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Nefopam Exerts Anticonvulsant and Neuroprotective Effects in Experimentally-Induced Status Epilepticus

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

Nefopam is a centrally acting, non-opiate analgesic drug used in the prevention of post-operative pain. In this study, the effect of nefopam on epileptic seizures and neuronal brain damage induced in the rat by pentylenetetrazole (PTZ) was examined. Status epilepticus was induced by repeated injections of PTZ and rats were treated with nefopam at 20 or 40 mg/kg, phenytoin at 30 mg/kg or both nefopam 40 mg/kg and phenytoin. Seizure scores, the latency and threshold dose of PTZ for status epilepticus were determined. Brain malondialdehyde, reduced glutathione, nitric oxide, and paraoxonase-1 activity were measured and histological study done. Results showed that the increase in malondialdehyde and nitric oxide levels and the decrease in reduced glutathione content and paraoxonase-1 activity caused by PTZ in the rat brain were significantly ameliorated by treatment with nefopam and nefopam/phenytoin. The mean epilepsy scores were significantly decreased, while the latency and threshold dose of PTZ for developing status epilepticus significantly increased by nefopam, phenytoin or their combination. Additionally, nefopam had no significant effect on the anti-convulsive action of standard antiepileptic drug phenytoin. The histological study demonstrated that the neurodegenerative changes induced by PTZ, namely, the dark shrunken cortical and hippocampal neurons were ameliorated by 40 mg/kg nefopam or nefopam/phenytoin administration. These data suggest that in experimentally-induced status epilepticus, nefopam decreases oxidative stress, exerts anticonvulsant and neuroprotective effects without influencing that of phenytoin. Nefopam, therefore, could be of value as an adjunct to phenytoin in the treatment of epilepsy in humans.

Keywords: nefopam; phenytoin; pentylenetetrazole; antiepileptic; status epilepticus; neurodegeneration; neuroprotection .

1. Introduction

Epilepsy is a common neurological disorder in which abnormally excessive, successive and synchronous firing of cerebral neuronal population results in the constellation of neurobehavioral changes or epileptic fits. It is widely accepted that the cause is an imbalance between γ-aminobutyric acid (GABA)-ergic inhibitory and glutamatergic excitatory neuronal transmission [1]. Seizures may be generalized, originating simultaneously in the two cerebral hemispheres (e.g., tonic-clonic seizures) or partial, arising in a discrete cortical area of one hemisphere, mostly the temporal lobe [2]. The disorder which affects about 1% of the population, starts during childhood, and unless properly managed can result in considerable negative impact on health and quality of life [3]. Unfortunately, one third of these patients will experience the so called “refractory epilepsy”, the form which is not controlled by two or more appropriately selected antiepileptic drugs or other therapies. This illustrates the need for finding or developing new therapeutics to control the disease [4].

Nefopam (© Acupan), 5-methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine, is a centrally acting non-opioid analgesic, which is structurally related to the antihistamine diphenhydramine and the antiparkinsonian drug orphenadrine [5,6]. It is effective when given by the oral and parenteral routes, while the initial oral dose is 30 mg, 3 to 4 times a day [5]. The drug is given in the sitting of multimodal analgesia in order to prevent mild to moderate postoperative pain, shivering and hiccups [7-9]. Several studies have dealt with the analgesic action of nefopam in combination with non-steroidal anti-inflammatory drugs such as nimesulide, acetylsalicylic acid, ketoprofen or acetaminophen [10]. The mechanism of action by which nefopam produces analgesia may be related to its ability to inhibit the reuptake of serotonin, norepinephrine and dopamine, interaction with α2–adrenoreceptors, or glutamatergic modulation [8, 11-13].

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The aim of this study was to examine the effect of nefopam on seizures and brain neuronal degeneration in a model of status epileptics evoked by pentylenetetrazole injection (PTZ) in rats. The study was extended to look for a possible modulatory action for nefopam on the anti-seizure effect of the antiepileptic drug phenytoin.

2. Materials and methods

2.1. 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).

2.2. Chemicals and reagents

Pentylenetetrazole (PTZ) was purchased from Sigma (St. Louis, USA). Nefopam hydrochloride was obtained from Medical Union Pharmaceuticals (Egypt). The drug was dissolved in saline and i.p. was administered. The rest of chemicals and reagents were purchased from Sigma (St. Louis, USA).

