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ORIGINAL ARTICLE

Elucidation of the Molecular Mechanism of Tempol in Pentylenetetrazol-induced Epilepsy in Mice: Role of Gamma-aminobutyric Acid, Tumor Necrosis Factor-alpha, Interleukin-1β and C-Fos

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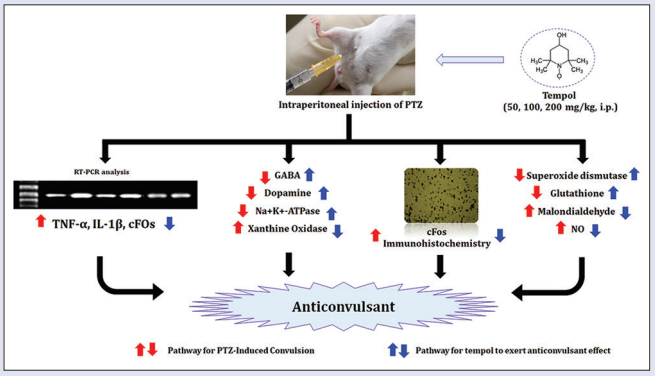
ABSTRACT

Background: Epilepsy is a chronic neurological disorder occurred due to periodic neuronal discharge and imbalance in brain electrical activity. 4-Hydroxy-TEMPO (Tempol) is a membrane-permeable radical scavenger moiety. **Aim:** The aim of this study is to evaluate the anticonvulsant potential of tempol against pentylenetetrazol (PTZ)-induced seizures in mice. **Materials and Methods:** Convulsion was produced in the male Swiss albino mice by administration of PTZ (90 mg/kg, i.p.). Mice were pretreated with either vehicle, tempol (50, 100 and 200 mg/kg, i.p.) or diazepam (5 mg/kg). Various behavioral, biochemical, molecular, and histological parameters were evaluated. **Results:** Mice pretreated with tempol (100 and 200 mg/kg) showed significantly ($P < 0.01$ and $P < 0.001$) delayed-onset on tonic-clonic convulsion, decrease the duration of convulsions and mortality in mice. Intraperitoneal administration of PTZ resulted in significant increase in oxido-nitrosative stress, whereas it significantly ($P < 0.01$ and $P < 0.001$) inhibited by the tempol administration. There was significant increased ($P < 0.01$ and $P < 0.001$) in the levels of brain monoamines (gamma-aminobutyric acid [GABA] and dopamine) and Na^+ K^+ ATPase activity, whereas significant decreased ($P < 0.01$ and $P < 0.001$) in xanthine oxidase activity in tempol pretreated mice. PTZ-induced up-regulated mRNA expressions of tumor necrosis factor-alpha, interleukin-1 beta , and c-Fos were significantly inhibited ($P < 0.01$ and $P < 0.001$) by tempol. It is also significantly down-regulated ($P < 0.05$ and $P < 0.001$) immunohistochemical c-Fos expressions. **Conclusion:** Pretreatment with tempol attenuates PTZ-induced tonic-clonic seizures via its anti-inflammatory, anti-oxidant and GABAergic potential.

Key words: 4-Hydroxy-TEMPO, c-Fos, convulsion, dopamine, epilepsy, gamma-aminobutyric acid, immunohistochemistry, interleukin-1 beta, pentylenetetrazol, tumor necrosis factor-alpha

SUMMARY

- Tempol is a membrane-permeable radical scavenger has an ability to crosses the blood-brain barrier. Pre-treatment with tempol attenuates PTZ-induced tonic-clonic seizures via its anti-inflammatory, anti-oxidant and GABAergic potential. This neuroprotective effect of tempol was exerted via inhibition of oxido-nitrosative stress, anti-inflammatory markers (TNF-α and IL-1β) and cFos expression as well as activation of brain neurotransmitter (GABA and dopamine) and membrane-bound inorganic enzymes in mice.



Abbreviations used: GABA: Gamma-aminobutyric acid; PTZ: Pentylenetetrazol; TNF-α: Tumor necrosis factor-alpha; 4-HT: 4-Hydroxy-TEMPO; DZP: Diazepam; GSH: Reduced glutathione; NO: Nitric oxide; MDA: Malondialdehyde; SOD: Superoxide dismutase; ROS: Reactive oxygen species; IL-1β: Interleukin-1 beta.

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INTRODUCTION

Epilepsy is a common chronic neurological disorder characterized by spontaneous recurrent seizures. This occurred due to periodic neuronal discharge, excessive cerebral neurons activation, and imbalance in brain electrical activity.[1] It has been reported as epilepsy is the second most chronic neurological disorder that affects 1%–2% of the world population.[2] The reported prevalence of chronic epilepsy is of 4–10/1000 people, whereas its incidence is common in children below the age of 7 years and individuals of above 55 years.[3] According to the World Health Organization, about 70 million people affected

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worldwide due to epilepsy among which 90% affected epileptic people present in developing countries such as India.[4] In India, it has been reported that overall prevalence of epilepsy is 3.0–11.9/1000 individual and incidence is of 0.2–0.6/1000 population in a year.[5] Thus, it is becoming the important medical problem and needs urgent medical attention.

