MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 4415-4425
DOI: 10.12659/MSM.897579

Received: 2016.01.14
Accepted: 2016.02.22
Published: 2016.11.17

Garcinol Upregulates $GABA_A$ and GAD65 Expression, Modulates BDNF-TrkB Pathway to Reduce Seizures in Pentylenetetrazole (PTZ)-Induced Epilepsy

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: Departmental sources

Background: Epilepsy is the most predominant neurological disorder characterized by recurrent seizures. Despite treatment with antiepileptic drugs, epilepsy still is a challenge to treat, due to the associated adverse effects of the drugs. Previous investigations have shown critical roles of BDNF-TrkB signalling and expression of glutamic acid decarboxylase 65 (GAD65) and $GABA_A$ in the brain during epilepsy. Thus, drugs that could modulate BDNF-TrkB signal and expression of GAD65 and $GABA_A$ could aid in therapy. Recent experimental data have focussed on plant-derived compounds in treatments. Garcinol (camboginol), is a polyisoprenylated benzophenone derived from the fruit of *Garcinia indica*. We investigated the effects of garcinol in pentylenetetrazole (PTZ)-induced epileptic models.

Material/Methods: Seizure scores were measured in epilepsy kindled mice. Neuronal degeneration and apoptosis were assessed by Nissl staining, TUNEL assay, and Fluoro-Jade B staining. Immunohistochemistry was performed to evaluate cleaved caspase-3 expressions. Expression of BDNF, TrkB, $GABA_A$, GAD65, Bad, Bcl-2, Bcl-xL, and Bax were determined by western blots.

Results: Significantly reduced seizure scores and mortality rates were observed with pretreatment with garcinol. Elevated expression of apoptotic proteins and caspase-3 in kindled mice were effectively downregulated by garcinol. Epileptogenic mice presented increased BDNF and TrkB with considerably decreased $GABA_A$ and GAD65 expression. Garcinol significantly enhanced $GABA_A$ and GAD65 while it suppressed BDNF and TrkB. Garcinol enhanced the performance of mice in Morris water maze tests.

Conclusions: Garcinol exerts neuroprotective effects via supressing apoptosis and modulating BDNF-TrkB signalling and GAD65/$GABA_A$ expressions and also enhanced cognition and memory of the mice.

MeSH Keywords: Garcinia Cambogia • Pentylenetetrazole • Receptor, trkB • Receptors, GABA

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/897579

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Background

Epilepsy characterized by recurrent seizures arising due to an imbalance between cerebral excitability and inhibition is a major complex neurological disorder affecting around 50 million people worldwide [1]. Epilepsy may be indicative of underlying ailments or could be idiopathic and is often associated with psychiatric disturbances [2–5]. Despite treatment with antiepileptic drugs (AED), epilepsy still presents a challenge due to the associated adverse drug effects [6]. Recent studies are focussing on the identification and development of more effective AEDs with negligible or no side effects.

Garcinol (camboginol) is found in Guttiferae Juss (also called Clusiaceae) plants native of South East Asia and India [7,8]. Garcinol is the major component of *Garcinia indica* fruit rind extract [9,10] and is structurally similar to known antioxidant curcumin [11]. Garcinol has a wide range of biological activities that include anti-cancer, anti-HIV, anti-inflammatory, antioxidant, and anti-ulcer properties [12–15]. Garcinol has been reported to reduce metastasis and also reduce MMP-7 levels [16] and activate mitochondrial apoptotic pathways in cancer cells [17]. However, studies on garcinol's antiepileptic properties are lacking. We attempted to investigate the protective effect of garcinol in experimental models of epilepsy and explore possible underlying molecular events.

Brain-derived neurotrophic factor (BDNF) and its TrkB receptor are known to critically regulate neurogenesis and synaptic plasticity [18,19]. The BDNF-TrkB signalling pathway is also known to have a crucial role in epileptogenesis [20]. Epileptic conditions have been reported to increase the expression level of BDNF [21–24]. Furthermore, infusion of BDNF into rat hippocampus has been reported to induce seizures [25]. BDNF binds to TrkB receptors and activates downstream protein kinases leading to raised presynaptic release of mediators due to phosphorylation of substrates, or alters the functions of postsynaptic receptors as $GABA_A$ receptors [26,27]. GABA is the main inhibitory neurotransmitter and GABA receptors mediate inhibitory neurotransmission and prevent the neurons from being overexcited [28]. Glutamic acid decarboxylase 65 (GAD65) is one of the major components through which glutamate is catalyzed to GABA through decarboxylation [29] and is a rate limiting step in GABA synthesis. GAD and GABA receptor expression in the brain are closely associated with epileptic conditions [30,31].

Material and Methods

Animals

Our research was carried out after obtaining ethical clearance from the University ethical committee and was executed in compliance with National Regulations of Laboratory Animal Management. The study C57BL/6 mice (20 per group), which weighed 20±2 g (4–6 weeks old) were collected and accommodated under standard laboratory conditions (25±1°C; humidity 40–60%; 12 hours light/dark cycle). The mice were provided with free access to food and water.

