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Website: www.ayujournal.org
DOI: 10.4103/0974-8520.93919
Quick Response Code:

Pharmacological Research

Psychotropic activity of *Argyreia speciosa* roots in experimental animals

Varsha J. Galani, Bharatkumar G. Patel

Department of Pharmacology, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar, Gujarat, India

Abstract

Argyreia speciosa (L.f.) Sweet (convolvulaceae) commonly known as *Briddhadaraka* is regarded as a "*Rasayana*" drug in the ayurvedic system of medicine to cure diseases of nervous system. In this study, hydroalcoholic root extract of *A. speciosa* was subjected to evaluate psychotropic effects in classical experimental models. Effect of the extract on spontaneous motor activity, pentobarbital-induced sleeping time, motor coordination, exploratory behavior, and apomorphine-induced stereotypy were investigated in mice. Effect of the extract on catalepsy and haloperidol-induced catalepsy were studied in rats. Preliminary phytochemical and acute toxicity screenings were also performed. The extract (100, 200, and 500 mg/kg, p.o.) significantly decreased spontaneous motor activity, exploratory behavior, and prolonged pentobarbital sleeping time in mice. The extract also remarkably attenuated the intensity of apomorphine-induced stereotypy but had no effect on motor coordination. The extract produced catalepsy and potentiated haloperidol-induced catalepsy in rats. These results provide evidence that the hydroalcoholic extract of *A. speciosa* roots may contain psychoactive substances that are sedative in nature with possible neuroleptic properties.

Key words: Apomorphine-induced stereotypy behavior, *Argyreia speciosa*, exploratory activity, haloperidol-induced catalepsy, psychotropic activity, pentobarbitone, spontaneous motor activity

Introduction

Argyreia speciosa (L.f.) Sweet is commonly known as *Briddhadaraka*, classified in *Ayurveda* as a *rasayana*, a group of plant-derived drugs reputed to promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalize the body in debilitated conditions and increase longevity. A. *speciosa* (L.f.) Sweet (convolvulaceae) known as "elephant creeper" in English and "*Vardharo*" in Gujarati is a woody climber distributed throughout India up to an altitude of 300 m. It is cultivated in the gardens as an ornamental plant for its green leaves and beautiful rose purple flowers. The plant is extensively used in the indigenous system of medicine. The roots of this plant have been regarded as tonic, aphrodisiac, and bitter. The root of this plant is also used in rheumatism, gonorrhea, chronic ulcer, and diseases of the nervous system.[1]

Address for correspondence: Dr. Varsha J. Galani, Department of Pharmacology, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar - 388 120, Gujarat, India.
E-mail: vrp173@yahoo.com

Previous phytochemical studies reveal the presence of lipids, flavonoids, triterpenes, steroids, phenylpropanoids, and coumarins in the plant. The major constituents isolated from the plant are friedelin, ergine, agroclavine, penniclavine, chanclavine, ergometrine, quercetin, kaempferol, scopoletin, and hexadecanyl p-hydroxycinnamate. Several investigations have proposed that this plant possesses nootropic, aphrodisiac, immunomodulatory, hepatoprotective, antioxidant, antinflammatory, analgesic, antihyperglycemic, and anticonvulsant activities.[2] Traditional and reported uses suggested that roots of A. *speciosa* might have action on the central nervous system. In the light of above information and folklore uses, this study was designed to evaluate the psychotropic activity of roots of A. *speciosa* using various experimental models.

Materials and Methods

Animals

Albino mice (25–30 g) and Wistar rats (200–250 g) of either sex bred in central animal house facility of the institute were used. The animals were housed under standard conditions, maintained on 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. All experiments were conducted during the light period (08.00–16.00 h). All the protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

Plant material and preparation of extract

The roots of A. *speciosa* were collected from Balasinor, Gujarat. Their authenticity was confirmed by a Taxonomist, 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 qualitative chemical investigation of hydroalcoholic extract was carried out to check the presence of various phytoconstituents.[3] It revealed the presence of carbohydrates, proteins, steroids, saponins, tannins, flavanoids, coumarins, triterpenes, and essential oil.

Drugs

Diazepam (Campose® inj., Ranbaxy Laboratories) was used as reference drug (positive control) for spontaneous motor, muscle relaxant, exploratory, and sedative activities. Haloperidol (Torrent Pharmaceuticals, India) was used as positive control for apomorphine-induced stereotypy in mice and cataleptic response in rats. It was administered in the form of suspension using Tween 80 (0.2% v/v) as the suspending agent. Apomorphine hydrochloride (Sigma, St. Louis, MO, USA) and sodium pentobarbital (Sigma, St. Louis, MO, USA) were used for induction of stereotypy and sleep, respectively. They were dissolved in 0.9% saline solution prior to administration.

