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Effect of Hydroalcoholic Extract of *Argyreia speciosa* Roots against Experimentally-induced Anxiety, Depression and Convulsions in Rodents

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ABSTRACT

Argyreia speciosa (*Convolvulaceae*) is regarded as a 'Rasayan' drug in the Ayurveda system of medicine to cure diseases of the nervous system. This work researched the action of the hydroalcoholic extract of *A. speciosa* root (ASE) on experimentally-induced anxiety, depression and convulsion in rodents. ASE (100, 200, 500 mg/kg, p.o.) was tested for the elevated plus maze, open field, forced swimming and tail suspension tests in mice. ASE at the same doses was also tested for its anticonvulsant activity by pentylenetetrazole (PTZ)-induced convulsions in mice and maximal electroshock (MES)-induced convulsions in rats. ASE at the tested doses did not cause a significant increase in the open arm entries and time spent in the open arms indicating the absence of an anxiolytic effect in the elevated plus maze test. However, the total number of entries in the open and enclosed arms was reduced indicating a reduction of locomotor activity in the elevated plus maze test. ASE did not affect the emotional activity parameters in the open field test significantly. ASE also decreased locomotor activity in the open field test suggesting a possible central depressant action. In addition, ASE increased the immobility time in the forced swimming and tail suspension tests, which further confirmed a probable central depressant effect. ASE protected rats against maximal electroshock-induced convulsions and mice against PTZ-induced convulsions indicating an anticonvulsant action. The results of the study suggest that the hydroalcoholic extract of *A. speciosa* roots contained phytochemically active ingredients with central nervous depressant and anticonvulsant effects.

Keywords: elevated plus maze test; open field test; forced swimming test; tail suspension test; maximal electric shock induced convulsions

INTRODUCTION

Argyreia speciosa (L.f.) Sweet (Convolvulaceae), commonly known as 'elephant creeper', is a woody climber distributed throughout the India up to an altitude of 300 m (Anonymous 1985). *A. speciosa* is regarded as a 'Rasayan' drug in the Ayurvedic system of medicine. The root of *A. speciosa* is known as an alternative, tonic and useful in rheumatism and diseases of the nervous system (Kirtikar and Basu 1981). Previous phytochemical studies revealed the presence of lipids (Batra and Mehta 1985), flavonoids (Ahmad *et al.* 1993), triterpenes (Khan *et al*. 1992), steroids (Chandler and Hooper 1979), phenylpropanoids (Shrivastava and Shukla 1998) and coumarins (Shukla *et al*. 2001) in the plant. Several investigations have proposed that this plant possesses hypotensive (Bhukani *et al.* 1969), anti-inflammatory (Gokhle *et al*. 2002), immunomodulator (Gokhle *et al*. 2003), antiamnesic (Joshi *et al*. 2007) and aphrodisiac activity (Subramoniam *et al*. 2007). These reported activities confirm that the roots of *A. speciosa* are able to modulate the physiology of the central nervous system (CNS). However, no investigative reports exist pertaining to its effect on anxiety, depression and convulsion. Hence, the present study was designed to evaluate the effect of roots of *A. speciosa* on experimentally induced anxiety, depression and convulsions in the rodents.

MATERIALS AND METHODS

Experimental animals

Wistar albino mice (25-35 g) and rats (200-250 g) of either sex bred in the Central Animal House facility of the Institute were used. The animals were housed under standard conditions, maintained in a 12 h light/dark cycle and had free access to food and water up to the time of experimentation. The animals were acclimatized to the laboratory environment 1 h before the experiments. Animals were randomly distributed into groups of 10 animals each. Each animal was used only once. All experiments were conducted during the light period (08.00-16.00 h). All protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals).