Reagents and chemicals

Garcinol and pentylenetetrazole (PTZ) were procured from Sigma-Aldrich (St. Louis, MO, USA). PTZ was dissolved in freshly prepared saline prior to the injection. Antibodies against BDNF, TrkB, $GABA_A$, and GAD65 (Cell Signalling Technology, Danvers, MA, USA), caspase-3, Bcl-2, Bad, Bax, Bcl-xL, and TNF-α ((Santa Cruz Biotechnology, Santa Cruz, CA,USA) were used for expression analysis. All other standard chemicals used in this study were of analytical grade procured from Sigma-Aldrich (St. Louis, MO, USA).

Kindling induction

Kindling is recognized as an experimental model for studying epilepsy-related alterations of behaviour and in assessing the effectiveness of antiepileptic drugs (AED) [32,33]. In our study, the mice were kindled with sub-effective dose of PTZ. On alternating days, PTZ (37 mg/kg, intraperitoneal.) was injected for a total of 14 times [34] and were monitored for 30 minutes after each administration. Separate treatment groups received garcinol in saline at 50, 100 or 200 mg/kg body weight, intraperineal, 30 minutes prior to each PTZ injection. Control mice that did not receive garcinol were kindled. Mice that were kindled with PTZ and not treated with garcinol served as epileptic controls. Valproate (150 mg/kg, intraperitoneal.) was administered to a separate group of PTZ-induced mice to serve as a standard drug control [35]. According to the modified Racine scale, seizures were classified as described by Becker et al. [36]. The seizures were scored: No response – Stage 0; Ear and facial twitching – Stage 1; Myoclonic jerks without rearing – Stage 2; Myoclonic jerks, rearing – Stage 3; Turning over into side position, clonic tonic seizures – Stage 4; Turning over into back position, generalized clonic-tonic seizures – Stage 5.

Histology – Nissl staining

The mice (n=6) were deeply anesthetized with isoflurane (0.75%) and perfused transcardially with 0.9% saline, followed by paraformaldehyde (4%). The brains were removed, post-fixed in 4% paraformaldehyde overnight, and then immersed in 30% sucrose solution at 4°C. After the brains sank to the bottom of the solution, coronal sections were made through the hippocampus tissue using a freezing microtome. Coronal sections of 7 μm thickness were exposed to Nissl staining with 1% toluidine blue as described by Hui et al. [37]. Nissl staining was used to assess the effect of garcinol on the architectural

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changes upon PTZ challenge. The number of viable cells in the hippocampal CA1 and CA3 pyramidal cells per 1-mm length of the bilateral hemispheres were determined by light microscopy under high magnification (400×).

Fluoro-Jade B staining

To identify neurons undergoing degeneration, Fluoro-Jade B (FJB) staining was performed. Hippocampal sections of 30 µm thickness were fixed on gelatin-coated slides and dehydrated overnight at room temperature. Slides were rehydrated and then incubated in potassium permanganate (0.06%% for 15 minutes), then rinsed in distilled H_2O. The slides were stained with FJB (Milipore, Chemicon International, Temecula, CA, USA) followed by incubation with acetic acid (0.1%% for 30 minutes) and observed under microscope.

Assessment of neuroapoptosis by TUNEL fluorescent assay

Neuroapoptosis following 12 h after last PTZ challenge was assessed by TUNEL assays as previously described by Li et al. [38]. Three sections of 5 µm thickness were sliced (200 µm apart) on the same plane of the hippocampi of each mouse and were subjected to TUNEL assay using DeadEnd™ Fluorometric TUNEL System kit (Promega, Madision, WI, USA). The slides were secured from exposure to direct light throughout the experiment. The hippocampal CA1 and CA3 region TUNEL positive cells were further analyzed with NIS-Elements BR imaging processing and analysis software (Nikon Corporation, Japan).

Immuno-histochemical staining

Cleaved caspase-3 expression is considered a marker of apoptosis. Caspase 3 expression in the hippocampi of PTZ kindled mice was assessed by immunohistochemistry as described previously by Li et al. [38,39]. In brief, the brain tissue sections were incubated overnight at 4 °C with anti-cleaved caspase-3 primary antibody followed by 40 minutes incubation with secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After incubation with avidin-biotinylated peroxidase complex (Vectastain ABC-Kit, Vector Laboratories, Burlingame, CA, USA) for 40 minutes, the tissue sections were treated with diaminobenzidine and analyzed with NIS-Elements BR imaging processing and analysis software.

Expression analysis by western blotting

For western blot studies, the mice (n=3) were anaesthetized with 0.75% isoflurane following 12 hours after last PTZ challenge and were sacrificed by decapitation. The hippocampi were immediately isolated on ice and stored at –80°C until used. To detect the level of expression of BDNF-TrkB, $GABA_A$, and GAD65, western blotting was performed. Hippocampal tissue was homogenized in lysis buffer and incubated on ice for 30 minutes. The protein contents were determined by BCA assay (BioRad, Hercules, CA, USA). The proteins were electrophoreitically separated by SDS-PAGE and were blotted on to PVDF membrane. The membranes were blocked in 3% BSA/TBST at room temperature for 60 minutes and incubated with specific primary antibodies at 4 °C overnight. After washing with TBST, the membranes were further incubated with HRP-labelled secondary antibody (1:2000). The blots were washed further and the immunoreactive bands were detected using an enhanced chemiluminescence method (GE Healthcare, Piscataway, NJ).

Determination of gamma-aminobutyric acid (GABA) and glutamic acid (Glu) in brain tissues

The mice (n=3) were sacrificed 50 minutes after administration of the last PTZ injection. The whole brains were collected as described for the western blotting procedure. The brain tissue was weighed and homogenized, and GABA and Glu contents were determined by HPLC method with reference standards [40].

