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Research Article

BEHAVIORAL AND BIOCHEMICAL INVESTIGATION OF *AMARANTHUS SPINOSUS* FOR ANTISTRESS AND NOOTROPIC ACTIVITY ON RATS

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ABSTRACT

There is a number of Synthetic (allopathic) drugs which are used in the treatment of mental disorders, they are helpful but synthetic drugs also have lots of side effects with monetary problem. The Ayurveda has a long tradition of treating mental disorders. Herbal drugs are playing a significant role in the health care agendas worldwide, mostly due to the general faith that they are without any side effects, besides being contemptible and locally available. Recently there is a recovery of an interest in herbal medicines for the management of different aliments including CNS disorders. Experimental screening of ethanolic extracts of Stem & root of *Amaranthus spinosus* for brain disorders was done by Anti-stress activity (Swimming endurance test, Anoxia stress tolerance test and Immobilization stress test) Nootropic activity (Elevated plus maze and the Morris water maze). It was concluded that the hydro alcoholic extract of *Amaranthus spinosus stem &* root (200, 400 mg/kg) has anti-stress and nootropic activity in dose dependent manner.

Keywords: *Amaranthus spinosus*, Antistress, Nootropic

INTRODUCTION

Stress is a state of threatened homeostasis or disharmony caused by intrinsic or extrinsic adverse forces and is counteracted by an intricate repertoire of physiologic and behavioural responses that aim to re-establish the challenged body equilibrium. There are a number of variety of brain disorders like depression, insomnia, anxiety, epilepsy, stress, Alzheimer's, Parkinsonism, schizophrenia, migraine, infection, Huntington's etc. The most prevalent disorders are stress and amnesia.

The adaptive stress response depends upon elaborate neuroendocrine, cellular, and Crucial functions of the stress system response are mediated by the hypothalamic-pituitary-adrenal (HPA) axis and the central and peripheral components of the autonomic nervous system (ANS)[1]**.** The Father of Stress Hans Selye made two observations, first the body has a set of similar responses to a broad assortment of stressors & under certain conditions, and the stressors will make you ill[2]**.** The World Health Organization (WHO) has declared in 2001 as the year for mental health in recognition of the burden that mental and brain disorders pose on people and families affected by them. Although most people encounter persons with mental disorders in families and neighbourhoods. Though the burden of illness resulting from psychiatric and behavioural disorders is enormous, it is grossly under- represented by conventional public health statistics, which have tended to focus on mortality rather than the morbidity or dysfunction within seconds of an acutely stressful event, norepinephrine is released from nerve endings in preparation for a rapid response & the adrenal glands release epinephrine & norepinephrine into the blood stream, resulting in the familiar fight or flight response. Within minutes of a stressful event (Possibly lasting for several hours) a much more complex interaction between the nervous & endocrine systems & other forms of internal communication occurs resulting in an intricate stress adaption response. During this time the adrenal glands release extra cortisol into the circulation. A wide range of events or conditions is considered physiologically stressful because the adrenals are stimulated to release stress hormones. These occurrences include calorie restriction, surgery, sleep deprivation, excessive exercise & various mental states- all of which can result in elevated cortisol & catecholamine stress hormones. Under health consequences of chronic stress, the Natural Killer cell activity, Secretory IgA & Bifidobacteria +Lactobacilli were decreased while Enterobacteria, *E. coli* & risk of myocardial infarction were increased[3].

The extracts of Ashwagandha produce GABA-like activity which may account for the herb's anti-anxiety effects. Its function is to decrease neuron activity & inhibit nerve cells from over-firing. This produces a calming effect. Excessive neuronal activity can lead to restlessness & insomnia, but GABA inhibits the number of nerves cells that the fire in brain & helps to induce sleep, uplift mood & reduce anxiety[4]**.** Stress has been postulated to be involved in the etiopathogenesis of a variety of disease states, viz hypertension, peptic ulcer, diabetes, immune suppression, reproductive dysfunctions, Central Nervous System (CNS) disorders, endocrine system and metabolic system[5]. Furthermore, an elevation of blood glucose, blood pressure, or lipids by stress stimuli results in the onset of lifestyle-related diseases such as diabetes, increased production of stress hormones and subsequent decrease in immune function appear to contribute to the stress-induced decline in health[6]. Similarly increased physical and psychological stress leads to an increased incidence of amnesia. There is increasing evidence that dementia disorder increases severe oxidative stress as a result of either beta

amyloid mediated generation of free radicals or perturbed ionic calcium balance within neurons and their mitochondria[7]. Recently, the use of complementary and alternative medicines is increasing over worldwide[8]. As Allopathic medicine have numerous side effects like benzodiazepine and anxiolytics despite having significant anti-stress activity, limits the clinical utility due to problem of tolerance and physical dependence on their prolonged use. Therefore, there is a need for a natural anti-stress agent in the therapy of stress induced disorders[9].