There is an array of ictogenesis pathologies responsible for induction of maintenance of various neurodegenerative diseases such as epilepsy, Parkinson's disease, Alzheimer's disease, and ischemia.[6] It includes imbalance among excitatory and inhibitory neurotransmitters, hyperexcitability, and altered function of synaptic junctions. It has been well documented that an imbalance in the excitatory (glutamate) and inhibitory (gamma-aminobutyric acid [GABA]) neurotransmitters are the most important mechanism responsible for cell death in epileptic seizures.[7] Thus, considering these multifactorial neurochemical abnormalities in epileptic seizures, the various researcher has attempted to develop the antiepileptic drugs (AEDs) that help in reducing the neuronal damage as well as delayed in the onset of epileptic seizures.[8]

Currently, available synthetic AEDs includes sodium channel blocker, GABA-A receptors antagonist, glutamate blockers, etc. have been implicated in the management of epilepsy.[9] However, these available synthetic AEDs are associated with unwanted adverse events, high risk of toxicity, and drug interactions. Furthermore, they can provide adequate control for epileptic seizures in the only significant proportion of patients.[10] In addition, these associated side effects also produce significant economic burden in the healthcare management of epilepsy.[11] In spite of tremendous advances in the pharmaceutical drug industry, the availability of drugs capable of ameliorating epilepsy is still limited. Thus, there is an urgent need for developing newer AEDs with lower side effect and cost.

Animal models play a significant role in the development of new AEDs.[12] Generalized-onset motor seizures or tonic-clonic seizure is the most common type of clinical epilepsy.[13] Hence, pentylenetetrazol (PTZ)-induced seizures in mice the most common, reliable, reproducible, and widely used animal model to understand the pathogenesis as well as to develop new AEDs against the seizures.[14] In the last couple of decades, the considerable progress has been occurred in determining the active ingredients from the herbal origin for the treatment of different human ailments. One such frequently used moiety is Tempol, i.e., 4-Hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl (4-Hydroxy-TEMPO). Researchers have determined the potential of tempol for their pharmacological activities against the various disease state including oxidative stress, chronic kidney disease, diabetes, nephropathy, neuropathy, obesity, hyperlipidemia, anxiety, colitis, cardiac fibrosis, hypertension, and nonalcoholic fatty liver disease.[15,16] The presence of tempol radicals have been documented in the leaves of *Vitis vinifera*.[17] It has been revealed that tempol can increase the antioxidant potential of various herbal medicines from natural sources including white seaberry (*Hippophae rhamnoides*), *Chrysophyllum inornatum, Machaerium villosum, Pterogyne nitens, Pera glabrata, Iryantera juruensis, Copaifera langsdorffii*, etc.[18] Numerous evidence showed that tempol is a low molecular weight (172 g/mol) moiety;[19] thus, it is a membrane-permeable radical scavenger has an ability to crosses the blood-brain barrier.[20] The researcher showed its neuroprotective potential for brain trauma and cerebral ischemia.[21] To the best of our knowledge, however, studies of anticonvulsant activity of tempol against PTZ-induced seizure have not been carried out. Hence, this study aimed to evaluate the anti-convulsion potential of tempol by using PTZ-induced convulsions by evaluation of various behavioral, biochemical, molecular, and histological parameters in mice.

MATERIALS AND METHODS

Experimental animals

Adult male Swiss albino mice (18–22 g) were purchased from the National Institute of Biosciences, Pune (India). They were maintained at 24°C ± 1°C with a relative humidity of 45%–55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro-industries Ltd., Sangli, India) and water throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The experiment was performed by the guidelines of Committee for Control and Supervision of Experimentation on Animals, Government of India on animal experimentation. Animals were brought to the testing laboratory 1 h before the experimentation for adaptation purpose. The experimentation was carried out in noise-free area.

Experimental design

A previously reported protocol was followed to induced convulsion using PTZ (Sigma Chemical Co., St Louis, MO, USA).[14] Mice were randomly divided various groups (n = 6), namely, normal (distilled water (DW), 200 mg/kg, p.o.), PTZ control (DW, 10 mg/kg, p.o.), diazepam (DZP) (5 mg/kg, i.p.), and tempol (50, 100, and 200 mg/kg, i.p.).[22] All animals (except normal received saline [10 mg/kg, i.p.]) received PTZ (90 mg/kg, i.p.) 45 min after administration treatment. Immediately after PTZ administration mice were observed for next 30 min for symptoms such as the onset of convulsion, duration of clonic convulsion, duration of tonic convulsion, and incidence (number of mice showing convulsions) and mortality. Locomotor activity was determined by using actophotometer according to the previously reported method.[14]

