Metabolic Brain Disease (2019) 34:991–999
https://doi.org/10.1007/s11011-019-00414-3

ORIGINAL ARTICLE

Neuro-pharmacological evaluation of anticonvulsant and neuroprotective activity of *Cocculus laurifolius* leaves in wistar rats

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Received: 18 December 2018 / Accepted: 28 March 2019 / Published online: 16 April 2019

Abstract

The aim of the study was to evaluate the anticonvulsant and neuroprotective activity of *Cocculus laurifolius D.C* leaves in albino wistar rats against strychnine induced convulsions. Initially the extract was investigated for acute oral toxicity testing in order to examine any signs of toxicity and mortality. For anticonvulsant activity, the ethanolic extract was evaluated at doses 200 and 400 mg/kg, *p.o.* against strychnine induced convulsions model, at 1, 7, 15 and 30th day of treatment. Meanwhile, the neuroprotective effect of the extract was investigated via histopathological assessment. *Cocculus laurifolius* (200 and 400 mg/kg, *p.o.*) exhibited anticonvulsant activity as indicated by significant delay in the onset of convulsions and time to death after strychnine induced convulsions. Similarly, significant reduction in the duration of convulsions and percentage of mortality was observed by ethanolic extract (200 and 400 mg/kg *p.o.*) at 1, 7, 15 and 30th day of test sessions. Furthermore, *Cocculus laurifolius* leaves (200 and 400 mg/kg *p.o.*) also exhibited neuroprotective effect with considerable preserved neuronal structures and significant decrease in neuronal apoptosis, in comparison with control. The results obtained from the present study indicate that ethanolic extract of *Cocculus laurifolius* leaves possess potential anticonvulsant and neuroprotective effect against strychnine induced convulsions. Therefore, it can be concluded that *Cocculus laurifolius* leaves may be a valuable in management of epilepsy, however further studies are required on large number of animals to confirm these findings.

Keywords *Cocculus laurifolius* · Anticonvulsant · Neuroprotective

Abbreviations

ECL	Ethanolic extract of *Cocculus laurifolius*
CNS	Central nervous system
GABA	Gamma aminobutyric acid

Chemical compound studied in the article
Strychnine (PubChem CID: 441071), diazepam (PubChem CID: 3016)

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Introduction

Epilepsy is a chronic neurological disorder that includes wide range of conditions which results in dysfunction of brain and spinal cord (Dennis et al. 2003). Approximately 50 million of global population suffers from epilepsy and it is estimated to contribute about 0.75% among global disease burden (Scott et al. 2001). This medical complication has been characterized by seizures (a condition with episodes of uncontrolled, involuntary movement of body with loss of consciousness). Seizures can occur from abnormal and excessive electrical discharge in neurons results from imbalance between excitatory and inhibitory state that may lead to abnormal electrical activities (Badawy et al. 2009; Watemberg 2000). The inhibition of GABAergic and excitation of glutamatergic system in central nervous system may also leads to epileptic state (Schneider and de Lores

Arnaiz 2006). This excitation of glutamatergic system leads to the accumulation of intracellular calcium ions that activates proteolytic enzymes, which ultimately results in neuronal cell death (Henshall and Murphy 2008).

Despite the availability of conventional antiepileptic drugs, treatment of epilepsy is still disappointing. Most of the antiepileptic drugs are only effective for shorter duration and resistance may develop on long term treatment. Furthermore, the major concerns are the adverse effects and interaction associated with these antiepileptic drugs that results in treatment failure (Perucca and Gilliam 2012). Therefore, people are switching towards safe and effective alternative options. In investigation of newer treatment option, phytochemicals can also contribute an important role for the development safe and effective antiepileptic drugs (Nsour et al. 2000).

The medicinal active plant *Cocculus laurifolius* belongs to the family Menispermaceae, commonly known as laurel leaf snail seed. This plant has been widely distributed Hunan, Taiwan, Xizang (Gyirong), Indonesia, Japan, Laos, Malaysia, Myanmar, Thailand, Nepal, India (Tamil Nadu) & South East Asia (Kottaimuthu 2008), 2 of the species have also been reported in Pakistan (Siddiqi 1974). *Cocculus laurifolius* has been traditionally used as a diuretic, muscle relaxant, in the treatment of hypertension, rheumatic pain and epilepsy (Kaur et al. 2019).

