EGYPTIAN PHARMACEUTICAL JOURNAL EPJ

Original Article 181

Protective effects of inositol on penicillin G-induced epileptiform activity in rats: Modulation of gamma-amino butyric acid, excitatory amino acid receptors and N-methyl-D-aspartate

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Received: 4 August 2024
Revised: 2 October 2024
Accepted: 3 October 2024
Published: 29 January 2025

Egyptian Pharmaceutical Journal 2025, 24: 181-188

Background
Inositol acts as gamma-amino butyric acid (GABA) receptor agonist and has been proven to be effective in treating panic and obsessive-compulsive disorders.
Objective
The present work investigated the protective effect of inositol against epileptiform activity induced by G-penicillin in rats.
Materials and methods
Wistar albino rats were divided into four groups (six rats each) as follows: group I: the normal control group received saline. Group II: penicillin group treated daily with intraperotineal (*i.p.*) injection of saline for 14 days before the induction of epileptiform activity by a single dose of penicillin G. Groups III-IV: Inositol groups received daily inositol at a dose of 0.625 and 1.25 mg/kg body weight, orally for 14 days before penicillin G injection.
Results and conclusion
Administration of inositol (0.625 and 1.25 mg/kg) attenuated the severity of the behavioral changes as crying, myoclonic twitching of the eyelids, facial muscles, forelimbs rearing, and myoclonic jerk. Inositol, also, reduced serum levels of calcium and N-methyl-D-aspartate, decreased brain contents of malondialdehyde and nitric oxide as well as elevated brain contents of reduced glutathione and total antioxidant capacity. In addition, both doses of inositol decreased brain contents of interleukin-1β by 12% and 24% and C-reactive protein by 59% and 77%, and elevated brain contents of GABA by 62% and 133%, glutamic acid decarboxylase antibody by 5 fold and 17 fold, and excitatory amino acid transporter 2 by 27% and 37%, respectively, as compared with epileptic group.
Inositol ameliorated epileptiform activity induced by penicillin G through its antioxidant effect, anti-inflammatory effect, and restoring neurotransmitters GABA, glutamic acid decarboxylase antibody, excitatory amino acid transporter 2, and N-methyl-D-aspartate receptors.

Keywords:
Epilepsy, glutamic acid decarboxylase, inositol, N-methyl-D-aspartate, penicillin, rats

Egypt Pharmaceut J 24:181–188

1687-4315

Introduction

Epilepsy is a brain disease associated with chronic recurrent seizures [1] Epileptic seizures are due to excessive discharge in a population of hyperexcitable neurons [2] inducing physical and mental dysfunction and disrupting the nervous system [3], especially subcortical structures that are involved in some seizure type resulting from excessive discharges generated in hippocampal and cortical structures [4].

Epilepsy is characterized by loss of consciousness and involuntary movement, which are the results of excessive neuronal activities in different parts of the brain [5,6]. The time of most of activities last less than 2 min but the time of the activities that last longer than 5 min are considered status epilepticus [7]. Approximately 70 million people worldwide are affected by epilepsy [8]. Epilepsy is a common disorder in children, about 4–10% of children 16 years old suffer from one seizure [9].

Penicillin G is a chemical convulsant and its administration induces epileptic activity [10]. Its single microinjection into the left sensorimotor cortex induces intracortical epileptiform activity within 2–5 min, progressing to full seizure activity lasting ~3–5 h [2]. In addition, Patwardhan *et al.* reported that the intraperitoneal and cortical injections of penicillin were described as methods for inducing seizures [11]. Induction of a seizure by penicillin is easy and is not resistant to

anticonvulsants [12,13]. Penicillin is a gamma-amino butyric acid (GABA) channel inhibitor and can also cause paroxysmal depolarization shifts and epileptiform activity field potents [14]. On this basis, the level of excitation would be expected as a result of the reduced amount of GABA in the brain or the decrease in inhibitory effectiveness. Also, over release of the excitatory neurotransmitter glutamate can cause excitation in the brain [15]. GABA receptor antagonist is used as a convulsant drug by the caudal hypothalamus, di-encephalic region, blockade of receptors involving the reticulate formation [16]. The antiepileptic compounds which affect T-type calcium channels and GABAergic mechanisms inhibit seizure threshold [17]. GABA is synthesized from glutamate by two isoenzymes in mammalians, namely, GAD1 and GAD2. GAD2 plays an important role in synaptic inhibition, so it is localized primarily in the inhibitory synapse [18].