2.3. Experimental groups

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

Group 1 received i.p. saline prior to the start of PTZ injections and served as PTZ control. Groups 2, 3,4 and 5 were treated with nefopam at 20 or 40 mg/kg, phenytoin at 30 mg/kg or both nefopam 40 mg/kg and phenytoin before PTZ injections.

Seizures scores were recorded for each rat, and thereafter, rats were quickly euthanized by decapitation, performed under light ether anaethesia, 2 hours after the last dose of PTZ. The brain of each rat, was then quickly removed out, 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 histological processing.

2.4. Pentylenetetrazole-iduced seizures

Pentylenetetrazole (PTZ) was i.p. injected at an initial starting dose of 30 mg/kg. This is followed by repeated doses of 10 mg/kg every 10 min, until status epilepticus developed. Seizure behaviors were classified into 5 stages and scored according to Sefil et al. [14] as follows:

Stage 0: no response; stage 1: ear and facial twitching; stage 2: convulsive waves through the body; stage 3: myoclonic jerks, rearing; stage 4: turn over onto one side position; stage 5: turn over onto back position, generalized tonic-clonic seizures. The average score of each stage was determined and compared between the PTZ control group and the different treated groups. In addition, the average latency time, and dose of PTZ which is required by each treated group to reach status epilepticus was determined.

2.5. Biochemical analyses

2.5.1. Lipid peroxidation assay

Lipid peroxidation was measured by determining the level of malondialdehyde (MDA), a lipid breakdown end product according to the method described by Nair and Turne [15]. In this method, thiobarbituric acid reactive substances (TBAS) react with thiobarbituric acid forming TBA-MDA adduct and the absorbance is read at 532 nm using spectrophotometer.

2.5.2. Nitric oxide assay

The level of nitric oxide, measured as nitrite, was determined using the Griess reagent. Nitrite, a stable end-product of nitric oxide, is used as an indicator of the production of nitric oxide. In this assay, nitrate is converted to nitrite by nitrate reductase. The Griess reagent then reacts with nitrite forming a deep purple azo compound. The absorbance is read at 540 nm using a spectrophotometer [16].

2.5.3. Reduced glutathione assay

Reduced glutathione (GSH) was determined in homogenates according to Ellman [17]. Briefly, DTNB (5,5'-dithiobis (2-nitrobenzoic acid) or Ellman´s reagent is reduced by the free sulfhydryl group on GSH molecule to generate the yellow colored 5-thio-2-nitrobenzoic acid which can be determined by reading absorbance at 412 nm.

2.5.4. Paraoxonase-1 assay

The arylesterase activity of paraoxonase-1 was determined by a colorimetric method using phenyl acetate as a substrate. Paraoxonase-1 catalyzes the cleavage of phenyl acetate, resulting in phenol formation. The rate of formation of phenol was measured by monitoring the increase in absorbance at 270 nm at 25°C. The working mix consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM $CaCl_2$ and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer were added to the

above mix and the changes in absorbance were recorded following a 20s lag time. One unit of arylesterase activity is equal to 1 µmole of phenol formed per min. Paraoxonase-1 activity is expressed in kU/l, based on the extinction coefficient of phenol of 1310 $M^{-1}cm^{-1}$. Blank samples containing water were used to correct for the spontaneous hydrolysis of phenyl acetate [18].

2.6. Histological 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 histological study [19].

2.7. Statistical analyses

Results are expressed as mean ± SEM. Biochemical data were statistically analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for multiple group comparison. 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.

3. Results

3.1. Effect of nefopam and/or phenytoin on brain oxidative stress in PTZ-treated rats

3.1.1. Brain lipid peroxidation

The administration of PTZ resulted in significantly increased MDA level by 61.4% as compared to saline control (30.92 ± 1.3 *vs.* 19.16 ± 0.22 nmol/g.tissue). Nefopam given at 20 or 40 mg/kg caused a significant decrease in MDA level by 14.2% and 30.4% as compared to the PTZ only group (26.54 ± 0.37 and 21.52 ± 0.46 *vs.* 30.92 ± 1.3 nmol/g.tissue). A significant decrease in MDA by 17.7% and 29.5% was also observed after treatment with phenytoin or nefopam/phenytoin as compared to the PTZ control (25.44 ± 0.24 and 21.79 ± 0.53 *vs.* 30.92 ± 1.3 nmol/g.tissue) (Fig. 1).

