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Antiamnesic Activity of Extracts and Fraction of *Desmodium Gangeticum*

KRITIKA MAHAJAN, DEEPAK KUMAR AND SURESH KUMAR*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala-147 002, Punjab, India

E mail: thakur_pu@yahoo.com

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Abstract Desmodium gangeticum D.C. (Salpani; family – Papilionaceae) has been traditionally used in the treatment of various ailments especially in dementia. Thus, it was planned to screen antiamnesic activity of D. gangeticum to validate its traditional claims. Properly identified powdered plant material was extracted successively using solvents in increasing order of polarity viz., n-hexane, chloroform, methanol and water. All the extracts were administered at the doses of 200 or 400 mg/kg, p.o. for six successive days to mice. The antiamnesic activity of crude extracts was evaluated against scopolamine (0.6 mg/kg, i.p.) induced amnesia using well established exteroceptive behavioural model, i.e., elevated plus maze (EPM). The efficacy of test drugs was statistically compared with the standard memory enhancing drug, piracetam (100 mg/kg, p.o.). A standardized procedure was adopted to prepare alkaloidal fraction from D. gangeticum roots, which was also evaluated for antiamnesic activity at the doses of 25 or 50 mg/kg, p.o. The chloroform extract and alkaloidal fraction of the plant significantly reversed the amnesia induced by the scopolamine at the dose of 400 and 50 mg/kg, respectively, with respect to control. The antiamnesic activity shown by the chloroform extract and alkaloidal fraction of the plant was statistically equivalent to the standard drug. It is concluded that alkaloids are responsible for antiamnesic activity of D. gangeticum roots.

Keyword: Amnesia, Desmodium gangeticum, Elevated plus maze, Piracetam, Scopolamine.

1. INTRODUCTION

Dementia is a chronic or persistent mental disorder characterized by decline in mental ability severe enough to interfere with daily life. Dementia is of several

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types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas (Baddeley, 1988; Drachman & Leavitt, 1974). The elder persons are at increased risk to suffer from dementia, which has the clinical features of impaired cognition (Chertkow *et al*., 2008; Hart *et al*., 2007). The prevalence of the disease increases exponentially with age; it is estimated to increase from 10 % at the age of 65 years to nearly 50 % at 85 years (Cacabelos, 2008). The central cholinergic pathways play a prominent role in learning and memory process (Nabeshima, 1993). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals and human beings (Higashida & Ogawa, 1987; Sitaram *et al*., 1978). Cognition enhancers are used to attenuate the impairment of cognitive functions associated with age and age-related pathologies (Parnetti *et al*., 1997). A number of side effects like lever damage and mutagenesis are associated with the use of cognition enhancers obtained from synthetic sources (Witschi, 1986). Thus, researchers are exploring natural resources to find out newer and safer natural memory enhancing agents. Since allopathic system of medicine is yet to provide a radical cure, it is worthwhile to look for new directions, which would minimize the memory loss seen in elderly patients. In the Ayurvedic system of medicine, many herbs and plants are used to treat various ailments. 'Madhya' drugs mentioned in Ayurvedic texts are a group of herbal medicines, used to improve mental abilities (Rai *et al*., 2001). These herbal drugs include extracts from *Clitoria ternatea, Celastrus panniculatus, Acorus calamus, Centella asiatica, Withania somnifera, Guduchi and Areca* (Anonymous, 2001). A large number of plants have long tradition of use in treatment of mental disorders but have not been validated scientifically, *Desmodium gangeticum* D.C. is one of such plants.

D. gangeticum, commonly known as Salpani, belongs to family Papilionaceae. It is widely distributed mainly in the Himalayan territory at elevations upto 5,000 feet. It is also distributed in the China, Philippine and tropical Africa (Sagar *et al*., 2010). Traditionally, the plant has been used as antipyretic, diuretic, astringent, anthelmintic, laxative, and in the treatment of dementia (Ma *et al*., 2011). The plant has been reported to exhibit anti-inflammatory, antibacterial, antidiabetic, hepatoprotective, antiulcer, locomotor and wound healing activities (Bhattacharjee *et al*., 2013). *D. gangeticum* has been reported to contain alkaloids, flavonoids, steroids and terpenoids (Bhattacharjee *et al*., 2013).

Though few studies have shown antiamnesic activity of the plant but employed crude aqueous extract, which showed antiamnesic activity at the doses of 100 or 200 mg/kg, *p.o.* against scopolamine induced amnesia in mice using elevated plus maze and passive avoidance paradigm (Joshi & Parle,

2006; 2007). The present study was designed to explore systematically the antiamnesic activity of various extracts and fraction of *D. gangeticum* roots to validate its traditional claims.

