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Research Article

A promising cognition boosting effect of leaves of *Abrus precatorius*

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ABSTRACT

Objective: To assess the leaves of *Abrus precatorius* had earlier show cognition boosting effect against dementia in rodents. **Methods:** The methanol extract was fractionated into ethyl acetate and aqueous fractions and assessed for its nootropic and learning activity in albino rodents. The effect of triterpenoids containing ethylacetate soluble fraction of methanol extract significant enhancement in the retention ability of amnesic mice as compared to their respective controls using elevated plus maze and Cook and Weidley's pole apparatus. Subsequently, the extracts were further studied for its *in vitro* acetylcholinesterase (AChE) inhibitory potential which can correlate with an improvement in cholinergic transmission. Piracetam was used as the standard drug while scopolamine hydrobromide served as the amnesic agent. **Result:** *A. precatorius* ethylacetate (APEA) soluble fraction of leaves produced a significant acquisition, retention and retrieval at dosed 150 and 300 mg/kg in interoceptive as well as exteroceptive memory models of rodents. While *A. precatorius* aqueous fraction (APAQs) of methanol extract not enhances both the acquisition as well as the retention of memory of learned task when administered at a dose of 300 mg/kg for a period of 7 days. It was found that APEA fraction potentially inhibits AChE with percentage inhibition of 91.33 ± 0.33 . Furthermore, both fraction were found safe with no deaths in mice treated orally with 2000 mg/kg.

Conclusion: APEA showed to be a useful memory restorative agent in the treatment of dementia from the experiments performed.

Keywords: *Abrus precatorius*, Piracetam, Scopolamine, Dementia

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1. INTRODUCTION

According to a recent National Mental Health survey in India approximately 150 million people affect mental disorders and need care for their socio-economic burden on society. Cognitive impairment is a major neurodegenerative disorder affecting large number of population in the world [1]. Cognitive-enhancing drugs are well-known compounds or supplements recover the patients with neuropsychiatric disorders and brain injury. It may also helpful in neuropsychiatric disorders such as schizophrenia, depression and anxiety disorder [2]. Cognitive-enhancing drugs, also recognized as smart drugs, "brain booster," or "nootropic drugs," are widespread use compound responsible for the enhancement of memory performance. The term nootropic make two word noos means "mind" and tropein mean towards thus drugs belong to the subclass of psychotropic agents with that increases mental functions including memory, motivation, concentration, and attention [3]. Ayurvedic medicines are collected from traditional plant native to India and have played an important role in support

and maintain equilibrium in various phase of human life. These include *Moringa oleifera* [4], *Asparagus recemosus* [5], *Bacopa monnieri* [6], *Convolvulus pluricaulis* and *Evolvulus alsinoides* [7], *Centella asiatica* [8] and *Butea frondosa* [9] have been exploited for the cure and management of acute and chronic neurological diseases. *A. precatorius*, locally known as Indian licorice is one of the crucial herbs commonly known as belonging to family Fabaceae, native to the Indian subcontinents. It is valuable source of unique natural compounds with documented biological activity against various diseases like anti-bacterial [10], immunopotentiating [11, 12], anti-fertility [13, 14], anti-asthmatic [15], anti-inflammatory [16, 17], anti-diarrhoeal [18], anti-diabetic [19, 20], anti-convulsants [21] anti-oxidant and anti-ulcer activities [22, 23].

Abrus precatorius, locally known as gunchi and rati, is one of the most deadly plants known to man. It is a beautiful, perennial, deciduous, prickly twining herb with delicate feathery leaves with greenish yellow branches [24]. In the Indian traditional system leaves of *A. precatorius* have been

used of medicine for the treatment of leucoderma spot, itching, urticaria, stomatitis, conjunctivitis, alopecia, migraine, lymphomas/ leukemia and dysmenorrhoea [25, 26].