The excitatory amino acid transporter 2 (EAAT2) regulates GABA and glutamate levels in the brain [19]. Improved expression of EAAT2 can inhibit seizure-iduced neuronal death [20].

N-methyl-D-aspartate receptors (NMDARs) play critical roles in neuronal excitability in the central nervous system. Both preclinical and clinical studies have revealed that the abnormal function or expression of these receptors can underlie the pathophysiology of epilepsy and seizure disorders. Accordingly, in various preclinical models of seizures as well as in patients with epilepsy, NMDAR modulators have been shown to exert anticonvulsant effects [21].

Epileptogenesis prevention is a subject of intensive research. Currently, drugs that can act as preventive medication are not clinically approved. While many drugs can prevent seizures, they have a limited impact on preventing or curing the disease [8,22]. In addition, the use of antiepileptic drugs is limited due to the vast array of adverse effects, such as cognitive impairment, affective disorders, and recurrent seizures [23,24].

Inositol is a ubiquitous component in all eukaryotic cells, in some foods, and in all animal tissues especially, the brain and the heart. It can be synthesized [25] by numerous biological processes and kinases [26]. Inositol is important for all essential biological activities [27] and regulates the neurotransmitter levels of glutamate, dopamine, and serotonin in the brain [28]. Low levels of inositols were found in the frontal cortex of depressed patients [29]. Therefore, it is used as a therapy for different neurological disorders [30]. The current study was conducted to evaluate the protective effect of inositol against penicillin G-induced epileptiform activity in rats. This aim can be achieved by measuring the effect of inositol on oxidative stress, inflammmation, changes in neurotransmitters GABA, glutamic acid decarboxylase antibody (GAD-ab), EAAT and N-methyl-D-aspartate (NMDA) receptor.

Materials and methods

Animals

Total of 24 male Wistar albino rats, weighing 230–250 g, were used in this work. The Animal House of the National Research Centre provided them. The animals were maintained under temperature and light-controlled conditions (normal 12 h light/dark cycle). The animals were provided with water *ad libitum* and standard laboratory rodent chow. All procedures were approved by the Ethics Committee of the National Research Centre, Giza, Egypt, with approval number 18174.

Chemicals and drugs

Penicillin G was purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). It was dissolved in saline. Myo-inositol was obtained from Fluka AG, CH-9470 Buchs, Switzerland.

Experimental design

Intraperitoneal injection (*i.p.*) of a single dose of penicillin G (3 million international units/kg, body weight) caused the epileptiform activity according to Marangoze *et al.* [31]. The animals were divided into four groups (six rats each) as follows: group I: control group received *i.p.* injection of saline for 14 consecutive days and served as normal control. Group II: penicillin group received a daily *i.p.* injection of saline for 14 days before the induction of epileptiform activity by a single dose of penicillin G (3 million international units/kg, body weight. Group III-IV: inositol groups received daily oral inositol at a dose of 0.625 and 1.25 mg/kg body weight, for 14 days [32], respectively, before penicillin G injection.

Behavioral changes

Behavioral observations such as crying, myoclonic twitching of the eyelids, facial muscles, forelimbs rearing, and myoclonic jerk were observed by the naked eye immediately after penicillin injection [33].