Assessment of learning and memory

The Morris water maze (MWM) test was used for assessing learning and memory behaviour [41]. The MWM test was performed 24 hours after the final PTZ injection. The apparatus consisted of a water pool (120-cm diameter) that was virtually distributed into four quadrants and filled with water (22±1°C). A colourless escape platform (10 cm in diameter) was submerged in a labelled target quadrant. The pool was placed in a noiseless room and was enclosed with several visual cues that could aid spatial orientation by the mice. The experiment involved two parts: learning trials (existed platform) and probe trials (non-existed platform). Prior to the experiment, mice were trained consecutively for five days with about four trials per day. Mice were allotted 60 seconds to trace the hidden platform; those that were unable to locate the platform within 60 seconds were directed and allowed to sit on the platform for 15 seconds. The escape latency – the time taken by each mouse to identify the platform was recorded. On the sixth day, the platform was removed from the target quadrant and the time spent by the mice in the target quadrant looking for the platform was recorded.

Statistical analysis

The results are expressed as mean ±SD, obtained from three or six experiments. The results were analyzed statistically by using SPSS statistical package (SPSS, version 22.0). Values of $p<0.05$ were considered statistically significant as determined by ANOVA (one-way analysis of variance) at $p<0.05$ by DMRT (Duncan's Multiple Range Test) for posthoc analysis.

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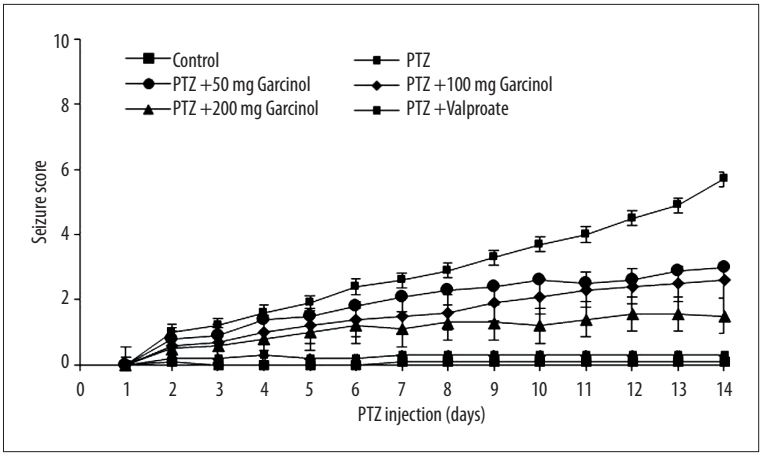

Figure 1. Garcinol reduced PTZ induced seizure scores. Data expressed as mean ±SD with n=6.

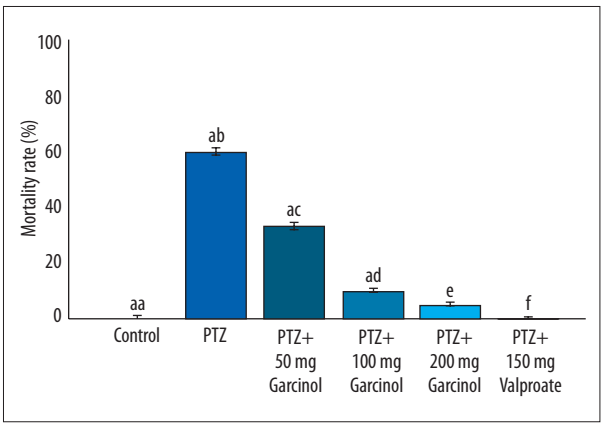

Figure 2. Garcinol reduced PTZ-induced mortality rate. Note that $p<0.05$ indicates statistical significance of comparison of the control group (denoted by [a]). Note also that [b–f] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

Results

Protective effect of garcinol against PTZ-induced kindling

In our study, repeat administration of subconvulsive doses of PTZ (37 mg/kg) on every alternative day (14 injections) resulted in a steady increase in seizure score, subsequently resulting in generalized clonic-tonic seizures. Pretreatment with garcinol (50, 100 or 200 mg/kg) prior to each PTZ injections was found to suppress the progression of kindling significantly ($p<0.05$) as demonstrated by the decrease in seizure scores in comparison with the PTZ control group (Figure 1). Further, the mortality rate after PTZ injections was reduced with garcinol treatment, with 200 mg/kg doses exhibiting no mortality (Figure 2).

Garcinol enhanced cognitive impairment brought by epileptic seizures

MWM test is an established method to assess learning and memory. PTZ kindling resulted in a progressive decline in the escape latency with each training session (Figures 3, 4). Mice in the PTZ kindled group showed sustained escape latency in comparison to the control group ($p<0.05$). Furthermore, this behavior was alleviated upon garcinol pretreatment. In the probe trial, the PTZ kindled mice spent less time in the target quadrant compared to the control group ($p<0.05$) that were not induced by PTZ. The garcinol pretreatment (50, 100 or 200 mg/kg) group significantly ($p<0.05$) improved their performance. The mice supplemented with garcinol spent a significantly longer time in the target quadrant looking out the platform than the mice in the PTZ control group ($p<0.05$). These observations suggest that garcinol improved learning and memory of the mice.

