ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 6, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

NEUROPROTECTIVE EFFECT OF HYDROALCOHOLIC EXTRACT OF *AMARANTHUS TRICOLOR* LEAVES ON EXPERIMENTAL ANIMALS

VIDHAN CHAND BALA*, MOHD ABID

Department of Pharmacology, School of Pharmacy, IFTM University, Moradabad, Uttar Pradesh, India.
Email: vidhanchandbala07@gmail.com

Received: 18 February 2020, Revised and Accepted: 20 February 2020

ABSTRACT

Objective: The research work deals with the screening of hydroalcoholic extract of *Amaranthus tricolor* (HAEATL) leaves for central nervous system activity (anti-stress, nootropic, and anti-cataleptic activity).

Methods: The screening of scopolamine and different model-induced neurodisorder rats were treated with 200 and 400 mg/kg body weight dose of hydroalcoholic extract of *A. tricolor* lives in 7 days' experimental schedule. It has been reported that the antioxidant, hepatoprotective, hematological, and antimicrobial activities.

Results: The result of the study reflected that HAEATL (200 and 400 mg/kg) was effective in all methods and showed anti-stress, nootropic, and anti-cataleptic activity in a dose-dependent manner. The results are represented that the HAEATL produce the significant decreased the swimming time, increased the anoxia time, decreased level of biochemical parameters such as glucose and cholesterol except blood urea nitrogen, decreased level of white blood cell and red blood cell, and more significantly decreased the catalepsies score on 7th day compared to the control group.

Conclusion: The above valuable animal study, we concluded that the HAEATL showing significantly affect compeer to the disease control group on neuroprotective activity.

Keyword: *Amaranthus tricolor*, Neuroprotective effect, Scopolamine, Anti-stress activity, Nootropic activity, Anti-cataleptic activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i6.37181

INTRODUCTION

Stress is simply a reaction to a stimulus that disturbs our physical or mental equilibrium. Acute stress can be exciting; it keeps us active and alert, but chronic stress can have detrimental effects on health [1-3]. Memory function is at risk to many pathological conditions, including many neuropsychiatric and neurodegenerative diseases like Alzheimer's disease [4,5]. Nootropic drugs such as piracetam and cholinesterase inhibitors are clinically used to develop learning and memory capacity, mood, and behavior in those neurodegenerative diseases [6-8].

Parkinson's disease (PD) is a neurodegenerative disorder caused by the progressive loss of mesencephalic dopaminergic neurons in the substantia nigra innervating the striatum [9]. Recent research works demonstrated the development of PD up to a greater extent in animals [10].

Amaranthus tricolor L. is an adornment plant known as Tandalja or Tandalja Bhaji in India. In Andhra, it is ordinarily known as "Perugu Thotakura." The nutrients in the leaves are carbohydrates, protein, Vitamin A, Vitamin C, riboflavin (Vitamin B2), thiamine (Vitamin B1), niacin, and minerals such as Ca^{++}, Fe, and fiber [11]. The presence of betalain pigments such as amaranthine, β-xanthin, methyl derivative of arginine β-xanthin, and betalamic acid has been reported in leaves [12,13].

The full plant of *A. tricolor* is conventionally used as astringent [14]. *A. tricolor* root combination with *Cucurbita moschata* used to manage post-abortion bleeding [15]. The cooked *A. tricolor* plant is utilized to strengthen the liver and promote the vision. The experimental research advises that it can inhibit calcium retention [16]. It was found that the plant possesses antioxidant [17], hepatoprotective [18], antinociceptive, and anti-inflammatory activities [19]. It is also reported for *in vitro* and *in vivo* anticancer effects [6], and *in-vitro* antioxidant, anti-amylase, anti-arthritic, and cytotoxic activity [20].

MATERIALS AND METHODS

Selection and verification of plant material

The raw *A. tricolor* (Amaranthaceae) plant was selected from self-cultivation in Moradabad region (Uttar Pradesh) India and verified by the Department of Botany, IFTM University, Moradabad - 244 001, Uttar Pradesh, India. No. – 2016/SOS/BOT/33.

Investigation of plant material

The dehydrated powder of *A. tricolor* was extracted with petroleum ether 60–800 C to 72 h. After petroleum ether extraction, marc is separated and dried at room temperature and then extracted with hydroalcoholic solvent (99% v/v alcohol + distilled water) to 72 h in Soxhlet apparatus. The extract was evaporated under reduced pressure which obtained a brownish-black color and a sticky residue. The sticky residue was used for identifying its activity on learning and memory activity.