Brain gamma-aminobutyric acid, dopamine, oxido-nitrosative stress, Na-K-ATPase, and xanthine oxidase estimation

Mice were sacrificed as soon as the onset of convulsions occurs, and the brain was isolated immediately. Brain GABA, dopamine, superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (malondialdehyde [MDA] content), nitric oxide (NO content), Na^+ K^+ ATPase, and xanthine oxidase (XO) as described previously.[14,23,24]

Determination of tumor necrosis factor-alpha, interleukin-1 beta, and c-Fos by real-time polymerase chain reaction in mice brain hippocampus

The levels of mRNA were analyzed in brain hippocampus tissue using real-time polymerase chain reaction. Single-stranded cDNA was synthesized from 5 μg of total cellular RNA using reverse transcriptase kit (MP Biomedicals India Pvt. Ltd., India). The primer sequence was selected on the basis of the previous study.[25] Expression of all the genes was assessed by generating densitometry data for band intensities in different sets of experiments and was generated by analyzing the gel images on the Image J software (Version 1.33, Wayne Rasband, National Institutes of Health (NIH), Bethesda, MD, USA) semiquantitatively.

Determination of c-Fos by immunohistochemistry in mice brain hippocampus

The flash frozen brain stored at −80°C and immunohistochemistry for c-Fos protein was carried out as described previously.[26] The c-Fos antibody (Santa Cruz Biotechnology, USA) was a rabbit polyclonal

antibody raised against a peptide mapping at the amino terminus of human c-Fos p62. The c-Fos-positive neurons were identified by the presence of dense immunohistochemical staining within the dentate gyrus of the hippocampus and medial prefrontal cortex under a light microscope.

Statistical analysis

Data were expressed as mean ± standard error mean and analyzed using GraphPad Prism 5.0 (GraphPad, San Diego, USA). The gel image and immunohistochemistry were analyzed by using the Image J program (Version 1.33, Wayne Rasband, NIH, Bethesda, MD, USA) semiquantitatively. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of tempol on pentylenetetrazol-induced alteration in duration and onset of tonic-clonic convulsion as well as mortality in mice

The onset of convulsion was increased significantly ($P < 0.05$) and duration of tonic-clonic convulsion in decreased significantly ($P < 0.05$) in DZP (5 mg/kg) pretreated mice as compared to PTZ control mice. Tempol (100 and 200 mg/kg) pretreatment also showed significant protection ($P < 0.05$ and $P < 0.01$) against PTZ-induced onset of convulsion and duration of tonic-clonic convulsion when compared with PTZ control mice [Table 1].

Administration of PTZ resulted in mortality (100%) in mice, whereas administration of normal saline did not show any mortality in normal mice. When compared with PTZ control mice, DZP (5 mg/kg) pretreated mice did not show any death in mice and thus it significantly ($P < 0.05$) protected PTZ-induced mortality in mice. Tempol (50, 100, and 200 mg/kg) pretreatment significantly ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively) and dose-dependently inhibited PTZ-induced mortality as compared to PTZ control mice [Table 1].

Effects of tempol on pentylenetetrazol-induced alteration in locomotor activity in mice

There was no significant difference in the locomotor activity of normal mice before intraperitoneal injection of saline as compared to after injection of saline. Furthermore, the locomotor activity did not differ significantly before treatment in normal, PTZ control as well as DZP (5 mg/kg) and tempol (50, 100, and 200 mg/kg) treated mice. Administration of DZP (5 mg/kg) resulted in significantly decreased ($P < 0.05$) locomotor activity as compared to normal mice. Tempol (50, 100, and 200 mg/kg) pretreated mice also showed significant and dose-dependent ($P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively) in the locomotor activity as compared to normal mice [Table 1].

Effect of tempol on pentylenetetrazol-induced alteration in oxido-nitrosative stress in mice

There was statistically significant decrease ($P < 0.001$) in brain SOD and GSH levels whereas significant increased ($P < 0.05$) in brain MDA and NO levels in the PTZ control mice as compared to normal mice. Pretreatment with DZP (5 mg/kg) significantly ($P < 0.001$) attenuated PTZ-induced elevated oxidative stress as compared to PTZ control mice. Tempol (100 and 200 mg/kg) pretreatment significantly and dose-dependently ($P < 0.001$) inhibit PTZ-induced increased brain oxido-nitrosative as compared to PTZ control mice [Table 2].

Effect of tempol on pentylenetetrazol-induced alteration in brain gamma-aminobutyric acid, dopamine, Na^+ K^+ ATPase, and xanthine oxidase level in mice

PTZ caused a significant decrease ($P < 0.001$) in brain GABA, dopamine and Na^+ K^+ ATPase activity whereas significantly ($P < 0.001$) increased in XO activity in PTZ control mice as compared to normal mice.

