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ORIGINAL PAPER

Proanthocyanidins alleviate pentylenetetrazole-induced epileptic seizures in mice via the antioxidant activity

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Abstract

The role of oxidative stress in the initiation and progress of epilepsy is well established. Proanthocyanidins (PACs), a naturally occurring polyphenolic compound, have been reported to possess a broad spectrum of pharmacological and therapeutic properties against oxidative stress. However, the protective effects of proanthocyanidins against epilepsy have not been clarified. In the present study, we used the pentylenetetrazole (PTZ)-induced epilepsy mouse model to explore whether proanthocyanidins could help to reduce oxidative stress and protect against epilepsy. Mice were allocated into four groups (n = 14 per each group): control, PTZ (60 mg/kg, intraperitoneally), PACs + PTZ (200 mg/kg, p.o.) and sodium valproate (VPA) + PTZ (200 mg/kg, p.o.). PTZ injection caused oxidative stress in the hippocampal tissue as represented by the elevated lipid peroxidation and NO synthesis and increased expression of iNOS. Furthermore, depleted levels of anti-oxidants, GSH, GR, GPx, SOD, and CAT also indicate that oxidative stress was induced in mice exposed to PTZ. Additionally, a state of neuroinflammation was recorded following the developed seizures. Moreover, neuronal apoptosis was recorded following the development of epileptic convulsions as confirmed by the elevated Bax and caspase-3 and the decreased Bcl2 protein. Moreover, AChE activity, DA, NE, 5-HT, brain-derived neurotrophic factor levels, and gene expression of Nrf2 have decreased in the hippocampal tissue of PTZ exposed mice. However, pre-treatment of mice with PACs protected against the generation of oxidative stress, apoptosis, and neuroinflammation in the PTZ exposed mice brain as the biomarkers for all these conditions was bought to control levels. In addition, the gene expression of Nrf2 was significantly upregulated following PACs treatment. These results suggest that PACs can ameliorate oxidative stress, neuroinflammation, and neuronal apoptosis by activating the Nrf2 signaling pathway in PTZ induced seizures in mice.

Keywords Epilepsy · Proanthocyanidins · Pentylenetetrazole · Oxidative stress · Neuroinflammation · Nrf2

Introduction

Epilepsy is the most common chronic neurological disorder characterized by recurrent spontaneous seizures. People of all ages, gender, ethnicity and social classes are affected (Beghi 2020). Globally, nearly 65 million people have epilepsy; with the increase in life expectancy, the burden of such population is increased as it correlates with a high number of incidences, such as infections of the brain, stroke, and traumatic brain injury (Billakota et al. 2020; Khan et al. 2020). Several possible pathogeneses for epilepsy include neurotransmitters, synapses, receptors, inflammatory cytokines, oxidative stress, apoptosis, and mitochondrial dysfunction (He et al. 2021). The critical pathogenic mechanism involved in epilepsy and most other neurodegenerative disorders is oxidative stress (Reynolds et al. 2007). Epilepsy patients (Dickstein et al. 2019; Gershen et al. 2015) and animal models of the disease have been frequently reported to suffer neuroinflammation (de Zorzi et al. 2019; Vezzani et al. 2019). Thus, anti-inflammatory drugs are used for the treatment of epilepsy and the prevention of its longstanding outcomes, such as cognitive decline or the progression of seizures (Dhir 2020; Terrone et al. 2019). However, long-term clinical use of these anti-antiepileptic chemicals may

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produce some adverse side effects (Perucca and Gilliam 2012). Thus, further research that focuses on treatment that modifies the underlying neuropathological process of epilepsy and has minimum side effects is urgently required. Natural products derived from drugs with lower side effects and lesser chances of drug interaction are currently being explored to manage epilepsy in a better way (Chen et al. 2020; Khoei et al. 2020).

Studies have shown that natural drugs containing compounds such as catechin (Ahmad et al. 2020), epigallocatechin gallate (Qu et al. 2019), luteolin (Tambe et al. 2017), and Curcumin (Kiasalari et al. 2013) have been used to treat epilepsy by regulating oxidative stress (OS). Proanthocyanidins (PACs) are ubiquitous naturally occurring hydrophilic phenolic phytochemicals with plausible health benefits, including protection against oxidative stress, cytotoxicity, inflammation, and cancer. Similar to vitamins C and E, PACs can help to efficiently scavenge reactive oxygen species (ROS) (Bagchi et al. 2003). Hence, numerous studies, *in vitro* and *in vivo*, have demonstrated that these agents may be helpful in the prevention and treatment of OS-related cardiovascular, neurodegenerative, metabolic, and inflammatory diseases, as well as various cancers. Furthermore, PACs are relatively less expensive than synthetic compounds and show low toxicity and side effects (Yang et al. 2018). Recently, the neuroprotective effects of Procyanidin B2 (a type of PACs) were examined in primary cultures of rat cerebellar granule neurons exposed to various stressors. It was confirmed that PACs provide neuronal protection against degenerative diseases by scavenging ROS (Sutcliffe et al. 2017). Proanthocyanidins have also been shown to protect ischemic neurons effectively. Furthermore, growing evidence has suggested the effect of PACs on improving blood circulation, visual protection, and edema elimination (Pons et al. 2014; Yang et al. 2016). These observations strongly suggest PACs to be a potential agent for the treatment of epilepsy.

For understanding the mechanism of epilepsy to monitor the effect of antiepileptic drugs, pentylenetetrazole (PTZ) is commonly used to establish experimental seizure models in animals (Yuskaitis et al. 2021). In addition, PTZ is a chemoconvulsants known to reduce GABA and hence induce epileptic activity by inhibiting chloride channels associated with GABAA receptors (Sefil et al. 2014). Thus, the experiments were designed to evaluate the efficacy of PACs in alleviating PTZ-induced epileptic seizures and inspect the mechanism that leads to epileptic seizures to identify potential treatment selection biomarkers.

Materials and methods

Chemicals

Pentylenetetrazole (PTZ) and sodium valproate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Proanthocyanidins were purchased from Bronson (Bronson, MI, USA). PCs extracted from grape seeds (purity > 95%).

Experimental protocol

Adult male Swiss mice weighing 25–32 g were provided from the Animal House Zoology Department at King Saud University under standard laboratory conditions at 22–25 °C and a 12 h artificial light/dark cycle. Mice were given water and commercial pelleted rodent feed *ad libitum* free of access.