Garcinol pretreatment reduced the neuronal loss caused by PTZ induced seizures

Mice were sacrificed 24 hours after the last PTZ injection using cervical decapitation; brain tissue was harvested and analyzed for neuronal apoptosis and protein expressions. Nissl staining was performed to study neuronal loss in the CA1 and CA3 hippocampal regions. PTZ-induced seizures showed severe cell death after 24 hours of seizures. The viable cell counts were observed to be drastically decreased ($p<0.05$) in PTZ control mice compared to treatment groups. However, garcinol pretreatment significantly ($p<0.05$) attenuated the neuronal loss induced by seizures ($p<0.05$) in a dose-dependent way, with the highest dose exhibiting maximal protective effects. Further, no significant differences in neuronal loss were observed between the control groups and groups treated with 200 mg/kg garcinol (Figure 5). Valproate treated PTZ-induced mice also exhibited negligible neuronal loss.

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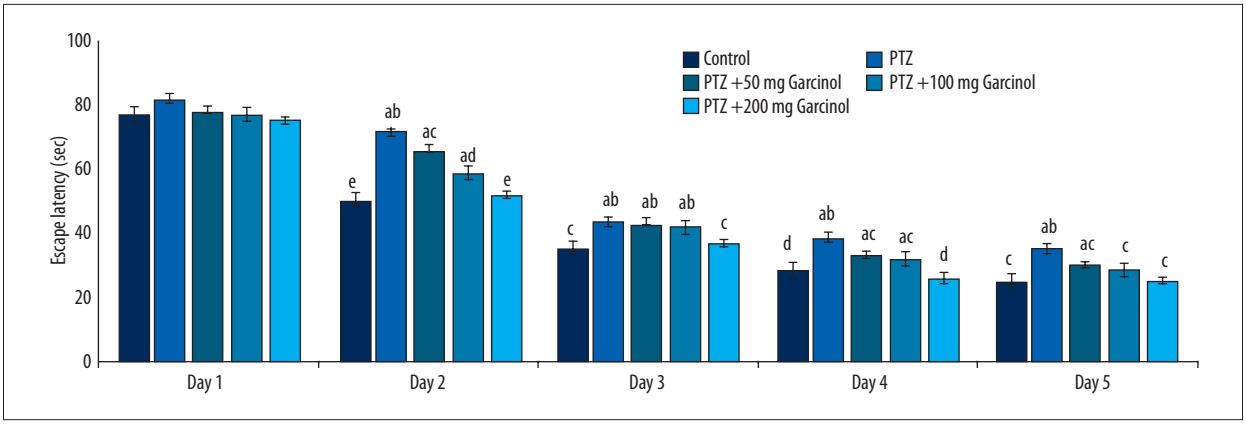

Figure 3. Garcinol improved learning and memory of PTZ kindled mice in MWM tests. Escape latency of PTZ kindled and garcinol-treated mice. Data expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to the control group (denoted by [a]). Note also that [b–d] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

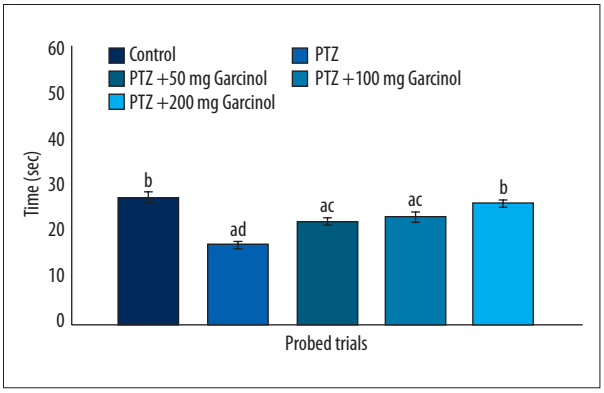

Figure 4. Garcinol improved learning and memory of PTZ kindled mice in MWM tests. Learning and memory of mice following PTZ injections as determined by probe trials with MWM tests. Data expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to the control group (denoted by [a]). Note also that [b–d] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

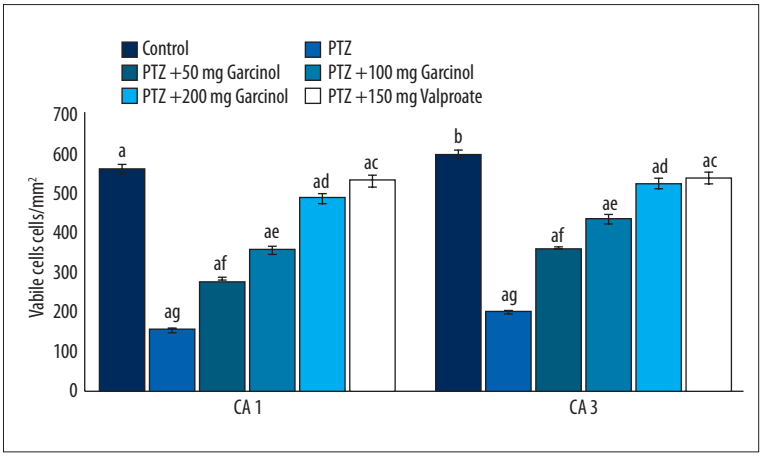

Figure 5. Garcinol improved viability of neurons in the hippocampal regions following PTZ injections. Data expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to the control group (denoted by [a]). Note also that [b–g] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