Chemicals and reagents

All the chemicals and reagents used in this study were of analytical grade. Organic and inorganic solvent used for extraction of the plant material included: Chloroform, petroleum ether, potassium bismuth iodide solution, concentrated hydrochloric acid, sodium hydroxide, potassium mercuric iodide solution, copper sulphate, glacial acetic acid, sulfuric acid, n-butanol, a-napthol, n-hexane, and ethyl-acetate were collected in standard deviation (SD) fine chemicals Pvt. Chennai.

Determination of extraction yield (%yield)
The yield (% w/w) from the dried extracts was calculated as:

$$\text{Yield}(\%)=\frac{W1}{W2}\times 100$$

Where, w_1 is the weight of dried extract after evaporation of solvent and w_2 is the weight of plant powder.

Phytochemical analysis
Phytochemical screening test was done on a hydroalcoholic extract of *A. tricolor* leaves (HAEATL) extract that was determine by flavonoids, saponins, alkaloids, steroids and sterols amino acids, carbohydrates, protein, and tannin [21-23].

Thin-layer chromatography (TLC)
TLC is a separation, identification, and purification procedure that gives the chemist a quick answer as to how many components are in a mixture. It is also used to base on the identity of a compound in a mixture when the retention factor (RF) of a test compound is compared to the RF of a standard compound (preferably both run on the same TLC plate).

Drugs and chemicals
All conventional drugs like scopolamine hydrochloride, Bacopa manniri, sodium carboxymethyl cellulose (CMC Sodium), saline, test drugs and amnesia-inducing drugs (suspended in 0.5% w/v sodium CMC) were administered in the morning session, i.e. from 10 am to 11 am on each day of the test period.

Selection of experimental animals
For both sexes healthy, young, 10–12-week-old Wistar albino rats weighing about 130–190 g, obtained from the animal house facility used for *in vivo* studies. The animal room should be maintained under normal ambient conditions (temperature 25°C and relative humidity 40–80%) for 12/12 h of day/night cycle and fed with rat pellet and water *ad libitum*. Experimental protocol approved by the Institutional Animal Ethics Committee (IAEC) under IAEC Number - 837/PO/Re/S/04/CPCSEA.

Experimental model
Anti-stress activity
Different methods were used to evaluate the anti-stress activity of *A. tricolor* leaves.

Grouping of animals
Following group division will be considered for all the screening activities:
Group-I – Control group (vehicle 10 ml/kg pre os [p.o.])
Group-II – Stress control group (scopolamine 0.4 mg/kg intraperitoneal [i.p.])
Group-III – Low dose HAEATL (200 mg/kg p.o. + scopolamine 0.4 mg/kg i.p.)
Group-IV – High dose HAEATL (400 mg/kg p.o. + scopolamine 0.4 mg/kg i.p.)
Group-V – Standard drug group (*Withania somnifera* 100 mg/kg p.o. + scopolamine 0.4 mg/kg i.p.).

Swimming endurance test (SET)
Stress was imposed on the rats by keeping them in cylindrical vessels (length 48 cm and width 30 cm) filled with water to a height of 25 cm and the total swimming time for individual rats was noted, the rats were allowed to swim daily until exhausted, Wister albino rats (150–200 g) were divided into four groups of six animals each. Group I is control, treated with vehicle (5 ml/kg p.o.), Group II and III treated with HAEATL (200 and 400 mg/kg p.o.), and Group IV treated with standard drug *W. somnifera* (100 mg/kg p.o) was given to rats once daily for 7 days. On the 7th day, 1 h after treatment, all the rats were subjected to SET. The rats were allowed to swim individually in a propylene tank, filled with water to a height of 25 cm maintained at room temperatures. The mean swimming time for each group was calculated [24].

Anoxia stress tolerance test (ASTT)
Wister albino rats (150–200 g) were divided into five groups of six animals each, grouping and treatment were similar as in SET. HAEATL or standard drug was given to the rats once daily for 7 days. On the 7th day, 1 h after the treatment, stress was induced in all rats by placing each animal individually in a hermetic vessel of 500 ml capacity to record anoxia tolerance time. The moment when the animal showed the first convulsions, it was immediately removed from the vessel and resuscitated if needed. The time duration between animal entry into the hermetic vessel and the appearance of the first convulsion was taken as the time of anoxia tolerance. Appearance of convulsion was a very sharp endpoint [25].