According to the Research Ethics Committee (REC), the animals were treated with approval no. KSU-SE-21-78. Using epileptic mouse mode mediated by PTZ, we explore the neuroprotective efficiency of PACs in mice were allocated randomly into four groups (n = 14) after seven days of acclimatization as follows:

1. Control group (Cont): The mice were gavaged with normal saline (0.9% NaCl) daily. Then, Onwere injected intraperitoneally (i.p.) on the 10th day with normal saline after the oral administration.
2. PTZ-injected group (PTZ): The mice were gavaged with normal saline daily for 10 days. On the 10th day, these animals received a single i.p. dose of PTZ (60 mg/kg) according to the previous studies (Al Omairi et al. 2022; Ilhan et al. 2005; Kapucu et al. 2020; Seo et al. 2020; Yuan et al. 2020) 1 h after the oral administration of saline.
3. PACs + PTZ-treated group (PACs + PTZ): This group received daily oral administration of PACs (200 mg/kg) and a single i.p. dose of PTZ (60 mg/kg) on the 10th day. In this investigation, both PTZ and PACs were dissolved in normal saline.
4. Sodium valproate + PTZ treated group: PTZ (60 mg/kg) was i.p. injected to induce epileptic convulsions in mice. In addition, VPA at 200 mg/kg (orally) was administered 30 min prior to PTZ injection.

- The seizure score was carefully monitored and recorded for 40 min after PTZ administration according to the modified Racine scale as follows: Phase 0: no seizure; Phase 1: twitching in ear and face; Phase II: myoclonic jerks without rearing; Phase III: myoclonic jerks with rearing; Phase IV: tonic-clonic seizures as the mouse turning over onto side position; Phase V: generalized

tonic-clonic seizures as the mouse turning over onto back position.

Biochemical markers

Mice were euthanized decapitated post-PAC last treatment by 24 h. The hippocampus was immediately extracted and separated from the mice's brains and washed with isotonic saline. The hippocampal tissue of seven mice was used to evaluate Nrf2 mRNA expression and the different biochemical parameters. First, the tissue was homogenized in ice-cold 10 mM phosphate buffer (pH 7.4) to produce a 10% (w/v) homogenate. Meanwhile, hippocampal tissue of the remaining seven mice was homogenized in 75% methanolic HPLC (10% w/v) and was at 4,000 rpm for 10 min to estimate the monoamines.

Estimation of oxidative stress indices

The hippocampal level of lipoperoxidation (LPO) targeted by malondialdehyde (MDA) was assessed using the thiobarbituric acid method at 535 nm and then existing in terms of MDA produced, as illustrated by Ohkawa et al. (1979). Next, the hippocampal nitric oxide (NO) of the hippocampal level was determined using the Griess reagent (sulfanilic acid and N-(1-naphthyl)ethylenediamine), and the formed azo dye was evaluated at 540 nm (Green et al. 1982). Finally, the glutathione (GSH) level was demonstrated using Ellman's reagent, and the developed yellow chromagen was estimated at 412 nm (Ellman 1959).

Estimation of antioxidant enzyme activities

The activities of hippocampal glutathione reductase (GR) and glutathione peroxidase (GPx) were investigated based on the protocols illustrated by (Factor et al. 1998; Paglia and Valentine 1967), respectively. Additionally, catalase (CAT) activity was estimated according to the method illustrated by Aebi (1984). Meanwhile, hippocampal superoxide dismutase (SOD) activity was determined based on the ability of SOD to suppress nitroblue tetrazolium reduction Sun et al. (1988).

Estimation of pro-inflammation cytokines

Hippocampal level of interleukin-1β (IL-1β; ThermoFisher Scientific, Catalogue number BMS6002), interleukin-6 (IL-6; ThermoFisher Scientific, Catalogue number EMIL6RA), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB; Cusabio, Catalogue number CSB-E12108m), tumor necrosis factor-α (TNF-α; ThermoFisher Scientific, Catalogue number BMS607-3) were measured using enzyme-linked immunosorbent assay kits based on the manufacturer's recommendations.

Table 1 Primer sequences of genes analyzed in real time PCR

Name	Accession number	Forward primer (5'---3')	Reverse primer (5'---3')
GAPDH	NM_001289726.1	GGGTCCCAGCTTAGGTTCATC	TACGGCCAAATCCGTTCACA
Nrf2	NM_001399226.1	CCTCTGTCACCAGCTCAAGG	TTCTGGGCGGCGACTTTATT

The abbreviations of the genes; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Nrf2, nuclear factor erythroid 2-related factor 2

Estimation of apoptotic proteins

Enzyme-linked immunosorbent assay kits of Bax (BioVision, Inc., Catalogue number E4513) and Bcl-2 (BioVision, Inc., Catalogue number CSB-E08854r) were used to detect these apoptotic proteins extracted from the hippocampal tissue. In contrast, caspase-3 activity was measured using a colorimetric kit (Sigma-Aldrich, Catalogue number CASP3C-1KT).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

The total RNA was isolated from the hippocampal using the standard TRIzol® method (Invitrogen, Carlsbad, CA, USA). Then cDNA was created using the RNA. All the primer sequences are listed in Table 1, according to Abdel Moneim (2016). Power SYBR® Green Master Mix was used to perform the RT-qPCR in triplicate. RT-qPCR cycling conditions were 10 min at 95 °C followed by 40 cycles involving denaturation at 94 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s. After normalizing the data with housekeeping genes, the gene expression in the experimented groups was expressed as a fold change relative to the control group. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene; its expression remained unaltered throughout the experiment.