Plant material and preparation of extract

The roots of *A. speciosa* were collected from Balasinor (Gujarat). Their authenticity was confirmed by Dr. A. S. Reddy, Department of Bioscience, Sardar Patel University, Vallabh Vidyanagar, Gujarat. A specimen of the plant is kept in the herbarium of our institute (Voucher No. ARGH8). The roots were completely dried in the sunlight and powdered. Root powder was extracted exhaustively with 50% ethanol by maceration for 2 days at room temperature with frequent shaking. Crude (hydroalcoholic) extract was filtered and dried under reduced pressure at 40°C (yield = 9.3% (w/w) of dried plant material).

Preliminary phytochemical screening

The hydroalcoholic extract of *A. speciosa* roots was tested for the presence of carbohydrates, proteins, alkaloids, flavanoids, glycosides, saponins, tannins and essential oils using standard procedures (Kokate 1994).

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Original Research Paper

Experimental protocol

Freshly prepared aqueous solution of dried extract of *A. speciosa* roots (ASE) in suitable dilution was administered to test animals. Distilled water as a vehicle (10 ml/kg) was administered per oral (p.o.) to the control animals. Diazepam (Calmpose® injection, Ranbaxy, India) was used as a reference drug for elevated plus maze test (1 mg/kg, i.p. (intraperitoneal)), open field test (1 mg/kg, i.p.) and Pentylenetetrazole (PTZ; Sigma, St. Louis, MO, USA) induced convulsion (4 mg/kg, i.p.). Imipramine (Torrent Pharma, India) was used as a reference drug for antidepressant action (10 mg/kg, i.p.) in the forced swimming and tail suspension tests. Phenytoin (Abbot, India) was used as a reference drug (25 mg/kg, i.p.) for maximal electroshock-induced convulsion. For the present experimental study, animals were divided in to five groups, each group consisting of 10 animals. Group 1 served as the control group and received distilled water (vehicle) 10 ml/kg, p.o., groups 2-4 served as test groups and received ASE (100, 200 and 500 mg/kg, p.o.) while group 5 served as the positive control and received reference drug mentioned above. 1 h after oral and 30 min after i.p. administration, the animals were submitted to various behavioural tests.

Elevated plus maze test

The elevated plus maze used in this study was modified from Lister (1987). The plus maze consisted of two opposite arms, 25 cm × 5 cm, crossed with two closed arms of the same dimensions with 30 cm high walls. The arms were connected with a central square, 7.5 cm × 7.5 cm, to give an apparatus in the shape of a plus sign. The whole apparatus was elevated 25 cm above the floor in a dimly illuminated room. Rodents have a natural aversion for high and open spaces and prefer enclosed arms, which have a burrow-like ambience and therefore spend a greater amount of time in the enclosed arm. When exposed to the novel maze alley, the animals experience an approach-avoidance conflict, which is stronger in the open arm than in the enclosed arms. Rodents have aversion for high and open space and prefer enclosed arm and therefore, spend greater amount of time in enclosed arms (Pellow *et al*. 1985). When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements. Animals were placed individually in the centre of the maze facing a closed arm, and thereafter the number of entries and time spent in the enclosed and open arms were recorded during the next 5 min. An arm entry was defined as all four feet in the respective arm. A selective increase in open arm exploration is observed as a consequence of anxiolytic drug administration (Thakur and Mengi 2005). The maze was cleaned after each trial to remove any residue or animal odor.

Open field test

The apparatus consisted of a dimly lit area of 96 × 96 cm, divided in to 16 squares. Mice were placed individually at one corner of the apparatus and observed for a period of 3 min for the number of peripheral squares crossed, number of central squares crossed, periods of immobility, number of rearings and faecal pallets (Novas *et al*. 1988).

Forced swimming test

Mice were made to swim individually in a polypropylene vessel (30 × 15 × 30 cm) with a water level of 15 cm at 25 ± 2°C. The mouse was initially allowed to swim for 10 min and thereafter, the total periods of immobility, characterized by complete cessation of swimming with the head just floating above water level, was determined during the subsequent 5 min period (Porsolt *et al*. 1978).

