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Original Article

Anticonvulsant mechanism of saponins fraction from adventitious roots of *Ficus religiosa*: possible modulation of GABAergic, calcium and sodium channel functions

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ABSTRACT

In our previous studies, quantified saponins-rich fraction from adventitious root extract of *Ficus religiosa* L., Moraceae, showed anticonvulsant effect in acute, as well as chronic mice models of epilepsy. The present study was designed to reveal putative anticonvulsant mechanism of quantified saponins-rich fraction using target specific animal models. The anticonvulsant effect of quantified saponins-rich fraction was initially studied in maximal electroshock and pentylenetetrazol test at 1, 2 and 4 mg/kg; *i.p.* doses. Based on the results of initial anticonvulsant testing, different groups of mice were injected with vehicle or quantified saponins-rich fraction (4 mg/kg; *i.p.*), 30 min prior to an injection of *N*-methyl-D-aspartic acid (100 mg/kg; *s.c.*), bicuculline (5 mg/kg; *i.p.*), strychnine hydrochloride (2 mg/kg; *i.p.*), BAY k-8644 (37.5 μg; *i.c.v.*), veratridine (500 μg/kg; *i.p.*) and the convulsive episodes were studied. Treatment with the extract (1, 2 and 4 mg/kg) showed significant protection in maximal electroshock and pentylenetetrazol-induced convulsion tests, in a dose-dependent manner. Moreover, quantified saponins-rich fraction at 4 mg/kg dose showed significant increase in latency to clonic convulsions, decrease in seizure severity and increase in average wave amplitude in bicuculline, BAY k-8644 and veratridine tests, respectively, as compared to vehicle control. However, SRF treatment failed to abolish *N*-methyl-D-aspartic acid and strychnine-induced convulsions, indicated by insignificant change in the appearance of turning behavior and onset of tonic extension, respectively, as compared to vehicle control. From the results of present study, it is concluded that quantified saponins-rich fraction suppress maximal electroshock, pentylenetetrazol, bicuculline, BAY k-8644 and veratridine-induced convulsions, indicating its GABAergic, Na^+ and Ca^{2+} channel modulatory effects. Further it can be correlated that quantified saponins-rich fraction causes deactivation of voltage-gated Na^+ and Ca^{2+} channels, without effecting ligand-gated Na^+ and Ca^{2+} channels. More studies are required at molecular levels using *in vitro* techniques to understand the exact molecular interactions of quantified saponins-rich fraction with these pathways.

Introduction

Ficus religiosa L., Moraceae, is a medicinally important plant of the genus *Ficus*, and has been extensively used in traditional medicine for a wide range of ailments. Its different botanical parts have been used for the ethnomedical treatment of epilepsy. Many of its traditional uses have been validated in different experimental studies throughout the globe. Its different parts have shown a variety of neurological effects including antiamnesic, acetylcholinesterase inhibitory, parasympathetic modulatory, antianxiety and reversal of reserpine-induced behavioral effects (Singh et al., 2011a). Apart from these pharmacological effects, it has also been studied for its anticonvulsant potential in different experimental studies.

In our previous study, the crude fruit extract of *F. religiosa* have shown anticonvulsant activity, which was found to be due to modulation of serotonergic functions of the brain (Singh and Goel, 2009; Goel and Singh, 2013). The flavonoid-rich fraction of the fruit extract in combination with phenytoin also showed protection in kindling mice model, along with attenuation of associated cognitive and behavioral impairments (Singh et al., 2014a). Its leaf extract was studied in acute animal models of convulsion, but was found to be ineffective (Singh et al., 2011b). In a recent study the crude bark extract of the plant also showed

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protection in acute animal models of convulsions, which was found to be due to GABA aminotransferase inhibitory activity of its bioactive metabolites (Singh et al., 2014b). The crude adventitious root extract of *F. religiosa* has also been studied for its anticonvulsant activity (Patil et al., 2011). When partitioned, only the saponins-rich fraction (SRF) of the extract retained anticonvulsant activity, rest all other fractions were found to ineffective. The study indicated saponins present in the adventitious root extract to be responsible for its activity (Singh et al., 2012). SRF *per se* also prevented behavioral impairments associated with kindling in mice, but failed to prevent cognitive deficit (Singh et al., 2013).