Serum and tissue biochemical analysis

Blood samples were collected from the retro-orbital venous plexus under isoflurane anesthesia then allowed

to stand for 15 min. Blood samples were centrifuged at 3000 r/min for 10 min. The separated sera were stored at −20°C [34] to be used for the estimation of calcium and NMDA. Determination of serum calcium was done according to Gindler and King [35] using commercially available kits (Biodiagnostic, Egypt) and NMDA level using Sunlog Biotechnology Inc. ELISA kit, China [36].

Immediately, the brains were removed and washed in ice-cold saline solution. After that, homogenization was carried out in 0.1 mol/l potassium phosphate buffer (pH 7.4) using tissue master TM125 (Omni International, Kennesaw United States). After centrifugation at 3000 r/min for 10 min, the clear supernatant was stored at −80°C to be used for estimation of glutathione (GSH), total antioxidant capacity (TAC), malondialdehyde (MDA), NO using diagnostic kits (Biodiagnostic) GABA, GAD-ab and interleukin-1β (IL-1β) were estimated using SunLong Biotechnology Inc. ELISA kits, China, EAAT2 using NOVA, ELISA kit, Beijing, China and C-reactive protein (CRP) using SunRed, ELISA kit, Shanghai, China [36].

Statistical analysis

All values are presented as mean±SD. One-way analysis of variance followed by Tukey's multiple comparisons test between different groups. GraphPad Prism software, version 5 (GraphPad Software Inc., San Diego, California, USA), was used to carry out these statistical tests. The difference was considered significant when P was less than 0.05.

Results

Injection of penicillin-induced typical behavioral disturbances displayed as crying, myoclonic twitching of the eyelids, facial muscles, forelimbs rearing and myoclonic jerk in most animals. However, inositol injection (0.625 and 1.25 mg/kg) decreased behavioral changes significantly from penicillin G such as crying by 27 and 53%, myoclonic twitching of the eyelids and facial muscles by 16 and 45%, forelimbs rearing by 20 and 43% and myoclonic jerk by 24 and 49%, respectively, as compared with penicillin G group (Table 1).

Effect of inositol on calcium and N-methyl-D-aspartate (NMDA)

Induction of epilepsy by penicillin significantly increased serum calcium and NMDA levels by 56% and 56%, respectively, as compared with the normal control group. Rats receiving inositol (0.625 and 1.25 mg/kg) showed significantly decreased serum levels of calcium by 16% and 33% and NMDA by 28% and 36%, respectively, as compared with the epileptic group. Inositol (1.25 mg/kg) restored calcium and NMDA levels (Fig. 1).

Effect of inositol on total antioxidant capacity (TAC), reduced glutathione (GSH), malondialdhyde (MDA) and nitric oxide (NO) brain contents

Brain contents of TAC and GSH were decreased in penicillin group by 60% and 52%, respectively. On the other hand, MDA and NO brain contents were elevated by 130% and 67%, respectively, as compared with the normal control group. Rats receiving inositol (0.625 and 1.25 mg/kg) had significantly increased brain content of TAC by 42% and 102% and GSH by 63% and 108%, respectively, as well as decreased MDA content by 33% and 53% and NO by 24% and 39%, respectively, as compared with epileptic group. In addition, inositol (1.25 mg/kg) restored GSH, MDA, and NO levels (Fig. 2).

Effect of inositol on interleukin-1β (IL-1β) and C-reactive protein (CRP) brain contents