Determination of acetylcholinesterase and monoamines

The acetylcholinesterase (AChE) activity in the hippocampal homogenate was assessed based on the procedure described by Elman et al. (1961). The concentration of hippocampal dopamine (DA), norepinephrine (NE), and serotonin (5-HT) were estimated by HPLC using an electrochemical detector

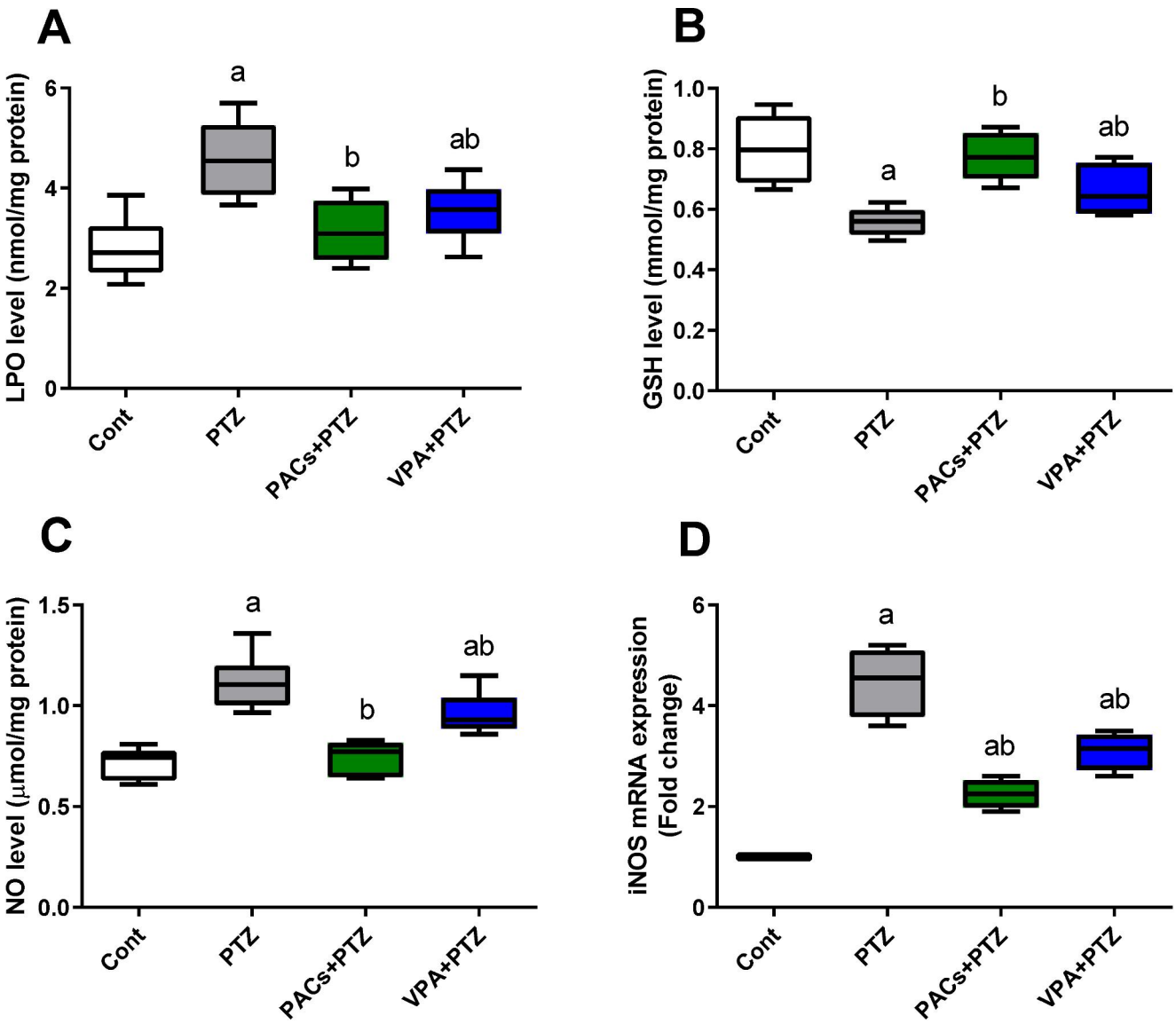

Fig. 1 Effect of proanthocyanidins on the modified Racine scale following pentylenetetrazole (PTZ) injection. Results are figured as mean ± SD (n = 14); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice. [c] represents a significant change against the VPA-treated mice

according to the protocol described by Jamwal et al. (2015). In addition, Brain-derived neurotrophic factor (BDNF) was estimated using an ELISA kit obtained from MyBioSource (San Diego, CA, USA; Catalogue number MBS355435).

10. Statistical analysis

Data are illustrated as means ± standard deviation (SD). The recorded results from the performed measurements were examined using One-way ANOVA analysis of variance and *post hoc* Tukey's test using a statistical package program (SPSS version 14.0), while Mann Whitney U and Student's *t*-tests were employed to the analysis of seizure score data; *P* values < 0.05 represent statistical significance.

Results

Epileptic seizures general observations

PTZ administration in mice induced epileptic seizures with different degree based on the type of the treatment (Fig. 1). In mice received vehicle, the modified Racine scale recorded 4.43 ± 0.79. On the other hand, PACs and VPA administrations significantly decreased the score to 3.14 ± 1.22 and 2.29 ± 1.11, respectively, reflecting the anticonvulsant effect of PACs against PTZ-induced seizure. However, the data showed a significant difference between PACs and VPA treatment groups.

