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Anticonvulsant activity of alcoholic root extract of *Cardiospermum halicacabum*

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Abstract: The aim of the present study was to evaluate the anticonvulsant effects of alcoholic root extract of *Cardiospermum halicacabum* L., Sapindaceae (ARECH), on the various murine models of epilepsy. The root extract of the plant was administered *p.o*. to male swiss albino mice at doses of 30, 100 and 300 mg/kg before evaluation. The brain monoamine levels were determined after two days administration. ARECH at doses of 100 and 300 mg/kg significantly delayed the onset of clonus and tonus in pentylenetetrazol, isoniazid and picrotoxin-induced convulsions. Tonic hind limb extension was also decreased at doses of 100 and 300 mg/kg as compared to vehicle control in maximal electroshock model. No significant motor toxicity was observed even at a highest dose administered, *i.e*. 900 mg/kg. Brain monoamine analysis by HPLC revealed a significant increase in GABAergic activity in C+ (in cerebellum) and C- (except cerebellum). These results suggested that ARECH possesses a significant anticonvulsant activity with a low motor toxicity profile. This activity may be attributed to an increase in GABAergic activity.

Article

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Introduction

Epilepsy is a common neurological disorder affecting an estimated 40-50 million people worldwide (Rudiger, 2002). The incidence of epilepsy is highest among children below 7 years-of-age and in individuals of above 55 years. The prevalence of epilepsy in India has been reported to be about 5.5 to 7.9 per 1,000 people, which is approximately about 1/18th of the world population (Nag, 2000). Anticonvulsant drugs (ACD) are the most commonly prescribed drugs for epilepsy and seizure disorders due to their high efficacy in the treatment of these disorders (Liow et al., 2007). Many of the existent ACD produce a host of undesirable side-effects including teratogenesis, drowsiness, mental dullness, nausea, ataxia, paresthesia, hematologic changes, hirsutism, weight gain, hypertrophy of gums and congenital malformations. For these reasons, new ACD are needed to improve seizure control and reduce the side-effect profile (Gasior et al., 1997).

The Ayurvedic system of medicine has a quite sophistical classification of medicinal plants as per the dominant pharmacological/therapeutic activity of mental functions (Vaidhya, 1997). Ethnopharmacological approaches have provided leads to identify potential new drugs from plant sources, including targets for neuronal disorders (Howes & Houghton, 2003).

Cardiospermum halicacabum L., commonly known as 'Kanphuti', from family Sapindaceae, is an annual or perennial climber, widely distributed in tropical and subtropical Asia and Africa, and often found throughout India (Sheeba & Asha, 2009). The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs and snake bite whereas the root alone has been used for curing diseases related to the nervous system (Muthu et al., 2006; Subramanyam et al., 2007). The root is mucilaginous and considered emetic, laxative and anti-rheumatic and the seeds are used as a tonic for fevers and as a diaphoretic (Joshi et al., 1992). Traditional Indian folklore describes the use of root for the treatment of anxiety and epilepsy (Venkteshbabu & Krishnakumari, 2006).

This study has been undertaken to investigate the pharmacological basis for the use of the alcoholic root extract of *Cardiospermum halicacabu*m and to understand the mechanisms following its anticonvulsant activity.

Material and Methods

Chemicals

The drugs used were diazepam (DZ) (Ranbaxy Lab. Ltd., Thane), phenytoin (Zydus Neuroscience, Ahemedabad) and isoniazid (Radicura Pharmaceuticals, New Delhi). Picrotoxin, strychnine and pentylenetetrazol (PTZ) were purchased from Sigma Aldrich, USA. Other chemicals used for extraction and phytochemical investigation were of analytical grade from S.D. Fine Chemicals, Mumbai, India.

Plant material

Whole plant of *Cardiospermum halicacabum* L., Sapindaceae, was collected from the villages in and around Kuppam in Chittoor district in the Indian state of Andhra Pradesh during the month of July, 2007. The plant material was authenticated by Dr. K. Lakshman, Professor and Head, Department of Pharmacognosy, P.E.S. College of Pharmacy, Bangalore and a specimen was preserved for future reference, bearing voucher number CIP-01 at the same institute. The plants were uprooted, roots collected, washed thoroughly to remove the debris, dried in shade and powdered. The powder was subjected for extraction using different polarities of solvents in a soxhlet apparatus.