Garcinol effectively reduced neuronal degeneration and apoptosis following PTZ induction

At 24 hours after the last PTZ injection, the number of FJB positive cells was increased significantly ($p<0.05$) in the PTZ control group, while garcinol treated rats exhibited reduced FJB positive cells, suggesting that garcinol treatment prevented PTZ-induced neurodegeneration (Figure 6). Further, TUNEL assays revealed raised TUNEL positive cell counts in the CA1 and CA3 hippocampal regions upon PTZ-induced seizures, while garcinol supplementation, interestingly, reduced TUNEL positive cell counts, indicating inhibition of neuronal apoptosis (Figure 7). Moreover, the decrease in apoptotic counts was

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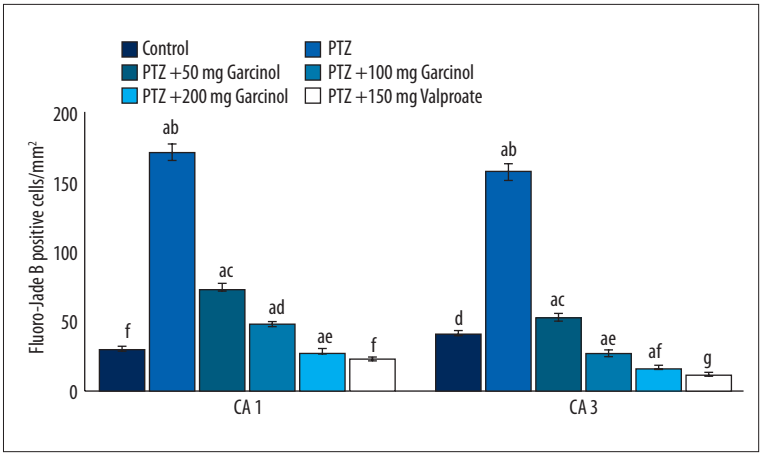

Figure 6. Garcinol effectively reduced apoptosis. Garcinol treatment effectively reduced neuronal apoptosis in PTZ kindled mice as observed by Fluoro-Jade B staining. Data expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to the control group (denoted by [a]) Note also that [b–f] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

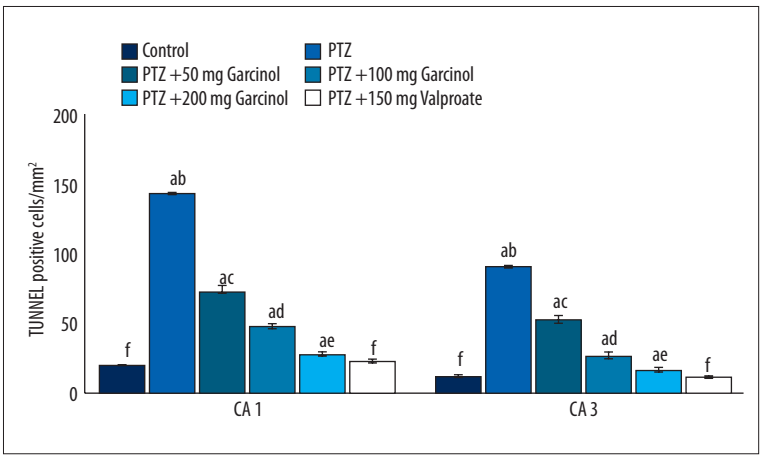

Figure 7. Garcinol effectively reduced apoptosis. Garcinol treatment effectively reduced neuronal apoptosis in PTZ kindled mice as observed by TUNEL assay. Data are expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to control group (denoted by [a]). Note also that [b–f] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

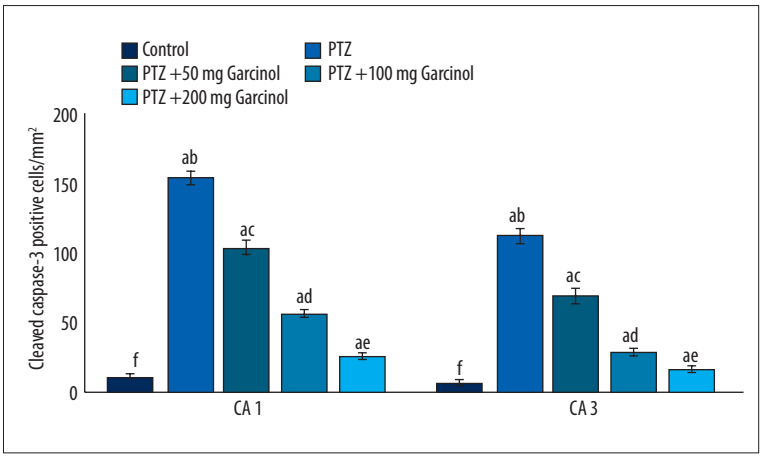

Figure 8. Influence of garcinol on Cleaved caspase-3 expressions. Garcinol treatment effectively downregulated cleaved caspase-3 expressions in a dose-dependent manner thus aiding in reduction of apoptosis. Data are expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to the control group (denoted by [a]). Note also that [b–g] denotes mean values within the same group that differ from each other at $p < 0.05$ as derived from ANOVA (one-way) and DMRT analysis.

observed to be dose-dependent with the effects of 200 mg/kg dose comparable to valproate.