Treatment

Freshly prepared aqueous solution of dried extract of roots of A. *speciosa* in suitable dilution was administered orally in the test animals. A. *speciosa* root extract was also administered intraperitoneally in the mice for the acute toxicity test. For the neuropharmacological activity, animals were divided in to five groups each group consisting of ten animals. Group 1 served as control group received distilled water as vehicle (1 ml/kg) per oral (p.o.), group 2–4 served as test groups received A. *speciosa* root extract (100, 200, and 500 mg/kg, p.o.) and group 5 served as positive control received reference drugs. Diazepam (2 mg/kg) and Haloperidol (1 mg/kg) were administered intraperitoneally (i.p.) in a positive control group for above-mentioned experimental models. One hour after oral and 30 min after intraperitoneal administration, each animal was submitted to various behavioral testing.

Acute toxicity test

Different doses (100–5000 mg/kg) of A. *speciosa* root extract were administered intraperitoneally to five groups of mice (six in each) and orally to another five groups of mice (six in each). Mortality within 24 h was recorded.[4] The LD_{50} was estimated from the graph of probit against log-dose of the extract.

Neuropharmacological screening

Spontaneous motor activity

The spontaneous motor activity of mice was recorded before and after treatments as activity score using an actophotometer.[5]

Effect on motor coordination

(a) Rotarod test: The effect on motor coordination was examined by the rotarod apparatus.[6] Fall off time was recorded for mice after treatments. The test was considered positive if a mouse is unable to remain on the rod during the 3 min trial.

(b) Traction test: Forepaws of a mouse were placed on a small twisted wire rigidly supported above a bench top. Inability to put up at least one hind foot constituted failure to traction.[7] After treatments mice were again placed on twisted wire and tested for traction.

Pentobarbitone induced hypnosis

Pentobarbitone sodium (35 mg/kg, i.p.) was administered to all pretreated animals. The onset of sleep and duration of sleep were recorded.[8]

Exploratory activity

(a) Head dip test: Exploratory activity was measured using the hole-board apparatus. The number of head dips in to hole by each mouse during 5 min period was noted.[9]

(b) Evasion test: Groups of mice (10 in each) were kept in a rectangular box having an inclined plane by which the mice could escape from the box. Mice, which escaped by 5 min of placing them in the box were selected for further testing. Numbers of mice remaining in the box after 5 min in each treatment group were noted.[10]

Body temperature

The rectal temperature of each mouse was recorded just before and after 15, 30, 60, 120, and 180 min of the treatment using a digital telethermometer.[11]

Catalepsy in rats

Rats were tested for catalepsy after 15, 30, 60, 90, 120, 180, 240, and 300 min of treatments by placing both front paws over an 8 cm high horizontal bar.

For observing the effect of A. *speciosa* root extract on the Haloperidol-induced catalepsy, Haloperidol (1 mg/kg, i.p.) was injected to control and A. *speciosa* root extract treated and positive control animals. Catalepsy score (descend latency) in seconds of each animal in the group at the respective testing time interval was measured.[12]

Apomorphine induced stereotypy

Stereotypy was induced by apomorphine hydrochloride (2 mg/kg, i.p.) to all groups of mice after treatment. The mice were individually placed in glass containers of 250 ml capacity. Continuous sniffing, rearing, licking, and gnawing were observed as stereotypic behavior at 0, 15, 30, 45, and 60 min after apomorphine administration. The intensity of stereotypy was recorded by the scoring system. For each mouse, a global score was calculated by averaging the five stereotype scores obtained at mentioned time interval.[12]

Statistical analysis

Data were expressed as mean ± S.E.M. The statistical significance of differences between groups was evaluated by

one-way analysis of variance (ANOVA) followed by the post hoc Dunnet's test. A probability level of 0.05 or less was accepted as significant.

Results

Acute toxicity test

Oral administration of hydroalcoholic extract of A. *speciosa* root did not show any toxic symptoms up to 5 g/kg dose in mice. The intraperitoneal LD_{50} of A. *speciosa* root extract was found to be 630 mg/kg.

Spontaneous motor activity

As shown in the Figure 1, the A. *speciosa* root extract (100, 200, and 500 mg/kg, p.o.) produced a significant ($P<0.05$) and dose-dependent decrease in spontaneous motor activity. Similarly, positive control diazepam (2 mg/kg, i.p.) also produced significant reduction in spontaneous motor activity.