Table 1: Effects of 4-hydroxy-TEMPO and diazepam on pentylenetetrazol-induced duration and onset of tonic-clonic convulsions and locomotor activity in mice

Treatment	Onset of convulsion (sec)	Duration of convulsion (sec)		Number convulsed/ number of used	Mortality (% death)	Locomotor activity (before treatment)	Locomotor activity (after treatment)	Reduction in activity (%)
		Tonic	Clonic					
Normal	-	-	-	-	-	531.40±1.80	530.20±2.85	0.22
PTZ control	7.36±0.93	57.79±1.43	85.54±1.4	6/6	6/6 (100.00)	-	-	-
DZP (5)	29.19±1.32***	12.49±1.84***	25.64±1.07***	0/6[b]	0/6 (0.00)[$$$]	528.20±2.78	83.80±2.35***	84.13
4-HT (50)	11.51±0.84	55.07±1.75	80.41±5.56	6/6	4/6 (66.67)[$]	536.00±1.34	440.60±2.78*	17.79
4-HT (100)	17.04±1.54*	43.15±1.21**	52.38±2.07**	3/6[a]	2/6 (33.33)[$$]	532.60±2.42	332.20±2.59***	37.62
4-HT (200)	27.07±1.36***	30.1±1.36***	32.68±1.07***	0/6[b]	0/6 (0.00)[$$$]	532.00±2.28	233.60±2.82***	56.09

Data are expressed as mean±SEM (n=6) in each group. Data of "incidence of convulsion" and "mortality" were analyzed by χ^2 [$]$P<0.05$, [$$]$P<0.01$ and [$$$]$P<0.001$ as compared to PTZ treated mice. [a]$P<0.01$ and [b]$P<0.001$ as compared to PTZ treated mice. Data of duration, the onset of tonic-clonic convulsions and locomotor activity was analyzed by one-way ANOVA followed by Dunnett's test *$P<0.05$, **$P<0.01$, and ***$P<0.001$ as compared to normal mice. PTZ: Pentylenetetrazol; DZP: Diazepam; 4-HT: 4-hydroxy-TEMPO; SEM: Standard error of the mean

Table 2: Effects of 4-hydroxy-TEMPO and diazepam on pentylenetetrazol-induced alteration in brain oxido-nitrosative stress, gamma-aminobutyric acid, dopamine, Na^+K^+-ATPase, and xanthine oxidase activity in mice

Treatment	SOD (U/mg of protein)	GSH (µg/mg of protein)	MDA (nM/mg of protein)	NO (mg/mL)	GABA (ng/g of brain tissue)	DA (ng/g of brain tissue)	Na^+K^+ATPase (µmol/ mg of protein)	XO (U/g of protein)
Normal	13.40±0.20	2.68±0.03	3.02±0.23	0.14±0.004	56.30±1.66	73.78±1.27	19.23±0.24	3.15±0.18
PTZ control	4.38±0.33[###]	0.70±0.03[###]	8.83±0.31[###]	0.24±0.005[###]	17.46±2.27[###]	37.60±1.69[###]	5.04±0.26[###]	5.04±0.20[###]
DZP (5)	11.32±0.16***	2.27±0.03***	3.84±0.34***	0.16±0.005***	49.86±3.32***	56.92±2.20***	7.59±0.22***	3.79±0.28***
4-HT (50)	6.12±0.36*	0.96±0.02**	8.19±0.31	0.222±0.008	22.90±1.96	41.92±1.91	4.62±0.17	4.91±0.14
4-HT (100)	8.19±0.40***	1.89±0.03***	7.07±0.35**	0.21±0.007*	35.41±1.59**	51.51±0.59**	9.35±0.26***	4.32±0.20
4-HT (200)	10.93±0.34***	2.19±0.01***	4.42±0.21***	0.168±0.004***	52.27±3.28***	57.20±2.78***	13.52±0.21***	3.77±0.15***

Data are expressed as mean±SEM (n=6) in each group. Data were analyzed by one-way ANOVA followed by Dunnett's test [###]$P<0.001$ as compared to normal mice whereas *$P<0.05$, **$P<0.01$ and ***$P<0.001$ as compared to PTZ control mice. PTZ: Pentylenetetrazol; DZP: Diazepam; 4-HT: 4-hydroxy-TEMPO; SOD: Superoxide dismutase; GSH: Reduced glutathione; MDA: Malondialdehyde; NO: Nitric oxide, DA: Dopamine; GABA: Gamma amino butyric acid; XO: Xanthine oxidase; SEM: Standard error of the mean

When compared with PTZ control mice, DZP (5 mg/kg) pretreated mice showed statistically significant ($P < 0.001$) increase in brain GABA, dopamine, and Na^+ K^+ ATPase activity as well as statistically significant ($P < 0.001$) decrease in brain XO activity. Tempol (100 and 200 mg/kg) pretreated mice also showed statistically significant and dose-dependent ($P < 0.01$ and $P < 0.001$) attenuation in alterations of brain GABA, dopamine, Na^+ K^+ ATPase and XO activity as compared to PTZ control mice [Table 2].