Influence of garcinol on the raised caspase-3 levels following PTZ kindling

Caspase-3 is a marker for apoptosis following seizures [42] and caspase-3 and Bcl-2 are known to critically regulate apoptotic process in neurons of the brains of chronic epileptic patients [43]. We observed raised caspase-3 positive cells in the hippocampus of PTZ kindled mice, while garcinol significantly ($p<0.05$) supressed activated caspase-3 expressions in line with the standard AED valporate (Figure 8). Our study revealed that garcinol effectively downregulated caspase-3 expression, thus supressing apoptosis. Garcinol 200 mg/kg exhibited high levels of inhibition of caspase-3 in line with

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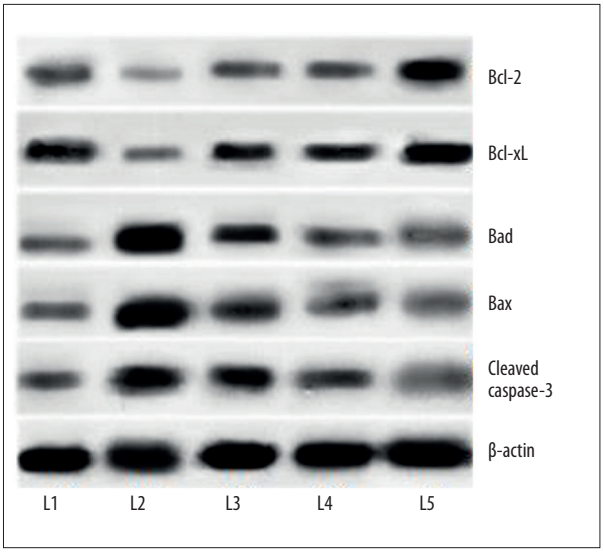

Figure 9. Garcinol modulates the expressions of apoptotic pathway proteins. Garcinol markedly upregulated anti-apoptotic proteins (Bcl-xL and Bcl-2) while downregulating Bad and Bax. Garcinol significantly reduced caspase-3 expressions following PTZ injections. (L1 – Control; L2 – PTZ; L3 – PTZ + 50 mg/kg garcinol; L4 – PTZ + 100 mg/kg garcinol; L5 – PTZ + 200 mg/kg garcinol).

standard AED (valproate) as compared to lower doses of garcinal (50 and 100 mg/kg).

Garcinol modulated the expressions of apoptotic pathway proteins in epileptic rats

Previous studies have suggested that PTZ kindling induces neuronal cell death in the CA1 and CA3 hippocampal regions [44,45]. However, in our study we observed that garcinol was able to effectively supress neuronal loss in the hippocampus of PTZ kindled mice. To assess the molecular events, we studied the influence of garcinol on the expression of apoptotic proteins. We observed multi-fold increases in the expression of pro-apoptotic proteins (Bad and Bax) in the PTZ control group (Figure 9). Interestingly, we also found that mice that were PTZ kindled and received garcinol prior to each PTZ dose, exhibited decreased expression of Bad and Bax levels. Further, suppression of anti-apoptotic proteins Bcl-xL and Bcl-2 in epileptic mice were found to be upregulated with garcinol treatment, indicating inhibition of apoptosis. These results showed that garcinol significantly ($p<0.05$) regulated the expression of these proteins and inhibited apoptosis of the neuronal cells, indicating neuroprotective effects.

Garcinol regulated the BDNF-TrkB signalling PTZ kindled mice

Involvement of BDNF–TrkB signalling is known to be critical in epilepsy. BDNF exerts its effects via binding to receptors TrkA

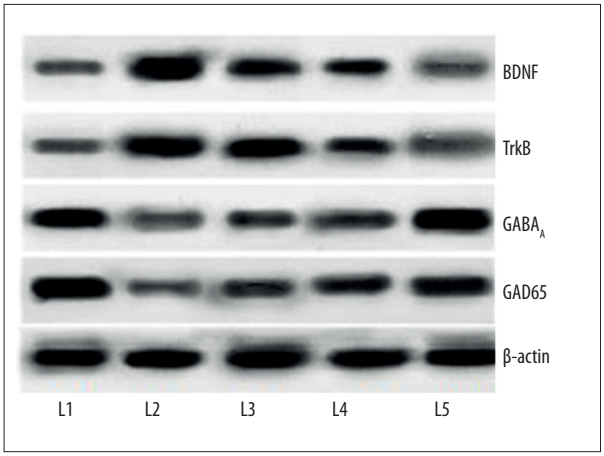

Figure 10. Garcinol regulates the expressions of BDNF-TrkB pathway and proteins $GABA_A$ and GAD65. (L1 – Control; L2 – PTZ; L3 – PTZ + 50 mg/kg garcinol; L4 – PTZ + 100 mg/kg garcinol; L5 – PTZ + 200 mg/kg arcinol).

and TrkB. Experimental models of epilepsy have revealed a BDNF-TrkB pathway in epileptogenesis [20,46,47]. It is known that BDNF potentiates glutamatergic neurotransmission while inhibiting GABAergic neurotransmission [48,49]. Thus, the excessive BDNF and TrkB expression induced by PTZ as observed in our study further enhances the excitability of the neurons and promotes epilepsy (Figure 10). Garcinol pretreatment before each PTZ injection downregulated the expression of BDNF and TrkB in a dose-dependent manner. The 100 and 200 mg/kg doses of garcinol were more effective in inhibition of BDNF-TrkB expression than the 50 mg/kg dose. This garcinol-mediated suppression of BDNF-TrkB signalling could have possibly contributed to the decrease in neuronal loss and the seizure scores.