3.1.2. Brain nitric oxide

In PTZ-treated rats, brain NO was increased significantly by 54.3% (32.38 ± 0.79 *vs.* 20.98 ± 0.84 µmol/g.tissue). The administration of nefopam to PTZ-treated rats at 20 or 40 mg/kg, caused a significant decrease in NO level by 16% and 28.2% after (27.19 ± 0.52 and 23.24 ± 0.43 *vs.* 32.38 ± 0.79 µmol/g.tissue). The level of brain NO was also significantly decreased by 16.6% and 31% after treatment with either phenytoin or nefopam/ phenytoin compared with the PTZ control (27.0 ± 0.66 and 22.37 ± 0.72 *vs.* 32.38 ± 0.79 µmol/g.tissue) (Fig. 2).

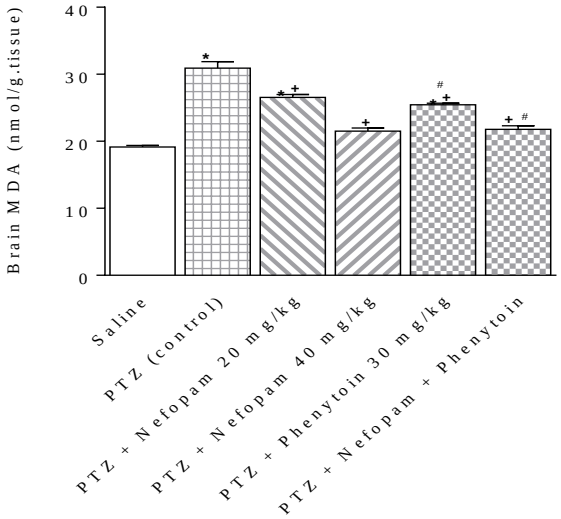

Figure 1. Effect of nefopam, phenytoin or nefopam/phenytoin on brain malondialdehyde (MDA) in pentylenetetrazole (PTZ)-induced status epilepticus. *: p< 0.05 *vs.* saline-treated group. +: p< 0.05 *vs.* PTZ control group. #p<0.05 *vs.* PTZ + nefopam 20 mg/kg.

3.1.3. Brain reduced glutathione

A significant decrease in GSH by 36% (2.10 ± 0.09 vs. 3.28 ± 0.11 µmol/g.tissue) was observed in the brain of PTZ-treated rats. GSH levels were increased by 23.8% and 38.1% after treatment with 20 or 40 mg/kg nefopam as compared to PTZ controls (2.6 ± 0.05 and 2.9 ± 0.1 vs. 2.10 ± 0.09 µmol/g.tissue). A significant increase in brain GSH by 17.7% and 39% was also observed after treatment with either phenytoin or nefopam/phenytoin compared with the PTZ control (2.7 ± 0.03 and 2.92 ± 0.07 vs. 2.10 ± 0.09 µmol/g.tissue) (Fig. 3).

3.1.4. Brain paraoxonase-1

In PTZ control group, the activity of PON-1 was significantly lower by 50.3% compared with the saline control (5.93 ± 0.41 vs. 11.94 ± 0.59 kU/l). This decrease in PON-1 activity induced by PTZ was attenuated by 30.7% and 80.4% by treatment with nefopam at 20 or 40 mg/kg, respectively (7.75 ± 0.44 and 10.70 ± 0.40 vs. 5.93 ± 0.41 kU/l). There was also a significant increase in brain PON-

1 activity by 42.7% and 70.3% after phenytoin or nefopam/ phenytoin administration compared with the PTZ control (8.46 ± 0.5 and 10.1 ± 0.31 *vs.* 5.93 ± 0.41 kU/l) (Fig. 4).

