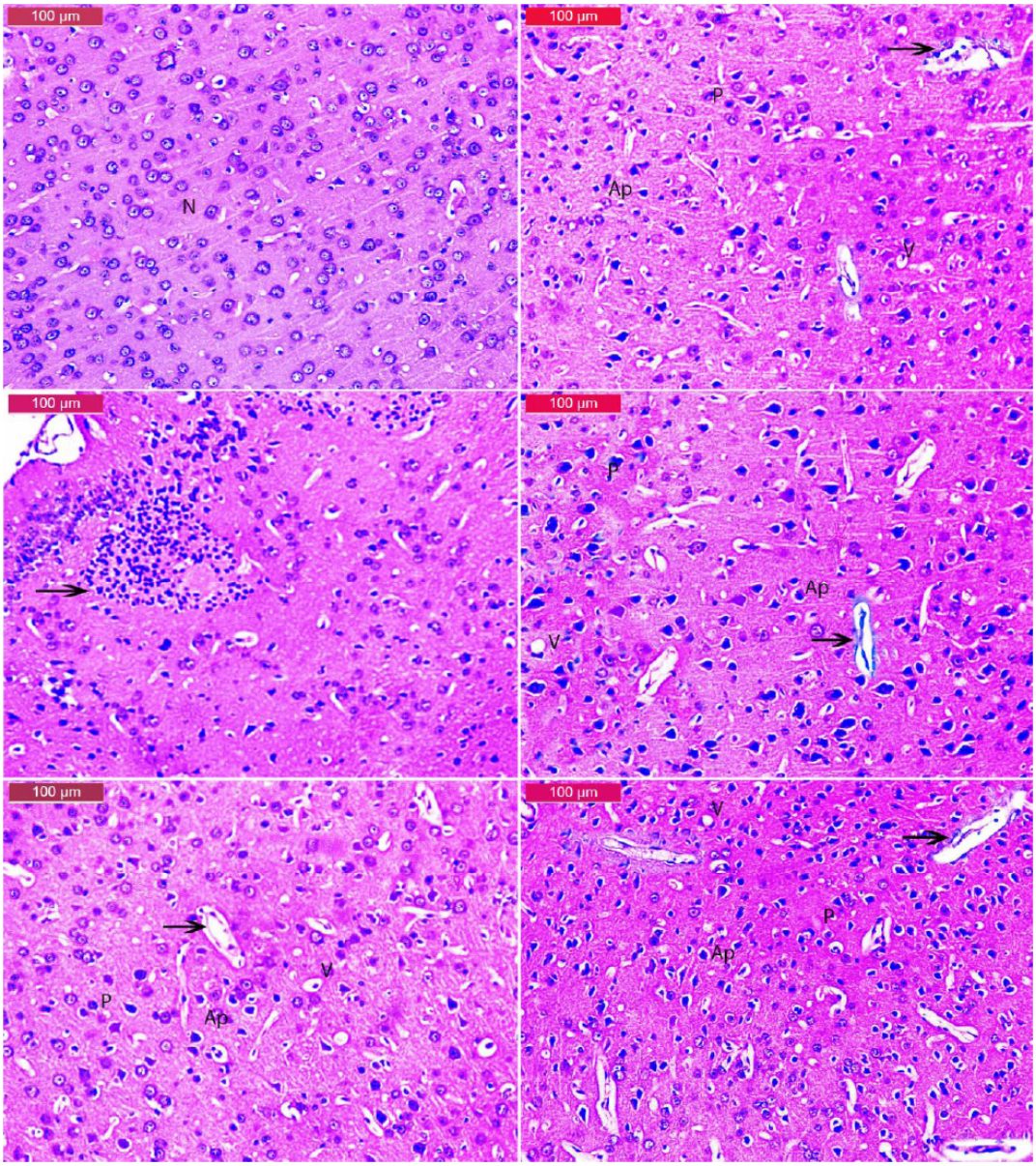

Figure 4: Representative photomicrograph of Hx & E stained sections of rat cerebral cortex after treatment with: **(A)** Saline showing normal neurons with large vesicular nuclei and well-defined nucleoli. **(B)** PTZ showing apoptotic cells (Ap), darkly stained neurons (P) and vacuolated cells (V). **(C)** PTZ showing gliosis (arrow). **(D)** PTZ + tramadol 30 mg/kg showing apoptotic cells (Ap), darkly stained neurons (P) and vacuolated cells (V). **(E)** PTZ + tramadol 60 mg/kg showing apoptotic cells (Ap), many darkly stained neurons (P), vacuolated cells (V) and dilated blood vessels with wide perivascular space (arrow). **(F)** PTZ + tramadol 90 mg/kg showing apoptotic cells (Ap), many darkly stained neurons (P), vacuolated cells (V) and dilated blood vessels with wide perivascular space (arrow).

Cortex

Brain sections from the saline group showed normal architecture in cerebral cortex with neurons being arranged in neat rows with abundant cytoplasm, and the nuclei are round and basophilic (Figure **4A**). The group treated with PTZ only showed neurodegeneration with presence of apoptotic cells, many darkly stained neurons and vacuolated cells (Figure **4B** & **C**). Sections of the cerebral cortex of group treated with PTZ and tramadol (30, 60 & 90 mg/kg) revealed loss of organization of layers, degeneration of neurons with acidophilic cytoplasm. Cells appeared apoptotic, with pyknotic deeply stained nuclei and vacuolated cells. Dilated blood vessels with wide perivascular space were observed. The higher dose of 90 mg/kg caused more damage (Figure **4F**) as compared to lower doses of 30 or 60 mg/kg (Figure **4D** & **E**).

Rats treated with PTZ and Vit E showed mildly affected cerebral cortex architecture with nearly normal