Preparation of extracts of Cardiospermum halicacabum

About 200 g of the root powder of *C. halicacabum* was successively extracted. Extraction with petroleum ether was carried out at 60-80 ^{o}C for 72 h using soxhlet apparatus. The dried marc obtained after petroleum ether extraction was subjected to further extraction with 95% alcohol. The aqueous extract was prepared by maceration for 72 h with distilled water containing chloroform (0.25% v/v) as preservative. All the extracts obtained were concentrated and dried by vacuum distillation till the solvents were completely removed. The percentage yield of ARECH was reported to be 11.9.

Preliminary phytochemical investigations

The ARECH was subjected to preliminary qualitative investigations (Khandelwal, 2007).

Animal care

Adult male Wistar rats (160-180 g) and male Swiss albino mice (18-22 g) were selected. Animals were housed under an alternative 12 h light/dark cycle in polypropylene cages with softwood granulate bedding. Three animals were housed in a single cage. Pelleted food and water were made available *ad libitum*. Animals used in these studies were maintained in facilities fully accredited by the CPCSEA and all experiments were performed under protocols approved by the Institutional Animal Ethics Committee (PES IMSR/Pharma/IAEC/002).

Acute toxicity

The LD50 value of ARECH was determined by using female, nulliparous and non pregnant mice weighing 18-22 g. The animals were fasted for 3 h prior to the experiment. Animals were administered with single dose of extract and observed for its mortality during 48 h (short term toxicity). Different doses of the extracts were administered to different animals based on the short term toxicity till the stop criteria was met as per OECD guideline 425. LD50 was calculated by using AOT 425 software provided by Environmental Protection Agency, USA (OECD, 2001).

Anticonvulsant tests

Maximal electroshock induced seizures (MES)

Tonic convulsions of the hind extremities of male mice were induced by passing alternating electrical current (50 Hz, 60 mA, 0.2 s) through ear clip electrodes by a Rodent shocker generator (Inco Electroconvulsometer model# 100-3). For each experiment, one group served as the control (3% tween 80, 10 mL/kg, *p.o*.) and one group as the standard (Phenytoin, 25 mg/kg, *p.o*.). The test extract, ARECH was also administered at various doses (30, 100 and 300 mg/kg, *p.o*.).The number of animals protected from tonic hind limb extension seizure and the latency of onset were determined in each dose group. Percentage protection against mortality was also calculated (Wamil et al., 1994).

PTZ Seizure test

Convulsions were induced in male mice by injecting PTZ (80 mg/kg, *i.p*.). The time of onset of clonic and tonic convulsions was noted. ARECH was tested at various doses (30, 100 and 300 mg/kg, *p.o*.) along with vehicle control (3% tween 80, 10 mL/kg, *p.o*.) and a standard, DZ (10 mg/kg, *p.o*.) (Nassiri Asi et al., 2009).

Picrotoxin (PT) induced seizures

PT (3.5 mg/kg, *i.p.*) was injected (n=6) in male mice, pre-treated 60 min prior with varying *p.o*. doses of ARECH (30, 100 and 300 mg/kg), vehicle (3% tween 80, 10ml/kg, *p.o*.) and diazepam 10 mg/kg (standard group). Latency to clonic and tonic convulsions was noted in all groups. All the extract treated groups were compared with control in order to determine the significant anticonvulsant activity (Leewanich et al., 1996).

Strychnine induced convulsions

Male mice were randomly allotted to the different control and test groups. Convulsions were induced in male mice by the *i.p*. injection of 3.5 mg/kg strychnine (STN). Graded doses (30, 100 or 300 mg/kg, *p.o*.) of ARECH were given to the test groups 60 min before strychnine. The latency of onset of tonic extensor convulsions was recorded (Adeyemi et al., 2007).