Effect of tempol on pentylenetetrazol-induced alteration in tumor necrosis factor-alpha, interleukin-1 Beta and c-Fos mRNA expression in mice

The mRNA expression of brain tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and c-Fos in PTZ control mice was up-regulated significantly ($P < 0.001$) as compared to normal mice. DZP (5 mg/kg) pretreated mice showed a significant down-regulation ($P < 0.01$, $P < 0.001$ and $P < 0.001$) in brain TNF-α, IL-1β and c-Fos mRNA expression as compared to PTZ control mice. When as compared to PTZ control mice, tempol (100 and 200 mg/kg) pretreated mice showed statistically significant, and dose-dependent down-regulation ($P < 0.01$ and $P < 0.001$) in brain TNF-α, IL-1β, and c-Fos mRNA expression [Figure 1].

Effect of tempol on pentylenetetrazol-induced histological alteration in mice brain

Histological analysis of brain tissue from normal mice showed the normal architecture of neurons without any inflammation and necrosis. It is devoid of any pyknosis reflected by the presence of darkly-stained nucleus and cytoplasm [Figure 2a]. Intraperitoneal administration of PTZ resulted in inflammatory infiltration with the presence of necrosis. It showed the presence of pyknosis with a reduced number of neurons. The arrangement of neurons was irregular in the brain of PTZ control mice [Figure 2b]. Histology of brain tissue from DZP (5 mg/kg) and tempol (100 and 200 mg/kg) pretreated mice showed the presence of mild inflammatory infiltration and necrosis [Figure 2c, e and f, respectively]. It did not show the presence of any pyknosis, and also the neuronal arrangement was linear. Tempol (50 g/kg) pretreated mice showed the distorted architecture of brain tissue reflected by the presence of inflammatory infiltration, pyknosis, and necrosis [Figure 2d].

Effect of tempol on pentylenetetrazol-induced alteration in c-Fos expression in mice hippocampus

Figure 3 depicts the effect of Tempol and DZP on PTZ-induced alteration in c-Fos expression in mice hippocampus. The c-Fos expression was up-regulated significantly ($P < 0.001$) in PTZ control mice as compared to normal mice. Pretreatment with DZP (5 mg/kg) showed significant attenuation ($P < 0.05$) in this PTZ-induced up-regulated in c-Fos expression in mice hippocampus as compared to PTZ control mice. The up-regulated c-Fos expression in mice hippocampus was significantly and dose-dependently ($P < 0.05$ and $P < 0.001$) down-regulated by pretreatment with tempol (100 and 200 mg/kg) as compared to PTZ control mice [Figure 3].