Effect on motor coordination

The A. *speciosa* root extract (100, 200, and 500 mg/kg, p.o.) did not exhibit significant effect on the rota-rod performance of the mice as all the animals stayed on the rod for 180 sec without falling.

In the traction test, the mice treated with A. *speciosa* root extract did not show significant failure in traction at all doses tested. However, diazepam (2 mg/kg, i.p.) treated mice reduced fall off time in the rotarod test and failed to produce traction in traction test indicating skeletal muscle relaxing action (data not shown).

Pentobarbitone-induced hypnosis

The results are summarized in Table 1. The A. *speciosa* root extract (100, 200, and 500 mg/kg, p.o.) significantly ($P<0.05$) prolonged the duration of pentobarbital sleeping time in mice, with no effect on the onset of sleep. Positive control, Diazepam-reduced onset of sleep and potentiated duration of sleep in significant manner.

Exploratory activity

As shown in the Table 2, A. *speciosa* root extract (100, 200, and 500 mg/kg, p.o.) produced reduction of exploratory activity as indicated by significant ($P<0.05$) and dose-dependent decrease in the number of head dips. Similarly, Diazepam caused a significant decrease in the number of head dips.

Mice treated with A. *speciosa* root extract caused significant ($P<0.05$) and dose-dependent inhibition of residual curiosity in the evasion test [Table 3]. This reduction of exploratory activity was also found to be time dependent. Positive control, diazepam (10 mg/kg, i.p.) inhibited total residual curiosity.

Body temperature

In the control animals, no significant variations of rectal temperature were observed. However, treatment with the A. *speciosa* root extract (100, 200, and 500 mg/kg) produced significant and dose-dependent fall in rectal temperature of mice at 60, 120, and 180 min [Figure 2].

Catalepsy in rats

As shown in the Figure 3, A. *speciosa* root extract in all the doses showed catalepsy at 60 and 90 min of time intervals. A. *speciosa* root extract treatment also showed catalepsy at 120 min (200 mg/kg and 500 mg/kg, p.o.) and 180 min (500 mg/kg, p.o.)

Table 1: Effect of hydroalcoholic extract of *A. speciosa* roots on pentobarbital (35 mg/kg, i.p.) induced hypnosis in mice

Treatment	Dose (mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Control	-	3.9 ± 0.33	26.9 ± 2.3
ASE	100	3.2 ± 0.24	41.1 ± 4.4*
ASE	200	5.2 ± 0.56	53.3 ± 6.5*
ASE	500	4.3 ± 0.15	80.3 ± 4.6*
Diazepam	2	2.5 ± 0.16*	100.7 ± 2.94*

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *$P<0.05$ when compared with the control group, ASE - *Argyreia speciosa* roots

Table 2: Effect of hydroalcoholic extract of *A. speciosa* roots (ASE) on exploratory behavior (Head dip test) in mice

Treatment	Dose (mg/kg)	Number of head dips
Control	-	13.9 ± 0.82
ASE	100	9.2 ± 1.3*
ASE	200	6.2 ± 0.72*
ASE	500	4.9 ± 0.69*
Diazepam	10	2.9 ± 0.50*

Values are expressed as mean ± SEM (n=10). One way ANOVA followed by Dunnett's test, *$P<0.05$ when compared with the control group, ASE - *Argyreia speciosa* roots

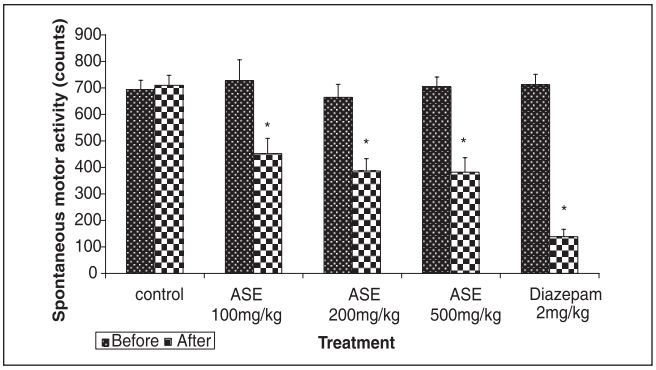

Figure 1: Effect of the hydroalcoholic extract of *A. speciosa* roots (ASE) on spontaneous locomotor activity. Each bar represents the mean ± SEM (n = 10). One way ANOVA followed by Dunnett's test, *$P<0.05$ when compared with control group