Isoniazid induced convulsions

Male mice were injected with isoniazid (INH), 300 mg/kg, *i.p*. one hour after the administration of the ARECH. The time of onset of clonic or tonic seizures was recorded (Bernasconi et al., 1988). Data of the control group (3% tween 80, 10 mL/kg, and *p.o*.) were compared to data of the group treated with the ARECH. The standard group received DZ, 10 mg/kg, *p.o.*

Motor toxicity test

Pre-trained male mice were subjected to rota rod test after 1 h administration of the control (3% tween 80, 10 mL/kg, and *p.o*.), standard (DZ, 10 mg/kg, and *p.o*.) or ARECH treatment (30, 100, 300, 600 and 900 mg/kg). The time taken for the animals to fall off from the rota rod was recorded in seconds. Inability to stay on the rota rod for less than 1 minute was considered as motor impairment (Sun et al., 2006).

Determination of brain monoamine and GABA

Adult male Wistar rats weighing 160-180 g were treated with the test or standard drug and control group was treated with equimolar quantity of saline for two days. Rats were sacrificed by decapitation 1 h after treatment of last dose and heads were dropped in ice cold 0.1 M perchloric acid. Immediately the brain was removed, weighed and homogenized in 2 mL of 0.1 M perchloric acid. After centrifugation at 14000 x *g* for 15 min at 4 ^{o}C, the supernatant was filtered through 0.45 µm membrane and 100 µL of the filtrate was injected into HPLC column. After separation, noradrenaline (NE), dopamine (DA), serotonin (5HT) and GABA were detected at the excitation wavelength of 280 nm and emission wavelength 350 nm. The mobile phase used for the determination, consisted of sodium acetate (0.02 M), methanol (16%), heptane sulphonic acid (0.055%), EDTA (0.2 mM) and dibutyl amine (0.01%v/v). The solution was adjusted to pH 3.92 with orthophosphoric acid and filtered through 0.45 µm membrane (Madepalli et al., 1997). Brain GABA level was estimated using paper chromatography as described by Maynert et al, 1962.

Statistical analysis

Assessment of the acute oral toxicity and their LD50 values (convulsant drugs) with 95% confidence limits was calculated using a computerized version of the AOT 425 software provided by Environment Protection Agency, USA. The latency of onset of clonic-tonic convulsions and duration of hind limb extensions values were expressed as mean±SEM from six animals. Statistical differences in means were calculated using one way ANOVA followed by Dunnett's post hoc test using GraphPad Prism 5.02 software.

Results

Phytochemical investigations

ARECH was found to contain fixed oils, fats, proteins, flavonoids, phenolics and tannins, carbohydrates, saponins, phytosterols and triterpenoids.

Acute oral toxicity

ARECH, when administered to mice at dose level of 550 mg/kg, showed no mortality. However at 2000 mg/kg, 67% of the animals died. The LD50 was found to be 1098 mg/kg, probably suggesting that the plant's extract is relatively safe in mice.

Table 1. Effect of alcoholic root extract of *Cardiospermum halicacabum* on maximal electroshock induced seizures induced convulsions.

Treatement	Duration of tonic flexion (s)	Duration of tonic extension (s)	Latency onset of clonus (s)	Percentage protection against mortality
Control (3% tween 80)	0	15.66± 1.14	3±0.57	0
Phenytoin (25 mg/kg)	6.16±1.07**	0	15.17±0.94**	100
ARECH 30 mg/kg	0	11.5± 1.17	4.16±0.60	0
ARECH 100 mg/kg	0	7.34± 0.67**	10.17±0.98*	34
ARECH 300 mg/kg	0	6.17± 0.47**	9.67±1.05*	50

Values are expressed as mean±SEM from six mice. Significant at *p<0.05 and **p<0.01 as compared to control group. One way ANOVA followed by Dunnett's multiple comparison post hoc test.

Table 2. Effect of alcoholic root extract of *Cardiospermum halicacabum* on PTZ induced convulsions.