Tail suspension test

This test is a variant of the forced swimming test in which immobility is induced by suspending a mouse by its tail. Individual mice were hung on a wire in an upside down posture so that their nostrils thus touched the water surface in a container. After initial vigorous movements, mice that assumed immobility during a 5 min observation period were noted (Bhattacharya *et al*. 1999).

Maximal Electric shock (MES) induced convulsions

Albino rats of either sex were given a supramaximal electroshock of 150 mA for a period of 0.2 sec through a pair of conreal electrodes, using an electroconvulsiometer (Techno., India). Animals, which showed a positive hind limb extensor response during prescreening were selected. These animals were treated as per the experimental protocol described above. On the next day, the test was repeated after drug treatments. In all electrically induced convulsions the rats are manually restrained and released immediately. After stimulation, the seizure was observed throughout its course. The severity of convulsions was assessed by duration of tonic flexion, tonic extensor, clonus and stupor phase for each animal. The duration of each phase for each animal (sec) was measured by using stopwatch. The criterion for anticonvulsant activity and protection against MES induced seizures was abolishing hind limb tonic extension (HLTE), which was taken as the end point of the test (Sudha *et al*. 2002).

Pentylenetetrazole-induced convulsions

The test was conducted in mice 1 h after vehicle (1 ml/kg, p.o.) or ASE (100, 200 and 500 mg/kg, p.o.) or diazepam (4 mg/kg, i.p.) treatment. PTZ was injected i.p. (50 mg/kg) into groups of mice (Speroni and Minghetti 1988). Mice were observed for the incidence of convulsions, latency to first convulsion and duration of convulsions.

Statistical analysis

The data was expressed as mean ± S.E.M. Statistical analysis was performed in one-way analysis of variance (ANOVA) followed by Dunnett's test using software sigma stat version 2.03. Results were considered significant at $P<0.05$.

RESULTS

Preliminary phytochemical screening

Phytochemical screening revealed the presence of carbohydrate, proteins, flavanoids, triterpenes, saponins, phenols, tannins, coumarins and essential oil in the hydroalcoholic extract of *A. speciosa* roots.

Elevated plus maze test

Results of the effect of ASE on entries and time spent in both the arms (open and enclosed) of elevated plus maze are shown in **Table 1**. In this test, number of entries and time spent on the open arms parameters were considered for the analysis of anxiolytic activity. While, total number of entries in both the arms (enclosed and open arms) was considered for the evaluation of locomotor activity of animals. Mice treated with ASE (100, 200 and 500 mg/kg) decreased number of entries in the open arms but not found statistically significant as compared to control. ASE reduced time spent by mice in the open arms significantly at the doses of 100, 200 and 500 mg/kg. Total number of entries in both the arms was reduced significantly by ASE indicating reduction in locomotor activity of mice at the doses (100, 200 and 500 mg/kg) tested. While total time spent in the arms was not changed by any dose of ASE. Positive control, Diazepam (1 mg/kg, i.p.) significantly increased the number of entries in the open arms as well as duration of stay in the open arms, indicating anxiolytic activity. Diazepam also increased total number of entries in the elevated plus maze.

Open field test

The overall results of the open field test are summarized in **Table 2**. As expected, control animal when released in to

Table 1 Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on elevated plus maze test in mice.

Treatment	Dose (mg/kg)	Time spent on (sec)		Enteries on	
		Enclosed arms	Open arms	Enclosed arm	Open arm
Control	-	197.7 ± 14.4	28.3 ± 6.5	6.8 ± 0.58	1.5 ± 0.32
ASE	100	235.7 ± 14.7	0.7 ± 0.32*	4.2 ± 0.89*	0.5 ± 0.21
ASE	200	224.4 ± 9.95	4.6 ± 2.07*	4.1 ± 0.49*	0.9 ± 0.39
ASE	500	194.8 ± 17.0	13.6 ± 6.15*	3.7 ± 0.76*	1.0 ± 0.38
Diazepam	1	145.7 ± 11.3	41.4 ± 3.73*	9.5 ± 0.57*	4.9 ± 0.57*

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

Table 2 Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on open field test in mice.