The saponins are a type of naturally occurring surface-active glycosides, which are generally produced by plants with an exception of some lower marine animals and bacteria (Francis et al., 2002). The therapeutic role of saponins has been suggested in several central as well as peripheral pathological conditions like, neurodegeneration, epilepsy, cognitive impairments, hypertension, atherosclerosis, inflammation, allergic reactions, cancer, hyperglycemia and many more (Radad et al., 2004; Nah et al., 2007). The saponins isolated from other plants have been found to interact with all the pathological processes involved in epilepsy. They showed GABAergic agonist (Kimura et al., 1994; Kim et al., 2001; Choi et al., 2003), glutamatergic antagonist (Kim and Rhim, 2004; Peng et al., 2009), glycinergic agonist (Noh et al., 2003; Kim et al., 2004), Ca^{2+} (Zhong et al., 1995; Kim et al., 2008) and Na^{+} channel blockade effects (Liu et al., 2001; Kim et al., 2005; Chindo et al., 2009). Due to wide spectrum of neuronal pathway interaction by saponins, the present study was envisaged to understand the putative anticonvulsant mechanism of SRF of adventitious root extract of *F. religiosa*, by using animal models of epilepsy involving primarily modulation of calcium, sodium, glutamate and GABAergic pathways.

Methods

Plant material, extraction, fractionation and quantification

SRF was prepared and quantified as described in our previous study (Singh et al., 2012). Briefly, the adventitious roots of *Ficus religiosa* were collected, cleaned, shade-dried, powdered and subjected to repetitive extraction with 50% ethanol in a percolator. After drying the combined percolate, the resultant extract was dispersed in water and fractionated with hexane, chloroform, ethyl acetate and butanol. The butanol fraction was precipitated and the total saponins content was determined in the precipitates collected using colorimetric method with vanillin-sulphuric acid system, as discussed in our previous study. The doses were prepared freshly before use and was injected intraperitoneally (*i.p.*). The doses of SRF were selected based on the results of our previous study (Singh et al., 2012) and was administered at 1, 2 and 4 mg/kg.

Animals

Male Swiss albino mice, weighing 20–30 g obtained from CCS Haryana Agricultural University, Hisar, were employed in the present study. The animals were housed in standard cages and maintained at room temperature with natural day and night cycles. The animals were allowed free access to food (standard laboratory rodent's chow) and water during the study period. All the experiments were conducted between 9 am and 4 pm. All procedures were carried out according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India and approved by the Institutional Animal Ethical Committee (no.: 107/99/CPCSEA-2009-4.1).

Drugs and chemicals

Pentylenetetrazol (PTZ) (dissolved in normal saline), strychnine hydrochloride (dissolved in normal saline), bicuculline (dissolved in minimum 0.1 N HCl, volume was make up with normal saline, pH was adjusted to 6.65 with NaOH), *N*-methyl-D-aspartic acid (NMDA) (dissolved in normal saline) and veratridine (dissolved in minimum 0.1 N HCl, volume was make up with normal saline, pH was adjusted to 6.65 with NaOH) were purchased from Sigma–Aldrich (St. Louis, MO). Reference drugs, diazepam and phenytoin were obtained locally from Jackson Laboratories Ltd. (Amritsar, India) and Cadila Laboratories (Ahmedabad, India), respectively.

Maximal electroshock (MES)-induced convulsions

Mice in different groups were injected with the varying doses of SRF (1, 2 and 4 mg/kg; *i.p.*), vehicle (10 ml/kg; *i.p.*) and phenytoin (25 mg/kg; *i.p.*). After 30 min of these treatments, all the groups were delivered a calibrated (through a current calibrator [Rolex, Ambala, India]) transauricular electroshock of 56 mA for 0.2 s using a convulsiometer (Rolex, Ambala, India), *via* a pair of crocodile ear clips. The duration of tonic hindlimb extension (seconds) was noted and was compared with that of vehicle control (Swinyard et al., 1952).

PTZ-induced convulsions

PTZ at a dose of 75 mg/kg was given to five different groups of mice pretreated 30 min prior with the varying doses of SRF (1, 2 and 4 mg/kg; *i.p.*), vehicle (10 ml/kg; *i.p.*) and diazepam (5 mg/kg; *i.p.*). Latency to clonic convulsions (min) was noted and was compared with that of vehicle control.