Bark and leaves of the plant are rich source of alkaloids, flavanoids, tanins, saponins and phenolic compounds (Ajaib et al. 2017). *Cocculus laurifolius* leaves are found to have Erythrina alkaloids, apoporphines, quaternary and secondary alkaloids. In vitro studies on *Cocculus laurifolius* leaves revealed its antimicrobial and antioxidant properties. While the major alkaloids of *Cocculus laurifolius* leaves have been reported to possess neuromuscular blocking and ganglionic blocking activities (Bhakuni 1984; Mukherjee et al. 1984). Furthermore, some of the quaternary bases cocculidine and cocculine that exhibit hypotensive activity in dogs, cats and rabbits (Bhakuni 1984). Extract from these species have been reported for anticonvulsant activity (Kumar et al. 2012; Quintans Júnior et al. 2008). Although no such animal study has been reported on the anticonvulsant activity of *Cocculus laurifolius* leaves so far.

The present work aims to undertaken the anticonvulsant activity of ethanolic extract of *Cocculus laurifolius* leaves in rats. Initially, acute oral toxicity testing was carried out to investigate the safety profile of extract and selection of doses for neuropharmacological activity. Then, we have evaluated the anticonvulsant activity of ethanolic extract of *Cocculus laurifolius* leaves by strychnine induced convulsions after acute and chronic administration in different days of test session. Furthermore neuroprotective effect of the extract was also evaluated by histopathological examination after strychnine induced convulsions.

Material & methods

Plant material

Fresh leaves of plant *Cocculus laurifolius* D.C were collected from botanical garden of GC University, Lahore in October 2017. The plant was identified by Prof. Zaheeruddin Khan (Department of Botany, GC University Lahore, Pakistan) and deposited in herbarium department of GC University, Lahore under voucher specimen NO. GCU-Herb-bot 2997 for further reference.

Extract preparation

Leaves of plant (2Kg) were washed and dried for extraction. Dried leaves were then coarsely grounded and macerated in ethanol (98% *w*/*v*) for duration of 1 week. The liquid was decanted to remove all the debris and was concentrated on rotary evaporator at 30–40 °C. The concentrated extract of 14.8 g was obtained. The extract was stored at 4 °C in air tight container.

Chemicals

Ethanol was purchased from BDH laboratory supplies (England), Sodium chloride and Strychnine were procured from Sigma Chemical Company (USA). All other reagents and drugs used in the study are of pharmaceutical standards.

Animals

Wistar albino rats of both genders, weighing (200–250 g) were procured from Animal house of Hafiz Muhammad Ilyas Research lab (HMI), Hamdard University. The animals were housed under controlled environment at (50–60% humidity, 22–28 °C temperature 12 h light and dark cycles) in Plexiglas cages, with proper food and water supply. All procedures involving animals and their care were conducted in accordance with the Guide to the Care and Use of Experimental Animals (Olfert et al. 1993). An hour before conducting the experiment animals were kept in a laboratory for adaptation of environment. Animals were handled according to the guideline of National Institute of Health (NIH) guide for the care and use of Laboratory Animal (National Research Council 2010).

Acute toxicity study

The acute oral toxicity test was conducted according to Organization for Economic Co-operation and Development (OECD) guideline 423 on Wistar albino rats (200–250 g), both gender (OECD Guidelines for the Testing of Chemicals (No. 423)). The animals were divided into 5 groups control, 5,

50, 300 and 2000 mg/kg (each group contain $n = 3$ animals), where the limit test dose used for the study was 2000 mg/kg. Food was restricted for animals with free excess to water, a night before performing the test. All animals were weighed prior to dose administration. Doses were prepared by dissolving the extract in distill water according to 1 ml/100 g body weight of each animal. Control group was kept on normal saline. All doses were administered via oral intubation. After drug administration, animals were observed for 24 h for any mortality and toxic symptoms, with special observation during initial 4 h. Animals were observed for behavioral changes, fatigue, diarrhea, convulsion, drowsiness, sedation, changes in skin and eye color, hair loss and mortality.