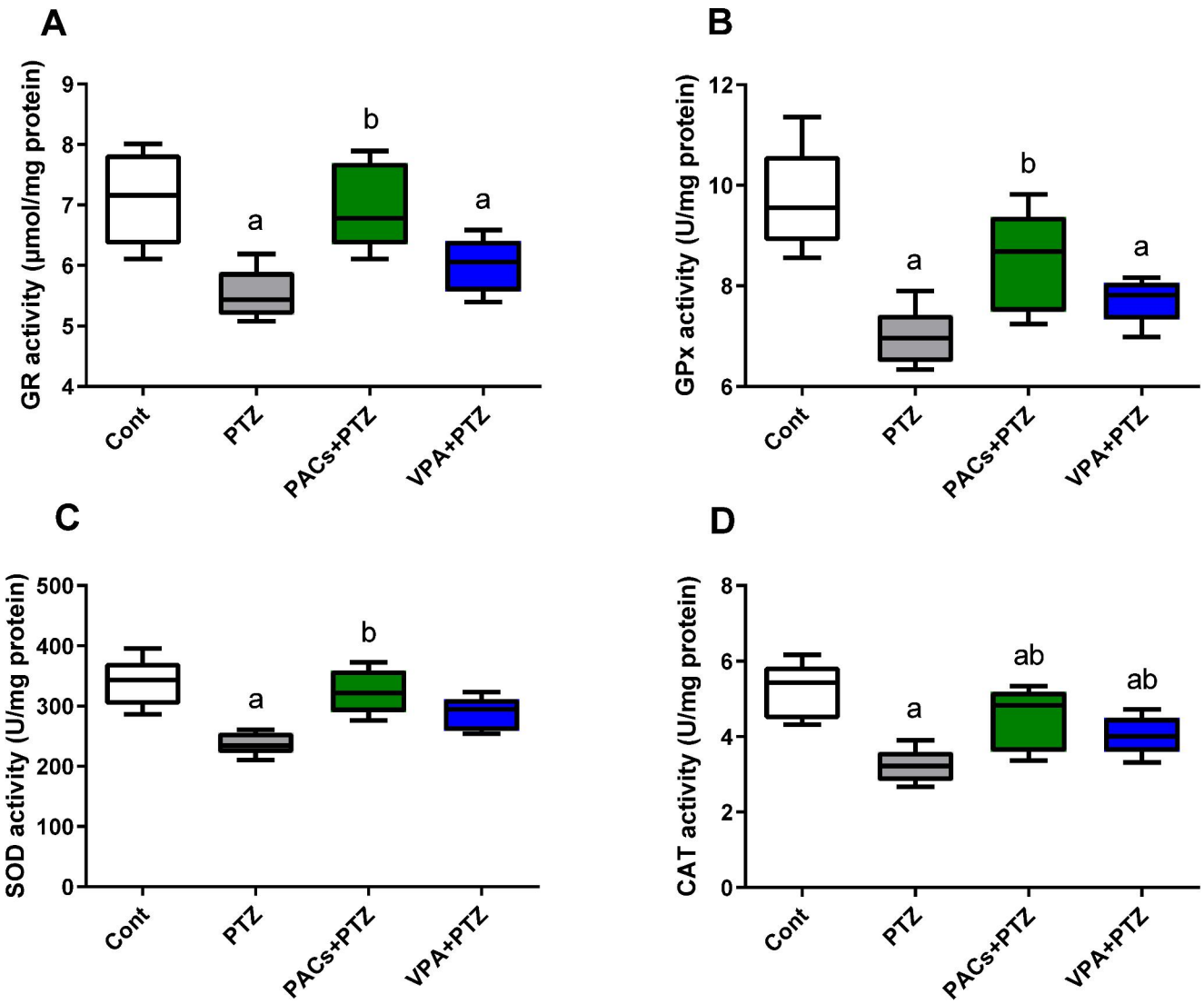

Fig. 2 Effect of proanthocyanidins on nitrosative and oxidative stresses (A) malondialdehyde (MDA) (B) glutathione (GSH) (C) nitric oxide (NO), and (D) inducible nitric oxide synthase (iNOS) levels in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Results are figured as mean ± SD (n = 7) while data of iNOS presented as mean ± SD (n = 3); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

3.2. Protective effect of PACs against oxidative stress and nitrosative stress generated in the hippocampal tissue following PTZ injection.

Compared to the control group, a significant (F = 8.544; p < 0.05) enhancement of LPO levels (Fig. 2 A) was found in the group exposed to PTZ alone and in the group treated with VPA before PTZ exposure. The group treated with PACs before being exposed to PTZ showed LPO levels similar to that in the control group and significantly attenuated compared to the untreated PTZ exposed group. On the other hand, compared to the control group, the levels of GSH were reduced significantly (F = 10.61; p < 0.05) in the only PTZ exposed group and the pre-treated VPA group followed by PTZ exposure pre-treated relatively to the control group. Meanwhile, the PTZ exposed group pre-treated with PACs exhibited the same GSH levels as the control group (Fig. 2B).

As expected, the analysis of NO levels in various study groups (Fig. 2 C) was significantly (F = 19.98; p < 0.05) higher in PTZ exposed animals than the control levels. In the group treated with PACs prior to exposure to PTZ, the NO levels were comparable to that in the control group. In the group treated with VPA prior to PTZ, the NO levels were significantly reduced compared to those exposed to PTZ alone, but it remained significantly higher than the control group.

The expression of iNOS mRNA is presented in Fig. 2D. Compared to the control group, there is significantly

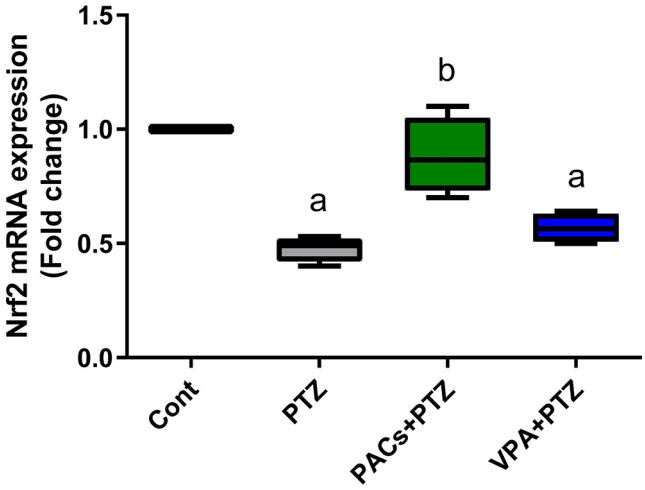

Fig. 3 Effect of proanthocyanidins on mRNA and activity of antioxidant enzymes [(A) glutathione reductase (GR), (B) glutathione peroxidase (GPx), (C) superoxide dismutase (SOD) and (D) catalase (CAT)] in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Biochemical results are figured as mean ± SD (n = 7); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

(F = 21.26; p < 0.05) enhanced expression of iNOS mRNA in all groups exposed to PTZ. Thus, it is evident that PTZ exposure had resulted in nitrosative stress in all study groups. The group of mice treated with either PACs or VPA before administration of PTZ showed significantly reduced expression of iNOS compared to the group exposed to PTZ alone. However, the reduction in iNOS mRNA expression was more prominent in the PACs treated group than the VPA treated group of mice.

The antioxidant enzymes GPx, GR, SOD, and CAT were measured in all study groups, shown in (Fig. 3). In general, exposure to PTZ caused a significant (F = 9.552; 13.46; 12.65; 10.82; p < 0.05) reduction in the activity of all the tested antioxidant enzymes compared to the control group. In the group where exposure to PTZ was preceded by treatment with PACs, the activity of GR was significantly elevated and was comparable to that in the control group. In contrast, pretreatment of PTZ exposed mice with VPA did not cause any significant increase in GPx activity. Figure 3 A presents the activity of GPx in various study groups. Although treatment with VPA did not increase the activity of GPx in mice exposed to PTZ, treatment with PACs caused a significant increase in GPx activity in the PTZ exposed group. The enzyme activity of SOD (Fig. 3 C) was significantly raised when PACs were used to treat the PTZ exposed mice. Contrary to this, VPA treatment failed to increase the activity of SOD in PTZ exposed mice. The activity of CAT was found to significantly increase when PTZ exposed mice groups were treated with VPA or PACs (Fig. 3D).