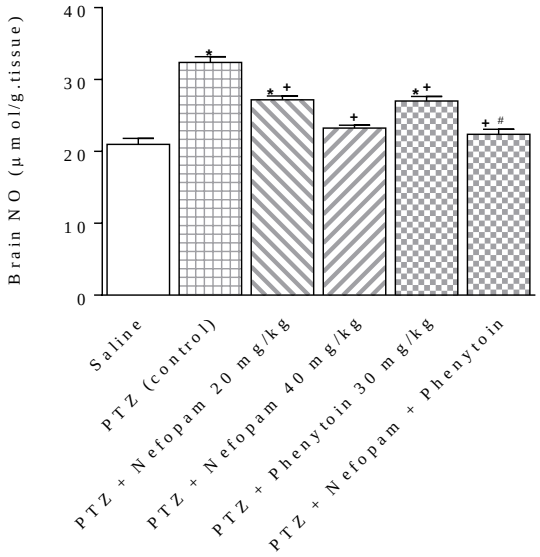

Figure 2. Effect of nefopam, phenytoin or nefopam/phenytoin on brain levels of nitric oxide (NO) in rats with pentylenetetrazole (PTZ)-induced status epilepticus. *: p< 0.05 *vs*. saline-treated group. +: p< 0.05 *vs*. PTZ control group. #p<0.05 *vs.* PTZ + nefopam 20 mg/kg.

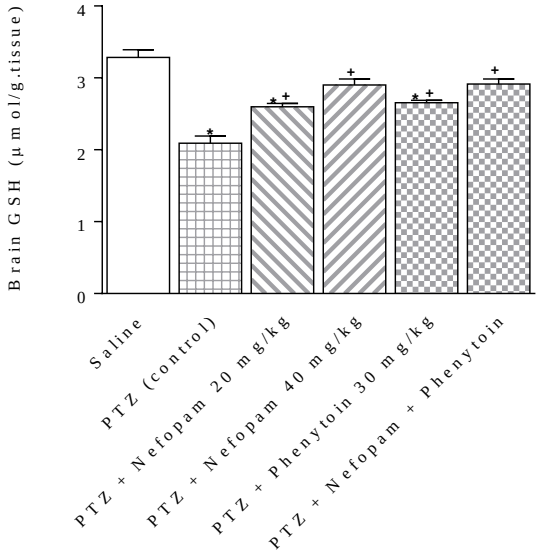

Figure 3. Effect of nefopam, phenytoin or nefopam/phenytoin on reduced glutathione (GSH) in brain of rats with pentylenetetrazole (PTZ)-induced status epilepticus. *: p< 0.05 *vs*. saline-treated group. +: p< 0.05 *vs*. PTZ control group.

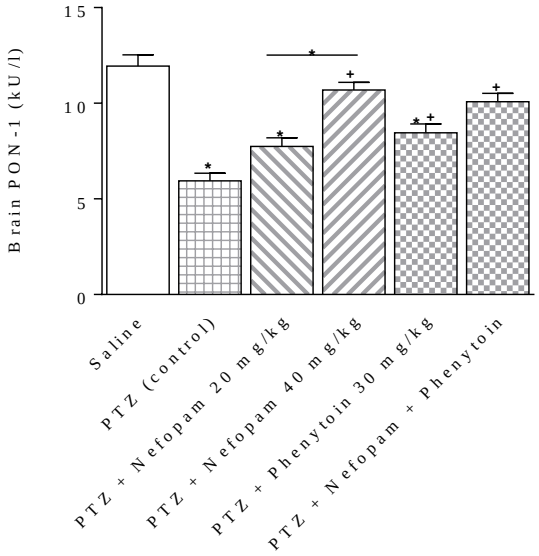

Figure 4. Effect of nefopam, phenytoin or nefopam/phenytoin on paraoxonase-1 (PON-1) activity in brain of rats with pentylenetetrazole (PTZ)-induced status epilepticus. *: p< 0.05 *vs*. saline-treated group. +: p< 0.05 *vs.* PTZ control group.

3.2. Effect of nefopam and/or phenytoin on PTZ-induced seizures

3.2.1. Effect of nefopam

The administration of nefopam at 20 and 40 mg/kg decreased the mean seizure scores over the study period by 47.5% and 57.5%, respectively. The mean seizure score decreased significantly from 4.0 ± 0.59 in the PTZ only group to 2.1 ± 0.64 and 1.7 ± 0.19 in the 20 and 40 mg/kg nefopam-treated groups, respectively (Fig. 5A). Meanwhile, the mean latency to develop status epilepticus increased by 147.8% and 244.4% by nefopam at 20 or 40 mg/kg from PTZ control value of 22.5 ± 2.2 to 55.75 ± 2.6 and 77.5 ± 0.78 sec, respectively (Fig. 5B). In addition, nefopam caused significant increases in the mean threshold dose of PTZ required to reach status epilepticus by 60% and 105% (80 ± 2.6 and 102.5 ± 1.6 *vs.* control value of 50 ± 2.7) (Fig. 5C).