Many species of *Amaranthus* are present in the world. They are used for different economical purpose when proceeds and dried *Amaranthus* are also used as leafy vegetables. *Amaranths spinosus* seeds are used as grain and its leaves which are used as a vegetable or green. The plant of *Amaranthus spinosus* leaves and seeds contain protein of an unusually high quality. In Indian traditional system of medicine (Ayurveda) the *Amaranthus spinosus* Linn. is used as febrifuge, antipyretic, laxative and diuretic. Besides its culinary value, it is a popular medicinal plant used to reputed for treat digestible, bronchitis, appetizer, biliousness, galactagogue, haematinic, stomachic effects, nausea, flatulence, anorexia, blood diseases, burning sensation, leucorrhoea, leprosy, piles and as a treatment for psychotic disorders/hallucination, healing of wounds and rheumatism, and to arrest the coughing up of blood. All parts of the plant are known to contain medicinally active constituents[10].

Amaranthus Spinosus (white) belong to family Amaranthaceae and is one of the important medicinal drugs used in indigenous medicine in India, the entire plant is reported to be useful in a variety of ailments[11]. Its claims many bioactive constituents, some of which are flavonoids, triterpenoids, alkaloids including delphocurarine, staphisagrine, delphine, condelphine, denudatin and a triterpenoid alkaloid identical to condelphine[12]. *Amaranthus Spinosus is* used as folk medicine in the treatment of various CNS disorders, although it is not reported in the literature. Based on the above information, it is planned to carry out the pharmacological evaluation of the *Amaranthus Spinosus* root for anti-stress and nootropic activities.

MATERIALS AND METHODS

Animals: Experiments were performed on either six of albino Wister rats (150-200g). Animals were procured from the animal house of the IFTM University, Moradabad and maintained on a natural day–night cycle (12h dark: 12h light) at room temperature of about 24-26°C, with free access to standard food pellets and water *ad libitum*. Animals were acclimatizing for at least ten days before exposure to behavioural experiments. Experiments were carried out between 10:00-17:00 hours. The study was carried out after Institutional Ethical Clerance.

Grouping of animals

Group- I – Control group (vehicles 10 ml/kg p.o)
Group- II – Low dose HAEASS (200 mg/kg. p.o)
Group- III – High dose HAEASR (200 mg/kg. p.o)
Group- IV –Low dose HAEASS (400 mg/kg. p.o)
Group- V- High dose HAEASR (400 mg/kg. p.o)
Group- VI – Compound standard drug group (*Withania Somnifera* 100mg/

Animal Treatment: All the groups will be treated for 7 successive days accordingly and test was performed on 7th day.

Acute oral toxicity study: The acute oral toxicity was performed according to the OPPTS (office and prevention pesticides and toxic substance) Guideline following up and down procedure[13]. Wister albino rats (150-200gm) were maintained under controlled standard animal house conditions with access to food and water *ad libitum.* The rats were acclimatized for 5 days and fasted overnight, food but not water was withheld. Animals were weighed; limit and main test were performed.

The limit test carried out first at (2000mg/kg) body weight for one animal and if animal dies, main test is performed. If the animal survives two more animals are dosed, if both survives the test should be terminated.

The main test is performed with an initial dose of 50mg/kg body weight.

The following sequence is followed.
50, 75, 175, 350, 750, 1000, 2000 mg/kg body weights

First one animal is dose with 50mg/kg body weight. If animal dies a much lower dose is tested. If animal survives, then the main test should be terminated. If animal dies, two more animals are dosed and observed.

3 consecutive animals survive at the upper end.
5 reversals occur in any 6 consecutive animals tested., At least 4 animals have followed the first reversal and the specified likelihood ratios exceed the critical value[14]. The control rats received vehicle (CMC, 1% w/v p.o.) only.