Immobilization stress test (IST)
In the immobilization stress model albino wistar rats were divided into five groups of six animals each. Group I is normal control, treated with vehicle (5 ml/kg p.o. control), Group II is stressed control treated with (10 ml/kg p.o.), Group III and IV treated with HAEATL (200 and 400 mg/kg p.o.), and Group V treated with standard drug *W. somnifera* (100 mg/kg p.o.) was given to rats once daily for 7 days. The stress was produced by restraining the animals inside an adjustable acrylic hemicylindrical plastic tube (4.5 cm diameter, 12 cm long). The rats were confined individually and exposed continuously for a period of 150 min once daily for 7 consecutive days. On the 7th day, immediately after the last exposure to stress, blood was collected from retro-orbital plexus under light ether anesthesia and serum and plasma were separated for biochemical estimation. The animals were sacrificed at the end of a specified period and the weights of organs were noted [13].

Nootropic activity
Different methods were used to evaluate the nootropic activity of *A. tricolor leaves*.

Grouping of animals
Following group division will be considered for all the screening activities:
Group-1 – Control group (vehicle 10 ml/kg p.o.)
Group-2 –Amnesia control group (scopolamine 0.4 mg/kg i.p.)
Group-3 – Low dose HAEATL (200 mg/kg p.o. + scopolamine 0.4 mg/kg i.p.)
Group-4 – High dose HAEATL (400 mg/kg p.o. + scopolamine 0.4 mg/kg i.p.)
Group-5 – Standard drug group (*B. monnieri* 100 mg/kg p.o. + scopolamine 0.4 mg/kg i.p.).

Elevated plus maze (EPM) test
An EPM comprising two open arms (40 cm×12 cm) crossed with two closed arms (40 cm×12 cm×40 cm) was used in this study to investigate the nootropic response. The arms were interconnected with a central square (12 cm×12 cm). The unit was raised to a height of 60 cm in dimly lit rooms. The EPM was placed inside the light and soundproofed room. Mice were placed separately on the open arm end of the EPM facing away from the midsection, and the time taken to move from the open arm to either of the closed arms was noted. Transfer latency (TL) was stored, which is considered as a parameter for evaluating the memory-enhancing property. TL was maintained over the period of time the mouse moved on one covered arm on all four legs, and TL was appoint for 90 s. The mouse was allowed to examine the EPM for 10 s and then arrived to the home cage. Memory retention was examined 24 h after the 1st day of the checkout (i.e., on day 2). On the 7th day, 60 min after treatment with the last dose, a first test is administered and after 24 h, a second test (i.e. on day 8) was observed. The inflection ratio (IR) was estimated using the formula.

$$\text{IR}=\frac{L0-L1}{L0}$$

Where L0 is the initial transmission latency (TL) per second for the first time, L1 is the TL per second for the second time. Decreased IR indicates the induction of amnesia and increased IR indicates an improvement in cognitive and memory impairment [5,26].

Morris water maze (MWM) test

The MWM presents a versatile element that can measure many different tasks for evaluating working memory in rats. MWM consists of a wide round container formed of black opaque PVC or solid cardboard, covered with fiberglass and resin, and then painted in white (2–2.5 m in diameter and 0.5–0.6 m in height). The pool is filled with water (20–25°C) to a depth of 0.5–0.6 m and made opaque by adding a limited amount of milk or milk powder or a non-toxic white color. The round tank floor is marked with four equal quarters, which are arbitrarily designed north, south, east, or west. The Escape Tray consists of plexiglass with a 12 cm square base fixed to a 34 cm long clear plexiglass, cylindrical base (5 cm diameter) mounted in 1 m^2 (5 mm thick) plexiglass. The top of the platform is covered with coarse material that provides the perfect grip for mice as they climb the platform. For the purpose of the hidden tray, water is added in a round container to a level 2 cm above the level.

The simplest measure of performance is the latency to escape from the water to a hidden substrate. The pad remains in a secure position during the exercise. Every animal is subjected to four consecutive examinations over a period of 4 days, during which they are granted to escape to a hidden level and remain there for 20 s.

Escape latency (EL) is the amount of time that the location of the hidden platform in the MWM is stored as an acquisition or learning index. If the animal cannot locate the hidden substrate within 120 s, it is carefully guided to the stage by hand and granted to remain there for 20 s. On the 9th day, 60 min after the last dose, the vessel is removed and each animal spent in the target quadrant searching for the hidden medium is recorded as a search index [27,28].

Cataleptic activity

Grouping of animals

Following group division will be considered for all the screening activities:

Group-I – Control group (vehicles 10 ml/kg p.o.)