The effect of nefopam on the individual stages of epilepsy is shown in Fig. 6. Compared with the PTZ only group, the scores for stage 1 was significantly decreased by 70.6% after 40 mg/kg nefopam (2.5 ± 0.19 *vs.* 8.5 ± 0.42). When given at 20 or 40 mg/kg, nefopam significantly decreased the scores for stage 2 by 68.8% and 87.5% (1.25 ±0.16 and 0.5 ± 0.19 *vs*. 4.0 ± 0.46). Myoclonic jerks and rearing (stage 3) decreased by 37.5% by the highest dose of the drug (1.25 ± 0.16 *vs*. 2.0 ± 0.27), while stage 4 decreased by 73.3% and 80% following treatment with nefopam at 20 and 40 mg/kg,

respectively (1.0 ± 0.26 and 0.75 ± 0.16 *vs.* 3.75 ± 0.31). Nefopam given at 40 mg/kg inhibited generalized tonic-clonic seizures (stage 5) by 90% (0.25 ± 0.16 *vs.* PTZ control value of 2.5 ± 0.19).

3.2.2. Effect of phenytoin

The administration of phenytoin at 30 decreased the mean seizure scores over the study period by 66.3% (1.35 ± 0.44 *vs.* 4.0 ± 0.59) (Fig. 5A). The mean latency to develop status epilepticus increased by 191.1% (65.5 ± 2.0 *vs.* 22.5 ± 2.2 sec) (Fig. 5B) and mean threshold dose of PTZ increased by 85% (92.5 ± 1.6 *vs.* 50 ± 2.7) (Fig. 5C). The effect of phenytoin on the individual stages of epilepsy is shown in Fig. 6.

3.2.3. Effect of nefopam/phenytoin combination

The combined administration of nefopam/ phenytoin decreased the mean seizure scores over the study period by 66.3% (1.35 ± 0.25 *vs.* 4.0 ± 0.59) (Fig. 5A) and the mean latency to develop status epilepticus by 194.1% (66.25± 2.0 *vs.* 22.5 ± 2.2 sec). However, this effect of nefopam/phenytoin was not significantly different from phenytoin alone (Fig. 5B). The mean threshold dose of PTZ decreased by 80% compared with the control value (90.0 ± 2.7 *vs.* 50 ± 2.7). This effect of nefopam/ phenytoin combination was not significantly different from that of phenytoin alone (Fig. 5C). The effect of nefopam/phenytoin combined administration on the individual stages of epilepsy is shown in Fig. 6.

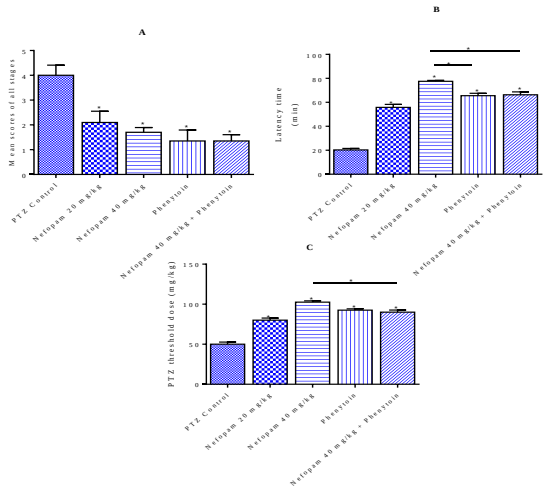

Figure 5. Effect of different treatments on the mean total epilepsy scores, threshold dose of PTZ and mean latency time for developing status epilepticus. Each bar represents mean ± S.E. of 7 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 *vs.* PTZ control. +: p<0.05 *vs.* PTZ and between different groups as indicated in the graph.