Experimental protocol

Animals were divided into four groups ($n = 7$) rats in each group:

Control group: Normal saline 0.9% treated (10 ml/kg, *p.o*)
Standard group: Diazepam treated (1 mg/kg, *p.o*) (Rajput et al. 2013)
ECL I: Ethanolic extract of *Cocculus laurifolius* leaves (200 mg/kg, *p.o*)
ECL II: Ethanolic extract of *Cocculus laurifolius* leaves (400 mg/kg, *p.o*)

Animal groups were subjected to 30 days of treatment with oral intubation. Anticonvulsant effect was observed at 1st, 7th, 15th and 30th day of treatment. All treatment groups were administered 30 min prior to administration of strychnine (Raygude et al. 2012). Strychnine 5 mg/kg *i.p* was used to induce convulsions in each test session.

Anticonvulsant activity

For chemoconvulsant activity, animals were administered orally with (control: 10 ml/kg, standard: 1 mg/kg, test groups: 200 and 400 mg/kg) 30 min before experiment. Thirty minutes later, each rat was administered with strychnine 5 mg/kg intraperitoneally. Immediately after strychnine administration, animal was individually placed in a cage and observed about an hour for the onset of convulsion, duration of convulsions, time to death of animal after convulsions and percentage of mortality rate (Quintans-Júnior et al. 2008). Strychnine exerts its anticonvulsant effect by inhibiting glycinergic pathway with an increase in brain glutamic acid levels, which innervates excitatory nerve impulses and produce skeletal muscle hyper excitability (Gupta et al. 2019). Animals did not convulse within 30 min after strychnine administration were considered as protected (Patil et al. 2011). Number of animals survived after 1, 7 and 15 day of experiment were continued with ECL and diazepam administration.

Histopathological examination

After completion of 30th day test session, animals from each group (control, standard, ECL 200 and 400 mg/kg) were sacrificed to extract brain. Animals that remained protected from strychnine poisoning, were sacrificed by cervical dislocation and then dissected to extract complete brain tissues. These samples were then kept in 10% buffered PFA for overnight for fixation and then processed for histological studies. Samples were embedded in paraffin wax and sectioned by using microtome. Sectioned tissues were exposed to water bath at 46–48 °C and placed on positively charged slides. Afterwards tissues were stained with hematoxylin and eosin stains. Slides were examined with light microscope and digital photographs were taken (Karimzadeh et al. 2012).

Statistical analysis

The statistical analysis was carried out using SPSS version 20.0. All results were presented as mean ± SEM. One-way ANOVA and post-hoc Tukey's was used for analysis. A probability value <0.05 was considered as significant between control and treated groups. Percentage of mortality was analyzed by Fisher's exact test.

Results

Effect of ECL on acute oral toxicity testing

The acute oral administration of ECL was well tolerated by animals up to dose 2000 mg/kg. The ethanolic extract did not showed any physical, behavioral and physiological changes, whereas no any signs of toxicity and mortality was observed in any administered dose. The LD_{50} of the extract was not calculated in this study due to no observable signs of toxicity. To evaluate the pharmacological effect of ECL 1/5th and 1/10th dose of 2000 mg/kg; 200 and 400 mg/kg respectively were calculated.

Strychnine induced convulsions

ECL 200 and 400 mg/kg significantly elicited the onset of convulsions ($P < 0.01$) at day 1 and ($P < 0.001$) at day 7th, 15th and 30th test sessions, in comparison with control. Similar results were observed with Diazepam 1 mg/kg with significant delay in onset of convulsion ($P < 0.01$) at day 1 and ($P < 0.001$) at different days of experiment (Table 1). Both doses of extract showed delayed in onset of convulsion with an increase in number of days of treatment.