2. MATERIALS AND METHODS

2.1. Collection of plant material

Fresh leaves of *A. precatorius* were collected from the medicinal garden of Dr. Harisingh Gour Vishwavidyalaya, Sagar, India, in the month November 2016 and washed properly and air-dried. The collected plant material was authenticated by botanist Dr. P. K. Khare, Professor of Botany, Dr. Harisingh Gour Vishwavidyalaya, Sagar, India. A herbarium specimen bearing voucher No. Bot/Her/01/2017 has been deposited in the Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar, India.

2.2. Preparation of extracts

The shade-dried leaves of the herb ground into a fine powder. Powdered material (80 g) was first defatted with petroleum ether and extracted successively with methanol in Soxhlet's extractor. Solvent was evaporated in rotary evaporator dryness under reduced pressure to obtain the methanol extract (4.9 % w/w). The methanol extract was further suspended in distilled water and fractioned by using ethylacetate to obtain ethylacetate soluble fraction and remaining aqueous fraction. Both fraction were concentrated under reduced pressure and tested for neuropharmacological study.

2.3. Chemicals

Piracetam, (Cerecetam, 400 mg/tablet, Intas Laboratories, India), Tween 80 (oxford laboratory, India) were used in this study. Acetylthiocholine chloride (ATCC) and 5, 5 -dithiobis [2- nitrobenzoic acid] (DTNB) were purchased from Fine Chem Industries Mumbai. Scopolamine hydrobromide and Acetylcholinesterase (*Electrophorus electricus*) were obtained from Sigma Ltd. (Mumbai, India). All other reagents and chemicals used were of analytical grade.

2.4. Phytochemical investigation

The ethylacetate soluble and aqueous fractions are subjected to phytochemical analysis using conventional protocol [27].

2.5. Animals and housing

Healthy Swiss albino mice (20–25 g) and Wistar albino rats of (180–200 g) of either sex were used for the study. All animals were maintained in standard household conditions of temperature, light and humidity were used for study. All animals were stored in standard cage and maintained at (27±2) °C under 12 h dark/light cycle. They were feed with standard rodent diet food and water freely available. Animals were fasted overnight prior to drug administration. The Institutional Animal Ethical Committee of BTPC, Sagar, India (0604/IAEC/2017/252), approved the study.

2.6. Acute toxicity and effect on gross behavior

The acute toxicity tests were performed according to the OECD-425 guidelines. Both fractions were orally administered to Swiss mice (n = 3) of either sex preferred by random sampling manner were employed in learning [28]. Both fractions were found safe up to the dose of 2000 mg/kg and from the results 300 mg/kg was selected as the maximum dose for further experimentation on rodent in the present study [29].

2.7. Administration of the extracts

In the present investigation, employing various interoceptive as well as exteroceptive memory models of rodents. Amnesia was provoked in separate group's mice by scopolamine hydrobromide (0.4 mg/kg, *i.p.*) in interoceptive models. Prepared suspensions of the ethylacetate and aqueous fractions in distilled water using Tween 80 (0.2% v/v) as the suspending agent. Both fractions were administered in dose of 150 and 300 mg/kg, *p.o.*, 45 min before the test procedures. Control groups were given only the vehicle (0.2% v/v Tween 80 solution) in volume equivalent to that of the plant fractions.

2.8. General behavioral tests

Swiss albino mice were categorized into three groups (6 in each group). The aqueous and ethyl acetate soluble fraction were administered orally in doses of 300 mg/kg and third group received Tween 80 as vehicle. The activities were confirmed in the initial phase, observed for body positions, locomotion, tremors, rearing, gait, and in the later phase, the effect on passivity, pain response grip strength, stereotypy, vocalization and righting reflex were noted.