2. MATERIALS AND METHODS

2.1 Plant material

D. gangeticum roots were procured from Himalaya Herbs Store, Madhav Nagar, Saharanpur, (Uttar Pradesh), India in September, 2014. The plant was identified by Dr. Avneet Singh, Assistant Professor, Department of Botany, Punjabi University, Patiala, India (Reference No. – SPL-103, dated 15-10-2014).

2.2 Solvents, chemicals and reagents

Calcium oxide, hydrochloric acid, sodium hydroxide, methanol (S.D. Fine Chemicals, Mumbai, India), chloroform, *n*-hexane (E Merck, Delhi, India), of LR grade, were used for the preparation of various crude extracts and fraction of *D. gangeticum* roots.

2.3 Preparation of various extracts

D. gangeticum roots were dried under sunlight and powdered in a grinder. The plant material (1 kg) was exhaustively extracted in a Soxhlet apparatus successively using solvents in order of increasing polarity *viz., n*-hexane, chloroform and methanol. The marc of plant material was dried and boiled with distilled water for 2 h on a hot plate to get water extract. The solvents from crude extracts were recovered under reduced pressure using rotary vacuum evaporator. Various extracts were screened for detection of different classes of phytoconstituents using specific standard reagents (Farnsworth, 1966).

2.4 Preparation of alkaloidal fraction

Dried powdered roots (1 kg) of *D. gangeticum* were moistened, treated with calcium oxide, dried, and then exhaustively extracted with chloroform in a Soxhlet apparatus. The chloroform extract was then concentrated to one-fourth of its original volume under reduced pressure. It was then partitioned in a separator using 5×50 ml of 2% acidulated (hydrochloric acid) water. The aqueous fraction was basified with 20% sodium hydroxide solution to pH 8-9 followed by partitioning with chloroform (5×50 ml). The chloroform fraction was rich in alkaloids (Madaan & Kumar, 2012).

2.5 Animals

Laca mice (either sex) of body weight 20-25 g purchased from the Central Research Institute, Kasauli, India were used for antiamnesic activity. The animals were fed with normal laboratory pellet diet and water *ad libitum*. The animal study was approved from Institutional Animal Ethics Committee of Punjabi University, Patiala (107/99/CPCSEA/2014-01, dated 11/10/2014). The animals were acclimatized to laboratory conditions daily for 1 h for continuous seven days before the start of experiment. All the experiments were performed from 9 AM to 12 PM as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals. Groups of six animals were used in all sets of experiments. The test drugs were administered orally with the help of an oral cannula fitted on a tuberculin syringe.

2.6 Vehicle

Distilled water + Tween 80 (2% v/v) was used as vehicle for preparing various doses of test samples in such a concentration as to administer a volume ranging 0.2 to 0.25 ml to the mice.

2.7 Standard drugs

Piracetam (UCB India Pvt. Ltd., Mumbai) (100 mg/kg, *p.o.*) and scopolamine (German Remedies, Mumbai) (0.6 mg/kg, *i.p.*) were used as antiamnesic and amnesic standard drugs, respectively.

2.8 Evaluation of antiamnesic activity

The elevated plus maze (EPM) method modified by ltoh *et al*. for the testing of memory enhancing drugs was used (Itoh *et al*., 1991). The apparatus consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, and similar arms faced each other. The maze was placed at a height of 25 cm from the floor. During the entire experiment, the animals were allowed to socialize. Elevated plus-maze test is a sensitive behavioural test which has been extensively validated for studying memory modulatory actions of drugs in animals. The test measures the transfer latency (TL), i.e., the time in which the mice move from open arm to the enclosed arm. An increase in the acquisition/learning processes is defined as decreased TL on the seventh day (2^{nd} day trial) relative to the sixth day (1^{st} day trial). Failure to decrease the TL on the 2^{nd} day trial is interpreted as an impairment of learning process. TL on EPM was used as an index of learning and memory process. The time taken by each mouse to move from the end of open arm to any enclosed arm of

EPM was measured on 6th day and 7th day of drug treatment. The animals were treated with control, standard and test drugs for 6 days. The last dose is given 45 min prior to the test on day 6 followed by i.p. administration of 0.6 mg/kg scopolamine. The TL of animals on day 6 and 7 was recorded. The results were expressed as percent retention calculated as:

Percent retention = (TL on 6th day – TL on 7th day / TL on 6th day) × 100.

2.9 Experimental protocol

Two experimental protocols were designed. Each group comprised 6 animals.

Experimental protocol I, comprising 10 groups, was designed to assess antiamnesic activity of various crude extracts of *D. gangeticum* roots.