On the other hand, standard 1 mg/kg considerably inhibit the duration of convulsions ($P < 0.001$) at day 1, 7, 15 and

Table 1 Effect of ECL on onset of convulsions (min)

S. No	Groups	Doses	DAY 1*	DAY 7*	DAY 15*	DAY 30*
1	Normal Saline	10 ml/kg	1.30 ± 0.22	3.29 ± 0.4	3.45 ± 0.3	4.07 ± 0.5
2	Diazepam	1 mg/kg	2.56 ± 0.29**	8.75 ± 0.5***	9.58 ± 0.8***	22.36 ± 2.3***
3	ECL I	200 mg/kg	2.9 ± 0.5**	7.43 ± 0.6***	10.12 ± 0.6***	29.78 ± 3.1***
4	ECL II	400 mg/kg	2.8 ± 0.7**	5.58 ± 0.3***	8.12 ± 0.5***	10.85 ± 1***

Data are expressed as mean ± S.E.M. $n = 7$ in each group. Comparison was made with control group for each test. Data of latency to onset of seizures was analyzed by one-way ANOVA followed by Post hoc Tukey test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) when compared with control groups. F-statistics: Day 1; $F_{(3,24)} = 4.680$, $P \leq 0.01$, Day 7; $F_{(3,24)} = 22.65$, $P \leq 0.001$, Day 15; $F_{(3,22)} = 25.05$, $P \leq 0.001$, Day 30; $F_{(3,22)} = 32.03$, $P \leq 0.001$

30 day of test session in comparison with control, represented in (Table 2). Meanwhile. ECL I and II noteworthy decline in duration of convulsions ($P < 0.001$). The results obtained from the leaves extract at both doses were comparable with the standard anticonvulsant diazepam 1 mg/kg.

Table 3 depicted the time to death after strychnine induced convulsions in rat. The acute administration of ECL on day 1 significantly prolong the time to death after convulsions ($P < 0.001$). In addition, statistical analysis revealed subsequent augmentation in time to death of animals after chronic administration of ECL I and II ($P < 0.001$) at 7th, 15th and 30th day test sessions in comparison with control. Meanwhile, diazepam 1 mg/kg remarkably increased the time to death of animals after convulsions as compared with control; with no animal death at 7th and 30th day of treatment. However, no animal death was observed with ECL 200 mg/kg at day 15 of test session.

Furthermore, ECL I and II ($P < 0.001$) produce significant decline in the percentage of mortality after strychnine induced convulsions in comparison with control at different days of experiment. No mortality was observed with ECL I at 15th day of test session. On the other hand, diazepam 1 mg/kg also illustrated protected effect against strychnine with considerable decrease in mortality rate ($P < 0.001$) at 1, 7, 15 and 30 day of test sessions. Although no animal death was observed by standard at 7th and 30th day of treatment. However, the results obtained from ECL 200 mg/kg were comparable with diazepam 1 mg/kg Represented in Table 4.

Histopathological evaluation

Results obtained from the histopathological evaluation of rat brains after strychnine induced seizures, illustrated that the shrunken neuron and eosinophilic necrosis with proliferated blood vessels were observed in hippocampus after strychnine administration (Fig. 1a). Similarly few healthy neurons can be seen in (Fig. 1b) with numerous pyramidal neuron and edematous tissues after strychnine administration in control (normal saline) group. Treatment with diazepam 1 mg/kg showed relatively healthier neuron with few reactive astrocytes in comparison with control (Fig. 1c). However, considerably preserved neurons were observed with few pyknotic cells after ECL I (Fig. 1d) and ECL II (Fig. 1e) treatment in comparison with control group.