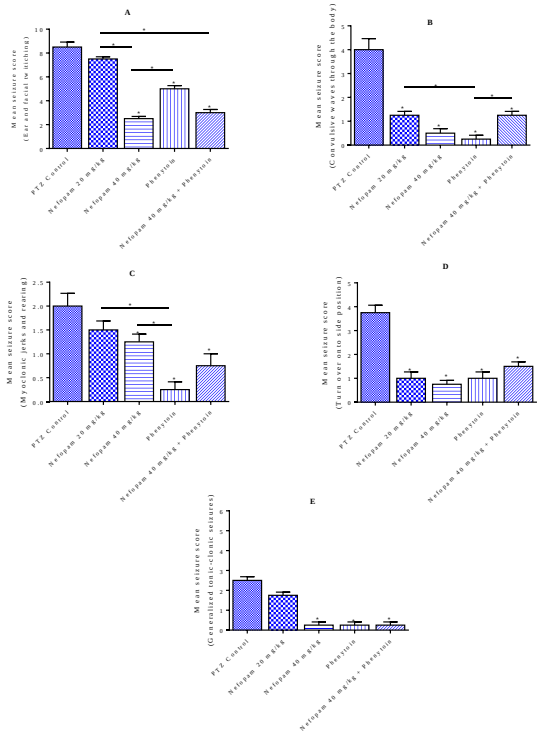

Figure 6. Mean scores of individual epilepsy stages 1-5 in PTZ only, PTZ + nefopam, PTZ + phenytoin and PTZ + nefopam/phenytoin-treated groups. Each bar represents mean ± S.E. of 7 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 *vs.* PTZ control and between different groups as indicated in the graph.

3.3. Effect of nefopam on histologic damage in rats with status epilepticus

3.3.1. Cerebral cortex

The cerebral cortex of the saline control group showed normal histological structure and well-organized of brain tissue, normal neurons with glial cells that had lightly stained nuclei (Fig. 7A). Sections of from PTZ control group showed degenerated neurons, with dilated and congested blood vessels. It demonstrated dark shrunken cortical neurons that had deeply stained pyknotic nuclei, apoptotic cells with pericellular vacuolations and many dark glial cells' nuclei. Moderate normal cortical neurons were seen (Fig. 7B). In the group treated with PTZ and nefopam 20 mg/kg moderate improvement was seen. However, perivascular vacuolation, pyknotic nuclei, apoptotic cells, glial cells with either many lightly or dark stained nuclei and slightly dilated blood vessels were observed (Fig. 7C). Rats treated with PTZ and nefopam 40 mg/kg showed few histopathological changes such as minimal pyknotic nuclei, apoptotic cells and glial cells with either many lightly or dark stained nuclei

and slight dilated blood vessels (Fig. 7D). In the group treated with PTZ and nefopam/phenytoin combination there was noticeable improvement in most neuronal cells of the cortex with few histopathological changes such as minimal pyknotic nuclei, apoptotic cells, and glial cells with either many lightly or dark stained nuclei (Fig. 7E).

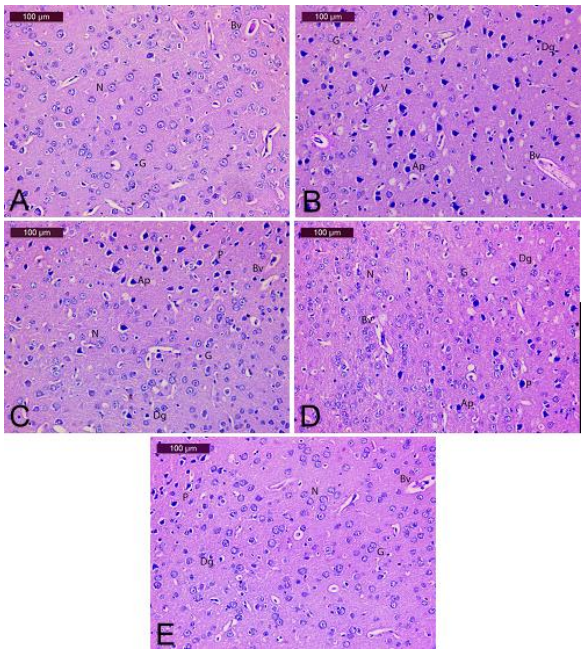

Figure 7. Representative photomicrographs of the cerebral cortex after treatment with (A) Saline: shows normal histological structure and well-organized brain tissue with normal neurons (N) with glial cells with lightly stained nuclei (G) and blood vessels (Bv); (B) PTZ: shows degenerated cerebral cortex neurons, with dilated and congested blood vessels (Bv), deeply stained pyknotic nuclei (P), apoptotic cells (Ap), with pericellular vacuolations and many dark glial cells' nuclei were noticed; (C) PTZ and nefopam 20 mg/kg: shows moderate improvement. However, perivascular vacuolation (V), pyknotic nuclei (P), apoptotic cells (Ap) and glial cells with either many lightly (G) or dark (Dg) stained nuclei and slight dilated blood vessels (Bv) were seen; (D) PTZ and nefopam 40 mg/kg: shows improvement of most of cortex with few histopathological changes such as minimal pyknotic nuclei (P), apoptotic cells (Ap) and glial cells with either many lightly (G) or dark (Dg) stained nuclei and dilated blood vessels (Bv); (E) PTZ and nefopam 40 mg/kg and phenytoin: shows noticeable improvement in almost neuronal cells of cortex with few histopathological changes such as minimal pyknotic nuclei (P), apoptotic cells (Ap) and glial cells with either many lightly (G) or dark (Dg) stained nuclei and normal blood vessels (Bv).