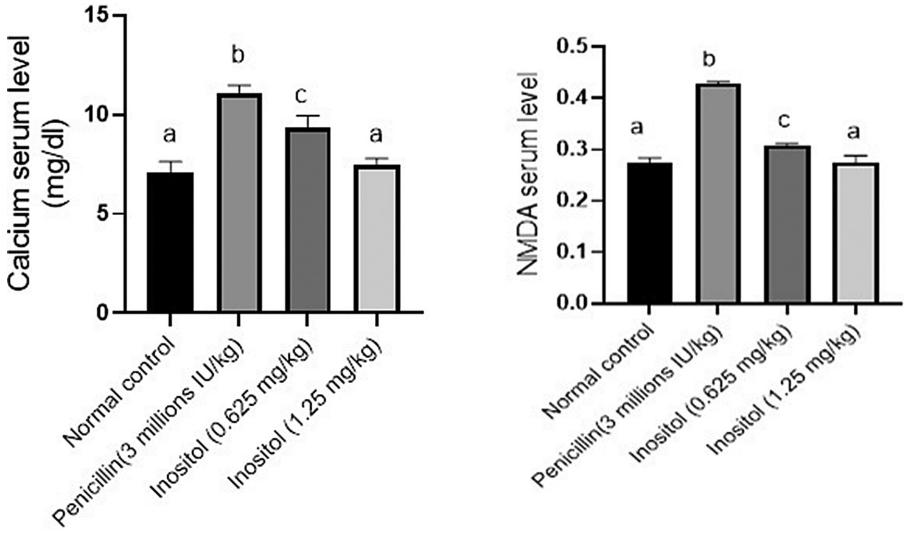
Induction of epilepsy by penicillin significantly increased brain IL-1β and CRP brain contents by 38% and 598%, respectively, as compared with the normal control group. Treatment with inositol (0.625 and 1.25 mg/kg) significantly decreased brain contents of IL-1β by 12% and 24% and CRP by 59% and 77%, respectively, as compared with the epileptic

Table 1 Effect of inositol on the behavioral symptoms of epilepsy induced by penicillin

	Crying		Myoclonic twitching of the eyelids, facial muscles, forelimbs		Rearing		Myoclonic Jerks	
	Number of rats	Latency	Number of rats	Latency	Number of rats	Latency	Number of rats	Latency
Penicillin	6	486.6±7.1[a]	6	565.2±13.8[a]	6	658±19.2[a]	6	863.6±25.1[a]
Inositol (0.625 mg/kg)	5	354.4±5.9[b]	4	474±12.9[b]	5	528.6±6.1[b]	5	658±12.9[b]
Inositol (1.25 mg/kg)	4	227.4±5.7[c]	3	310±9.5[c]	2	376±14.1[c]	3	443±35.6[c]

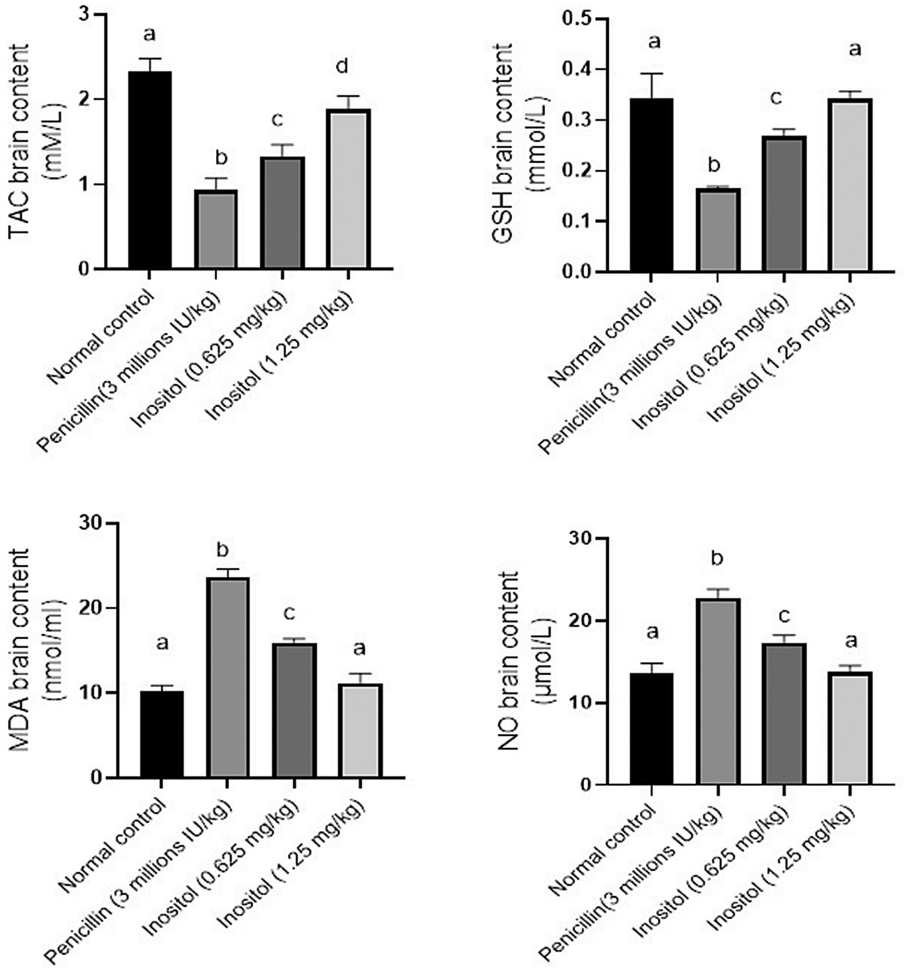
Data were expressed as mean±SD (n=6). Statistical analysis was carried out by one-way analysis of variance followed by the Tukey high significant difference (HSD) test for multiple comparisons. Same letter means nonsignificant difference, while a different letter means significant difference at P less than 0.05.