The protective effects of PACs involved the activation of the Nrf2 pathway

- The Nrf2 mRNA expression was found to be significantly (F = 13.68; p < 0.05) downregulated in the hippocampal tissue following PTZ injection (Fig. 4). However, pretreatment of PTZ exposed mice with PACs resulted in a significant up-regulation of Nrf2 mRNA. Furthermore, pretreatment of PTZ exposed mice with VPA also caused Nrf2 mRNA to be slightly upregulated, but this result failed to reach significance (Fig. 4).

Effect of PACs on inflammatory markers

- In general, all studied inflammatory markers (TNF-α, IL-6, IL-1β, and NF-κB) levels were significantly (F = 27.70; 39.83; 32.72; 10.01; p < 0.05) elevated after PTZ exposure compared to the control group (Fig. 5). Treatment with PACs and VPA before exposure to PTZ effectively suppressed NF-κB concentration to a level equivalent to that in the control group. For IL-1β, it was noted that for PTZ exposed mice, pretreatment with PACs reduced this cytokine level to a concentration similar to that in the control group. Although pretreatment of PTZ exposed mice to VPA, causing IL-1 β concentration to reduce, the level remained meaningfully higher than that in the control group. Pretreatment of PTZ exposed mice with either VPA or PACs helped reduce cytokines TNF-α and IL-6. However, it was interesting that neither VPA nor PACs could effectively restore these two cytokine levels to a concentration similar to that recorded in the control group.

Protective effects of PACs on apoptosis

- Another variable studied was the potential of PACs to modulate apoptosis. In comparison to the control group, the levels of Bax were found to be significantly (F = 6.214; p < 0.05) elevated on exposure to PTZ. The pre-treated PACs group was found the restore the level of Bax to that which was in the control group. Pretreatment of the PTZ exposed group with VPA also demonstrated similar results (Fig. 6 A). Caspase-3 activity was also measured in all study groups. The activity was highest (F = 8.093; p < 0.05) in the PTZ group compared to all other groups. Pretreatment with PACs restored the activity of caspase-3 (Fig. 6B). Pretreatment of

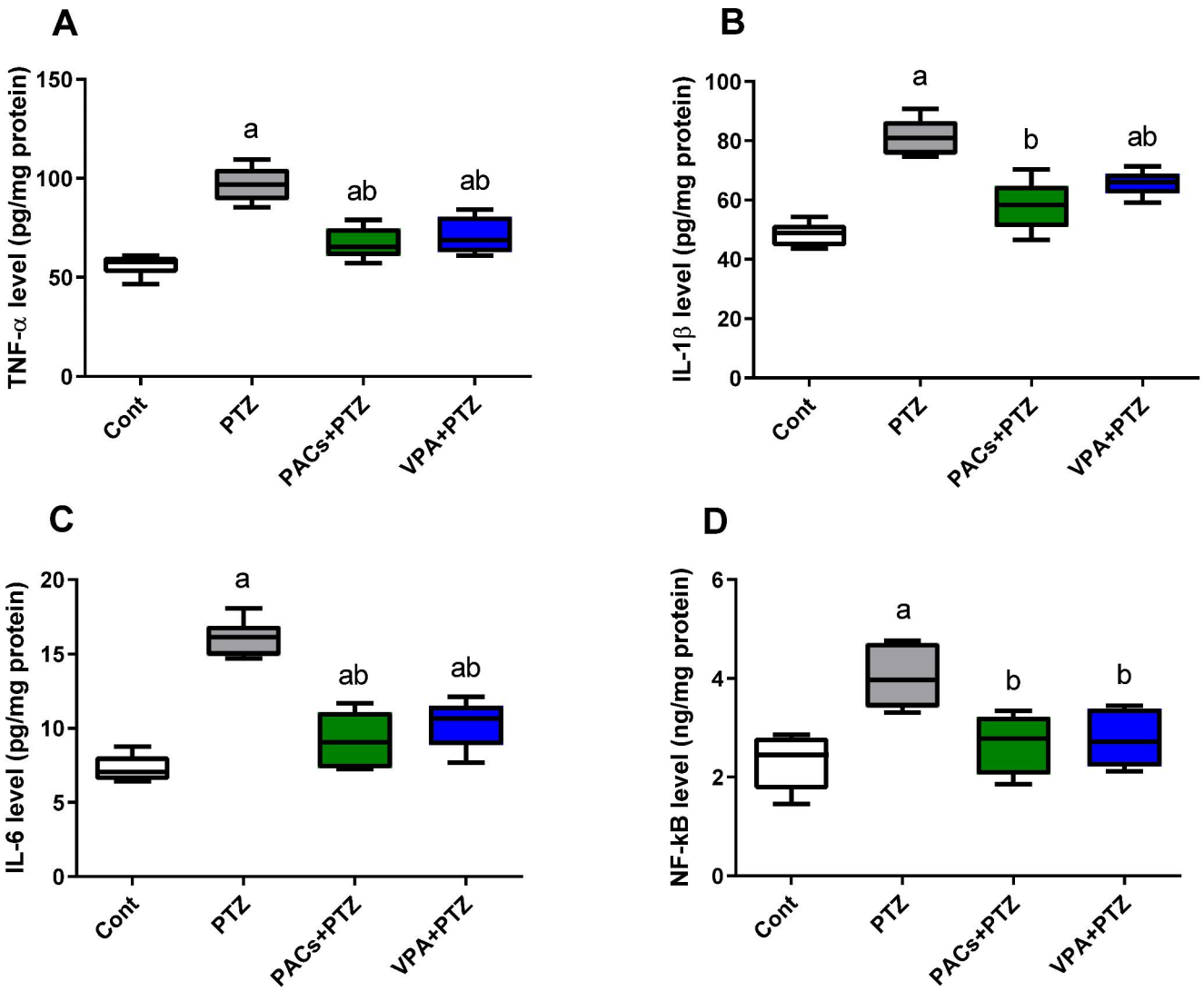

Fig. 4 Effect of proanthocyanidins on the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Data presented as mean ± SD (n = 3); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