3.3.2. Hippocampus

The hippocampus from the saline group showed the pyramidal layer illustrated the pyramidal cells with prominent nucleoli (Fig. 8A). Sections of the hippocampus from PTZ control group showed neurodegeneration in the pyramidal layer. Some cells appeared with normal nuclei and others were shrunken pyramidal cells which have dark eosinophilic cytoplasm and pyknotic nuclei (Fig. 8B). The group treated with PTZ and nefopam 20 mg/kg, showed moderate improvement of pyramidal layer with prominent nuclei and mild pyknotic nuclei (Fig. 8C). Treatment with the higher dose of nefopam (40 mg/kg) resulted in nearly normal histological structure of pyramidal cells with prominent nuclei and very few pyknotic nuclei (Fig. 8D). Sections of the hippocampus from the PTZ and nefopam/phenytoin group showed more or less nearly normal histological structure of preserved pyramidal cells with prominent nuclei and few pyknotic nuclei (Fig. 8E).

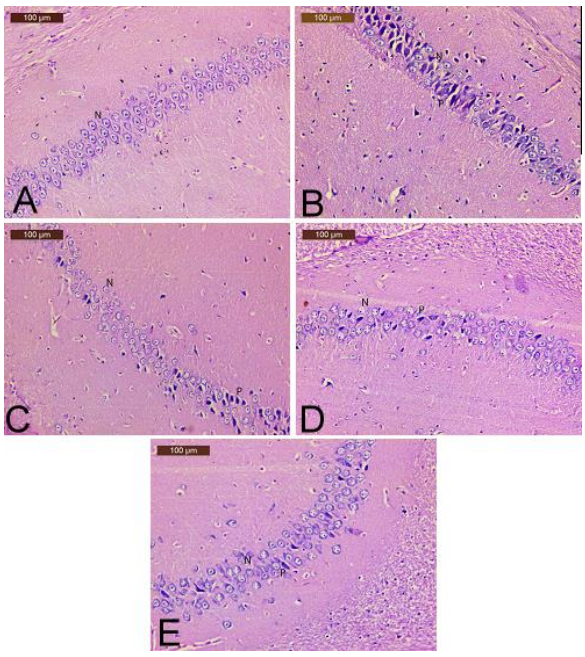

Figure 8. Representative photomicrographs of the cerebral cortex after treatment with (A) Saline: shows well organized the pyramidal cells with prominent nuclei (N); (B) PTZ group shows neurodegeneration in the pyramidal layer with some cells appearing with normal nuclei (N) and other shrunken pyramidal cells which have dark eosinophilic cytoplasm and pyknotic nuclei (P); (C) PTZ and nefopam 20 mg/kg shows moderate improvement of pyramidal layer with prominent nuclei (N) and mild pyknotic nuclei (P); (D) PTZ and nefopam 40 mg/kg shows nearly normal

histological structure of pyramidal cells with prominent nuclei (N) and very few pyknotic nuclei (P). (E) PTZ and nefopam 40 mg/kg and phenytoin shows more or less nearly normal histological structure of preserved pyramidal cells with prominent nuclei (N) and few pyknotic nuclei (P).