Discussion

Epilepsy is the second most common neurological disorder among the population. Despite of advancement in research, treatment of epilepsy is still in effective, due to lack of understanding of pathology (Gourie-Devi et al. 2004). Due to this reason approximately 35% of epilepsy patient have seizures that does not respond to commercially available antiepileptic drugs, which results in treatment failure (Reddy 2005). To eradicate these issues, researchers are investigating for the development of novel treatment options with minimum complications. One of the approaches to search for new antiepileptic

Table 2 Effect of ECL on Duration of convulsions (min)

S. No	Groups	Doses	DAY 1*	DAY 7*	DAY 15*	DAY 30*
1	Normal Saline	10 ml/kg	3.8 ± 0.3	3.3 ± 0.2	4.8 ± 0.7	3.7 ± 0.3
2	Diazepam	1 mg/kg	2.04 ± 0.24***	1.9 ± 0.1***	2.0 ± 0.2***	1.8 ± 0.2***
3	ECL I	200 mg/kg	1.3 ± 0.1***	1.9 ± 0.2***	2.1 ± 0.2***	1.5 ± 0.2***
4	ECL II	400 mg/kg	2.6 ± 0.1***	2.3 ± 0.2***	2.2 ± 0.3***	1.9 ± 0.3***

Data are expressed as mean ± S.E.M. $n = 7$ in each group. Comparison was made with control group for each test. Data of latency to onset of seizures was analyzed by one-way ANOVA followed by Post hoc Tukey test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) when compared with control groups. F-statistics: Day 1; $F_{(3,24)} = 20.777$, $P \leq 0.001$, Day 7; $F_{(3,24)} = 12.186$, $P \leq 0.001$, Day 15; $F_{(3,22)} = 13.495$, $P \leq 0.001$, Day 30; $F_{(3,22)} = 19.750$, $P \leq 0.001$

Table 3 Effect of ECL on time to death after convulsions (min)

S. No	Groups	Doses	DAY 1*	DAY 7*	DAY 15*	DAY 30*
1	Normal Saline	10 ml/kg	1.19 ± 0.18	3.4 ± 0.5	1.9 ± 0.9	2 ± 0.2
2	Diazepam	1 mg/kg	5 ± 0***	0***	16 ± 0***	0***
3	ECL I	200 mg/kg	6 ± 0***	6.8 ± 0***	0***	16.3 ± 0***
4	ECL II	400 mg/kg	4.9 ± 0.5***	4.5 ± 0.4***	12 ± 0.5***	14 ± 0.9***

Data are expressed as mean ± S.E.M. $n = 7$ in each group. Comparison was made with control group for each test. Data of latency to onset of seizures was analyzed by one-way ANOVA (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) when compared with control groups. F-statistics: Day 1; $F_{(3,10)} = 68.870$, $P \leq 0.001$, Day 7; $F_{(2,8)} = 3.522$, $P \leq 0.08$, Day 15; $F_{(2,8)} = 292.872$, $P \leq 0.001$, Day 30; $F_{(2,8)} = 189.398$, $P \leq 0.001$

drugs is the investigation of naturally occurring compounds; including those obtained from traditional medicinal plants (Sahranavard et al. 2014; Muazu and Kaita 2008). Considering the fact, the present study was focused to evaluate anticonvulsant and neuroprotective activity of ethanolic extract of *Cocculus laurifolius* leaves by strychnine induced convulsions after acute and chronic administration.

Phytomedicines have been extensively used traditionally and over the counter medication for the treatment of different ailments in under privilege countries. Despite of numerous medicinal benefits, the complex chemical nature of plants may produce some harmful effects and toxicity (Mythilypriya et al. 2007). In the current study, ethanolic extract of *Cocculus laurifolius* leaves were investigated for acute oral toxicity testing; no neurobehavioral changes and mortality was observed at maximum dose (2000 mg/kg) after 24 h of observation. On the basis of results, optimal doses of ECL 200 and 400 mg/kg were selected for neuropharmacological evaluation.

Chemoconvulsants are widely used to induce convulsions in experimental animals for the development of novel antiepileptic drugs (McNamara 1994). Strychnine is a poisonous indole type alkaloid obtained from the seed of *Strychnos nux vomica* (Volfova and Patocka 2003) and act at central nervous system by blocking inhibitory effect of glycine that lead to excitation, muscle contractions and convulsions followed by death (Brams et al. 2011). Strychnine induced convulsions model, represent generalized tonic clonic convulsions (Amabeoku and Chandomba 1994; Philippe et al. 2004). Many conventional drugs and plant extracts have been reported to abolish generalized tonic clonic seizure induce by strychnine administration (Billimoria et al. 1981; Rafeeq et al. 2017). In our study the ethanolic extract of *Cocculus laurifolius* significantly increased the onset time of convulsions after acute and chronic administration of dose 200 and 400 mg/kg at 1, 7, 15 and 30 day test session. These results were comparable with reference standard diazepam 1 mg/kg. Previous studies reported that drugs capable to delay in onset of convulsions and decrease in episodes of convulsions are capable of reducing seizures in epileptic brains (Corda et al. 1982).