2.9. Elevated plus maze test

Elevated plus-maze act as the exteroceptive behavioral method to assess memory and learning progression in mice by measure transfer latency. The instrument was fabricated of two open arms (30 × 05 cm), with central platform (5×5 cm) and two enclosed arms (30 ×05 × 25 cm). From first day to day six, each mouse was put at the end of an open arm, facing away from the central display place. Transfer latency (TL) was measured as the time duration by the mice to shift into any one of the enclosed arms. TL was trace for six succeeding days as training section. If the mice did not come inside the closed arms within 90 s, it was softly pushed into one of the two enclosed arms and TL allotted as 90 s. The mice were permitted to explore the maze and then returned to its home cage. Memory retention of this learned-task was scrutinized 24 h after the 6th day trial. Overnight fasted mice were divided into seven groups containing six animals in each group. Group I- received only 0.2% v/v Tween 80 solution volume equivalent. Group II- received scopolamine 0.4 mg/kg, *i.p* single dose on day seventh. Group III- Piracetam (100 mg/kg, *p.o.*) was received to mice for 7 successive days and group IV–VII received APEA and APAqs (150 and 300 mg/kg *p.o.*) were administered orally for 7 successive days to mice. Retention of this learned-task was examined after 24 h (on eighth day) [30, 31].

2.10. Active avoidance paradigm

To assess number of active avoidance responses (ARs) using Cook and Weidley's pole instrument [32]. The apparatus consisted of Electric shock (20 V, A.C.) was transmitted to the grid floor with soundproof chamber and with a buzzer tone. Experimental chamber had sliding transparent door, through which the animal could well observe. A wooden pole attached to internal face of lid of the chamber performed as the shock-free zone. The 6 mA foot shock stimuli delivered for a period of 10 s on the electrified grid floor for assessment of nootropic activity. Rats were previously trained to escape the shock by climbing on to wooden pole. Beginning trial was carried out by having three trial session interspersed with period of 10 s. Only those rats were selected in the study, which were responsive to the foot shock and could ascend the pole. The rats were divided into six groups, containing six animals in each group. Group II Piracetam (100 mg/kg, *p.o.*) was received to rat for 7 successive days and Group III–VI received APEA and APAqs

fractions of leaf (150 and 300 mg/kg p.o.) were administered orally for 7 successive days to rat following which the training trial (TT) was conducted. Totally 10 trial sessions interspersed with a period of 30 s. During every trial rat was permitted to walk around the equipment for 10 s, follow by 50 Hz buzzer tone (conditioned stimulus) followed by the foot shock for 10 s. The rat seeking to coalesce the buzzer tone with the impede foot shock and keep away from the foot shock on hearing the buzzer warning. Avoidance responses (AR) mean jump onto the wooden pole, previous to the shock phase. One day later, a relearning trial (RT) compiled of number of ARs in the 10 trial sessions computed.

2.11. Assay of AChE enzyme activity by microplate reader

The determine AChE activity of the extracts were measured by using microplate reader based on Ellman's method [33]. The enzyme AChE hydrolyses the substrate acetylthiocholine chloride resulting thiocholine formed due to hydrolysis which reacts with Ellman's reagent (DTNB) to produce a yellow 3-thio-2-nitrobenzoic acid anion which can be detected at 415 nm. In brief a 96 well plate was placed first take 125µL of 3 mmoles/L Ellman's reagent in buffer A (50 mmoles/L Tris-HCl containing 0.1 mol/L NaCl and 0.02 mol/L MgCl₂·6H₂O and keep pH 8), 50µL of buffer B (50 mmoles/L containing 0.1 % bovine serum albumin keep pH 8), 25µL of 15 mmoles /L ATCC in water, and 25 µL of herb leaves fraction (25 – 400 µg/ mL). After that, AChE (0.2 U/mL) was insert to the wells and the absorbance measured by microplate reader (i Mark Microplate Reader, Bio-Rad Laboratories, UK) at 415 nm each 10 sec for 3 mins, for a blank with buffer as a substitute of enzyme solution was used. Experiments were performed in triplicate. The percentage enzyme inhibition was calculated from the rate of absorbance alter with time [34].

$$\text{Inhibition(\%)} = 100 - \frac{\text{Change of sample absorbance} \times 100}{\text{Change of blank absorbance}}$$

Statistical analysis

All the results are given as mean $\pm$ standard error (SEM). The data obtained was analyzed using one-way ANOVA followed by Dunnett's t-test. *p <0.05, **p <0.01 were considered significant.