Collection, identification and authentication of plant material

Fresh plant material: The stem and root were collected and rinsed ten times of any debris or dirt, distilled water. The Stem and root were then patted dry with blotting paper and used for the experiments

Preparation of Extract*:* The fresh stem and root were separated and dried in room temperature. The dried plant materials were coarsely powdered and pass through a 20-mesh sieve. The coarsely powdered materials of plant were taken. The coarsely powder were defatted with petroleum ether and then extract with successively by a Soxhlet apparatus. The extracts were undergo filtration and concentrated by distilling off the solvents and evaporate to dryness using rotary vacuum evaporator

Drugs and Chemicals

Withania Somnifera (Himalaya pharmaceutical Ltd tab.250mg)
Levodopa (Sun pharma Ltd.)
Bacopa monnieri (Himalaya pharmaceutical Ltd.)
Scopolamine (Merck KGaA Darmstadt Germany),
CMC (SD fine chemicals)

Experimental model

The Anti stress activity: Different methods were used to evaluate the anti-stress activity of *Amaranthus spinosus* stem and root.

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 (150-200g) were divided into four groups of six animals each. Group I is control, treated with vehicle (5ml/kg, p.o), Group II and III treated with HAEASS and Group IV& V treatment with HAEASR (200 and 400 mg/kg, p.o.) Group VI treated with compound standard drug compound *Withania somnifera* (100 mg/kg p.o) was given to rats once daily for 7 days. On the seventh

day, one hour after treatment, all the rats were subjected to swimming endurance test. 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[15].

Anoxia stress tolerance test (ASTT): Wister albino rats (150-200g) were divided into four groups of six animals each, grouping and treatment was similar as in swimming endurance test. HAEASS and HAEASR or compound standard drug was given to the rats once daily for 7 days. On the 7th day, one hour 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. The appearance of convulsion was a very sharp end point[16]**.** Grouping & treatment remained same as SET.

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 (5ml/kg, p.o., control), Group II is stressed control treated with (10 ml/kg, p.o), Group III and IV treated with HAEASS(200 and 400mg/kg p.o) Group V and VI HAEASR (200 and 400 mg/kg, p.o.) Group VII treated with compound standard drug *Withania 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 hemi-cylindrical plastic tube (4.5 cm diameter, 12 cm long). The rats were confined individually and exposed continuously for a period of 150 minutes once daily for seven 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 [17].

Nootropic activity: Different methods were used to evaluate the nootropic activity of *Amaranthus spinosus* stem and root.

Elevated plus maze model: The elevated plus maze (EPM) consisting of two open arms (50x10 cm) crossed with two closed arms (50x10x40 cm) was used in this study to evaluate nootropic activity. The arms were connected together with a central square (10x10 cm). The apparatus was elevated to the height of 70 cm in a dimly illuminated room. The EPM was placed inside a light and sound attenuated room. Mice were placed individually at the end of an open arm of EPM facing away from the central platform and note the time it took to move from the end of open arm to either of the closed arms. Transfer Latency (TL) was recorded which is used as a parameter for estimation of memory enhancing property.

The Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs and the TL was assigned as 90 sec. The mouse was allowed to explore the EPM for 10 sec and then returned to its home cage. Memory retention was examined 24 h after the first day trial (i.e., on 2nd day). On the 9th day, 60 min after the treatment of last dose first trial is given and after 24 hr TL was noted for second time (i.e. on 20th day). The inflexion ratio (IR) was calculated by the formula.

$$IR = (L0-L1) / L0$$

Where, L0 is the initial transfer latency (TL) in Sec on first time, L1 is the transfer latency (TL) in Sec on 2nd time. Decreased IR indicates the induction of amnesia and increased IR indicates improvement in cognition and memory impairment**.**

The Morris water maze (MWM): The Morris water maze (MWM) represents a versatile tool in which a number of distinct tasks can be measured to evaluate working memory in rats. MWM consists of large circular tank made of black opaque PVC or hard board coated with fiber glass and resin and then surface painted white (1.8-2.0m in diameter and 0.4-0.6m height). The pool is filled with water (20-220C) to a depth of 0.3-0.4m and rendered opaque by the addition of small quantity of milk or milk powder or non-toxic white colour.

The floor of circular tank is marked off into four equal quadrants arbitrarily designed north, south, east or west. Escape platform is made up of Plexiglas's with a 13cm square platform attached to a 34cm long clear Plexiglas's, cylindrical pedestal (3cm diameter) mounted on a 1sq. m (5mm thick) Plexiglas's base. The top of the platform is covered with a coarse material that provides a good grip for the mice when climbing on a platform. For the hidden plate form task, water is added to circular tank to a level 2cm above the top of the platform. The simplest measure of performance is the Latency to escape from the water on to the hidden platform.