NMDA-induced convulsions

Mice in two different groups were treated with either vehicle (10 ml/kg) or SRF (4 mg/kg, *i.p.*) 30 min prior to a subcutaneous injection of NMDA (100 mg/kg). Thereafter, the mice were observed for the appearance of turning behavior for next 30 min. Turning behavior was characterized as two consecutive 360 cycles completed by the same animal (Bhutada et al., 2010). The test was performed to determine the role of glutamatergic processes for the anticonvulsant effect of SRF.

Bicuculline-induced convulsions

Bicuculline was administered (5 mg/kg; *i.p.*) in two different groups of mice, 30 min after treatment with vehicle (10 ml/kg; *i.p.*) or SRF (4 mg/kg; *i.p.*). The mice were observed for the appearance of clonic–tonic seizures and death for a period of 30 min after bicuculline injection. Antagonism of bicuculline seizures was defined as the absence/delay of clonic–tonic seizures for 30 min (Irifune et al., 2003). The test was performed to examine the GABAergic effects of SRF.

Strychnine-induced convulsions

The test was performed to investigate the involvement of glycinergic pathway for the protective effect of SRF. Two groups of mice were injected with vehicle or SRF (4 mg/kg, *i.p.*), 30 min prior to the injection of strychnine hydrochloride (2 mg/kg; *i.p.*). The onset of tonic extension and mortality was determined (Löscher and Schmidt, 1988).

BAY k-8644-induced convulsions

BAY k-8644 was used to induce convulsions as described by VonVoigtlander et al. (1987) with slight modifications. A free hand *i.c.v.* injection of BAY k-8644 (37.5 μg/10 μl) was made in conscious mouse, 30 min after vehicle (10 ml/kg; *i.p.*) or SRF (4 mg/kg; *i.p.*) treatment by visual location method described by Haley and McCormick (1957). Briefly, each animal was firmly griped by tautly pulling the loose skin behind the head, a midline was drawn using a marker through the anterior base of the ears and the injection was made 2 mm on either side the line. A cross between the left eye–right ear and right eye–left ear was drawn to locate the midpoint to draw midline. The injections were made with hypodermic needle of 0.4 mm external diameter attached to a 10 μl Hamilton syringe (Hamilton, Bonaduz, Switzerland). The needle was covered with a polypropylene tube, leaving 3 mm of the tip region, so as to insert this portion through the skull into the brain of mouse. The syringe was held at an angle of around 45 degree to the skull, with the bevel of needle facing up, pointing in the direction of the tail. Induced seizures were rated according to a scale devised as stage 1, scratching and twisting of the forelimbs (score 1); stage 2, rearing and walking (score 2); stage 3, intermittent clonic jerks of limbs with tonic flexion of fore limbs and tail flexion; stage 4, head bobbing with complex grooming actions (licking of fur and scratching); stage 5, jumping, squeaking and tonic extension of hind limbs; stage 6, barrel rolling (Kaur and Goel, 2011). The occurrence of seizure signs and latency were recorded for 1 h. The test was performed to investigate the role of Ca^{2+} channels.

Veratridine-induced seizure

Veratridine seizures in mice were induced by the methods described by Otoom et al. (2006) and Otoom and Sequeira (2011), with slight modifications. Two different groups of mice were treated with vehicle (10 ml/kg; *i.p.*) or SRF (4 mg/kg; *i.p.*), 30 min prior to an intraperitoneal injection of veratridine (500 μg/kg). After 15 min of veratridine administration, electroencephalogram (EEG) recording was performed using a Digital Polygraph (PC-2004, Medicaid System, Chandigarh, India) by the method described by Otoom and Sequeira (2011), but using surface electrodes. Average wave amplitude (μV/min) and wave pattern in vehicle control animals were compared with that of normal (naïve) and SRF treated animals.

Statistical analysis

All the results were expressed as mean ± SEM. The significance of difference between means was determined using one-way analysis of variance (ANOVA) followed by Tukey's test in MES, PTZ and veratridine tests. Unpaired Student's *t*-test was employed to determine significance of difference between mean in NMDA, bicuculline, strychnine and BAY k-8644-induced convulsion tests. Statistical analysis for the percentage mortality and percentage of animals showing turning behavior per group was performed by Chi-square test. The results were regarded as significant at $p < 0.05$.