3. RESULTS

3.1. Phytochemical investigation

Phytochemical studies of the APEA and APAqs fractions of leaves exhibited various phytoconstituent as shown in Table 1.

3.2. Acute Toxicity Study

Both the fraction of *A. precatorius* was found to be safe up to oral administer even with the highest dose (2000 mg/kg). Sound responses were no utter sound and vocalization noxious stimulus. Touch response and pain response touched with a forceps at various parts on the side of the neck, groin and abdomen. Their motor activity and grip strength were normal and the animals did not show staggering gait or contractions.

3.3. Effect on Elevated plus maze test

Significant decline in TL value of retention indicated improvement in memory. Piracetam (100 mg/kg, p.o.) and APEA fraction (150 and 300 mg/kg, p.o.) showed dose-dependent drop in TL on 7th day and 8th day in mice compared to respective control groups induced by scopolamine. APEA fraction treated mice showed remarkable decrease TL (p < 0.01) in seventh day as well as eighth day, demonstrating notable improvement in memory and

learning (Table: 2). APAqs fraction (150,300 mg/kg p.o.) do not give any significant result in the above parameter.

3.4. Active avoidance paradigm

The percentage avoidance response (AR) was evaluated by Cook and Weidley's pole apparatus for evaluating the cognitive enhancing activity in both fraction of *A. precatorius* (150 and 300 mg/kg, p.o.) and piracetam (100 mg/kg po). Our result showed that APEA fraction and piracetam administered for 7 days showed significant dose dependent increase in the percentage AR in the TTs as well as in the RTs, the results are given in Table: 3. On the other hand, the higher doses of APAqs fraction (150, 300 mg/kg p.o.) did not exert any significant effect on AR as compared to control group.

3.5. Effect on acetyl cholinesterase activity

The results obtained from the both fraction and piracetam against AChE enzyme showed percentage inhibitory activities were evaluated (Figure: 1). APEA fraction showed very potent inhibition (91.33 $\pm$ 0.33%) at the concentration of 400 µg/ml when compared with piracetam (94.10 $\pm$ 0.15%). The highest dose of APAqs fraction (400 µg/ml.) did not produce any significant inhibition of cholinesterase activity (40.34 $\pm$ 2.17%).

4. DISCUSSION

The present study investigated for the first time the CNS effects of methanolic extract of ethyl acetate soluble fractions of *A. precatorius* showed significant cognitive enhancing activities evaluated by elevated plus maze (EPM), Cook and Weidley's pole apparatus and assay of AChE enzyme activity. In addition to natural process ageing induced amnesia was also induced by intraperitoneal injection of scopolamine or diazepam in mice. Hence, the possibility of correlating performance with altering in the extracellular level of neurotransmitters in CNS regions involved in information transmission and modulation is immense role in the study of memory building. Cognitive dysfunction has been attributed due to impairment of acetylcholine neurotransmitter functioning. [35]. There are sufficient evidences that support central cholinergic system has a vital role in patients of memory impairments with the senile dementia [36]. There are extensive evidences linking the central cholinergic to memory observed reduction in symptoms of AD upon chronic use of AChE inhibitors drugs [37]. The cognitive enhancer symbolizes a category that initiate the integrative functions of the CNS, predominantly learning capacity, intellectual performance and memory. The findings from the present study are confirmed triterpenoids containing ethylacetate fraction of methanol extract boost up effect on learning and memory only after action for a period of 7 days. Their nootropic potentials claims probably may be attributed to the contribution of neurotransmitters augmented only when the repeated administration of the ethyl acetate fraction. The study reveals dose dependant evidence that the central cholinergic system effect in being able to produce vital role and even giving a closer comparison with the reference drug. [38].