Group-II – Catalepsia control group (haloperidol 1 mg/kg i.p.)

Group-III – Low dose HAEATL (200 mg/kg. p.o. + haloperidol 1 mg/kg i.p.)

Group-IV – High dose HAEATL (400 mg/kg. p.o. + haloperidol 1 mg/kg i.p.)

Group-V – Standard drug group (Levodopa 5 mg/kg p.o. + haloperidol 1 mg/kg i.p.).

Haloperidol-induced Catalepsy

Haloperidol causes the defective function of various neurotransmitters such as acetylcholine, Gama aminobenzoic acid, and serotonin. Pathological state of haloperidol indicated catalepsy underlying increased oxidative stress. It is an antipsychotic drug, blocks central dopamine receptors in striatum. It also the production of a behavioral state in animals such as mice and rats, in which they fail to correct externally imposed postures (called catalepsy); thus, keeping the above fact in mind, the haloperidol-induced catalepsy model was selected. In this study, the animals were divided into five groups (n =6). Group I served as vehicle control, Group II served negative control group no drugs, Groups III-VI served as test group treated with HAEATL (200 and 400 mg/kg p.o.), and Group V served as standard, levodopa (5 mg/kg p.o.), respectively. A standard bar test was used to measure the catalepsy. Catalepsy was induced by haloperidol (1 mg/kg i.p.) and examined at every 30 min interval for 90 min. The duration for which the rat retains the forepaws extended and resting on the elevated bar was considered as a cataleptic score. A cutoff time of 5 min was applied [29].

Behavioral studies

This scoring method was followed in three steps [30].

Step 1: The rat was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned.

Step II: The front paws of the rats were placed alternately on a 3 cm high block. If the rat failed to correct the posture within 15 s, a score of 0.5 for each paw was added to the score of step I.

Step III: The front paws of the rat were placed alternately on a 9 cm high block if the rat failed to correct the posture within 15 s a score

Table 1: Percent yield of *Amaranthus tricolor* leaf extracts

Extraction	Solvents used	Material	Extract yield (%)
HAE (maceration)	Ethanol	Dried powdered leaf	5.6%
HAE (soxhlation)	Ethanol	Dried powdered leaf	7.3%

Table 2: Analysis for the presence of the different phytochemical compound in HAEATL

Constituent	HAEATL
Flavonoids	+
Saponins	+
Alkaloids	+
Steroids and sterols	+
Amino acids	+
Carbohydrates	+
Protein	+
Tannins	+

+ = Present; − = Absent. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves

Table 3: The RF values of hydroalcoholic extract of *Amaranthus tricolor* leaves

No.	Band colour visible day light	RF values of test sample
1.	Yellow-green	0.45
2.	Green-brown	0.5

RF: Retention factor

Table 4: Effect of HAEATL on stress performance by swimming endurance test

S. No.	Treatment groups	Swimming endurance time in minutes
1.	Control group(vehicle 10 ml/kg)	18.50±0.806
2.	HAEATL (200 mg/kg)	15.67±0.333*
3.	HAEATL (400 mg/kg)	14.67±0.557**
4.	*Withania somnifera* (100 mg/kg)	13.50±0.806***

All values are mean ± SD error. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05, **p<0.01, and **p<0.001 when compared with control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Table 5: Effect of HAEATL on stress performance by anoxia stress tolerance test

S. No.	Treatment groups	Duration of anoxia stress tolerance in minutes
1.	Control group (vehicle 10 ml/kg)	18.83±0.401
2.	HAEATL (200 mg/kg)	21.67±0.614^{ns}
3.	HAEATL (400 mg/kg)	22.67±0.557**
4.	*Withania somnifera* (100 mg/kg)	25.00±1.291***

All values are mean ± SD error. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, **p<0.01, and ***p<0.001 when compared with control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Table 6: Effect of HAEATL on biochemical parameters in the immobilization stress test

S. No.	Treatment group	Biochemical parameter		
		Glucose	Cholesterol	Blood urea nitrogen
1.	Control group (vehicle 10 ml/kg)	62.00±0.730	67.53±0.111	19.27±0.381
2.	Stress control group	139.2±1.102	72.60±0.162	15.27±0.378
3.	HAEATL (200 mg/kg)	97.00±1.317***	69.81±0.166***	16.15±0.140^{ns}
4.	HAEATL (400 mg/kg)	72.67±0.918***	42.47±0.095***	16.57±0.080**
5.	*Withania somnifera* (100 mg/kg)	83.67±0.557***	66.63±0.145***	18.00±0.200***