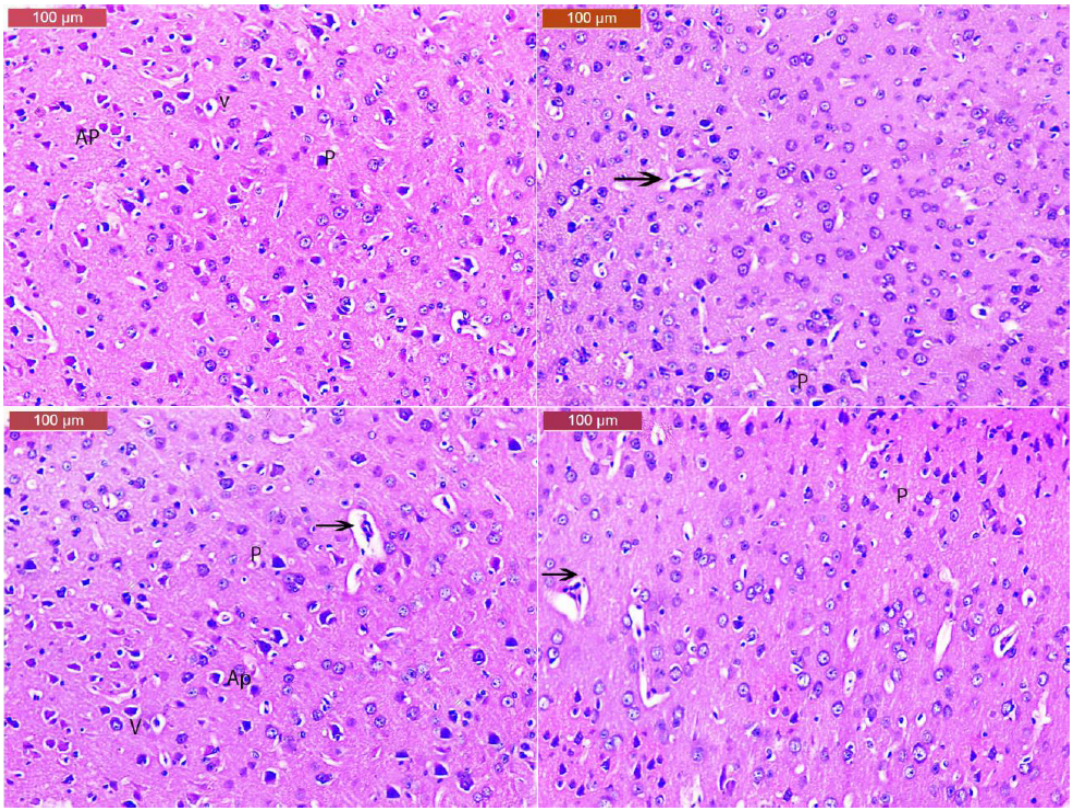

Figure 5: Representative photomicrograph of Hx & E stained sections of rat cerebral cortex after treatment with: **(A & B**) PTZ + Vit E showing amelioration of the PTZ damage, although some dark neurons are still observed. **(C & D)** PTZ + tramadol (90 mg/kg) + Vit E showing amelioration of many neurons, although some dark neurons are still observed.

arrangement of the cerebral cortex while other neurons were still pyknotic (Figure **5A** & **B**). The group treated with PTZ, tramadol (90 mg/kg) and Vit E showed moderate to mildly affected cerebral cortex architecture with nearly normal arrangement of the cerebral cortex. Other neurons were still pyknotic (Figure **5C** & **D**).

Hippocampus

The saline group showed normal neurons with prominent nuclei in hippocampal region (Figure **6A**). Following PTZ injections, hippocampal neuronal cells showed histopathological changes with mild degeneration, shrunken pyknotic nuclei and vacuolated cells (Figure **6B** & **C**). Sections from group treated with PTZ and different doses of tramadol showed mild to moderate degenerative changes, necrotic cells, shrunken neuronal cells with dense pyknotic nuclei and vacuolated cells (Figure **6D**, **E** & **F**).

Rats treated with PTZ and Vit E showed a decrease in this neuronal degeneration, with only small number of neuronal pyknotic cells (Figure **7A** & **B**). On the other hand, rats treated with PTZ + Vit E + tramadol (90 mg/kg) showed decreased neuronal degeneration, with only small neuronal pyknotic compared with the tramadol group (Figure **7C** & **D**).

DISCUSSION

The findings of the present study can be summarized as follows: (i) administration of tramadol in rats with PTZ-induced status epilepticus resulted in both anticonvulsant and proconvulsant effects. The anticonvulsant effects of tramadol were observed for facial twitching, convulsive body waves, and myoclonic jerks and rearing. In contrast, tonic-clonic convulsions were significantly increased by tramadol; (ii) the mean latency for developing seizures by PTZ as well as the threshold dose of PTZ required to reach status epilepticus were significantly decreased by tramadol; (iii) Vit E showed anti-seizure properties in this model and reduced neuronal damage in brain of rats with status epilepticus; (iv) these beneficial effects of Vit E decreased in the presence of tramadol.

Seizures are thought to result from an imbalance between the excitatory neurotransmitter glutamate relative to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Pentylenetetrazole which is