Results

Effect on MES-induced convulsions

Treatment with SRF showed significant ($p < 0.001$) dose-dependent decrease in the duration of tonic hind limb extension at 1 mg/kg (7.8 ± 0.86 s), 2 mg/kg (4.1 ± 1.2 s), and 4 mg/kg (1.4 ± 0.8 s), as compared to vehicle control, with maximum protection observed at 4 mg/kg. The anticonvulsant activity of SRF

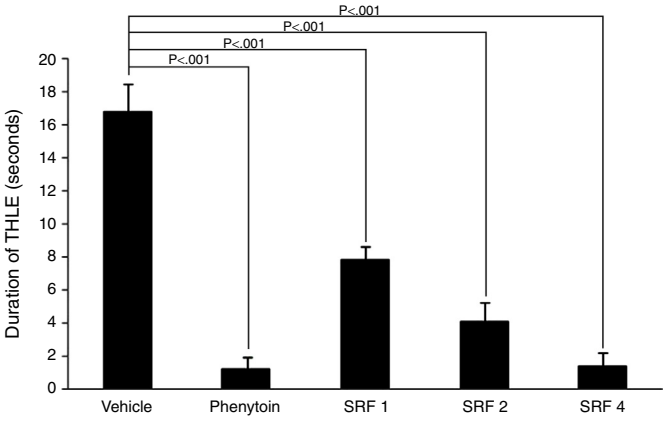

Fig. 1. Effect different doses of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on the duration of MES-induced tonic hind limb extension. THLE, tonic hind limb extension; SRF 1, 2 and 4, saponins-rich fraction 1, 2 and 4 mg/kg, respectively.

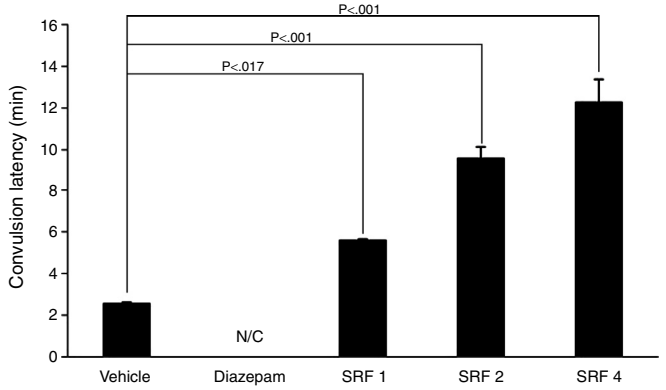

Fig. 2. Effect different doses of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on the latency to PTZ-induced convulsions. N/C, no convulsions; SRF 1, 2 and 4, saponins-rich fraction 1, 2 and 4 mg/kg, respectively.

at 4 mg/kg was found to be comparable to phenytoin treated (1.2 ± 0.8 s) group (Fig. 1).

Effect on PTZ-induced convulsions

In PTZ-induced convulsions test, treatment with SRF at 1 mg/kg ($p = 0.017$), 2 mg/kg ($p < 0.001$) and 4 mg/kg ($p < 0.001$) significantly increased the latency to clonic convulsions, as compared to vehicle control at 5.58 ± 0.14 min, 9.54 ± 0.64 min and 12.28 ± 1.12 min, respectively. Similarly, as in MES test, SRF showed dose-dependent anticonvulsant effect. SRF was found to be less effective as that of standard diazepam, as its treatment only delayed the clonic convulsions, were as diazepam pretreatment completely abolished the induction of PTZ convulsions (Fig. 2).

Effect on NMDA-induced convulsions

Treatment with SRF showed no protection against convulsions induced by NMDA, indicated by insignificant change in the onset and occurrence of turning behavior in mice as compared to vehicle control (Fig. 3A and B).

Effect on bicuculline-induced convulsions

In bicuculline test, treatment with SRF (4.12 ± 0.19 min) showed significant ($p < 0.001$) increase in the latency to clonic convulsions in mice, as compared to vehicle control (2.25 ± 0.25 min).