Treatment	Dose (mg/kg)	Peripheral square crossed (number)	Central square crossed (number)	Rearings (counts)	Fecal dropping (counts)	Time of Immobility (sec)
Control	-	28.8 ± 5.5	3.2 ± 1.40	26.3 ± 9.3	0.6 ± 0.4	15.0 ± 7.51
ASE	100	10.1 ± 4.6*	1.3 ± 0.55	25.7 ± 6.95	0.3 ± 0.19	116 ± 21.4*
ASE	200	9.7 ± 2.71*	0.4 ± 0. 25*	7.1 ± 1.87	0.5 ± 0.29	118.8 ± 25.8*
ASE	500	4.2 ± 1.52*	0.2 ± 0.13*	5.1 ± 2.27	0.3 ± 0.15	167.7 ± 18.1*
Diazepam	1	36.2 ± 5.28	4.1 ± 0.49	5.6 ± 1.68	0.6 ± 0.21	59 ± 9.17

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

Table 3 Effect of hydroalcoholic extract of *A. speciosa* root (ASE) in forced swimming test and tail suspension test in mice.

Treatment	Dose (mg/kg)	Immobility time in Forced swimming test (sec)	Immobility time in Tail suspension test (sec)
Control	-	137.4 ± 9.37	68.5 ± 10.86
ASE	100	154.0 ± 13.67	110.8 ± 5.3*
ASE	200	159.9 ± 12.08	111.1 ± 10.7*
ASE	500	187.0 ± 12.14*	130.8 ± 10.01*
Imipramine	10	71.5 ± 11.45*	26.8 ± 6.03*

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

Table 5 Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on pentylenetetrazole (50 mg/kg, i.p.)-induced convulsions in mice.

Treatment	Dose (mg/kg)	Pentylenetetrazole-induced generalized clonic seizures		
		Incidence	Latency (sec)	Duration (sec)
Control	-	10/10	116.7 ± 13.6	10.4 ± 0.54
ASE	100	10/10	152.8 ± 41.8	7.2 ± 0.65*
ASE	200	8/10	231.9 ± 52.8	7.3 ± 0.57*
ASE	500	6/10	174.4 ± 42.7	6.9 ± 0.3*
Diazepam	4	0/10	-	-

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

the open field started moving along the walls, with initial exploration being limited mostly to peripheral squares; the inner squares being explored only exceptionally. Therefore, peripheral square entry in control animals was high and central square entry was low. Mice treated with ASE (100, 200 and 500 mg/kg) reduced number of peripheral squares traversed in a significant and dose dependent manner. ASE at the dose of 200 and 500 mg/kg also reduced number of central squares crossing significantly. Treatments of ASE (100, 200 and 500 mg/kg) produced significant and dose dependent increase in immobility time of mice in the open field. Diazepam (1 mg/kg, i.p.) treated animals increased number of peripheral square crossing and central square crossing but did not found statistically significant. Diazepam treatment did not increase immobility of mice significantly in the open field test. No significant change was observed in the number of rearings and fecal bolus of mice treated with ASE (100, 200 and 500 mg/kg) and diazepam (1 mg/kg, i.p.).

Forced swimming test and tail suspension test

Effect of ASE on forced swimming test was measured by the time of immobility of mice during observation period. As shown in **Table 3**, mice treated with the dose of 500 mg/kg produced significant increase in the immobility time as compared to control. While, ASE at the dose of 100 and 200 mg/kg, did not produced significant increase in the immobility time of mice. All doses of ASE (100, 200 and 500 mg/kg) was significantly prolonged the immobility time of the mice in the tail suspension test (**Table 3**).