Effect of garcinol on expressions of GAD65 and $GABA_A$ in epileptic rats

GAD65 and $GABA_A$ are chief regulators in the production of GABA [40,50]. In PTZ-induced epileptic rats, supressed expression of GAD65 and $GABA_A$ were observed (Figure 11). Garcinol pretreatment interestingly caused a significant ($p<0.05$) upregulation in GAD65 and $GABA_A$ expression. Further, 100 and 200 mg/kg doses of garcinol were found to be more effective in enhancing GAD65 and $GABA_A$ expression. This significant upregulation aids in inhibition of PTZ-induced seizures and this could have possibly contributed to the reduction in seizure scores observed. These observations suggest the antiepileptic potential of garcinol.

Garcinol effectively modulated Glu/GABA levels in PTZ kindled mice

GABA is the chief inhibitory neurotransmitter, in contrast to Glu which is a key excitatory neurotransmitter and the imbalance

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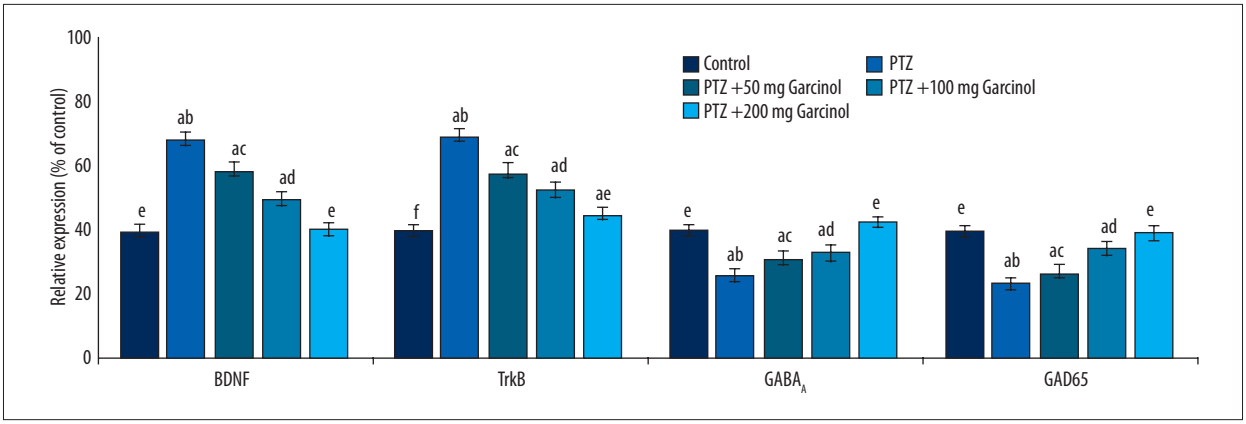

Figure 11. The expression of BDNF-TrkB pathway and proteins GABA$_A$ and GAD65 is regulated by garcinol. Data are expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance comparison to the control group (denoted by [a]). Note also that [b–f] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

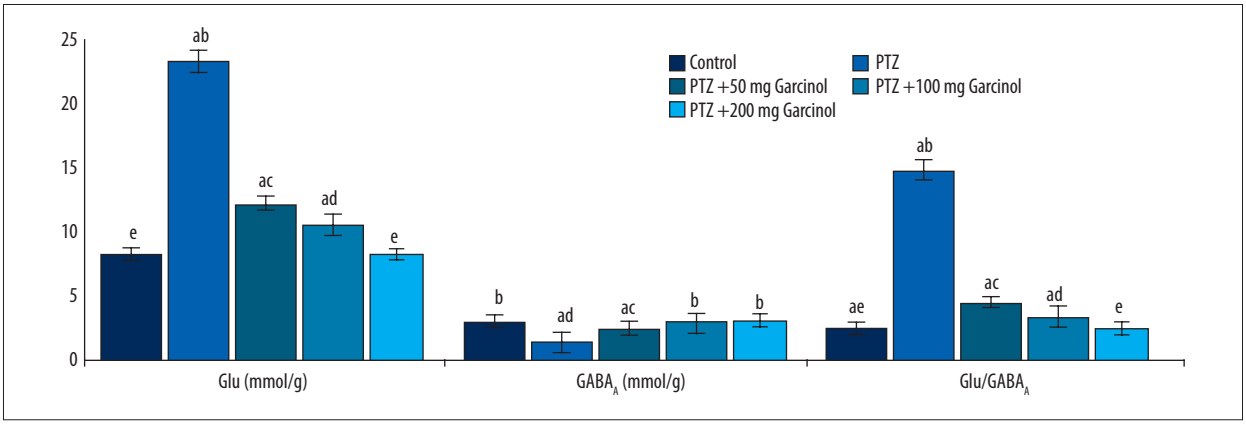

Figure 12. Garcinol restores Glu/GABA balance in PTZ kindled mice. Imbalance of Glu/GABA is an important factor in epiletogenesis. Garcinol treatment effectively upregulated GABA and suppressed Glu levels and restored the Glu/GABA balance. Data are expressed as mean ±SD with n=6. Note that $p<0.05$ indicates statistical significance of comparison to the control group (denoted by [a]). Note also that [b–e] denotes mean values within the same group that differ from each other at $p<0.05$ as derived from ANOVA (one-way) and DMRT analysis.

of Glu/GABA in the brain is a critical factor in epileptogenesis [30,50]. In our study, pretreatment with garcinol at 50, 100 and 200 mg/kg doses resulted in raised GABA levels while significantly reducing Glu levels in the brains of PTZ kindled mice. Although all of the three tested doses of garcinol reduced Glu levels, 200 mg/kg dose reduced the Glu levels more effectively. The results reveal that garcinol effectually normalised the Glu/GABA ratio (Figure 12).