All values are mean ± SD error. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05, **p<0.01, and ***p<0.001 when compared with stress control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Table 7: Effect of HAEATL on hematological parameters in the immobilization stress test

S. No.	Treatment	Hematological parameters	
		Total WBC	Total RBC
1.	Control group vehicle (10 ml/kg)	7445±115.30	6.107±0.03
2.	Stressed control group	11472±248.10	4.44±0.09
3.	HAEATL (200 mg/kg)	9157±64.01***	5.153±0.07***
4.	HAEATL (400 mg/kg)	8087±108.30***	5.400±0.11***
5.	*Withania somnifera* (100 mg/kg)	7777±60.09***	5.775±0.02***

All values are mean ± SD error. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05, *p<0.01, and **p<0.001 when compared with stress control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, WBC: White blood cell, RBC: Red blood cell, SD: Standard deviation

of 1 for each paw was added to the scores of steps I and II. Thus, the highest score for any animal was 3.5 (cut off score) and that reflects total catalepsy [31,32].

RESULTS

Determination of percentage (%) yield

Table 1 gives the percent yield of leaf extracts of *A. tricolor* obtained using various solvents/techniques. The hydroalcoholic (ethanol+distril water) extracts obtained were dark reddish-brown and semisolid in consistency.

Qualitative phytochemical analysis

The different phytochemical tests carried out for a screening of various chemical constituents of *A. tricolor* leaves. The phytochemical tests indicated the presence of proteins, amino acids, tannins, polyphenols, sterols, flavonoids, and polyphenol in the HAEATL as results are shown in Table 2.

Chromatographic studies

TLC

TLC of the hydroalcoholic extract in hexane: chloroform:ethyl acetate (1:3:3) solvent system yielded two visible spots. Distance travel by solvent 6.2 cm distances between first sport 2.8 cm and second sport 3.1 cm on exposure to visible daylight and iodine vapors a total of 2 spots could be visualized (Table 3).

Anti-stress activity

SET

In SET, HAEATL (200 mg/kg) significantly ($P < 0.05$) decreased the swimming time, whereas HAEATL (400 mg/kg) significantly ($P < 0.01$) and standard drug *W. somnifera* (100 mg/kg) more significantly ($P < 0.001$) decreased the swimming time compared to the control group as the results are shown in Table 4.

ASTT

The results are represented that the HAEATL (200 mg/kg) did produce a significant effect. HAEATL (400 mg/kg) significantly ($P < 0.01$) and standard drug *W. somnifera* (100 mg/kg) more significantly ($P < 0.001$) increased the anoxia time on 7th day compared to the control group. High dose (400 mg/kg) and standard drug (100 mg/kg) were found to be comparable effective in this test as the results are shown in Table 5.

Immobilization stress

Biochemical parameters

In this test, HAEATL (200 mg/kg) showed significant ($P < 0.001$) effect of glucose and cholesterol level but blood urea nitrogen (BUN) is did not significantly affect whereas HAEATL (400 mg/kg) and standard drug *W. somnifera* (100 mg/kg) more significantly ($P < 0.001$) decreased level of biochemical parameters such as glucose and cholesterol except BUN, 400 mg significantly ($P < 0.01$) and standard drug *W. somnifera* (100 mg/kg) more significantly ($P < 0.001$) increased as compared to the stressed control group. Both standard (100 mg/kg) and high dose (400 mg/kg) drug showed an almost similar effect as the results are shown in Table 6.

Hematological parameters

In this test, HAEATL (200 mg/kg) showed significant effect whereas HAEATL (400 mg/kg) and standard drug *W. somnifera* (100 mg/kg) more significantly ($P < 0.001$) decreased level of white blood cell and red blood cell more significantly ($P < 0.001$) increased, compared to the stressed control group. Both standard and HAEATL (400 mg/kg) drugs showed almost similar effects as the results are shown in Table 7.

Organ weights

In this test, HAEATL (200 mg/kg) did not significantly measure weight of liver and significantly ($P < 0.01$) decreased weight of adrenal gland but more significantly ($P < 0.001$) increased weight of spleen, whereas HAEATL (400 mg/kg) and standard drug *W. somnifera* (100 mg/kg) more significantly ($P < 0.001$) decreased the body organ weights of liver and adrenal gland but the more significantly ($P < 0.001$) increased weight of spleen as compared to the stressed control group, the results are shown in Table 8.