Treatment	Onset of clonus in s	Onset of tonus in s	Percentage protection against mortality
Control (3% tween 80)	46.33±1.67	138.16±12.19	0%
Diazepam 10 mg/kg	0	0	100%
ARECH 30 mg/kg	88.16±6.12*	303.25±13.56**	0
ARECH 100 mg/kg	167.5±16.42**	477.2±15.08**	17%
ARECH 300 mg/kg	210.16±13.04**	654.4±32.69**	17%

Values are expressed as mean±SEM from six mice. Significant at *p<0.05 and **p<0.01 as compared to control group. One way ANOVA followed by Dunnett's multiple comparison post hoc test.

Table 3. Effect of ARECH on picrotoxin induced convulsions.

Treatment	Onset of clonus in s	Onset of tonus in s	Percentage protection against mortality
Control (3% tween 80)	284.16±14.57	559.83±41.68	0
Diazepam 10 mg / kg	593.5±50.74*	1226±80.11*	34
ARECH 30 mg/kg	330.5±47.04	620.9±101.74	0
ARECH 100mg/kg	355.2±15.43	781.56±22.70	17
ARECH 300mg/kg	566.3±31.65*	1137.4±35.50*	17

Values are expressed as mean±SEM from 6 mice. Significant at p<0.05* as compared to control group. One way ANOVA followed by Dunnett's multiple comparison post hoc test.

Table 4. Effect of ARECH on strychnine induced convulsions.

Treatment	Onset of tonus in s	Percentage protection against mortality
Control (3% tween 80)	155±16.07	0
Diazepam 10mg/kg	147.66 ±16.73	0
ARECH 30mg/kg	190.66±22.46	0
ARECH 100mg/kg	220±11.10*	0
ARECH 300mg/kg	216±17.31	0

Values are expressed as mean±SEM from 6 mice. Significant at p<0.05* as compared to control group. One way ANOVA followed by Dunnett's multiple comparison post hoc test.

Effect of ARECH on MES induced seizures

ARECH exhibited dose dependent reduction in duration of tonic extension, tonic flexion and latency of onset of clonus compared to vehicle control. Doses of 100 and 300 mg/kg showed similar effect, while percentage protection against mortality was higher at 300 mg/kg dose level (Table-1).

Effect of ARECH on PTZ induced seizures

PTZ (80 mg/kg, *i.p.*) induced clonic and tonic convulsions in all the groups. The onset of clonic convulsions in control animals was 46.33±1.67 s while onset of tonic convulsions in the same animal was 138.16±12.19 s. ARECH (30, 100 and 300 mg/kg, *p.o.*) produced [(p<0.05), (p<0.01)] significant increase in the duration of onset of tonic-clonic convulsions in a dose dependent manner (Table 2). The reference anticonvulsant drug used, DZ, 10 mg/kg, p.o. protected all the animals from PTZ-induced clonic convulsions.

Effect of ARECH on PT-induced convulsions

Pre-treatment with ARECH at dose 300 mg/kg produced a significant increase in duration of onset of tonic-clonic convulsions (p<0.01). The reference anticonvulsant, DZ (10 mg/kg, *p.o.*), also increased the latency of tonic-clonic convulsions (p<0.01) compared to vehicle control (Table 3).

Effect of ARECH on STN-induced convulsions

Only at a dose of 100 mg/kg, ARECH significantly (p<0.05) produced prolongation of onset of tonic convulsion. None of the animals were protected from seizures or from death. Both diazepam and ARECH were ineffective in STN-induced convulsions (Table 4).

Effect of ARECH on INH-induced convulsions

In the case of INH-induced convulsions, ARECH 300 mg/kg delayed the onset of clonic and

tonic convulsions as compared to control. However DZ (10 mg/kg, *p.o.*) exhibited significant effect ($p<0.01$) in clonic and ($p<0.05$) tonic convulsions induced by INH (Figure 1).