Group 1 – Control group received vehicle.
Group 2 – Standard group received piracetam (100 mg/kg, *i.p.*).
Groups 3 & 4 – Test groups received 200 and 400 mg/kg doses of HE respectively.
Groups 5 & 6 – Test groups received 200 and 400 mg/kg doses of CE respectively.
Groups 7 & 8 – Test groups received 200 and 400 mg/kg doses of ME respectively.
Groups 9 & 10 – Test groups received 200 and 400 mg/kg doses of WE respectively.

Experimental protocol II, comprising 4 groups, was designed to assess antiamnesic activity of alkaloidal fraction (AF) of *D. gangeticum* roots.

Group 1 – Control group received vehicle.
Group 2 – Standard group received piracetam (100 mg/kg, *i.p.*).
Groups 3 & 4 – Test groups received 25 mg/kg and 50 mg/kg doses of AF respectively.

2.10 Statistics

The results were expressed as mean ± standard deviation. The test drugs were compared with standard drug and control by one way analysis of variance (ANOVA) followed by Student Newman Keul's test (Scheffer, 1980).

3. RESULTS

Yields of HE, CE, ME and WE were found to be 0.83, 0.33, 4.10 and 10.12% w/w, respectively. All extracts were screened for different classes of

phytoconstituents. The results of phytochemical screening showed presence of fixed oils in HE; alkaloids and steroids in CE; flavonoids, carbohydrates and tannins in ME; carbohydrates and proteins in WE.

All extracts of *D. gangeticum* roots were evaluated for antiamnesic activity against scopolamine-induced amnesia in mice using EPM. Table 1 shows the mean TL and percent reduction in TL in EPM by mice after administration, for six successive days, of 200 or 400 mg/kg, *p.o.* doses of crude extracts, piracetam (100 mg/kg, *p.o.*) and vehicle, *p.o.* Amongst various extracts, CE exhibited significant antiamnesic activity with respect to control. HE, ME and

Table 1: Effect of various extracts of *D. gangeticum* roots in scopolamine-induced amnesia using EPM model.

Sr. No.	Group*	Dose (mg/kg)	Mean[n] TL (sec) ± S.D.		Percent reduction in TL
			Day 6	Day 7	
1.	Control	Vehicle	25.56 ± 3.65	24.34 ± 4.12b	4.77
2.	Piracetam	100	16.50 ± 1.04	7.16 ± 0.73a	56.60
3.	HE	200	20.50 ± 0.54	18.50 ± 1.87ab	5.70
		400	19.50 ± 1.87	19.33 ± 0.80ab	5.12
4.	CE	200	15.83 ± 0.75	8.66 ± 0.81ab	45.29
		400	16.50 ± 1.64	7.33 ± 0.51b	55.57
5.	ME	200	14.83 ± 0.75	12.83 ± 0.71ab	13.48
		400	14.33 ± 1.03	12.33 ± 0.81ab	13.95
6.	WE	200	18.00 ± 0.89	16.50 ± 0.59ab	8.33
		400	13.66 ± 0.81	12.83 ± 0.85ab	6.07

n=6; The data is expressed as Mean ± S.D.; [a]$P<0.05$ *vs* control; [b]$P<0.05$ *vs* piracetam; one way ANOVA followed by Student-Newman-Keul's test.

*Scopolamine (0.6 mg/kg, *i.p.*) was given to all animals of control, standard and test groups on sixth day.

WE were found to be devoid of antiamnesic activity. CE showed maximum activity with 45.29 and 55.57 percent reduction in TL at the dose of 200 and 400 mg/kg, *p.o.*, respectively. The CE reversed scopolamine induced amnesia in mice at the dose of 400 mg/kg in the similar manner to the standard cerebroprotective drug, piracetam.

The CE of plant showed presence of alkaloids as major class of phytoconstituents, thus, alkaloidal rich fraction (AF) of the plant was separated using standard procedure. The yield of AF was found to be 0.067% w/w. AF was screened for antiamnesic activity against scopolamine-induced amnesia in mice using EPM. Table 2 shows the mean TL and percent reduction in TL in EPM by mice after administration, for six successive days, of AF (25 or 50 mg/kg, *p.o.*), piracetam (100 mg/kg, *p.o.*) and vehicle, *p.o.* AF exhibited significant antiamnesic activity at the dose of 50 mg/kg with respect to control**,** and the activity was also statistically equivalent to the standard drug. AF dose dependently increased activity from 48.78 percent reduction at 25 mg/kg to 53.37 percent reduction at 50 mg/kg.