Maximal electroshock (MES) induced convulsions

The effects of ASE in flexion and extension phases of maximal electroshock-induced convulsions in rats are shown in **Table 4**. ASE at the dose of 200 and 500 mg/kg produced a significant reduction of duration of hind limb tonic extension. The flexion phase of electrically induced seizures was also abolished significantly with ASE treatment (100, 200 and 500 mg/kg). The incidence of convulsions was reduced. Mice treated with phenytoin (25 mg/kg) were completely protected from MES induced convulsions as indicated by absence of all the phases of convulsions.

Pentylenetetrazole induced convulsions

Results are shown in the **Table 5**. All the doses (100, 200 and 500 mg/kg) of ASE produced significant and dose dependent reduction in the duration of first clonic convulsion in mice. However, ASE treatment (100, 200 and 500 mg/kg) did not affect significantly, the latency of onset of PTZ-induced convulsion in mice. Incidence of convulsion is reduced and no mortality was observed in the animal treated with ASE. As expected, diazepam (4 mg/kg, i.p.) treated mice did not have any convulsive episode and mortality,

Table 4 Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on maximal electroshock (MES) induced convulsions in rats.

Treatment	Dose (mg/kg)	Incidence	Phases of MES induced seizures (sec)			
			Tonic flexion	Hind limb tonic extension	Clonic	Stupor
Control	-	10/10	4.2 ± 0.42	9.2 ± 0.97	13.9 ± 2.57	36.1 ± 5.32
ASE	100	8/10	2.8 ± 0.31*	6.7 ± 1.16	10.8 ± 2.02	28.6 ± 4.6
ASE	200	7/10	2.5 ± 0.43*	3.8 ± 0.87*	11.3 ± 2.63	22.6 ± 2.93
ASE	500	6/10	2.1 ± 0.29*	3.0 ± 0.96*	7.5 ± 1.35	30.7 ± 3.35
Phenytoin	25	0/10	-	-	-	-

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *p<0.05 when compared with control group.

when treated with PTZ, presented 100% protection of animals as compared to control. Thus, anticonvulsant activity of diazepam was confirmed.

DISCUSSION

In the present study, the effect of hydroalcoholic extract of *A. speciosa* roots (ASE) on experimentally induced anxiety, depression and convulsions was evaluated.

Effect on anxiety

Elevated plus maze is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli that is the fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform (Kalueff and Tuohimaa 2004). Number of entries and time spent in open arms are increased by anxiolytics and reduced by anxiogenic agents (Pellow *et al*. 1985). Neither dose of ASE increased significant number of entries or time spent in open arms indicating the absence of anxiolytic effect. Dosage of the extract seems to be crucial to the type of effect obtained. Furthermore, under these conditions no anxiolytic or anxiogenic effects were observed with the extract treatment, since the locomotor activity was impaired after their administration. However, animals treated with diazepam (1 mg/kg) increased locomotor activity (Galani and Patel 2010). Diazepam as expected reduced the mouse's natural aversion to the open arms and promoted maze exploration thereof. Data in the literature relate that diazepam producing act as anxiolytic at low doses while producing anticonvulsants, sedation and myorelaxant effect at higher doses (Galani and Patel 2009a, 2010). Reduction in the locomotor activity by the hydroalcoholic extract of *A. speciosa* roots in the elevated plus maze test may correlate with central nervous depressant (Masur *et al*. 1971; de Sousa *et al*. 2005; Galani and Patel 2009b) and sedative action (Perez *et al*. 1998; Adeyemi *et al*. 2006; Galani and Patel 2009b).