Piracetam drug therapy to ameliorate cognitive deficits is a nontoxic compound and do not interfere with general behavior, limbic system, autonomic functions and level of wakefulness etc. The primary representatives of cognitive enhancer, piracetam facilitate intellectual performance and improve memory in pediatric and geriatric [39]. Recently, Itoh verified that the transfer latency of mice to enter in enclosed arm from open arm and then exposure of apparatus for 10s or more shortened the TL when trial were recurring

on 2nd day [40]. Scopolamine, a known muscarinic antagonist, displayed temporary loss of memory by interfering with cholinergic transmission [41, 42]. In the present study, ethyl acetate soluble fractions of *A. precatorius* orally administered for 7 days improved the memory of mice as indicated by diminished TL as compared to control suggest the possible neuroprotective role. Previous studies performed by researchers have exposed that numerous herbal plants possess cognitive enhancing drugs like *B. monniera* [43] and *A. recemosus* [44] including various Ayurvedic formulations such as Anwala churna (*E. officinalis*) have been significant effect to improve memory through amplification of CCS function [45]. Active avoidance learning is a fundamental behavior phenomenon performed in the Cook and Weidley's pole climbing apparatus. The first stage of avoidance rats were initially trained escape the shock by climbing onto the wooden pole. Our findings have also indicated that both the ethyl acetate fractions as well as piracetam provide a significant protection against shock consequence in less trials and time to reach the criterion of conditioned avoidance learning. Both doses of 150 and 300 mg/kg of ethyl acetate fractions were administered orally successfully reverse amnesia for seven successive potent indicating the dose dependent action on cognitive functions. Standardized extracts of *B. monniera* and *G. biloba* are eminent memory enhancers in Indian and Chinese

traditional medicine systems both show a dose-dependent inhibitory effect on AChE activity [46]. In the assay of AChE enzyme activity our result indicates significant (91.33±0.33) percentage AChE inhibitory activity as compared to piracetam. Already, Bhardwaj et al. had reported to separate non-glycosidic and glycosidic components of seed kernels of *A. precatorius* was extracted exhaustively with ethanol then repeatedly extracted with Diethyl ether and then Ethyl acetate solvent [47]. The plant leaves contain sweet-tasting unique phytoconstituent are abrusoside A to D and abrusogenin, a triterpene isolated from an n-butanol soluble extract of the leaves, as well as abrus agglutinin, isoflavanquinones including abruquinones D, E and F [48,49]. Preliminary phytochemical studies on ethyl acetate fractions rich presence of triterpenoids which have been reported to have anti-inflammatory activity [50]. From the present investigation it was concluded and validated the previous research for the utilization of gunchi as a nervine tonic in traditional Ayurvedic medicine is not invalid. The pharmacological actions of ethyl acetate fractions *A. precatorius*, justify the therapeutic uses nootropic activities in the models tested and these effects may probably be mediated through augmentation of cholinergic system due to its anti-cholinesterase activity. Thus, our conclusion validates the traditional claims of *A. precatorius* as a promising cognition boosting herb.

Table: 1 Qualitative analysis of ethyl acetate and aqueous fraction of *A. precatorius* leaves.

Phyto Constituents	Phyto chemical Test	APEA fractions	APAqs fractions
Carbohydrates	Molisch test	+ve	+ve
	Fehling test		
Proteins	Biurate test	-ve	+ve
Amino acid	Million's test	-ve	+ve
Tannins	Ferric chloride Test	-ve	+ve
Alkaloids	Hager test	+ve	+ve
	Wagner test	+ve	+ve
Saponins	Foam test	+ve	-ve
Flavonoids	Lead acetate test	+ve	+ve
Glycosides	Hydroxyanthraquinone test	+ve	-ve
Steroid	Liebermann Burchard test	-ve	-ve
Terpenoid	Salkowski Test	+ve	-ve

Table: 2 Effect of different fractions of *A. precatorius* and piracetam on transfer latency of mice on elevated plus maze apparatus.