Discussion

Epilepsy one of the most common neurological disorder and kindling is considered as a widely recognized experimental model for human epilepsy and is characterized by progressive and intense seizures evoked by sub-effective doses of chemicals or by electrical stimulus. PTZ is commonly used for kindling and is the most extensively acknowledged animal model used to study seizure mechanisms, understanding the neurobiology of epilepsy, learning and memory deficits induced by seizures and to evaluate the effectiveness of novel treatments [34] and cognitive deficits [51,52].

In this study, we found that repetitive administration of a subconvulsive dose of PTZ elicits severe seizures and also high mortality. Administration of garcinol effectively supressed the severity of seizures in PTZ kindled mice and drastically reduced the mortality rates. Higher dose of garcinol was more effective than the lower doses and its effects were in line with valproate. Furthermore, several studies have shown severe neuronal cell death in CA1 and CA3 regions of the hippocampus [44,45]. Becker et al. [53] suggested that cell death could be due to the

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enhanced glutamatergic systems. Furthermore, the seizures induce transient cerebral ischemia that also subsequently leads to death of the neurons in the brain [54–57]. We observed significantly ameliorated cell loss in the hippocampus of PTZ kindled mice upon garcinol treatment. The effects were, however, found to be dose-dependent. In addition, the extent of inhibition of neuronal cell death was in line with the intensity of seizures. To further analyse the possible mechanism involved in the protective effects of garcinol, we analyzed the expression of caspase-3 and proteins of the apoptotic pathway. PTZ induction caused a significant increase in the expression of caspase-3 and Bax and Bad proteins in the brain tissues of kindled mice. Garcinol downregulated caspase-3, Bad and Bax and upregulated Bcl-xL and Bcl-2 in a dose-dependent way. These observations suggest that garcinol modulated expressions could have supressed apoptosis and aided in part to the reduction in neuronal cell loss.

Studies have shown that BDNF strongly potentiates glutamatergic neurotransmission while inhibits GABAergic neurotransmission [48,49,58]. GABA is one the chief inhibitory neurotransmitters while glutamate is a chief excitatory neurotransmitter. BDNF exerts its effects by binding with TrkB receptors leading to activation of downstream protein kinases eventually causing enhanced presynaptic release of neurotransmitters and postsynaptic receptor functions [27,59]. In our study, PTZ induction resulted in markedly enhanced expression of BDNF and TrkB, suggesting enhanced glutamatergic neurotransmission resulting in seizures. While garcinol treatment potentially inhibited expression of BDNF and also TrkB, thus downregulating BDNF signalling, that could have aided in suppression of glutamate-mediated excitation. Moreover, the role of TrkB in mediating the effects of BDNF for epileptogenesis in various seizure models have been demonstrated [60–62]. Thus, garcinol by downregulating TrkB could have in part lead to suppression of seizures.

Balance between excitatory and inhibitory neurotransmitters crucially affects epileptogenesis [63]. Significantly raised levels of glutamate with reduced GABA levels were observed in PTZ kindled mice thus upsetting the Glu/GABA balance. Interestingly, we noticed restoration of this balance in PTZ kindled and garcinol treated mice. Garcinol dose-dependently increased levels of GABA while reducing glutamate levels. To explore further if garcinol regulated GABA contents at gene levels, expression of GAD65 were assessed. GAD65 is the key rate-limiting enzyme in GABA synthesis. GAD65 and $GABA_A$, the chief GABA receptor are the key targets in research for development of novel antiepileptic drugs due to their critical roles in epileptogenesis [30,50,63]. In the present study, results revealed upregulated expression of GAD65 and $GABA_A$ upon treating with garcinol. Hence the restoration of Glu/GABA balance could be possibly due to upregulation of GAD65 and also raised expression of $GABA_A$ could also be a possible mechanism of antiepileptic effects of garcinol.

Cognitive impairments have been well demonstrated in epilepsy [51,64]. In line with previous studies, we found PTZ kindling resulted in learning and memory deficits in mice as evidenced by a significant increase in escape latency in training sessions. PTZ kindled mice exhibited much shorter time spent in the target quadrant in the probe tests in MWM. Garcinol-treated PTZ kindled mice had improved performance in identification of submerged platform in the training sessions and also spent significantly longer time span in the target quadrant looking for the platform. These observations suggest that garcinol improved cognition of PTZ kindled mice. Thus garcinol-mediated reductions in seizure scores and neuronal loss could have contributed to better cognitive abilities.

Conclusions

Garcinol exerted protective effects by significantly reducing PTZ-induced seizures, improving cell viability, regulating the expressions of BDNF-TrkB signalling and restoring the Glu/GABA balance. Garcinol thus should be explored further as a potential candidate in treatment of epilepsy.

Conflicts of interest

None.

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