PTZ exposed mice to VPA also caused a reduction in caspase-3 activity; however, it was found to be less

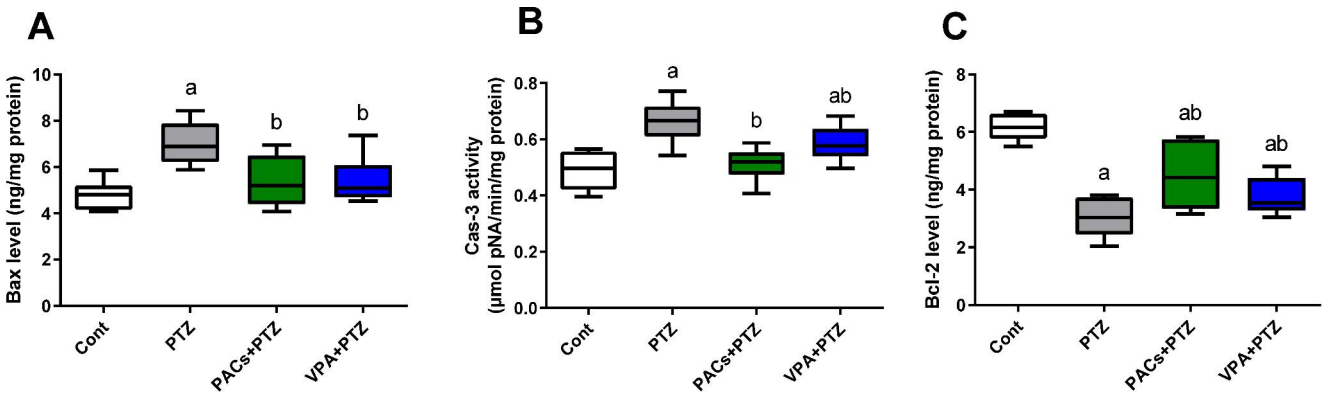

Fig. 5 Effect of proanthocyanidins on inflammatory markers (A) TNF-α, (B) IL-1β, (C) IL-6 and NF-κB in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Results are figured as mean ± SD (n = 7); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

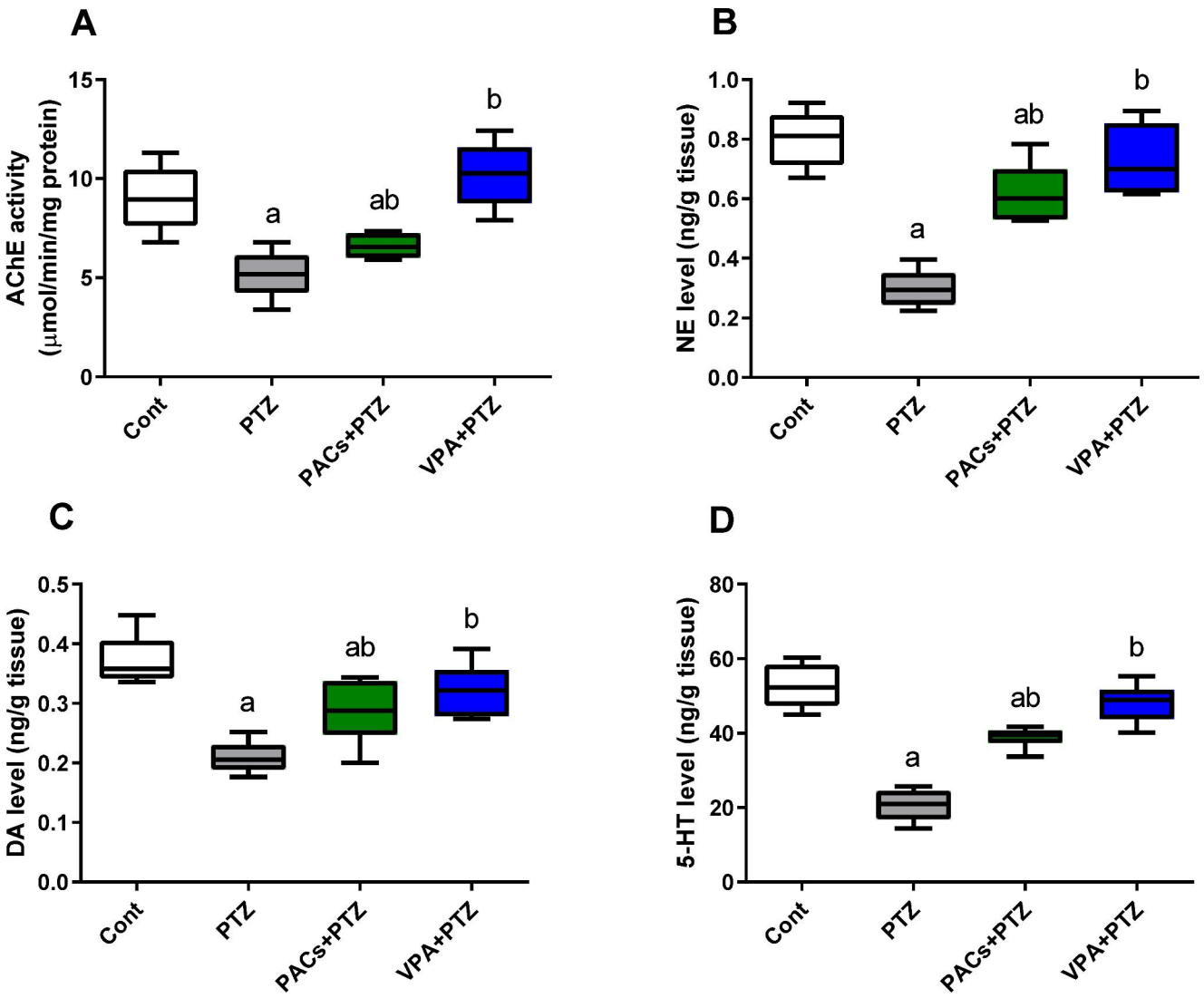

Fig. 6 Effect of proanthocyanidins on apoptotic markers (A) Bax, (B) caspase-3 and (C) Bcl2 in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Results are figured as mean ± SD (n = 7); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

effective than PACs. Furthermore, results (Fig. 6 C) show that compared to only PTZ exposed group, the pre-treated group with either VPA or PACs had higher levels of Bcl-2. However, neither VPA nor PACs could restore Bcl-2 levels to be equivalent to that recorded for the control group.