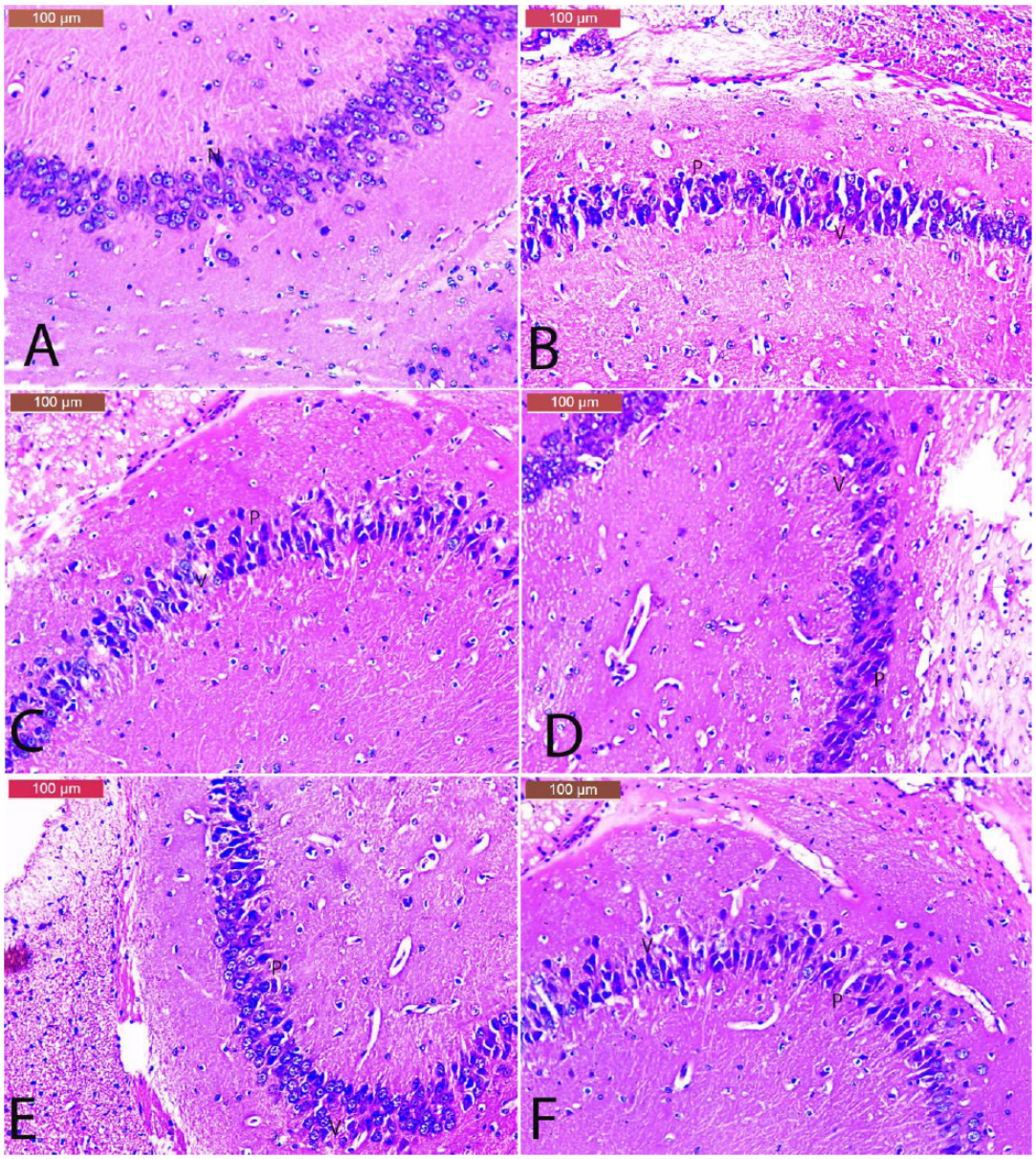

Figure 6: Representative photomicrograph of Hx & E stained sections of rat hippocampus after treatment with: **(A)** Saline showing normal neurons with vesicular nuclei and well-defined nucleoli. **(B & C)** PTZ showing pyknotic nuclei (P) and vacuolated cells (V). **(D)** PTZ + tramadol 30 mg/kg showing pyknotic nuclei (P) and vacuolated cells (V). **(E)** PTZ + tramadol 60 mg/kg showing pyknotic nuclei (P) and vacuolated cells (V). **(F)** PTZ + tramadol 90 mg/kg showing moderate degenerative changes, pyknotic nuclei (P) and vacuolated cells (V).

a non-competitive GABA-A receptor antagonist evokes generalized myoclonic and tonic-clonic by virtue of its ability to inhibit GABAergic function with the consequent increase in glutamatergic activity, making this model more relevant to the human epilepsy [19, 20]. Pentylenetetrazole-induced status epileptic is thus used in the current study in order to delineate the effect of tramadol on seizure development. The drug at doses of 30, 60 or 90 mg/kg showed anti-convulsant effects, markedly decreasing the scores for ear and facial twitches, convulsive waves throughout the body *i.e*., stages 1 and 2 as well as myoclonic jerks (stage 3). In addition, stage 4 *i.e*., turn over to one side was inhibited by 30 and 60 mg/kg of the drug. In contrast, at high doses of 60 or 90 mg/kg, did tramadol enhanced the development of generalized seizures caused by PTZ.