Nootropic activity

EPM

In this test, HAEATL (200 mg/kg) significantly ($P < 0.05$) and HAEATL (400 mg/kg) significantly ($P < 0.01$) reduced the memory of the standard group (*B. monnieri* 100 mg/kg) demonstrated a significant decrease in TL compared to the AC control group as the results are shown in Table 9.

MWM

In this test, HAEATL (200 mg/kg) did not significantly increase EL, whereas HAEATL (400 mg/kg) significantly ($P < 0.01$) and the standard group (*B. monnieri* 100 mg/kg) significantly ($P < 0.001$) increased EL compared to the AC control group because the results are presented in Table 10.

Table 8: Effect of HAEATL on immobilization stress-induced changes in organ weight

S. No.	Treatment groups	Organ weight (g/100 g body weight)		
		Liver	Adrenal gland	Spleen
1.	Control group (vehicle 10 ml/kg)	3.26±0.100	0.03±0.002	0.35±0.003
2.	Stressed control group	6.11±0.042	0.08±0.003	0.27±0.003
3.	HAEATL (200 mg/kg)	5.56±0.167^{ns}	0.06±0.002**	0.34±0.003***
4.	HAEATL (400 mg/kg)	4.98±0.336***	0.05±0.002***	0.32±0.004***
5.	*Withania somnifera* (100 mg/kg)	4.15±0.019***	0.05±0.003***	0.39±0.003***

All values are mean ± SD error. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05 significant, **p<0.01 more significant, and ***p<0.001 highly significant when compared with stress control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Table 9: Effect of HAEATL on learning performance by plus-maze test

S. No.	Treatment group	Transfer latency 7th day	Transfer latency after 24 h	Inflection ratio (IR)
1.	Control group (vehicle 10 ml/kg)	16.17±1.66	13.62±1.52	0.16±0.02
2.	AC control group (scopolamine)	23.50±1.68	30.33±1.85	0.29±0.01
3.	HAEATL (200 mg/kg + scopolamine)	16.50±0.42	14.17±0.60**	0.12±0.03
4.	HAEATL (400 mg/kg + scopolamine)	13.67±0.88	11.83±0.94***	0.13±0.03
5.	*Bacopa monnieri* (100 mg/kg) + scopolamine	12.50±0.61	10.67±0.42***	0.14±0.01

All values are means ± SD error. Statistical analysis of the input was performed by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05, **p<0.01, and ***p<0.001 compared to the AC control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Table 10: Effect of HAEATL on learning performance by water maze model

S. No.	Treatment group	Escape latency 7th day	Escape latency after 24 h	Inflection ratio (IR)
1.	Control group (vehicle 10 ml/kg)	15.83±0.07	11.00±0.25	0.28±0.04
2.	AC control group (scopolamine)	24.50±0.80	40.83±1.24	0.006±0.00
3.	HAEATL (200 mg/kg + scopolamine)	20.00±0.96	17.50 ±1.11***	0.12±0.02
4.	HAEATL (400 mg/kg + scopolamine)	19.17±0.60	15.83 ±0.74***	0.16±0.03
5.	*Bacopa monnieri* (100 mg/kg)+scopolamine	17.33±1.17	13.67 ±0.76***	0.19±0.04

All values are means ± SD error. Statistical analysis of the input was performed by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05, **p<0.01, and ***p<0.001 compared to the AC control group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Table 11: Effect of HAEATL on catalepsies performance by behavioral studies

S. No.	Treatment groups	Catalepsy score after haloperidol administration		
		30 min	60 min	90 min
1.	Control group (vehicle 10 ml/kg)	0.00±0.00	0.00±0.00	0.00±0.00
2.	Catalepsia control group (haloperidol 2 mg/kg)	3.5±0.00	3.5±0.00	3.5±0.00
3.	HAEATL (200 mg/kg + haloperidol 2 mg/kg)	2.25±0.11***	2.41±0.15***	2.85±0.10**
4.	HAEATL (400 mg/kg + haloperidol 2 mg/kg)	1.58±0.37***	2.16±0.10***	2.25±0.17***
5.	Standard group (levodopa + carbidopa 10 mg/kg + haloperidol 2 mg/kg)	1.33±0.24***	1.33±0.10***	1.97±0.15***

All values are mean ± SD error. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *p<0.05, **p<0.01, and ***p<0.001 when compared with AC group. HAEATL: Hydroalcoholic extract of *Amaranthus tricolor* leaves, SD: Standard deviation

Anti-cataleptic activity

Behavioral studies

The results are represented that the HAEATL (200 mg/kg) more significantly ($P < 0.001$) decreased the catalepsies score after 30 min, 60 min, and 90 min. Haloperidol administration HAEATL (400 mg/kg) and standard drug Syndopa (levodopa+carbidopa 10 mg/kg) more significantly ($P < 0.001$) decreased the catalepsies score on 7th day compared to the CC control group. High dose (400 mg/kg) and standard drug (100 mg/kg) were found to be comparable effective in this test as the results are shown in Table 11.