Effect of PACs against PTZ injection induced neurotransmitters disturbance

The administration of PTZ caused a significant reduction in the activity of AChE as compared to the control group. When VPA was given to the mice prior to exposure to PTZ, a significant ($F = 18.08$; $p < 0.05$) increase in AChE activity was noted, though the level of AChE activity remained significantly less than that observed in the control group. On the other hand, treatment with PACs prior to exposure to PTZ restored the activity of AChE to control levels. Nevertheless, the activity of AChE was restored to the control levels in the group that received PACs before exposure to PTZ (Fig. 7 A). Compared to the control, the levels of NE were significantly ($F = 32.91$; $p < 0.05$) less in mice treated with PTZ alone and in mice treated with PACs prior to PTZ exposure. PACs treatment did improve the NE level in PTZ exposed mice but failed to restore them to control levels. On the other hand, VPA treatment could effectively restore NE levels in PTZ treated animals (Fig. 7B).

The analysis of DA levels (Fig. 7 C) revealed that although PTZ exposure caused a significant fall in levels of

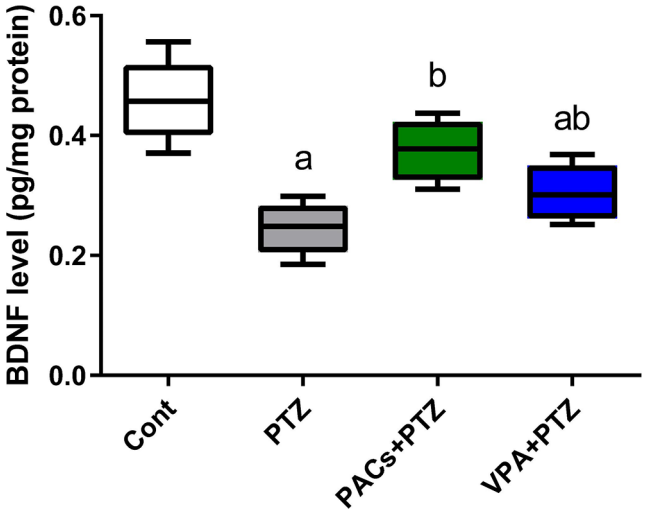

Fig. 7 Effect of proanthocyanidins (A) acetylcholinesterase activity (AChE), (B) norepinephrine (NE), (C) dopamine (DA) (D) and serotonin (5-HT) in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Results are figured as mean ± SD (n = 7); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

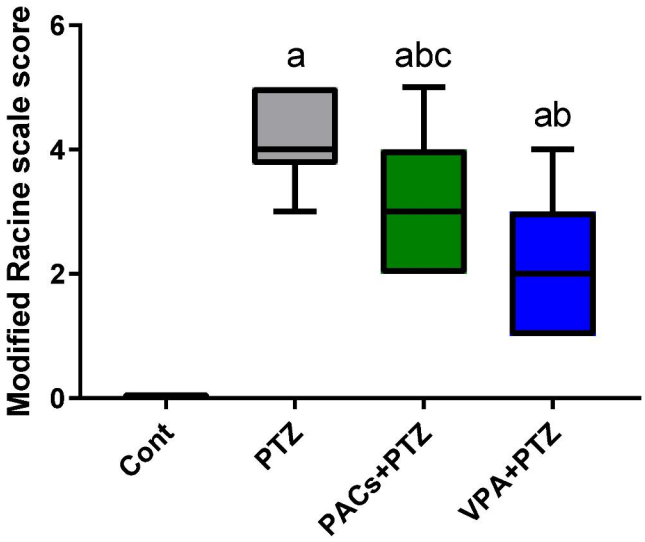

Fig. 8 Effect of proanthocyanidins brain derived neurotropic factor (BDNF) in the hippocampal tissue following pentylenetetrazole (PTZ) injection. Results are figured as mean ± SD (n = 7); $P < 0.05$ was considered significant. [a] represents a significant change against the control mice; [b] represents a significant change against the PTZ-injected mice

this neurotransmitter. Although PACs were given to the mice before exposure to PTZ, there was a significant ($F = 15.81$; $p < 0.05$) rise in DA level; pre-treatment with PACs could not restore the level of DP to be equivalent to its level in the control group. As presented in Fig. 7D, the levels of 5-HT were depressed in hippocampal tissue of mice exposed to PTZ alone. When PACs were given to the mice along with PTZ exposure, it was noted that the levels of 5-HT were significantly ($F = 55.79$; $p < 0.05$) greater than that when PTZ was given alone. Nevertheless, even in PACs, in PTZ exposed mice, the 5-HT levels remained significantly less than the control level. Conversely, when mice were administered VPA before exposure to PTZ, the level of 5-HT was comparable to that in the control group.

Finally, BDNF levels were also measured in all study groups, and results are presented in Fig. 8. Furthermore, the level of BDNF was reduced ($F = 18.31$; $p < 0.05$) under the influence of PTZ, but treatment with PACs and VPA caused a significant rise in BDNF levels. In addition, PAC treatment was more effective as it restored BDNF levels to become comparable to that in the control group, while VPA could not do so.

Discussion

Consistent with several previous reports (Shimada and Yamagata 2018; Yuan et al. 2020), exposure of mice to PTZ in the current study mimicked epilepsy condition and resulted in aggravation of inflammation and oxidative stress along with depletion of antioxidant enzyme and disturbed neurotransmitter levels.

The occurrence of oxidative stress in the PTZ-induced animal model of epilepsy in the presented research was confirmed by analysis of the status of antioxidants in the hippocampus tissue homogenate. The occurrence of oxidative stress in the PTZ-induced animal model of epilepsy in the presented research was confirmed by analysis of the status of antioxidants in the hippocampus tissue homogenate. The activities of all investigated antioxidant enzymes (GPx, SOD, CAT, and GR) were considerably reduced on exposure of the animals to PTZ, which was in line with previous reports (Rodrigues et al. 2012). The ability of PACs to alleviate oxidative stress was evident since our results demonstrated an enhancement in activity of GPx, SOD, CAT, and GR following treatment of PTZ-induced animal models of epilepsy with PACs. The effectiveness of PACs as an antioxidant has been confirmed in several *in vitro*, *in vivo*, and human studies. All these studies point out PACs' potential for prevention or treatment of oxidative stress-associated diseases (Eng et al. 2003; Galati et al. 2000; Gossé et al. 2005) though its mechanism remains poorly understood.