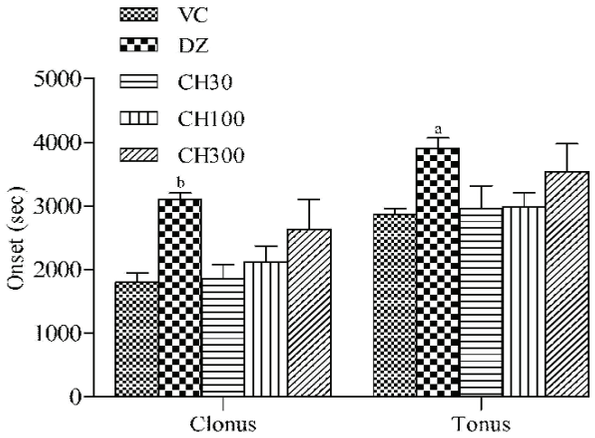

Figure 1. Effect of alcoholic root extract of *Cardiospermum halicacabum* on INH induced convulsions. Values are mean±SEM; n=6. Analysed by one way AN0VA followed by Dunnet's post hoc test at $^{a}p<0.05$ and $^{b}p<0.01$, as compared to VC.

Motor toxicity of ARECH

ARECH produced a dose-dependent decrease in time spent on the rota rod. However at all doses (100, 300, 600 and 900 mg/kg), the animals were able to walk on the rota rod for more than 1 min thus did not affect motor coordination in mice (Figure 2).

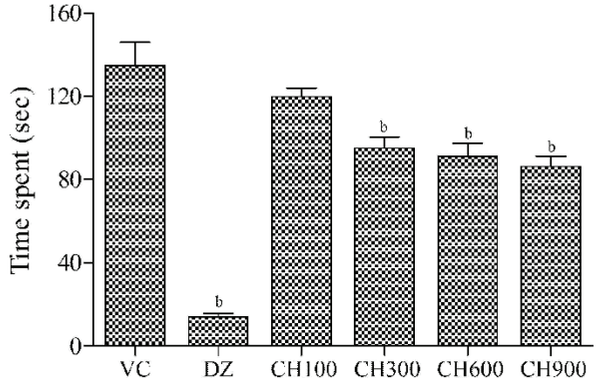

Figure 2. Effect of different concentration of alcoholic root extract of *Cardiospermum halicacabum* on motor toxicity. Values are expressed as mean±SEM; n=6. $^{b}p<0.01$, in comparison to VC. One way ANOVA followed by Dunnett's multiple comparison post hoc test.

Effect of ARECH on brain monoamine levels

The ARECH (100 and 200 mg/kg) significantly enhanced the GABA activity in cerebellum (C+) as well as except cerebellum (C-) when compared to control ($p<0.05$). However, there is no significant difference observed in other monoamine levels (5 HT, NE and DA) [Figure 3 and 4].

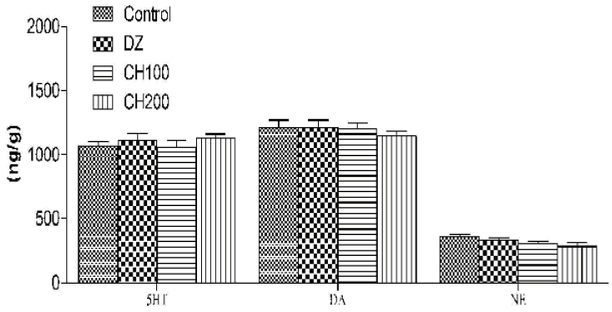

Figure 3. Effect of alcoholic root extract of *Cardiospermum halicacabum* on brain monoamine levels [5-HT, DA, NE]. One way AN0VA followed by Dunnett's multiple comparison post hoc test showed no significance observed in monoamine levels as compared to VC.