4. Discussion

The results of the present study provided the evidence that the non-opiate analgesic nefopam is able to efficiently inhibit the development of seizures in a model of status epilepticus in the rat. Nefopam and also phenytoin caused significant inhibition of the epilepsy scores in all stages of seizures. The effect of nefopam was dose-dependent. In particular, generalized tonic-clonic convulsions were markedly suppressed after nefopam at 40 mg/kg to the same degree as that caused by phenytoin or nefopam/phenytoin co-treatment. The threshold dose of the epileptogen that resulted in status epilepticus was also markedly increased by nefopam at 40 mg/kg. Nefopam in addition resulted in significantly more increase in latency time to reach status epilepticus compared with either phenytoin or nefopam/phenytoin co-treatment. Moreover, the associated neuronal injury was prevented by nefopam in a dose-dependent manner. These effects of nefopam were comparable with those produced by the standard antiepileptic drug phenytoin. The study in addition showed that the combined treatment of nefopam and phenytoin was not superior to that caused by phenytoin alone. This finding suggests that nefopam did not alter the anticonvulsive effect of phenytoin. Other studies have reported antiseizure effect for nefopam against electrically induced seizures in mice. Nefopam given at a dose of 5 mg/kg increased the threshold for electric seizures and enhanced the effect of phenytoin or phenobarbital [20].

Our findings demonstrated an increase in malondialdehyde, a marker for lipid peroxidation and a decrease in the level of antioxidant reduced glutathione in brain of PTZ-treated animals. There was also a marked rise in brain nitric oxide, the high level of which is neurotoxic and contributes to neurodegeneration in various brain pathologies, through the formation of the highly reactive species peroxynitrite (ONOO–) [21]. These observations are supported by other studies [22,23] and provide evidence that PTZ induces an increase in reactive oxygen/nitrogen metabolites and oxidative stress in the brain. This increase in reactive oxygen and nitrogen species contributes to the initiation of epileptic seizures as well as brain damage encountered in such conditions [24,25]. In the present study, we found that nefopam exerted an antioxidative action in TTZ-treated rats by decreasing MDA and nitric oxide, and increasing GSH levels. Results from the present study also indicated a significant inhibition of paraoxonase-1 activity by PTZ, a finding which is consistent with earlier observations [22,23,26]. Paraoxonase-1 has both anti-inflammatory and antioxidative properties [27,28] and is suggested to have a neuroptective effects [29]. It is inactivated by oxidative stress [30], which could explain the decrease in enzyme activity in brain of epileptic rats. Conversely, the restoration in enzyme activity by treatment with nefopam is indicative of a lowered level of oxidative stress.

The use of PTZ to induce epileptic seizures in experimental animals represents a useful model for screening newer compounds with potential anti-seizure activity [31]. The agent is an antagonist of the γ-aminobutyric acid (GABA)-A receptors and therefore may initiate seizures through an inhibitory effect on GABA-ergic neurotransmission [32]. The epileptogen may also up-regulates excitatory NMDA glutamate receptors in cerebral cortex and hippocampus [33]. Glutamate is the main excitatory neurotransmitter in brain and mediates its effects *via* ionotropic and metabotropic glutamate receptors. There is evidence that involves NMDA glutamate receptor subtype on glutamatergic neurons and GABA-ergic interneurons in the initiation and propagation of epileptic seizures as well as the neurodegeneration encountered in the epileptic brain [34,35]. Glutamate levels in the extracellular fluid are raised in temporal lobe epilepsy, which then *via* stimulation of *N*-methyl-D-aspartate receptors induces excitotoxic neuronal damage [36]. Infusion of NMDA into the hippocampal formation induced neuroinflammation and degeneration of hippocampal cells along with upregulation of GluN1 and GluN2B subunits of NMDA receptor [37]. Nefopam is structurally related to diphenhydramine and orphenadrine [5.6], which exert antagonistic activity at the phencyclidine binding site of NMDA receptors [38]. Nefopam does not alter GABA-ergic neurotransmission. The drug, however, has been shown to protect against NMDA-receppr mediated neurotoxicity]39]. It prevented calcium influx and NMDA-mediated excitotoxicity following stimulation of L-type voltage sensitive calcium channels *in vitro* [40]. Nefopam was found effective against the seizures induced in mice by agonists of *N*-methyl-D-aspartate receptors as well as against seizures caused by activation of kainate receptors. The drug may alter GABA-ergic neurotransmission *via* blocking the voltage-sensitive sodium channels, which then reduces glutamate release and decreases the neuronal excitability caused by the stimulation of the glutamate receptors [13].

5. Conclusions

This study demonstrates the suppressive effect of nefopam on the PTZ-induced epileptic seizures and neuronal injury. It is suggested that the anti-seizure actions of nefopam on PTZ-induced status epilepticus may be due a decrease in glutamatergic neurotransmission and consequently excitotoxic neuronal injury.

6. Conflicts of interest

There are no conflicts to declare.

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