Similarly, pretreatment of rats with ECL 200 and 400 mg/kg showed significant increase in the time to death after strychnine induced convulsions. In addition, the percentage of mortality was considerably decreased after acute and chronic administration of ECL I and II. The effect produce by ECL 200 mg/kg with noteworthy decrease in mortality rate and increase in time to death of animals, these results were comparable with diazepam 1 mg/kg. Meanwhile, the extract at dose 200 mg/kg showed more pronounced results than standard 1 mg/kg on onset and duration of convulsions. Kendall et al. 1981 stated that the anticonvulsant property of a drug is not only considered by its ability to prevent convulsions but also by its potential to delay the onset, decrease in death rate and frequency of convulsions. Thus, the ability of *Cocculus laurifolius* to delay the onset of convulsions, decrease the mortality rate, time to death and duration of convulsions, indicates that the extract possessive substantial anticonvulsant potential. However, the extract does not produce dose dependent anticonvulsant effect which might be link to the presence of alkaloids in the extract (Herrera-Calderon et al. 2018). The

Table 4 Effect of ECL on percentage of mortality rate (%)

S. No	Groups	Doses	DAY 1*	DAY 7*	DAY 15*	DAY 30*
1	Normal Saline	10 ml/kg	100 (7/7)	87.5 (6/7)	100(7/7)	100(7/7)
2	Diazepam	1 mg/kg	14.3 (1/7)***	0 (0/7)***	14.3 (1/7)***	0 (0/7)***
3	ECL I	200 mg/kg	28.6 (2/7)***	14.3 (1/7)***	0 (0/7)***	14.3 (1/7)***
4	ECL II	400 mg/kg	57.1 (4/7)***	42.9 (3/7)***	42.9 (3/7)***	42.9(3/7)***

Data for percentage of mortality was analyzed by Fisher's exact test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) when compared with control groups. N = 7, F-statistics: Day 1; [$\chi 2 = 4.680$ ($df = 3$), $P \leq 0.01$], Day 7; [$\chi 2 = 22.65$ ($df = 3$), $P \leq 0.001$], Day 15; [$\chi 2 = 25.05$ ($df = 3$), $P \leq 0.001$], Day 30; [$\chi 2 = 32.03$ ($df = 3$), $P \leq 0.001$] after an hour

