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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 as well as chronic mice models of epilepsy. The present study was designed to reveal the 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 the *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. 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 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 some bacteria (Francis et al., 2002). The therapeutic role of saponins has been suggested in several central as well as peripheral disorders like, neuroprotection, epilepsy, learning, memory, 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 this wide spectrum of saponin interactions the present study was envisaged to understand the putative anticonvulsant mechanism of SRF of adventitious root extract of *F. religiosa*, by employing animal models of epilepsy involving primarily modulation of calcium, sodium, glutamate and GABA.

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* L., Moraceae, were collected, cleaned, shade-dried, powdered and subjected to repetitive extraction with 50% ethanol in a percolator. After drying the combined percolate, the 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).

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