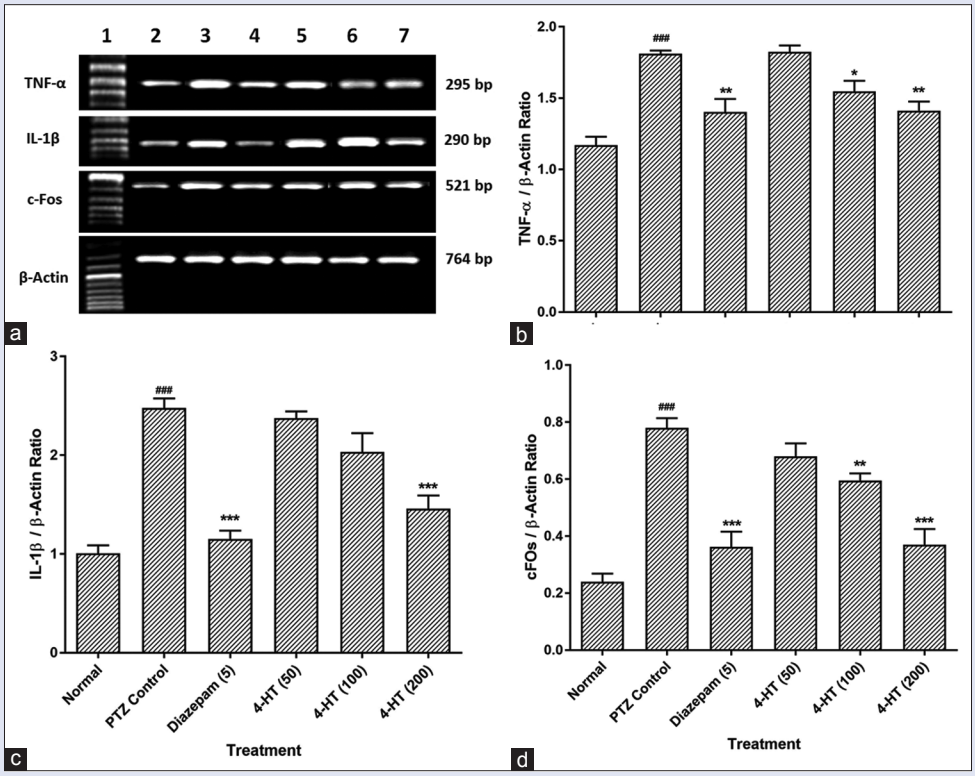

Figure 1: Effects of tempol and diazepam on pentylenetetrazol-induced alterations in tumor necrosis factor-alpha, interleukin-1 beta, and c-Fos mRNA expression in mice brain hippocampus (a), quantitative representation of the mRNA expression of tumor necrosis factor-alpha (b), interleukin-1 beta (c), and c-Fos (d). Data are expressed as mean ± standard error mean. $n = 4$ in each group. Data were analyzed by one-way ANOVA followed by Dunnett's test. *$P < 0.05$, and ***$P < 0.001$ as compared to pentylenetetrazol control mice. PTZ: Pentylenetetrazol; DZP: Diazepam; 4-HT: Tempol; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1 beta

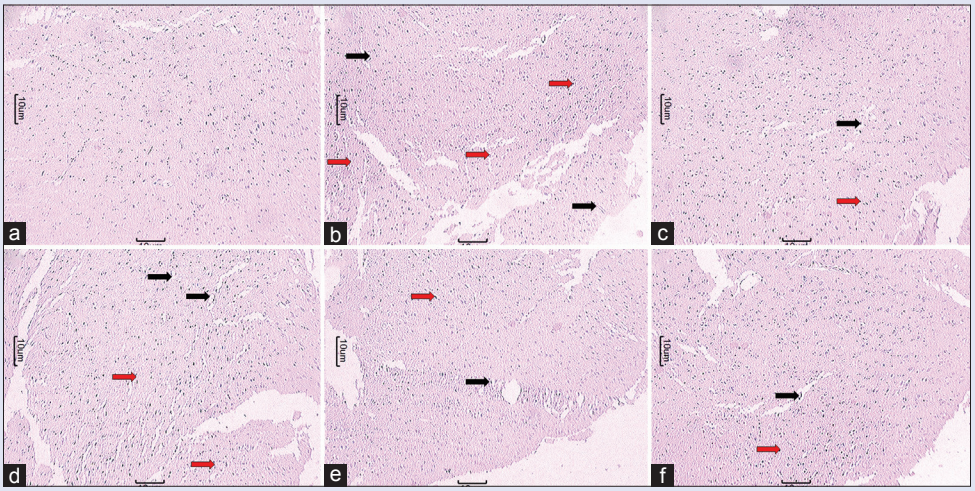

Figure 2: Effects of tempol and diazepam on pentylenetetrazol-induced alterations in brain histopathology in mice. Photomicrograph of sections of brain of normal (a), pentylenetetrazol control (b), diazepam (5 mg/kg) (c), tempol (50 mg/kg) (d), tempol (100 mg/kg) (e) and tempol (200 mg/kg) (f) treated group. Images at ×40. Edema (black arrow) and inflammatory infiltration (red arrow)

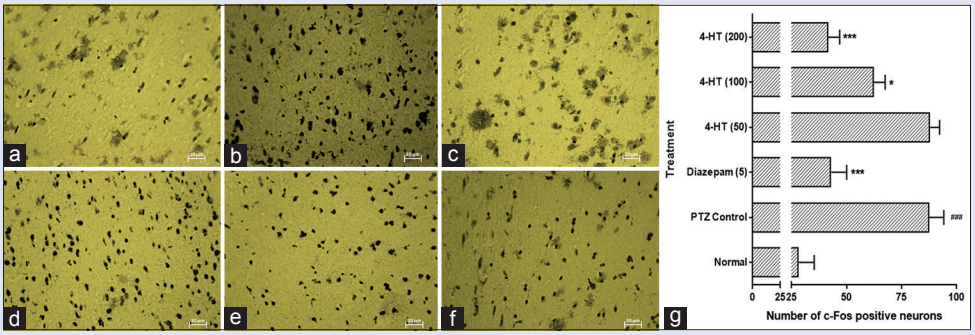

Figure 3: Effects of tempol and diazepam on pentylenetetrazol-induced alterations in c-Fos expression in mice brain hippocampus. Representative images of sections of brain of normal (a), pentylenetetrazol control (b), diazepam (5 mg/kg) (c), tempol (50 mg/kg) (d), tempol (100 mg/kg) (e) and tempol (200 mg/kg) (f) treated group. The quantitative representation of tempol and diazepam on pentylenetetrazol-induced alterations in c-Fos expression (g). Data are expressed as mean ± standard error mean. $n = 4$ in each group. Data were analyzed by one-way ANOVA followed by Dunnett's test. *$P < 0.05$, and ***$P < 0.001$ as compared to PTZ control mice. PTZ: Pentylenetetrazol; DZP: Diazepam; 4-HT: Tempol