The open field test is a paradigm used for evaluating the effect of drugs on the gross general behavior and is used for measuring the level of nervous excitability (File and Fernandes 1994). Animals removed from their acclimatized home cage and placed in a novel environment; express their anxiety and fear by showing decreases in ambulation and exploration, immobilization or freezing, reduction in normal rearing and grooming behaviour (Jayabalan *et al*. 2008). Increased micturation and defecation due to augmented autonomic activity is observed. These paradigms are attenuated by classical anxiolytics and potentiated by anxiogenic agent (Novas *et al*. 1988). A decrease in locomotor activity in the open field test of mice treated with ASE produced more evidence for the central nervous depressant activity (Masur *et al*. 1971; Gomes *et al*. 2008; Lima *et al*. 2010). Defecation is also a good indicator of emotionality in animals and research shows that high emotionality is related to an increase in defecation, with anti-anxiety drugs reducing defecation (Angrini *et al*. 1998). Fecal drop count change by the hydroalcoholic extract of *A. speciosa* roots was not found statistically significant in this test. Rearing is an aspect of exploratory behaviour and generally decreases when an animal is placed in a novel or stressful environment and may increase when anxiolytic drugs are given (Johansson and Ahlenius 1989). Rearings of mice was not significantly changed with the treatment of hydroalcoholic extract of *A. speciosa* roots. Thus, the results of open field test is also indicated that acute administration of hydroalcoholic extract of *A. speciosa* roots at these doses and route did not interfere with anxiolytic activity.

Effect on depression

Forced swim test and tail suspension test are widely used to screen new antidepressant drugs (Porsolt *et al*. 1978; Steru *et al*. 1985). In the results of forced swimming test and tail suspension test, significant increase in the immobility time was observed with treatment of hydroalcoholic extract of *A. speciosa* roots. In this way, the overall results seem to be predictive for central nervous depressant properties of hydroalcoholic extract of *A. speciosa* roots (Pandy *et al*. 2009).

Effect on convulsions

The criterion for anticonvulsant activity and protection against maximal electroshock induced convulsions was abolishing hind limb tonic extension. Significant reduction of hind limb tonic extensor phase in rat by prior administration of the hydroalcoholic extract of *A. speciosa* roots may relate with its anticonvulsant action (Sudha *et al*. 2002). PTZ is the most frequently used substance, as well as an acute experimental model in a preliminary screening to test potential anticonvulsant drugs. The induction of convulsions by PTZ is attributed to repression of gamma amino butyric acid type A ($GABA_A$) receptor Cl^- channel (Ramanjaneyulu and Ticku 1984). Anticonvulsant effect of hydroalcoholic extract of *A. speciosa* roots from PTZ-induced convulsions may be related to a facilitation of the GABAnergic transmission. Also, anticonvulsant property of the *A. speciosa* roots may be linked at least in part, to its ability to depress the central nervous system activity (Sudo *et al*. 2010).

The efficacy of most herbal remedies is attributed to various active principles in combination. The observed pharmacological actions of hydroalcoholic extract of *A. speciosa* roots may be due to the presence of steroids, saponins, tannins, flavanoids, coumarins, triterpenes and essential oil as indicated by the results of preliminary phytochemical screening (Galani and Patel 2009b). Since triterpenoids (Chattopadhyay *et al*. 2003; Datta *et al*. 2004), saponins (Wagner *et al*. 1983; Dubois *et al*. 1986), flavanoids (Datta *et al*. 2004; Fernández *et al*. 2006) and essential oil (Hendriks *et al*. 1981) from other plants have reported to display depression of central nervous system. It is therefore probable that the components that are present in abundance in the ASE might contribute in part for the observed central nervous system activity.

CONCLUSION

The results of the present investigation indicate that the hydroalcoholic extract of *A. speciosa* roots (ASE) has central nervous depressant activity. The investigation also highlights the fact that anxiolytic activity of ASE was not observed at the tested doses (100, 200 and 500 mg/kg, p.o.). Furthermore, the results obtained in the present study suggest that ASE has anticonvulsant activity, which lends pharmacological justification to the use of the plant extract by traditional medicine practitioners in the treatment of epilepsy. Thus, this study provides experimental support for the traditional medicinal use of this plant for nervous disorders.

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