Groups	Treatment	Dose mg/Kg	Transfer latency Day 7 th (sec)	Transfer latency Day 8 th (sec)
I	Positive Control	Vehicle p.o.	28.8±0.6	26.3±0.6
II	Negative Control (SCP)	0.4 mg/kg i.p.	42.6±0.8	38.3±0.4
III	Piracetam + SCP	100 mg/kg p.o.	16.5±0.4**	13.5±0.4**
VI	APEA + SCP	150 mg/kg p.o.	23.5±.4*	22.3±0.3*
V		300 mg/kg p.o.	20.5±.4**	18.5±0.5**
VI	APAqs + SCP	150 mg/kg p.o.	32.4±1.9	30.4±1.9
VII		300 mg/kg p.o.	31.1±3.7	28.1±0.3

Values are expressed in mean ± SEM (n = 6). *p < 0.05, **p < 0.01 One-way ANOVA followed by Dunnett's test.

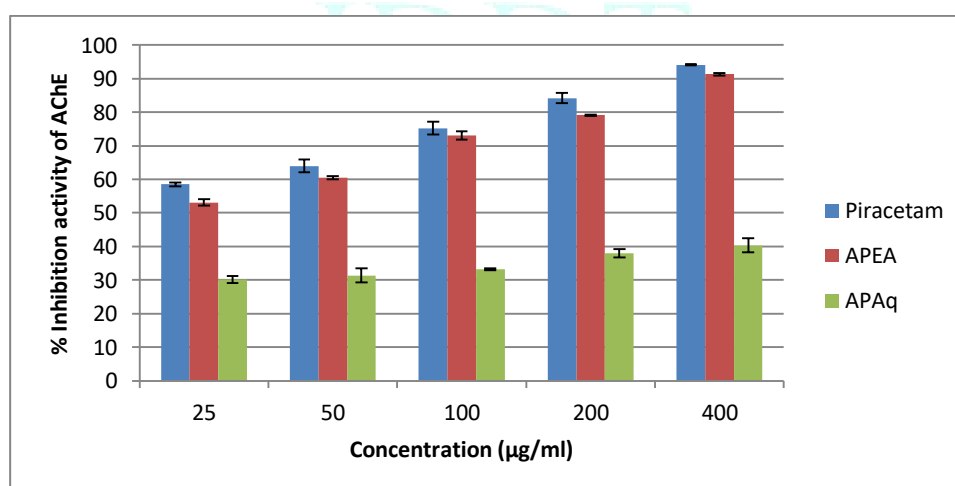
APEA: *E. A. precatorius* ethyl acetate fraction, APAqs: *A. precatorius* aqueous fraction Scopalamine (SCP)

Table: 3 Effect of the different fractions of *A. precatorius* and piracetam on nootropic activity in rats administered for a period 7 days using Cook and Weidley's pole apparatus.

Group	Treatment	Dose (mg/kg) p.o.	% Avoidance responses in TT (sec)	% Avoidance responses in RT (sec)
I	Control	Vehicle	21.6 ± 3.0	24.1 ± 1.0
II	Piracetam	100	66.6 ± 3.3**	79.2 ± 3.2**
III	APEA	150	45.0 ± 2.2*	59.2 ± 2.2*
IV		300	60.0 ± 3.6**	73.4 ± 2.1**
V	APAqs	150	28.9 ± 3.1	30.1 ± 3.0
VI		300	31.2 ± 1.2	33.12 ± 1.2

Values are expressed in mean ± SEM (n = 6). *p < 0.05, **p < 0.01 One-way ANOVA followed by Dunnett's test.

APEA: *A. precatorius* ethyl acetate fraction, APAqs: *A. precatorius* aqueous fraction, TT—training trial, RT—relearning trial.

Fig.:1 Effect of the different fractions of *A. precatorius* and piracetam percentage inhibition activity of acetylcholinesterase (AChE, electric eel) by Ellman's assay.

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The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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