The loss of antioxidant enzymes in hippocampus tissue also correlated with ROS overproduction, such as superoxide anion radicals, hydrogen peroxide, nitric oxide, and peroxide anions, leading to neuronal damage (Mori et al. 2004). Our results indicate an increase in NO levels and enhanced expression of iNOS mRNA in PTZ exposed animals. Furthermore, the iNOS enzyme is a major isoform of nitric oxide synthase that helps NO synthesis. NO expression

plays a critical role as a neuromodulator in various central and peripheral nervous systems. This gas is considered a major pathological factor in epilepsy (Asadi-Shekaari et al. 2012; Khoei et al. 2020). Excessive NO production can cause lipid peroxidation (Rodrigues et al. 2012). Thus the observed increase in lipid peroxidation could have been due to increase NO production in our study. We have shown that PACs protect against PTZ induced lipid peroxidation. PACs was also able to reduce this nitrosative stress successfully. We found PACs to reduce lipid peroxidation and nitrosative stress more effectively than VPA, a commonly used epilepsy medicine. Our study is in line with a previous study (Per et al. 2013) that reported the role of iNOS activity in the anticonvulsant effect of grape seed extract, a rich source of PACs (Unusan 2020).

It has been hypothesized that neuroinflammation can lead to atypical neural connectivity and hyper-excitable neural network, resulting in epilepsy (Musto et al. 2011, 2016). Neuroinflammation can either be caused directly due to brain injury or due to several common systemic inflammatory diseases when the blood-brain barrier gets compromised (Rana and Musto 2018). Furthermore, growing evidences considered inflammatory processes as a biomarker of epileptogenesis which further stimulate hyperexcitability by N-methyl-D-aspartate receptor and Toll-like receptor 4 and seizures. Furthermore, the discharge of pro-inflammatory cytokines further stimulates NF-kB and enhances cellular stress response (Alvi et al. 2021). Neuroinflammation is known to be activated by PTZ in rodents (Hoda et al. 2017; Liu et al. 2018); accordingly, we found that inflammatory biomarkers TNF-α, IL1β, IL-6, and NF-κB were increased post-PTZ treatment in mice. Furthermore, our results demonstrate that this PTZ kindled increase in inflammatory cytokines was reversed by PACs pre-treatment, consistent with previous findings in rat seizure models (Zhou et al. 2011).

Cellular apoptosis induced by OS is another important mechanism of neuronal damage, as seen in several neurodegeneration diseases such as Alzheimer's disease and Parkinson's disease (Rizk et al. 2021). Oxidative stress-induced apoptosis in the nerve cells occurs via the activation of the mitochondrial apoptotic pathways (Almeer et al. 2020). The apoptotic level of the brain was significantly increased in the PTZ exposed group compared with that in the controls in the present research. The increased expression of the Bax to Bcl2 content ratio in the PTZ exposed mice showed the activation of the mitochondrial apoptotic pathway. Furthermore, cleaved caspase-3 was the essential apoptotic initiator and was also activated in PTZ exposed mice. In an *in vitro* study, it was shown that PACs possessed a protective effect on apoptosis induced by oxidative stress (Ma et al. 2018). Consistently, we found that administration of PACs could significantly decrease the apoptotic level in the hypothalamus of the PTZ exposed group, significantly enhancing the activity of Bcl2 (anti-apoptotic protein) while reducing the activity of Bax and caspase-3 (pro-apoptotic protein). Thus, our findings suggested that PACs might protect against neuronal apoptosis induced by oxidative stress via decreasing the Bax/Bcl2 ratio.

PACs can attenuate oxidative stress by scavenging free radicals and modifying the signaling pathways, including those involving nuclear factor erythroid 2-related factor 2 (Nrf2), mitogen-activated protein kinase, NF-κB, and phosphoinositide 3-kinase (Puiggròs et al. 2014). The transcription factor Nrf2 binds to the antioxidant response element (ARE) to upregulate the antioxidative gene expression, thus regulating antioxidant response and preventing oxidative stress in various cells. In some other studies, the activation of the Nrf2 pathway could protect various cells against apoptosis(Jiang et al. 2014). Furthermore, the Nrf2 signaling pathway can be activated by phytochemicals and food polyphenols (Niture et al. 2014; Scapagnini et al. 2011). For example, a study on diabetic rats found that grape seed proanthocyanidin extract ameliorates diabetic bladder dysfunction via activating the Nrf2 Pathway (Chen et al. 2015). However, its protective effects against epilepsy have not yet been clarified. Therefore, to the best of our information, the present study is the first to provide supportive evidence for the protective effects of PACs against PTZ induced epileptic seizures in mice by enhancing the activity of the Nrf2 path. Significant up-regulation of Nrf2 after exposure to PACs in the current research suggests role of PACs in Nrf2 modulation. Further, as mentioned earlier, the changes in levels of apoptosis markers (Bax, Bcl2 and caspase-3) and of antioxidant enzymes (GPx, SOD, CAT and GR) is also in agreement with changes in Nrf2 expression. Thus, our findings suggested that the protective effects of PACs on the hippocampus tissue of PTZ exposed mice might be due to the activation of the Nrf2 signaling pathway.

Conclusion

Taken together, the data obtained from this study ascertains that pretreatment with PACs can effectively reduce oxidative stress, neuroinflammation, and neuronal apoptosis by activating the Nrf2 signaling pathway in PTZ induced epileptic mice models. Therefore, PACs have significant potential as a natural antiepileptic drug and could be competently employed in the future for clinical treatment and protection against epilepsy. However, future studies based on this preliminary study are required to establish PACs as the natural antiepileptic agent.

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Authors' contributions S. Abadi, N.M. Alyami and R.S. Almeer designed the project, performed the experiments, drafted and edited the manuscript. N.M. Alyami and H.M. Alyami analyzed the data, interpreted the data, drafted and edited the manuscript. H.M. Alyami supplied the chemicals and reagents. All authors approved the final draft.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate The animals were treated according to the Research Ethics Committee (REC), and the approval no. (KSU-SE-21-78).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests All authors declared no conflicts of interest in this manuscript.

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