DISCUSSION

Learning has been defined as the process of obtaining the knowledge while memory in mind retention of knowledge that can be saved. In the plus-maze acquisition (learning), it can be expected to be latent (TL) in the 7th-day assay and retention/consolidation (memory) is examined 24 h later, i.e., on the 8th day after treatment with the test compound. TL increased in AC control group, i.e., scopolamine treated group. Amnesia has been noticed after the treatment. After day 7 of treatment, HAEATL (200–400 mg/kg) had a significant ($P < 0.01$) reduction in memory transfer delay. The standard group (*B. monnieri* 100 mg/kg) showed a more significant ($P < 0.001$) decrease in transmission latency compared to the AC control group (IR 0.03 ± 0.006). Reduce TL (treatment group) showed protection against memory loss and impaired cognition. MWM learning is a reasonably good test for cognitive function. The exit delay was increased using MWM. Exit delay increased in extract and *B. monnieri* treated group due to cognitive function.

CONCLUSION

The above valuable animal study, we concluded that the HAEATL showing significantly affect compeer to the disease control group, on anti-stress, nootropic, and anti-Alzheimer activity. Hence, research calculates that *A. tricolor* has the capacity for management of central nervous system (CNS) disorder as it shows the effect on almost all the medals. It can be used for the reduction of side effects of drugs currently available in market.

However, further studies are necessary to examine underlining mechanisms of CNS effect of different fraction of potent extract and to isolate the active compound responsible for these pharmacological activities.

ACKNOWLEDGMENT

My work cannot be completed by the grace of God, so I pay deep sense of gratitude to the almighty who blessed me with patience and dynamism to complete this work. I would like to express my deep sense of gratitude to all those who helped me directly and indirectly in completing this thesis work. I am heartfelt thankful to my guide Mr. Mohd. Abid to given me his priceless time and support for awarding me supervision and real interest in my research work and completing my thesis nicely. I express my deep gratefulness to Prof. Dr. Ashok Kumar Ghosh Director of School of Pharmaceutical Sciences, for providing the facilities, co-operation guidance, and independence for my research work.

AUTHORS' CONTRIBUTIONS

All authors have an equal contribution.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

AUTHORS' FUNDING

The authors do not have any source of funding.