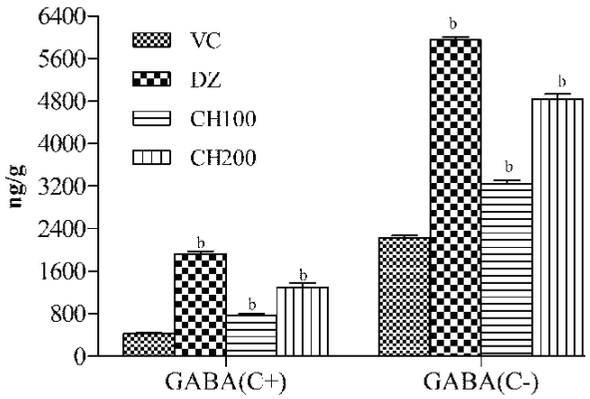

Figure 4. Effect of alcoholic root extract of *Cardiospermum halicacabum* on brain GABA levels [GABA C+ (in cerebellum) and GABA C-(except cerebellum)]. $^{b}p<0.01$, in comparison to VC. One way ANOVA followed by Dunnett's multiple comparison post hoc test.

Discussion

In the present study, our results demonstrate the anticonvulsant action of ARECH by using classical models for screening of ACD. The spectrum of its anticonvulsant activity was characterized further by performing various seizure models in comparison to the standard anticonvulsants. Protection against hind limb tonic extension in MES test predicted the ability of ARECH to prevent the spread of seizure discharge from the epileptic foci in brain. In addition, the effectiveness in MES test can be correlated with efficacy of drugs that suppress generalised tonic-clonic and partial seizures by causing use dependent blockage of voltage-sensitive sodium channels and by enhancing GABAergic mediated

neurotransmission (Krall et al., 1978; Loscher & Schmidt, 1988).

The activity of ARECH in PTZ test indicated the presence of anticonvulsant compounds that may produce an effect either by inhibiting low threshold T-type Ca^{2+} currents or by enhancing GABAA receptor mediated inhibition, as PTZ-induced seizures can be blocked by standard drugs that reduce T-type Ca^{2+} currents, such as ethosuximide, and drugs that enhance GABAA receptor mediated inhibition, such as benzodiazepines, phenobarbital and valproic acid (White, 1999; Sun et al., 2009). BZ_2 receptors may be primarily involved in the convulsant action of PTZ (Griebel et al., 1999). Hence, there may be a possible predominant involvement of α_2-containing GABAA receptors in the convulsant action of PTZ. GABAA receptors (primarily those containing α_1 and α_2 subunits), may explain the potency of ARECH against tonic rather than against myoclonic and clonic seizures as well as a dose-dependent difference in the potency of ARECH against different PTZ-induced seizures (Dhir et al., 2006).

In this work, ARECH offered protection to mice against PT-induced seizures. There was a significant (p<0.05) increase in seizure threshold with higher doses of the extract. PT, a pro-convulsant drug blocks chloride channel directly (Anthony & Walter., 1998). Thus the pathway of activity of the extract can be through GABA receptor activation. STN directly antagonizes the inhibitory spinal reflexes of glycine (Chen et al., 2007). ARECH did not exhibit its anticonvulsant effect on STN-induced convulsions thus indicating its glycine independent activity. INH is regarded as an inhibitor of GABA synthesis,thus produces the convulsion. ARECH 300 mg/kg delayed the onset of clonic and tonic convulsions as compared to control which may be due to the inhibition of the INH induced clonic-tonic convulsions indicating its GABA potentiating activity (Vogels, 2008). In addition, the analysis of monoamine levels in the brain has provided additional evidence for its GABA mediated activity.

Impaired locomotor activity and co-ordination are known problems with barbiturates and benzodiazepines, and are among the factors influencing poor compliance (Mattson, 1992). ARECH did not affect motor coordination in animals closer to its LD50 value. However further studies required to assess the therapeutic window of ARECH related to psychomotor dysfunction.

Conclusion

In conclusion, the present data indicates that ARECH is active in multiple seizure models, and may be efficient against both grandmal and partial seizures, as it prevents seizure spread by inhibiting the tonic seizure activity in well-established animal seizure models. ARECH has an acceptable safety profile as evidenced by acute toxicity and acute neurotoxicity tests. Although the exact mechanism of action of ARECH needs more investigation, the overall effect of ARECH in seizure models indicates that it mediates a part of its effect through GABA mediated neurotransmission.

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