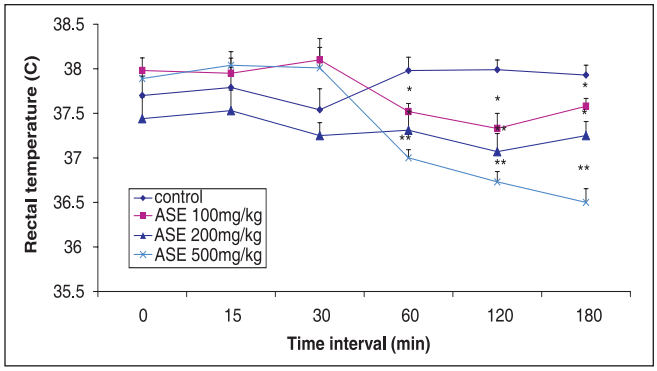

Figure 2: Effect of hydroalcoholic extract of *A. speciosa* roots (ASE) on rectal temperature of mice. Values are expressed as Mean ± S.E.M. (n = 10). One way ANOVA followed by Dunnett's test, *$P<0.05$, **$P<0.001$ when compared with control group

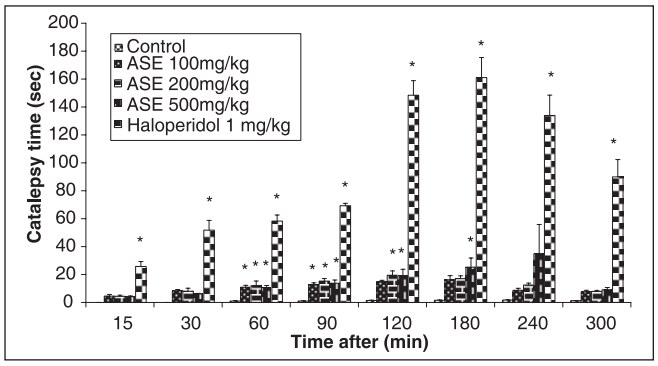

Figure 3: Effect of hydroalcoholic extract of *A. speciosa* roots (ASE) on catalepsy in rats. Each bar represents the mean ± SEM (n = 10). One way ANOVA followed by Dunnett's test, *P<0.05 when compared with control group

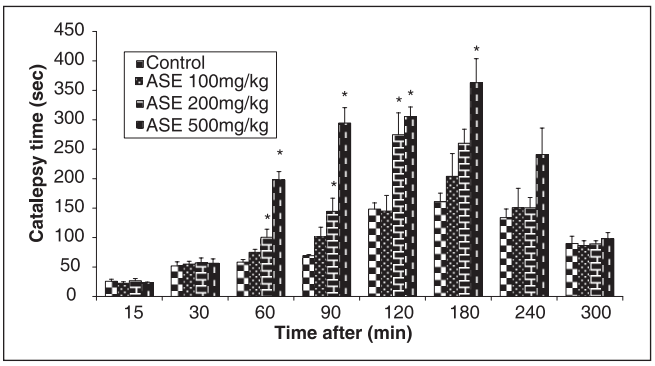

Figure 4: Effect of hydroalcoholic extract of *A. speciosa* root on haloperidol induced catalepsy in rats. Each bar represents the mean ± SEM (n = 10). One way ANOVA followed by Dunnett's test, *P<0.05 when compared with control group

Table 3: Effect of hydroalcoholic extract of *A. speciosa* roots (ASE) on residual curiosity (Evasion test) in mice

Treatment	Dose (mg/kg)	No. of mice	No. of mice remaining in box after 5 min			% mice showing curiosity		
			30 min	60 min	120 min	30 min	60 min	120 min
Control	-	10	0	0	0	100	100	100
ASE	100	10	4	5	7	60	50*	30*
ASE	200	10	5	6	8	50*	40*	20*
ASE	500	10	8	8	10	20*	20*	0*
Diazepam	10	10	10	10	10	0*	0*	0*

n=10. Chi square test. **P*<0.05 when compared with the control group, ASE - *Argyreia speciosa* roots

of time intervals. Positive control, Haloperidol showed catalepsy for all time intervals.

A. *speciosa* root extract treatment at the doses of 200 mg/kg and 500 mg/kg was also produced significant potentiation of haloperidol-induced catalepsy at 60, 90, and 120 min time intervals [Figure 4].

Apomorphine-induced stereotypy

As shown in the Table 4, A. *speciosa* root extract (100, 200, and 500 mg/kg, p.o.) significantly attenuated apomorphine-induced stereotyped behavior in mice dose dependently. This effect was similar to that produced by haloperidol (1 mg/kg).