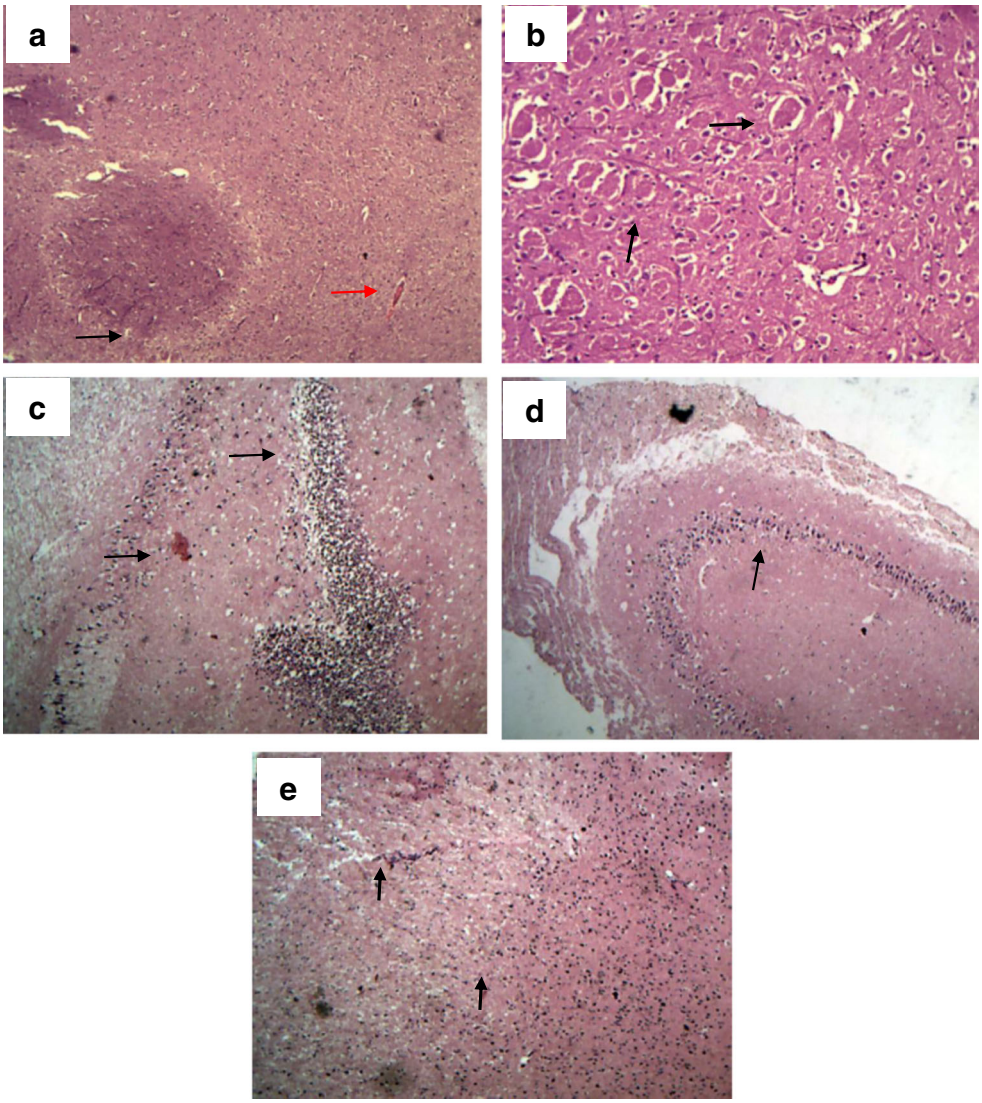

Fig. 1 Photomicrographs of representative section of hematoxylin and eosin staining of rat brain after strychnine induced seizure at day 30th test session. **a** Representative section of strychnine induced seizure with eosinophilic necrosis, edematous and proliferated blood vessels with few normal neuron (pointed by arrows). **b** Representative section of normal saline group, with shrunken neuron and inflammatory cells with blood vessels (pointed by black arrow). **c** Representative section of Diazepam, showing healthy neurons with few reactive astrocytes and blood vessels. **d** Representative section of ECL I, showing relatively healthy neurons with few pyknotic cells. **e** Representative section of ECL II, showing relatively healthy neurons

dose independent effect of the extract might be produce due to saturation of metabolism and excretion processes that leads to substantial changes in plasma levels of extract, which results in slightly reduce anticonvulsant effect at dose 400 mg/kg in comparison with 200 mg/kg of ECL (Lin 1994). However, further investigation are needed to prove these assumptions.