DISCUSSION

Epilepsy is a chronic disorder of abnormal and hypersynchronous release of cortical neurons. There is various underlying mechanism has been established for epilepsy which includes the imbalance between excitatory and inhibitory pathways of the brain, abnormalities in the voltage and ion gated channels, increase in the level of cyclic guanosine monophosphate (cGMP), the release of reactive oxygen species (ROS), etc. In the present investigation, epilepsy was induced in the mice by intraperitoneal administration of PTZ that resulted in the production of tonic-clonic seizures. Furthermore, anticonvulsant effect of tempol was evaluated against intraperitoneal administration of PTZ, results showed that tempol significantly attenuated the PTZ-induced convulsion by inhibiting elevated levels of oxido-nitrosative stress, inflammatory release (TNF-α and IL-1β) and improved release monoamines (GABA and dopamine) along with membrane-bound inorganic phosphate enzymes (Na^+ K^+ ATPase) to decrease incidence and mortality in PTZ-induced convulsion.

It has been well documented that, various neurotransmitter such as GABA, dopamine, serotonin (5-hydroxytryptamine) played a vital role in the induction, spread, maintenance and termination of epileptic seizures.[14] The alteration in the balance between excitatory and inhibitory neurotransmitter pathways in the brain resulted in epileptogenesis. The available evidence suggested that GABA, dopamine, serotonin, and noradrenaline involved in the pathways of epileptogenesis. Furthermore, PTZ-induced convulsion also associated with a decrease in these levels of neurotransmitters. Among various experimental seizure models, the levels of glutamate, serotonin, GABA, aspartate, and taurine been found to decrease in the brain region.[14] Various clinical studies also implicate the decrease in the activity of this neurotransmitter during the epileptic period.[27,28] Thus, most of the AEDs was developed to increase the potential of inhibitory monoamines such as GABA and dopamine as well as to reduce the levels of excitatory neurotransmission in the brain. In the present investigation, intraperitoneal administration of PTZ resulted in a decrease in the level of GABA and dopamine, whereas pretreatment with tempol showed significant protection against the PTZ-induced decrease in these levels of monoamines.

Furthermore, the recent clinical study suggested that the onset and duration of the acute convulsion are depended on the release of various neurotransmitter during the epileptogenesis.[29] The delayed-onset of convulsion is associated with the balanced level of both excitatory (GABA) and inhibitory (glutamic acid) neurotransmitter.[7] In the

present investigation pretreatment with tempol significantly delayed the onset of convulsion which may be due to its GABAergic potential. In addition, an elevated level of brain GABA is also associated with the decreased locomotor activity. Evidence suggesting that the locomotor activity serves as a notion for the alertness and decrease in its activity reflect the sedative effect.[30] Pretreatment with tempol also showed the decrease in the locomotor activity which may be due to an increase in the GABA level in the brain. Results of the present investigation are in line with the findings of the previous investigator where administration AEDs caused a significant decrease in the locomotor activity.[30]

The antioxidant defense system is consists of various endogenous anti-oxidative enzymes such as SOD, glutathione peroxidase (GPx), and substances like GSH.[31] These SOD, GPx, and GSH serve as a prominent cellular defense against oxidative stress by quenching the free radical and thus reduces the elevated ROS levels.[24] The neuroprotective role of this antioxidant is extensively studied during the epileptic seizures. Numerous scientific reviews and studies demonstrate that PTZ-induced seizures are mediated by increases in oxidative stress and a decrease in the levels of antioxidants such as SOD and GSH.[6,32] Findings of the present investigation are also in line with previous investigatory where intraperitoneal administration of PTZ resulted in decreased activities of SOD and GSH.[32] Mice pretreated with tempol showed increased activity of antioxidants suggesting its preventive role against deleterious effects induced by free radicals. Furthermore, a researcher reported that tempol is a cell membrane-permeable amphibolite and it can catalyzes to facilitates hydrogen peroxide metabolism which in turn increases the endogenous levels of SOD.[33] It is also reported to have an ability to reduce the concentration of toxic hydroxyl radicals via Fenton reactions.[33] Thus, tempol may serve as an important antioxidant to detoxifying these ROS, thereby an impairment of antioxidant defense system to alleviates PTZ-induced convulsions.

NO has been reported to play an important role in the pathophysiology of epilepsy via regulating the endothelial permeability to lipoproteins.[23] In addition, it also serves as a vital neuronal messenger or neurotransmitter thus it is an integral part of the central nervous system. It can cause DNA damage and also increases the cGMP level via activation of soluble guanylyl cyclase which played an important role in the regulation of seizure intensity.[34] XO is another important source of oxygen free radical. Increase in the level of cGMP stimulates the XO to release free radical in the tissue, thereby increasing the oxidative stress.[35] In the present investigation, administration of PTZ is associated with elevated levels of NO and XO, whereas administration of tempol attenuates these elevated levels. The previous researcher also showed that treatment with tempol reduces the NO formation in the brain region.[36] Results of the present study are in line with the findings of previous investigator[36] where administration of tempol decreases the release of NO that in turn might attenuate PTZ-induced release of XO to ameliorates convulsions.