Figure 1



Effect of inositol on Ca and N-methyl-D-aspartate serum levels of rat model of epielpsy. Data were expressed as mean±SD (*n*=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey HSD test for multiple comparisons. The same letter means nonsignificant difference, while a different letter means significant difference at *P* less than 0.05.

Figure 2



Effect of inositol on total antioxidant capacity, glutathione, malondialdehyde and nitric oxide brain contents of rat model of epilepsy. Data were expressed as mean±SD (*n*=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey HSD test for multiple comparisons. Same letter means nonsignificant difference, while a different letter means a significant difference at *P* less than 0.05.

group. Moreover, inositol (1.25 mg/kg) restored IL-1β level (Fig. 3).

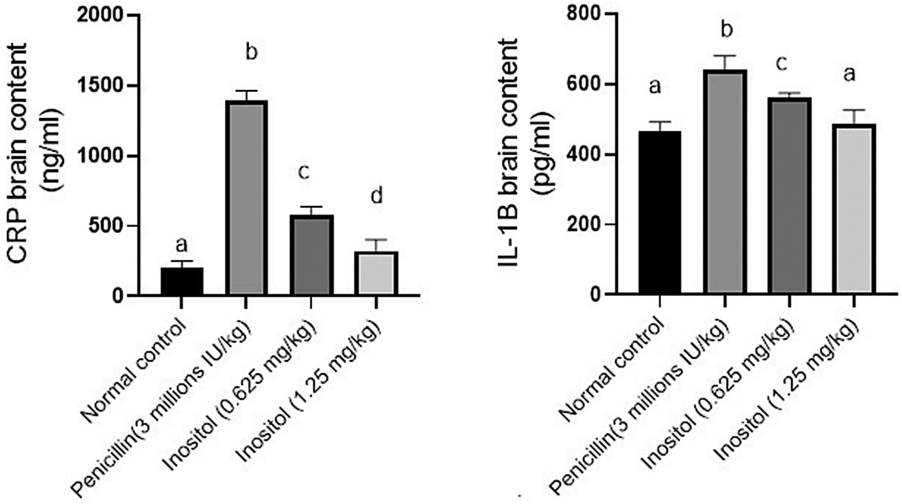
Effect of inositol on brain gamma-amino butyric acid (GABA), glutamic acid decarboxylase antibody (GAD-ab) and excitatory amino acid transporter 2 (EAAT2) brain contents

The present study showed a reduction in brain contents of GABA, GAD-ab, and EAAT2 by 63, 95, and 28%, respectively, in the penicillin control group. While treatment with inositol (0.625 and 1.25 mg/kg) elevated brain contents of GABA by 62% and 133%, GAD-ab by 5 fold and 17 fold, and EAAT2 by 27% and 37%, compared with penicillin control group. In addition, inositol (1.25 mg/kg) restored GAD-ab, and EAAT2 levels (Fig. 4).