REFERENCES

1. Pawar VS, Hugar S. Acurrent status of adaptogens: Natural remedy to stress. Asian Pac J Trop Dis 2012;1:480-90.
2. Kumar N. Developments in mental health scenario: Need to stop exclusion-dare to care. ICMR Bull 2001;31:1-10.
3. Gaziano T, Chaturvedi V, Raddy KS, Paccaud F, Horton S. Cardiovascular Disease. Disease Control Priorities in Developing Countries. 2nd ed. Oxford: Oxford University Press; 2006. p. 2012-18.
4. Dinesh D, Milind P, Kulkarni SK. Memory enhancing activity of *Glycyrrhiza glabra* in mice. J Ethnopharmacol 2004;9:361-5.
5. Hanumanthachar J, Milind P. Nootropic activity of calyces of *Hibiscus sabdariffa* Linn. Iran J Pharmacol Ther 2006;5:15-20.
6. Sani D, Sanni SN, Ngulde I. Phytochemical and antimicrobial screening of the stem aqueous extract of *Anisopus mannii*. J Med Plants Res 2009;3:112-5.
7. Hanumanthachar J, Milind P. Pharmacological evidences for the ant amnesic effects of *Desmodium gangeticum* in mice. Iran J Pharm Res 2007;l:199-07.
8. Muraleedharannair JM, Johnson MA, Mony M, Zachariah MP, Solomon J, Phytochemical studies on *Azolla pinnata* R. Br, *Marsilea minuta* L. and *Salvinia molesta* Mitch. Asian Pac J Trop Biomed 2011;l:26-9.
9. Alexi T, Borlongan CV, Faull LM, Williams CE, Clark GR, Gluckman PD. Neuroprotective strategies for basal ganglia degeneration, Parkinson's and Huntington's diseases. Prog Neurobiol 2000;5:409-70.
10. Przedborski S, Tieu K, Perier C, Vila M. MPTP as a mitochondrial neurotoxin model of Parkinson's disease. J Bioenerg Biomembr 2004;36:375-9.
11. Bala VC, Avid M, Kumar P, Sangam. A review on *Amaranthus tricolor* as a traditional medicinal plant. World J Pharm Res 2019;8:226-37.
12. Mousumi B, Satyahari D, Ramakrishna S. Betalains from *Amaranthus tricolor* L. J Pharmacogn Phytochem 2013;1:87-95.
13. Rai D, Bhatia G, Sen T, Palit G. Anti-stress effects of *Ginkgo biloba* and *Panax ginseng*. A comparative study. J Pharmacol Sci 2003;93:458-4.
14. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: Council of Scientific and Industrial Research; 1956.
15. Duke J, Ayensu ES. Medicinal Plants of China. Vol. 1. Algonac, Mich: Reference Publications, Inc.; 1985. p. 20-4.
16. Larsen T, Thilsted SH, Biswas SK, Tetens I. The leafy vegetable amaranth (*Amaranthus gangeticus*) is a potent inhibitor of calcium availability and retention in rice-based diets. Br J Nutr 2007;l:521-7.
17. Samsul K, Krupanidhi K, Rao KR. Evaluation of *in-vitro* antioxidant activity of *Amaranthus tricolor* Linn, Asian J Pharmacol Toxicol 2013;1:12-6.
18. Simran MV, Sushma A, Satish S. Phytochemistry and hepatoprotective activity of aqueous extract of *Amaranthus tricolor* (Linn.) roots. J Ayurveda Integer Med 2013;4:211-5.
19. Gopal VB, Subhash LB, Parag PK, Girish NZ. Anti-nociceptive and anti-inflammatory activity of hydroalcoholic extract of leaves of *Amaranthus tricolor* L. Scholars Res Lib 2013;5:48-55.
20. Kumar V. *In-vitro* anti-oxidant, anti-amylase, anti-arthritic and cytotoxic activity of important commonly used green leafy vegetables. Int J PharmTech Res 2011;3:2096-103.
21. Evans WC. Trease and Evans Pharmacognosy. 5th ed. Mumbai: Harcourt Publisher Limited; 2002. p. 262-3.
22. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 20th ed. Pune: Nirali Prakashan; 2002. p. 108-9.
23. Shil D, Laloo D, Das SR, Dash S. Pharmacognostical and phytochemical standardization of *Physalis minima* leaf. Int J Pharm Pharm Sci 2020;11:20-5.
24. Ahmad G, Amin KM, Khan NA. The anti-stress activity of a gem-containing unani formulation against diverse stressors. J Ethanopharmacol 1998;59:187-3.
25. Babu PV, Rao MA, Kumar SM, Rao NV. A study on adaptogenic activity of tuber extracts of *Pueraria tuberosa*. Indian Drugs 2006;43:486-2.
26. Rao VN, Pujar B, Nimbal SK, Shantakumar SM, Satyanarayana S. Nootropic activity of tuber extract of *Pueraria tuberosa* (roxb). Indian J Exp Biol 2008;46:591-8.
27. Rahman H, Muralidharan P. *Nardostacys jatamansi* protects from the loss of memory and cognition deficits in sleep deprived Alzheimer's disease mice model. Int J Pharm Sci Rev Res 2010;5:160-7.
28. Kshirsagar SN. Nootropic activity of dried seed kernels of *Caesalpinia crista* against scopolamine induced amnesia in mice. Int J PharmTech Res 2011;l:104-9.
29. Elliott PJ, Close SP. Neuroleptic-induced catalepsy as a model of Parkinson's disease. Effect of dopaminergic agents. J Neural Transm Park Dis Dement Sect 1990;2:79-89.
30. Kulkarni SK. Handbook of Experimental Pharmacology. 3rd ed. New Delhi: Vallabh Prakashan; 1999. p. 125-6.
31. Rout S, Rath B, Bhattamisra SK, Kumar A, Rath S. Neuroprotective effect of methanolic extract of *Sargassum wightii* on haloperidol induced catalepsy and tardive dyskinesia in albino rats. Int J Pharm Pharm Sci 2020;12:1-6.
32. Sharma N, Rana AC, Bafna P. Effect of aqueous extract of *Cynodon dactylon* on reserpine induced catalepsy. Int J Pharm Pharm Sci 2011;3:424-6.