Table 4: Effect of hydroalcoholic extract of *A. speciosa* roots (ASE) on apomorphine (2 mg/kg, i.p.) induced stereotypy in mice

Treatment	Dose (mg/kg)	Score for stereotypy behavior
Control		2.72 ± 0.09
ASE	100	1.34 ± 0.05*
ASE	200	1.22 ± 0.04*
ASE	500	0.66 ± 0.04*
Haloperidol	1	0.00 ± 0.0*

Values are expressed as mean ± SEM (*n*=10). One way ANOVA followed by Dunnett's test, **P*<0.05 when compared with the control group , ASE - *Argyreia speciosa* roots

Discussion

In this work, the neuropharmacological effects of the hydroalcoholic extract of A. *speciosa* roots were studied in several behavioral animal models for the evaluation of their possible psychotropic activity. The results of the present investigation showed that the hydroalcoholic extract of A. *speciosa* roots has some potent neuropharmacological activity.

Assessment of acute toxicity is the first step in the toxicological investigation of an unknown substance. The hydroalcoholic extract of A. *speciosa* roots was well tolerated by mice and there were no signs of acute (during 2 h observation period) or delayed (24 h after extract treatment) toxicity after oral administration. Increasing doses of the hydroalcoholic extract of A. *speciosa* roots up to 5 g/kg (p.o.) were not lethal, the LD_{50} values for the extract was estimated to be higher than 5000 mg/kg for oral administration. Thus, suggesting that this administration route is adequate and secure to produce its neuropharmacological effects.

Reduction in the spontaneous motor activity leads to sedation[13] as a result of reduced excitability of the central nervous system. Prolongation of pentobarbital hypnosis was due to sedative and/or hypnotic property[14] attributed to an action on the central mechanisms involved in the regulation of sleep[15] or an inhibition of pentobarbital metabolism.[16] The hydroalcoholic extract of A. *speciosa* roots significantly reduced spontaneous motor activity and prolonged pentobarbital induced hypnosis in mice. Thus, suggesting that the hydroalcoholic extract of A. *speciosa* roots might be acting as mild neurosedative agents. Hole-Board test is a measure of exploratory behavior[17] and an agent that decreases this behavior reveals sedative[18] activity. The hydroalcoholic extract of A. *speciosa* roots reduced exploratory behavior in the hole board test and evasion test, further confirming sedative or central nervous depressant nature of A. *speciosa*. Reduction

in the rectal temperature of mice by *A. speciosa* treatment also indicated probable central nervous depressant action.

Neuroleptics, which have an inhibitory action on the nigrostriatal dopamine system known to induce catalepsy[19] while neuroleptics with little or no nigrostriatal blockade produce relatively little or no cataleptic behavior.[20] Compounds which prevent apomorphine induced stereotypy also antagonize dopamine receptors in the nigrostriatal system.[21] Haloperidol induces catalepsy and antagonizes apomorphine-induced stereotypies by blocking the postsynaptic striatal D_2 and D_1 dopamine receptors.[22] The neuroleptic potential of the hydroalcoholic extract of *A. speciosa* roots was confirmed by the results in which it produced catalepsy, potentiated haloperidol-induced catalepsy and antagonized apomorphine induced stereotypies.

Furthermore, the inability of the hydroalcoholic extract of *A. speciosa* roots to affect motor coordination is additional evidence of centrally mediated actions and not blockade of neuromuscular system.[23] 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. In our previous work, central nervous depressant activity of both polar and nonpolar fractions of hydroalcoholic extract of *A. speciosa* roots was observed.[24] It is therefore suggested that components which are present in the hydroalcoholic extract of *A. speciosa* roots might contribute in providing observed psychotropic effects.

In conclusion, the results of present study provide evidence that the hydroalcoholic extract of *A. speciosa* roots may contain some psychoactive principles, which are sedative and neuroleptic in nature.

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हिन्दी सारांश

आरजीरीया स्पीसीओसा मूल की मन: प्रभावी क्रिया का प्रायोगिक अध्ययन

वर्षा जे. गलानी, भरतकुमार जी. पटेल

रसायन चिकित्सा में आरजीरीया स्पीसीओसा के मूल को मनोरोग में उपयोग में लिया जाता है। प्रस्तुत अध्ययन में इस वनस्पति के मूल का स्विस आल्बिनो चूहों में मानसभाव पर प्रभाव विभिन्न मापदंडो पर परीक्षित किया गया। जिसके परिणाम दर्शाते हैं कि मूल का उपशामक और तंत्रिका शामक प्रभाव है।