Discussion

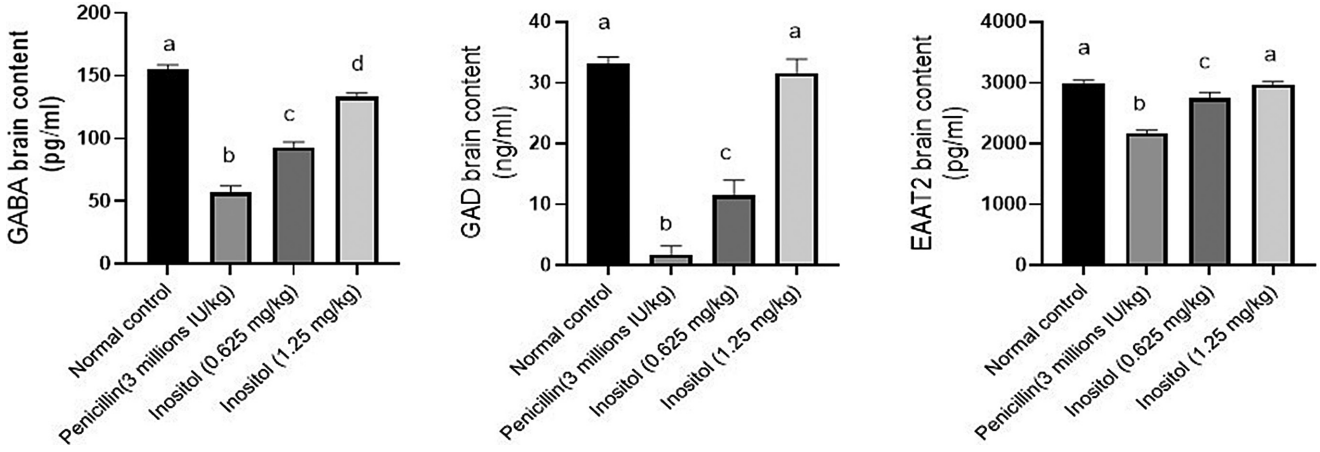
Inositol is considered as a vital compound having regulatory roles in some cellular processes such as maintaining cell function. Imbalance in inositol triggers molecular changes including calcium homeostasis, transcription, metabolism, autophagy, energy metabolism and ion channel. In addition, the reduction of inositol levels produced neurological disorders [37]. So, exogenous inositol is important for the brain activities [38].

Figure 3



Effect of inositol on interleukin-1β and C-reactive protein contents in the brain of rat model of epilepsy. Data were expressed as mean±SD (n=6). Statistical analysis was carried out by one-way analysis of variance followed by Tukey HSD test for multiple comparisons. The same letter means non-significant difference, while a different letter means a significant difference at P less than 0.05.

Figure 4



Effect of inositol on gamma-amino butyric acid, glutamic acid decarboxylase, excitatory amino acid transporter 2 contents hi the brain of rat model of epilepsy. Data were expressed as mean ± SD (n=6). Statistical analysis was carried out by one-way analysis of variance followed by the Tukey HSD test for multiple comparisons. The same letter means nonsignificant difference, while a different letter means significant difference at P less than 0.05.

In this study, injection of penicillin induced typical behavioral disturbances displayed by crying, myoclonic twitching of the eyelids, facial muscles, forelimbs rearing and myoclonic jerk in most animals. Consistent with our findings, Chen *et al.* found that the *i.p.* injection of penicillin G caused the same behavioral disturbance [39]. However, the treatment with inositol reduced the number of rats showing crying, myoclonic twitching of the eyelids, facial muscles, forelimbs rearing and myoclonic jerk compared with penicillin rats.