A growing body of evidence has suggested that Na^+ K^+ ATPase, a neuronal membrane-bound inorganic phosphatase enzyme plays crucial in the regulation of membrane potential and transmembrane Ca^{2+} fluxes, maintenance of cellular ionic gradient and cell volume.[37,38] The maintenance of Na^+ and K^+ gradients between the intracellular and extracellular compartments is important for basic cellular homeostasis. Imbalance in these results in increased neuronal excitability and convulsions.[37] The decrease in the level of Na^+ K^+ ATPase enzyme causes uncontrolled dendritic discharges in the rat cerebellum's Purkinje cells leads to epileptogenesis in mice.[38] Many preclinical and clinical studies reported that activity of Na^+ K^+ ATPase altered in experimental models of epilepsy[37,38] and postmortem epileptic human brain.[39] In the present investigation, the sensitivity of Na^+ K^+ ATPase enzyme activity impaired by intraperitoneal administration of PTZ injection resulted in hyperexcitability and convulsions. Administration of tempol showed significant improvement in the Na^+ K^+ ATPase enzyme activity in the brain which in accordance with the findings of the previous researcher.[40]

Inflammatory cytokines play a crucial role in mediating the development of epileptic seizures.[6,41] Proinflammatory cytokines such as TNF-α and IL's plays a decisive role in the release of glutamatergic neurotransmission.[42] Experimental studies showed that increased mRNA expression of inflammatory cytokines in rodent forebrain after epileptic seizures.[43] Clinically, human epileptic tissue also showed the elevated IL-1β immunoreactivity[44] and elevated level of cytokines was also reported in the serum and cerebrospinal fluid from epileptic patients.[45] It has been found that IL-1β increases the synthesis of other cytokines such as IL-6 and TNF-α that impaired GABAergic neurotransmission in microglia.[44] Induction of generalized tonic-clonic seizures by PTZ associated with elevated expression of TNF-α and IL-1β in the rat hippocampus after. In the present investigation, there was also an increase in the expressions of brain TNF-α and IL-1β followed by PTZ administration. However, administration of tempol significantly attenuated this PTZ-induced increase in TNF-α and IL-1β expressions in mice brain. These findings confirm the results of the previous study that showed the inhibition of elevated levels of TNF-α and IL's by administration of tempol.[46] The histopathological finding of mice brain also supports this notion where inflammatory infiltration was attenuated by tempol pretreated.

A previous study suggested that cFos expression is commonly implicated in the determination of neuronal activation patterns.[47] Activation of cFos expression also represents the intensity of neurons that undergo the seizure-induced depolarization.[48] Thus, induction of cFos expression is played a crucial role in seizures development.[49] Furthermore, cFos expression labeling has been used widely for determination of neuronal activation patterns in PTZ-induced convulsions.[26] In the present investigation, there was a significant increase in the cFos expression in mice brain after PTZ administration whereas pretreatment with tempol inhibited this PTZ-induced increased in cFos expression. These results of the present study agree with the findings of previous studies that have shown that administration of tempol decreases cFos expression in rodent brain.[15]

An array of plant-based bioactive moieties, such as chrysin, fisetin, rutin, vitexin, Eugenol, α-terpineol, have been shown to possess potent anti-convulsant activity. A recent study showed that the isolated moieties from the herbal origin had been implicated in the clinical management of epilepsy.[50] Various alternative and complementary medication have been clinically used for the treatment of epilepsy in children.[51] Furthermore, oral administration cannabidiol isolated from *marijuana* in a patient with drug-resistant seizures showed a significant reduction in convulsive-seizure frequency.[52] Due to the low molecular weight of tempol, it can be cross-linked with various potent antioxidant molecules derived from natural origin. Thus, this tailored tempol may serve as important therapeutic moieties for targeted drug delivery with site-specific binding in the management of epilepsy. Thus, finding from present investigation may open novel vistas as an alternative option with natural antioxidants like tempol to prevent tonic-clonic seizures.

CONCLUSION

Pretreatment with tempol attenuates PTZ-induced tonic-clonic seizures via its anti-inflammatory, anti-oxidant and GABAergic potential. This neuroprotective effect of tempol was exerted via inhibition of oxido-nitrosative stress, anti-inflammatory markers (TNF-α and IL-1β) and cFos expression as well as activation of brain neurotransmitter (GABA and dopamine) and membrane-bound inorganic enzymes in mice.

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Conflicts of interest

There are no conflicts of interest.

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