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

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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* leaves 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 reported 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.

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 Amaranth. 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], anti-inociceptive, and anti-inflammatory activities [19]. It is also reported for *in vitro* and *in vivo* anticancer effects [6], and *in vitro* antioxidant, anti-amylose, 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 hydride, potassium mercuric iodide solution, copper sulphate, glacial acetic acid, sulfuric acid, n-butanol, a-naphthol, 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:

\[
\text{Yield} = \left( \frac{W_1}{W_2} \right) \times 100
\]

Where, \( W_1 \) is the weight of the dried extract after evaporation of solvent and \( W_2 \) is the weight of plant powder.

Phytochemical analysis
Phytochemical screening was done on a hydroalcoholic extract of \( A. \text{tricolor} \) leaves (HAEATL) extract that was determined 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 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 monniri, 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 150–190 g, obtained from the animal house facility used for \textit{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 model

Anti-stress activity
Different methods were used to evaluate the anti-stress activity of \( A. \text{tricolor} \) leaves.

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

- Group-I – Control group (vehicle 10 ml/kg p.o.)
- Group-II – Stress control group (scopolamine 0.4 mg/kg 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 (B. monnieri 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. 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 \( W. \text{somnifera} \) (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 convolution was a very sharp endpoint [25].

Imobilization 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 \( W. \text{somnifera} \) (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 15 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 \( A. \text{tricolor} \) 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 from 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.

\[
\text{IR} = \frac{L_0 - L_1}{L_0}
\]

Where \( L_0 \) is the initial transmission latency (TL) per second for the first time, \( L_1 \) 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 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 m² (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 vehicle 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.

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     | +      |

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 I: 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 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**                               |
| 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<0.05, **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***      |
| 4.     | HAEATL (400 mg/kg)               | 72.67±0.918***          | 42.47±0.095***         | 16.57±0.080**       |
| 5.     | Withania somnifera (100 mg/kg)   | 63.67±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 (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***              | 5.15±0.07***             |
| 4.     | HAEATL (400 mg/kg)         | 8087±108.30***             | 5.40±0.11***             |
| 5.     | Withania somnifera (100 mg/kg) | 7777±60.09***           | 5.77±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.

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+distilled 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:methyl 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) decreased 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.
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 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 \(P < 0.001\) decrease in transmission latency compared to the AC control group. 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. TL (treatment group) showed protection against memory loss and impaired cognition. MWM learning was 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.

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

| S. No. | Treatment groups | Liver | Adrenal gland | Spleen |
|-------|------------------|------|---------------|--------|
| 1.    | Control group (vehicle 10 ml/kg) | 3.26±0.1 | 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 | 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) | 4.51±0.001*** | 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 t-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 | 15.62±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 t-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 | 11.0±0.25 | 0.28±0.04 |
| 2.    | AC control group (scopolamine) | 24.50±0.80 | 40.83±1.24 | 0.06±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 t-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) | 0.0±0.00 | 0.0±0.00 | 0.0±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*** | 2.05±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 t-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
However, further studies are necessary to examine underlying mechanisms of CNS effect of different fraction of potent extract and to isolate the active compound responsible for these pharmacological activities.

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AUTHORS’ CONTRIBUTIONS

All authors have an equal contribution.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

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