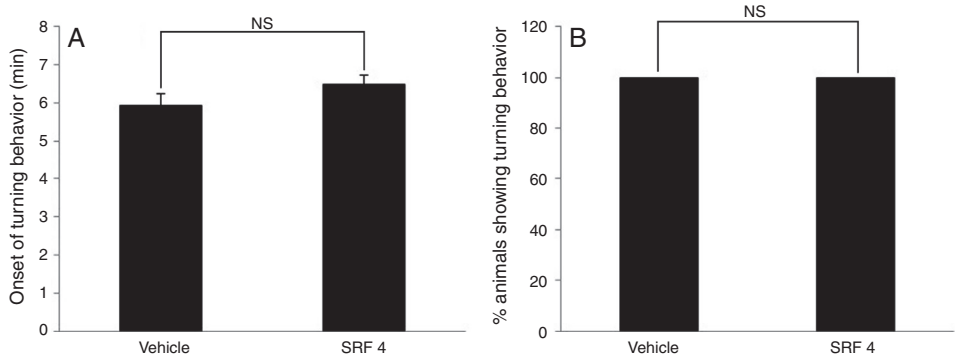

Fig. 3. Effects of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on NMDA-induced convulsions. (A) Effect of saponins-rich fraction on the onset of NMDA-induced turning behavior. (B) Effect of saponins-rich fraction on the percentage of animals showing NMDA-induced turning behavior; NS, not significant; SRF 4, saponins-rich fraction 4 mg/kg.

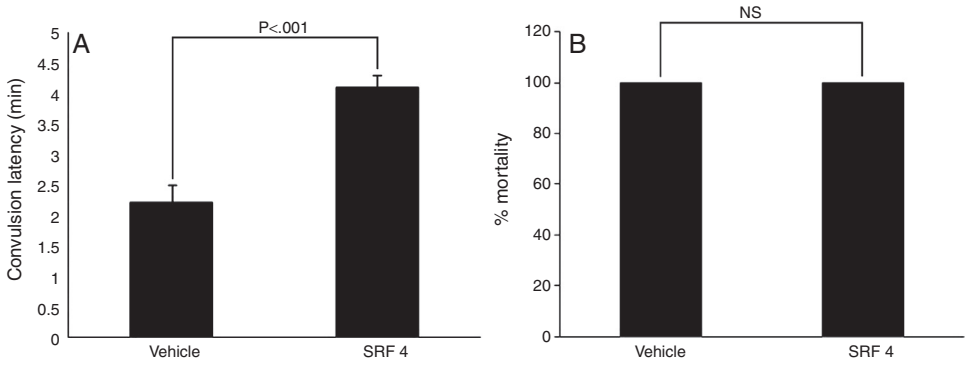

Fig. 4. Effects of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on bicuculline-induced convulsions. (A) Effect of saponins-rich fraction on the latency to convulsions induced by bicuculline; (B) Effect of saponins-rich fraction on the percentage mortality; NS, not significant; SRF 4, saponins-rich fraction 4 mg/kg.

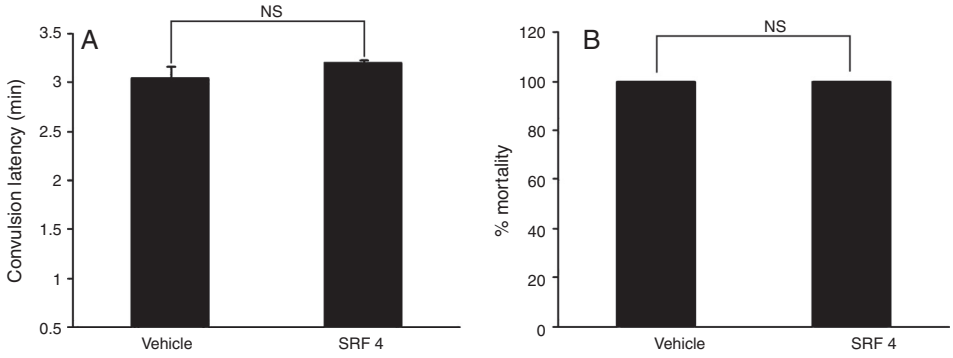

Fig. 5. Effects of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on strychnine-induced convulsions. (A) Effect of saponins-rich fraction on the latency to convulsions induced by strychnine. (B) Effect of saponins-rich fraction on the percentage mortality; NS, not significant; SRF 4, saponins-rich fraction 4 mg/kg.

However, no significant protection against mortality was observed after treatment with SRF as that of vehicle control (Fig. 4A and B).

Effect on strychnine-induced convulsions

Treatment with SRF showed no protection against convulsions induced by strychnine, indicated by insignificant change in the latency to convulsions, as compared vehicle control. Moreover, no significant protection was observed on the percentage mortality as that of control (Fig. 5A and B).