Potschka *et al*. [21] using the kindling model of temporal lobe epilepsy in the rat observed anti-seizure effect for tramdol at 5 mg/kg. A significant decrease in duration of seizures was also recorded for 10 mg/kg dose of tramadol. In contrast, marked decrease in seizure threshold occurred when the dose of tramadol

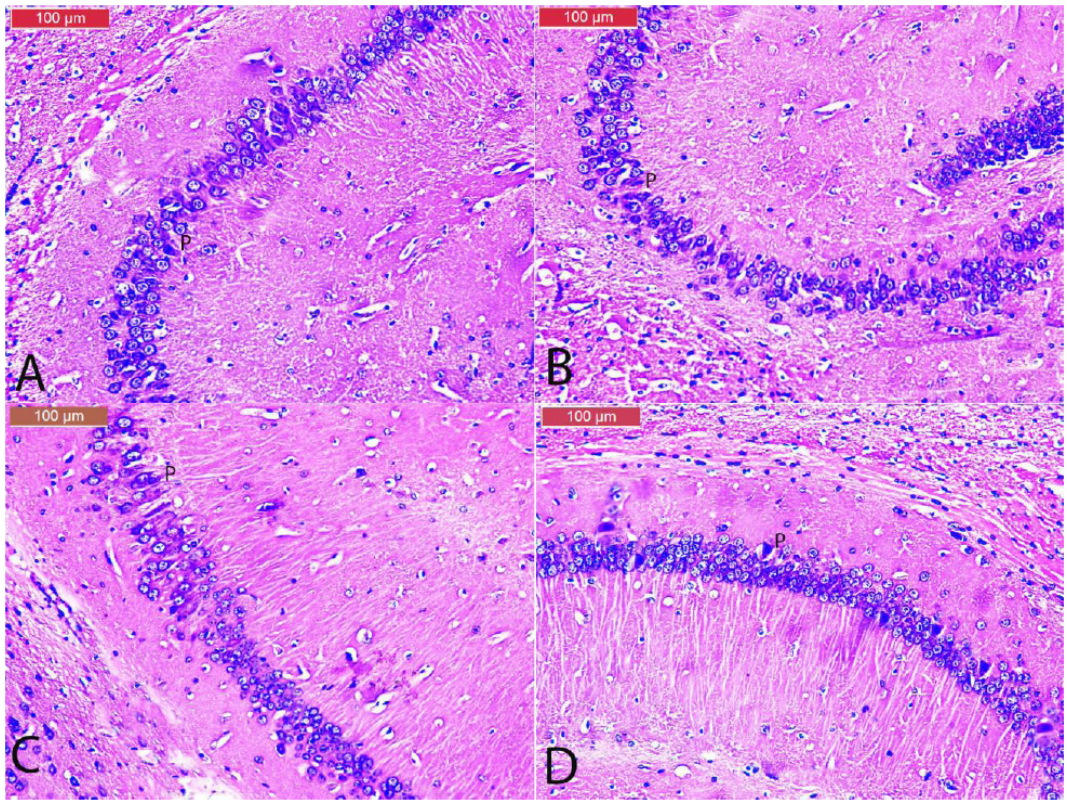

Figure 7: Representative photomicrograph of Hx & E stained sections of rat hippocampus after treatment with: **(A & B)** PTZ + Vit E showing moderate improvement of many neurons, some dark neurons are still observed (P). **(C & D)** PTZ + tramadol (90 mg/kg) + Vit E showing moderate normalization of almost all neurons. Some dark neurons are still observed (P).

was increased to 30 mg/kg with development of generalized fits in most of kindled rats. It would appear therefore that tramadol decreases the threshold mainly for generalized tonic-clonic seizures and this effect is evident with the high doses of the drug. It is also likely that the proconvulsant effect of tramadol occurs in the presence of increased cortical excitability, resulting for example in the present study from PTZ. In this respect, patients with previous trauma to the brain, history of epilepsy or on drugs that lower seizure threshold like antipsychotic drugs appear to be prone to the proconvulsant effects of tramadol [15]. Other studies indicated an increased risk of tramadol-induced seizures by the concomitant administration of drugs of abuse such as opiates and cannabis [22] or alcohol [23].