Our results clarified that the treatment with penicillin significantly increased serum calcium and NMDA levels. This result is in agreement with Salama and colleagues who reported that eplipsy caused massive influx of calcium in rats that is correlated with a hyperexcitability state originating from glutamate release which stimulates receptors of NMDA receptors [40]. On the other hand, animals receiving inositol reduced the penicillin effect through a reduction in serum levels of calcium and NMDA as compared with the penicillin group. Previous study has demonstrated that the abnormal metabolism of antioxidants, electrolytes, and trace elements may underlie the pathophysiology of severe neurologic and mental disorders, including epilepsy [41]. Massive influx of serum calcium in rats is correlated with the hyperexcitability state originating from the massive glutamate release which stimulates the receptor of NMDA [42] which in turn leads to production of free radicals and neuronal cell death [43].

In current study, penicillin induced epileptiform activity that was evident from reductions in brain contents of GSH and TAC and elevations of MDA and NO. These results are in line with Ayyildiz *et al.* who found that lipid peroxidation was increased [2], whereas GSH was reduced after penicillin-induce epileptiform activity [44,45]. Moreover a significant increase in NO content was found in the rats suffering from epilepsy and also a decrease in serum TAC level suggesting excessive release of free radicals [40]. However, the present data showed that inositol ameliorated the oxidative stress via increasing GSH and TAC as well as decreasing MDA and NO. These results are in a line with previous studies that showed a reduction of the levels of MDA and NO and an elevation of the levels of GSH and TAC brain contents with inositol treatment in depression model in rats [46,47].

There is accumulating evidence that the pathophysiology of epilepsy is affected by chronic inflammation [48]. After exposure to inflammatory signals, the liver sensitizes the acute-phase short pentraxin protein which is called CRP. Initially, it was considered as a marker of inflammation. However, there are not many studies of CRP in the context of seizures or epilepsy [49].

Our data revealed that epilepsy induced by penicillin caused an elevation in brain contents of IL-1β and CRP, corresponding to the normal control group. Phoswa and Mokgalaboni found that epilepsy performs complications that cause physical and psychological distress and inflammation through an increase in IL-1β levels [50]. Also, CRP is secreted in response to inflammation as an acute phase reactant [51]. On the other hand, pretreatment with inositol significantly decreased brain contents of IL-1β and CRP, as compared with the epileptic group. Our results are in agreement with Nozadze and colleagues who reported that inositol has been considered as a promising antiepileptic substance [52].

The present study reported, also, reduced brain content of GABA, GAD-ab, and EAAT2 in penicillin group. At the GABA receptor, it has been suggested that penicillin action in the central nervous system is based on its competition for GABA [52] and GABA transmission is inhibited. In the extracellular spaces of epileptic patients, accumulated excitatory neurotransmitter glutamate was removed by EAAT2 from the synaptic cleft [53]. In different animal models of epilepsy, changes in the expression of EAAT2 were shown [54]. Previously myo-inositol was an agonist of a GABA-A receptor [55] and had a seizure suppressant effect in the brain [56]. In rat brain membranes, inositol modulates GABA-A and NMDA receptors thereby exerting its anticonvulsant action. The osmotic activity of myoinositol may also be a contributing factor to its antiepileptic effects against a massive influx of sodium, calcium, Chloride, and water that occurs at the increased rates of neuronal activity minimizing swelling and preserving function of all essential enzymes.

Conclusion

The present findings revealed that inositol reduced serum levels of calcium and NMDA and inhibited oxidative stress via reducing MDA and elevating GSH level as well as inflammation (IL-1β and CRP). In addition, inositol maintains the balance of neurotransmitters GABA, GAD-ab, and EAAT2. So,

inositol can be used as a neuroprotective compound against penicillin-induced epileptiform activity.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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