Effect on BAY k-8644-induced convulsions

BAY k-8644, a Ca^{2+} channel agonist administration in mice resulted in convulsions with a latency of around 7.28 ± 0.27 min in vehicle control group. Treatment with SRF, significantly ($p < 0.001$) increased the latency to BAY k-8644-induced convulsions to 15.85 ± 0.89 min. SRF treatment also significantly ($p < 0.001$) reduced the seizure severity score to 1.83 ± 0.6, as compared to vehicle control (5.33 ± 0.21) group (Fig. 6A and B).

Effect on veratridine-induced seizure

The vehicle control animals after veratridine treatment showed significantly ($p < 0.001$) increased average wave amplitude of 77.42 ± 3.84 μV/min, as compared to normal (naïve) animals showing the amplitude of 59.11 ± 1.98 μV/min. Treatment with SRF significantly ($p < 0.001$) decreased the average wave amplitude to 51.68 ± 2.69 μV/min, as compared to vehicle control animals (Figs. 7 and 8).

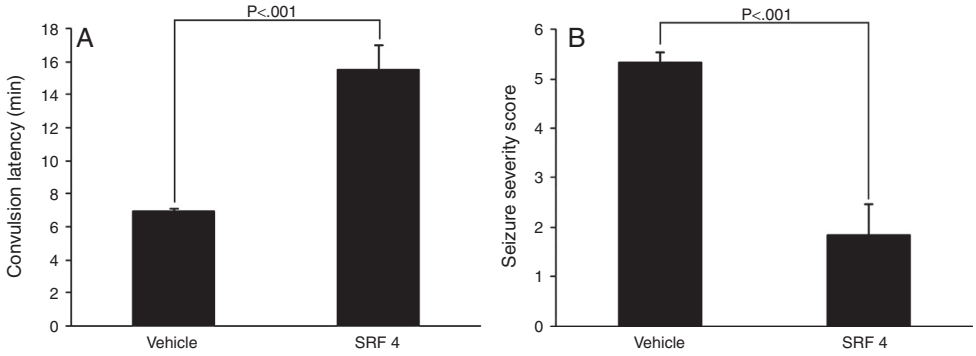

Fig. 6. Effects of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on BAY k-8644-induced convulsions. (A) Effect of saponins-rich fraction on the latency to convulsions induced by BAY k-8644; (B) Effect of saponins-rich fraction on the seizure severity score; SRF 4, saponins-rich fraction 4 mg/kg.

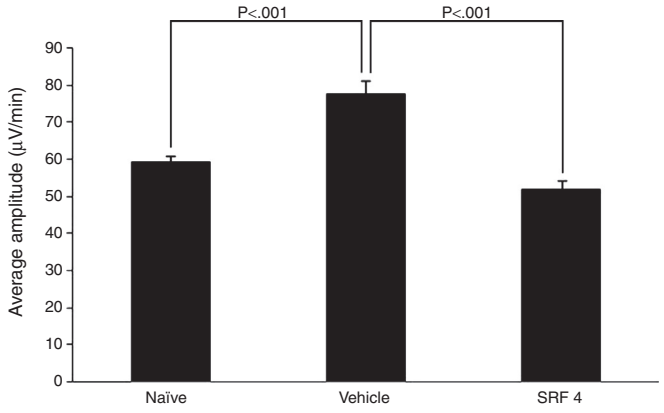

Fig. 7. Effects of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on veratridine-induced changes in wave amplitude. SRF 4, saponins-rich fraction 4 mg/kg.

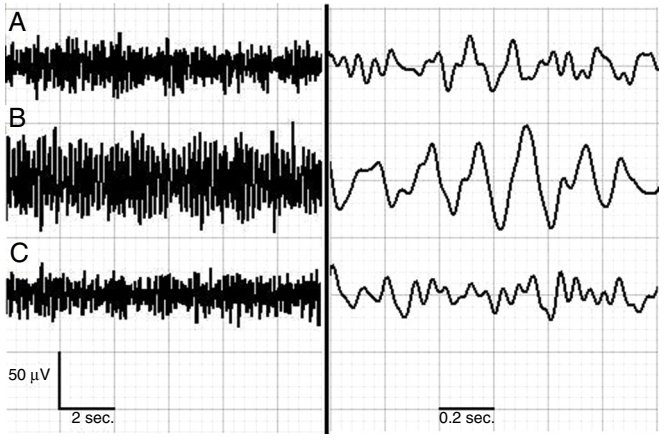

Fig. 8. Effects of saponins-rich fraction from the adventitious root extract of *Ficus religiosa* on veratridine-induced changes in wave pattern during seizures phase. (A) Naïve group; (B) vehicle control group; (C) saponins-rich fraction treated group (4 mg/kg).