Glycine is a major inhibitory neurotransmitter of central nervous system and strychnine is competitive antagonist of glycine receptor (Patil et al. 2012). The strychnine insensitive receptor glycine (Gly_2) have been also reported to allosterically link to excitatory amino acid NMDA receptor (Larson and Beitz 1988). However, some studies also suggested the suppression of seizures after glycine administration (Cherubini et al. 1981) while other illustrated the inhibition of convulsions after administration of GABA receptor antagonist (Nutt and Lister 1988). These finding are also supported by the current study on rat model, because ECL successfully inhibits the convulsions along with decrease in mortality rate in rats after acute and chronic administration. On this basis, it can be assumed that ECL exerts its anticonvulsant effect through its action on glycinergic or GABAergic pathway. On the other hand, flavanoids have also been found to possess significant role in inhibition of epilepsy via acting on different underlying mechanisms i.e. activation of Ca^{++} and K^{+} ions that results in decrease outflow of potassium in neurons, inhibition of Na^{+} ions (Rajput et al. 2017), activation of GABAergic system that results in neuronal hyperpolarization via chloride influx (Hanrahan et al. 2011) and reduction of calcium influx via inhibition of NMDA receptor (Subash and Subramanian 2009). So, it can be assumed that the anticonvulsant effect might be attributed due to presence flavanoids in *Cocculus laurifolius* plant (Diniz et al. 2015). The current work does not only reports the anticonvulsant effect of *Cocculus laurifolius* on different days of test sessions but it also showed enhancement in anticonvulsant potential of the extract with an increase in the number of days of treatment, as previously explained in different studies (Wannang et al. 2008; Viswanatha et al. 2009; Navarro-Ruiz et al. 1996; Muralidharan et al. 2009; Babu et al. 2018).

A part from subsequent inhibition in convulsions, epilepsy can also lead to neuronal damage and hippocampal structure impairment that may lead to memory loss in patients (Kohl et al. 2011). The results of the present study indicates the neuroprotective effect of ethanolic extract of *Cocculus laurifolius* leaves after neurotoxic strychnine administration. The histopathological examination showed pronounced effect in reduction of neuronal damage in hippocampus shown Fig. 1d and e in comparison with control. The photomicrographs obtained from ECL I and II were similar to that of diazepam 1 mg/kg, with preserved structures and healthy neurons with negligible blood vessels. It has been previously reported that free radicals and oxidative stress can lead to neuronal damage in brain by activation of reactive oxygen that ameliorates the process of apoptosis and results in hippocampal neuronal loss (Ankarcrona et al. 1996). Tannins and saponins present in the leaves of *Cocculus laurifolius* have been illustrated in previous studies to possess anticonvulsant and antioxidant effect by reducing free radicals involved in neuronal damage (Chindo et al. 2009; Hussain et al. 2016; Calis et al. 2016). However, oxidative stress can also be reduced by administration of a compound that possessed antioxidant properties. The plant *Cocculus laurifolius* has been previously reported for its in vitro antioxidant activity. Therefore, it can be presumed that neuroprotective effect attributed by ECL at dose 200 and 400 mg/kg might be due to its antioxidant potential (Ajaib et al. 2017; Liu et al. 2012).

Conclusion

The neuronal ability of epilepsy patients is poorly understood as detailed studies are difficult to carry out in population. However, animal based anticonvulsant have been widely used to investigate the therapeutic potential of novel compound. Our study demonstrate that the ethanolic extract of *Cocculus laurifolius* leaves possess anticonvulsant activity after acute and chronic administration against strychnine induced convulsions in rats. The effect may be mediated via GABAergic excitation or glycinergic inhibition. Meanwhile the extract also found to possess neuroprotective activity, which might be contributed due to antioxidant properties of the extract. Moreover, the acute toxicity profile of the extract did not show any toxicity or neurotoxic side effects. However, further investigations are require to fully understand the underlying mechanism of anticonvulsant and neuroprotective activity of ethanolic extract of *Cocculus laurifolius* leaves.

Acknowledgements Authors are grateful to Hafiz Muhammad Ilyas Research lab (HMI), Hamdard University, for technical support and assistance. The authors are thankful to HURC for financial assistance to conduct this research.

Compliance with ethical standards

Ethical approval All the research protocol, procedures, euthanasia as well as ethical clearance were approved by the Board of Advanced Studies and Research, Hamdard University, Karachi. The animals used in the study were handled as per specifications described in Helsinki Resolution 1964. The acute oral toxicity test was conducted according to Organization for Economic Co-operation and Development (OECD) guideline 423 on Swiss-albino rats of (200–250 g) (Class, 2001).

Conflict of interest Declared none.

Approval of project & ethical clearance In the present study, all research protocols, animal screening and euthanasia procedures are approved by the Ethical Review Board (ERB), Hamdard University. This study protocol was also approved by BASR (Board of Advanced Studies & Research), Hamdard University.

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