Table 2: Effect of alkaloidal fraction of *D. gangeticum* roots in scopolamine-induced amnesia using EPM model.

Sr. No.	Group	Dose (mg/kg)	Mean[n] TL (sec) ± S.D.		Percent reduction in TL
			Day 6	Day 7	
1.	Control	Vehicle	28.76 ± 3.00	27.34 ± 5.45[b]	4.90
2.	Piracetam	100	15.66 ± 2.33	7.33 ± 1.82[a]	53.19
3.	AF	25 50	19.00 ± 1.26 17.16 ± 1.16	9.73 ± 0.75[ab] 8.00 ± 0.90[b]	48.78 53.37

n=6; The data is expressed as Mean ± S.D.; [a]*P*<0.05 *vs* control; [b]*P*<0.05 *vs* piracetam; one way ANOVA followed by Student-Newman-Keul's test.

*Scopolamine (0.6 mg/kg, *i.p.*) was given to all animals of control, standard and test groups on sixth day.

4. DISCUSSION

Alzheimer and Dementia are progressive neurodenerative disorders. Synthetic antidementives (Tacrine, Donepezil, Rivastigmine, Galantamine, etc.) are commonly prescribed in the management of dementia but these synthetic drugs are associated with severe side effects such as hepatotoxicity, gastrointestinal side effects, nausea, diarrhoea and vomiting (Mimica & Presecki, 2009). Therefore, a traditionally used and medicinally promising plant, *Desmodium gangeticum*, has been selected for the present investigation to establish a safer and efficacious antiamnesic drug.

Antiamnesic activity of *D. gangeticum* roots, one of the highly reputed plants of Ayurveda, was evaluated employing widely used model, i.e., EPM. The EPM model was chosen since these are effective, cheap, simple, less time consuming, and require no preliminary training to the mice and do not cause much discomfort to the animals while handling (Kumar & Kumar, 2015).

Though few studies have shown antiamnesic potential of *D. gangeticum* but employed uncharacterized crude aqueous extract of the plant. Joshi & Parle (2006; 2007) have reported that aqueous extract of the plant exhibited significant antiamnesic activity at the doses of 100 or 200 mg/kg, *p.o.* against scopolamine induced amnesia in mice using elevated plus maze and passive avoidance paradigm through increased mice brain acetylcholine content and decreased acetylcholinesterase activity. Alkaloids have been suggested to possess memory enhancing activity as these compounds isolated from *D. gangeticum* have been reported to possess CNS activities (Ghosal & Bhattacharya, 1972), but no detailed systematic work was further carried out to validate these activities. In contrary to above reports, it is reported that Gangetin, a pterocarpan compound, isolated from *n*-hexane extract of the plant (Purushothman *et al*., 1971) significantly reversed scopolamine- as well as streptozotocin-induced learning and memory deficits along with rise in brain AChE activity and brain oxidative stress levels at the doses of 1, 2 or 3 mg/kg, *i.p.* (Made & Joshi, 2012). Thus, *n*-hexane extract of the plant was prepared by exhaustively extracting the plant material with *n*-hexane in a Soxhlet apparatus, and evaluated for antiamnesic activity in mice against scopolamine induced amnesia using EPM. It could not able to reverse scopolamine induced amnesia in mice. This observation suggests that Gangetin, which is present in *n*-hexane extract, is not responsible for antiamnesic activity of the plant. Therefore, the marc of the plant was further extracted successively with solvents in increasing order of polarity, viz., chloroform, methanol and water. All extracts were also screened for antiamnesic activity in mice against scopolamine induced amnesia using EPM. Only chloroform extract exhibited antiamnesic activity. Preliminary phytochemical screening showed presence of alkaloids as major

class of phytoconstituents, thus, a standard procedure was adopted to separate alkaloidal rich fraction from the plant material. AF exhibited significant antiamnesic activity in mice.

The results that AF of *D. gangeticum* roots exhibits antiamnesic activity are in agreement with the reported literature where a large number of alkaloids such as neferine (Jung *et al*., 2010), oxoglauidaline, protoberberine, pseudoberberine, pseudodehydrocorydaline (Hung *et al*., 2008), protopine (Kim *et al*., 1999), mahanimbine (Kumar *et al*., 2010) and vinconate (**Kinoshita** *et al*., 1992) have been reported to exhibit antiamnesic activity. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions, thus, it is suggested that AF of *D. gangeticum* roots exhibits antiamnesic activity by reducing acetyl cholinesterase enzyme activity and increasing level of acetylcholine in brain (Chuong *et al*., 2014). The research is in progress to isolate alkaloids responsible for antiamnesic activity of *D. gangeticum*.

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