Tramadol has a history of causing seizures, according to several case reports, but these usually occurred at high dosages of the drug, between 250 and 2500 mg [13, 22, 23], suggesting that the effect is dose-dependent. Contrarily, some studies revealed that tramadol users did not have a higher risk of seizures when compared to those taking other opioid or non-opioid analgesics [24] or when compared to those taking codeine [25].

Interestingly, in the present study, all the seizure stages were markedly inhibited by Vit E given at the dose of 70 mg/kg. Moreover, Vit E caused significant increase in the threshold dose for developing status epilepticus by 37.3% and increased the latency for status epilepticus by 90% compared to the PTZ controls, which raises the question as regards the role of oxidative stress in the development of seizures in this model. There is evidence from both clinical and experimental studies that favors oxidative brain injury as a mechanism that leads to neuronal hyperexcitability and neurodegeneration [26, 27]. In previous studies, we have shown the presence of markedly elevated oxidative stress levels in brain of PTZ kindled rats. This was evidenced by the increase in the lipid peroxidation end product malondialdehyde and the depletion of the antioxidant molecule reduced glutathione [28-30]. The latter is the most abundant non-protein thiol in the cell which has an essential function in maintaining its redox-balance [31]. Other studies found decreased antioxidant enzymes *i.e.*, superoxide dismutase and catalase in brain of PTZ-treated rats [32]. Vitamin E is a lipid-soluble chain-breaking antioxidant which functions to prevent the oxidative modification *i.e.*, lipid peroxidation of membrane and lipoprotein

polyunsaturated fat into lipid hydroperoxides [33]. Vitamin E, as a peroxyl radical scavenger maintains the integrity of long-chain polyunsaturated fatty acids in the cell membranes, and thus maintains their functionality [34]. Vitamin E is also important for neuronal integrity and its supplementation can interfere with one of the most important pathogenetic mechanisms underlying neurological dysfunction and neurodegeneration that is oxidative stress [35, 36]. Vitamin E by virtue of its antioxidant properties may act to reduce the neuronal hyperexcitability caused by the epileptogen PTZ and hence dampens seizure development.

Our histological study indicated the development of neuronal injury in the form of apoptotic cells, many darkly stained neurons and vacuolated cells in the cerebral cortex, hippocampus in PTZ-treated rats which is in agreement with previous observations [28, 30]. Previously, upregulation of *N*-methyl-D-aspartate (NMDA) glutamate receptors was observed in hippocampus and cortex after PTZ injection [37] which may account for the neuronal cell loss caused by the epileptogen [29, 38]. In the present study, the PTZ-induced neuronal damage was increased by the administration of tramadol. Previous studies indicated the development of neuronal injury eg., shrunken neurons in the brain of experimental animals treated with tramadol at doses of 5-20 mg/kg for 6 weeks [39]. Higher doses eg., 50 mg/kg of tramadol for 4 weeks resulted in neuronal disorganization, and apoptosis in rat brain [40, 41]. Our results also showed that neuronal injury was markedly alleviated after treatment with Vit E. It was also observed that the effect of Vit E/tramadol combination was less than that of Vit E alone, which provides another evidence for the adverse effect of tramadol on neuronal integrity and function.

CONCLUSIONS

The present study provided the first evidence that tramadol exerts distinct anticonvulsant and proconvulsant effects in PTZ-induced status epilepticus depending on the seizure stage. The proconvulsant action of tramadol was observed for tonic-clonic convulsions. Vitamin E conferred neuroprotection against PTZ or PTZ/tramadol-induced seizures and neuronal damage.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests.

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