Discussion

It is important to study the mechanism of a novel anticonvulsant component, as it might act on well-recognized targets in novel ways and/or by novel combinations of actions on well-recognized targets. In some cases, such a practice had revealed entirely new antiepileptic targets like topiramate and lamotrigine (Rogawski, 2006a). Hence, to study the anticonvulsant mechanism of SRF, the present study was carried out. Treatment with SRF resulted in suppression of MES and PTZ-induced convulsions in a dose-dependent manner. The results of this initial screening are in line with the results of our previous study (Singh et al., 2012). SRF treatment resulted in marked suppression of BAY k-8644 and veratridine-induced convulsions, and showed partial protection against bicuculline-induced convulsion. However it was found to be totally ineffective against strychnine and NMDA-induced convulsions. Since, MES and PTZ tests are considered to be the "gold standards" in early stages of drug testing (Rogawski, 2006b), hence initial confirmation of the anticonvulsant effect of SRF was carried in these tests at all doses. The other tests (NMDA, strychnine, bicuculline, BAY k-8644 and veratridine-induced convulsion tests) were performed to investigate its putative anticonvulsant mechanism and effect in different acute seizure models of varied phenotype. A single most effective dose was used to reduce the number of animals required in the study, due to ethical constraints.

Some of the previous *in vitro* studies revealed that saponins isolated from ginseng and other *Ficus* species causes blockade of voltage dependent Na^+ channels, hence decreases neuronal excitation (Liu et al., 2001; Kim et al., 2005; Chindo et al., 2009). Hence, to study the effect of SRF on Na^+ channels, veratridine-induced seizure model was used. Veratridine is a commonly used experimental tool in various electrochemical studies, it opens Na^+ channels during sustained membrane depolarization by inhibiting inactivation, leading to Na^+ influx, secondarily it causes Ca^{2+} influx, increased pump activity and in turn exocytosis, leading to experimental seizures in rodents (Ulbricht, 1998; Otoom et al., 2006). In the present study, administration of veratridine in vehicle control animals resulted in seizures, indicated by characteristic EEG changes, these changes are in line with a previous study (Otoom and Sequeira, 2011). Several clinically used antiepileptic drugs acting through blockade of Na^+ channels, like valproic acid, phenytoin and carbamazepine have been found to suppress veratridine-induced abnormal neuronal excitation in experimental studies (Otoom and Alkadhi, 2000a,b). Since in our study, pretreatment with SRF, abolished the veratridine-induced seizure activity indicated by normalization of EEG pattern, indicating its effect might be due to Na^+ channel blockade activity.

Panaxatriol saponins, the main constituents extracted from *Panax notoginseng* have been reported to possess an inhibitory action on voltage-gated Ca^{2+} channels, leading to shortening of their open time, prolonging the close time, and reducing their open-state probabilities (Zhong et al., 1995; Kim et al., 2008), suggesting Ca^{2+} channel antagonist effect of saponins. Ethosuximide, gabapentin, lamotrigine, oxcarbazepine, topiramate include the list of clinically used antiepileptics that act through blockade of Ca^{2+} channels (Czapiński et al., 2005). These literature finding suggests that, SRF might act through the blockade of Ca^{2+} channels. Hence the effect of SRF was studied against convulsions induced

by BAY k-8644, which induce convulsions through activation of voltage-gated Ca^{2+} channels. SRF pretreatment inhibited BAY k-8644-induced convulsion, thereby suggesting its effect to be due to inhibition of Ca^{2+} channels.

Several preclinical studies indicating the modulation of GABAergic functions by plant isolated saponins have been reported in literature. Saponins isolated from *Panax ginseng* showed $GABA_A$ receptor binding in the rat brain (Kimura et al., 1994; Kim et al., 2001). These saponins also increased the GABA-mediated inward peak current in *Xenopus oocytes* (Choi et al., 2003). Effectiveness of SRF in initial anticonvulsant screening in PTZ ($GABA_A$ receptor antagonist) test indicated its GABAergic modulatory effect. Since several other anticonvulsant molecules acting through other mechanisms have also shown activity against PTZ convulsions, hence to further confirm the GABAergic effect of SRF, its effect was studied in bicuculline ($GABA_A$ receptor antagonist) test. Treatment with SRF resulted in suppression of bicuculline-induced convulsions, indicated by increase in latency to clonic convulsions, but however it was found to be ineffective in reducing mortality. Further studies are required to understand the exact GABAergic modulatory effects of SRF.

Inhibition of NMDA-induced excitatory responses by plant isolated saponins has also been well documented in several studies. Saponins isolated from ginseng have shown inhibition of NMDA receptor-mediated epileptic discharges in cultured hippocampal neurons (Kim and Rhim, 2004). Ginsenoside Rb3 have shown neuroprotective role on hippocampal neurons, through facilitation of Ca^{2+}-dependent deactivation of NMDA receptors (Peng et al., 2009). Clinically used drugs like felbamate and lacosamide act through inhibition of NMDA receptor (Rogawski, 2006). Therefore, to study the effect of SRF on NMDA-mediated excitatory responses, NMDA-induced convulsions test was used. However SRF failed to suppress NMDA-induced convulsions, indicating lack of NMDA receptor interactions of SRF.

The role of glycinergic receptor mechanism in the pathogenesis of clinical and experimental epilepsies has been well established. In different studies, the glycinergic drugs have shown suppression of epileptic discharge (Laube et al., 2002; Chattipakorn and McMahon, 2003). Ginsenosides have shown enhancement of glycine-induced inward peak current in human glycine receptor channel activity expressed in *Xenopus oocytes* (Noh et al., 2003). They have also been found to antagonize NMDA receptors through the glycine modulatory site in rat cultured hippocampal neurons (Kim et al., 2004). These literature findings suggest the glycinergic effects of saponins. Hence to study the glycinergic modulation of SRF, its effect was studied against convulsions induced by strychnine (glycine receptor antagonist). Since, the SRF was found to be ineffective in suppressing strychnine-induced convulsions, thereby indicating lack of effectiveness as direct interaction with glycinergic pathway.

The results of the present study showed that SRF treatment inhibited MES-induced convulsions and suppressed BAY k-8644, veratridine and $GABA_A$ receptor antagonists (PTZ and bicuculline)-induced convulsions at tested doses, indicating it to be acting through multiple mechanisms as GABA agonist as well as Na^+ and Ca^{2+} channels inhibitor. Several clinically used drugs have also been found to be acting through multiple mechanisms (Köhling, 2002). Interestingly, SRF showed protection in BAY k-8644 and veratridine tests, which act through activation of voltage-gated Ca^{2+} and Na^+ channels, respectively, but was found to be ineffective against convulsions induced by NMDA (receptor-operated ion channels) receptor agonist, NMDA. The similar anticonvulsant profile is shown by ethosuximide, a clinically used antiepileptic (Turski et al., 1990; Otoom and Sequeira, 2011). These results indicated the effectiveness of SRF in blockade of voltage-gated ion channels and ineffectiveness toward ligand-gated Na^+ and Ca^{2+} channels. Since, veratridine act by depolarization of excitable cells, thereby preventing inactivation of Na^+ channels, leading to an increase in influx of Na^+ and Ca^{2+} (Jordán et al., 2000), therefore it is not exactly clear that the anticonvulsant effect of SRF is due to blockade of Na^+ or Ca^{2+} channels or both, as it has also shown protection against seizures induced by BAY k-8644. As, ethosuximide which shows anticonvulsant effect through the blockade of Ca^{2+} channels also suppress veratridine-induced seizures. More studies at molecular levels are required to understand these effects accurately.

Conclusion

The results of present study concluded that SRF exhibit its anticonvulsant effect through modulation of GABAergic, Na^+ and Ca^{2+} channel functions. Effectiveness of SRF in BAY k-8644 and veratridine tests indicated its voltage-gated Na^+ and Ca^{2+} channels inhibitory effects but not ligand-gated Na^+ and Ca^{2+} channels. More studies are required at molecular levels to understand the exact molecular interactions of SRF with these pathways.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

The first author conducted the experiments, and second author planned and supervised the study. Both the authors wrote the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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