The platform remains fixed in the position during the training session. Each animal is subjected to four consecutive trials for four days, during which they are allowed to escape on to the hidden platform and allowed to remain there for 20 sec.

Table 1: Effect of HAEASS on stress performance by swimming endurance test

S.N.	Treatment	Swimming endurance time in minutes
1	Control	12.33 ± 0.66
2	HAEASS (200 mg/kg)	15.00 ± 0.89^{ns}
3	HAEASS (400 mg/kg)	17.33 ± 1.22****
4	*W. somnifera* (100mg/kg)	19.67 ± 0.88*****

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test ns = not significant, (*P<0.05 and **P<0.01 and ***P<0.001) when compared with control group.

Table 2: Effect of HAEASR on stress performance by swimming endurance test

S.N.	Treatment	Swimming endurance time in minutes
1	Control	22.50±2.15
2	HAEASR (200 mg/kg)	13.33±0.84****
3	HAEASR (400 mg/kg)	12.67±0.98*****
4	*W. somnifera* (100mg/kg)	12.83±1.81*****

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. Ns = not significant, (*P<0.05, **P<0.01and ***p<0.001) when compared with control group.

Table 3: Effect of HAEASS on stress performance by anoxia stress tolerance test

S. No.	Treatment	Duration of anoxia stress tolerance
1	Control	7.83±0.55
2	HAEASS 200	10.83±0.600*
3	HAEASS 400	17.83±0.600***
4	W. somnifera	21.67±1.14***

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns not significant (*p<0.5, **p<0.01 and ***p<0.001) when compared with control group.

Table 4: Effect of HAEASR on anoxia stress tolerance test

S. No.	Treatment	Duration of anoxia stress tolerance
1	Control	8.25±7.50
2	HAEASR 200	0.88±1.04^{ns}
3	HAEASR 400	14.33±1.14***
4	W. somnifera	21.83±1.16***

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns not significant (*p<0.5, **p<0.01 and ***p<0.001) when compared with control group.

Table 5: Effect of HAEASS on learning performance by elevated plus maze test

Group No.	Elevated plus maze performance		Memory percentage retention
	After scopolamine		
	Transfer latency taken on 7th day (in seconds)	Transfer latency taken on 8th day (in seconds)	
Control (vehicle 5 ml/kg)	19.60±0.90	24.30±6.01	87.50
AC control group (Scopolamine)	23.15±3.15	69.80±6.10	33.10
HAEASS (200mg/kg+Scopolamie)	36.40±4.30	54.60±2.41*	67.00
HAEASS (400mg/kg+Scopolamine)	31.30±7.40	39.40±.18*	74.38
Bacopa monieri (100mg/kg)	32.40±1.42	38.90±3.55**	82.70

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *P<0.05 and **P<0.01 when compared with AC group.

Table 6: Effect of HAESR on learning performance by elevated plus maze test

Group No.	Elevated plus maze performance		Memory percentage retention
	After scopolamine		
	Transfer latency taken on 7th day (in seconds)	Transfer latency taken on 8th day (in seconds)	
Control (vehicle 5 ml/kg)	19.70±0.98	24.40±6.08	87.65
AC control group (Scopolamine)	24.16±4.16	70.80±7.10	34.10
HAEASR (200mg/kg+ Scopolamine	37.40±5.30	55.60±3.41*	68.00
HAEASR (400mg/kg + Scopolamine)	30.30±6.40	38.40±.17*	73.38
Bacopa monnieri (100mg/kg)	33.40±2.42	39.90±4.55**	83.70

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, (*P<0.05 and **P<0.01) when compared with AC group.

Table 7: Effect of HAEAS on learning performance by Morris water maze model

Group No.	Morris water maze performance		Memory percentage Rotation
	After scopolamine		
	Average time taken on 7th day (Seconds)	Average time taken on 8th day (Seconds)	
Control (5 ml/kg)	57.40±0.10	61.08±0.70	94.66
Amnesic control (AC) Scopolamine (1mg/kg)	22.500±1.50	59.00±12.70	37.80
AC+HAEASS (200 mg/kg)	32.40±1.25	65.15±9.30*	50.09
AC+HAESS (400 mg/kg)	29.10±1.05	56.40±4.90**	51.85
Bacopa monieri (100mg/kg)	43.80±1.20	51.90±9.92**	80.93

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *P<0.05 and **P<0.01 when compared with AC group.

Table 8: Effect of HAEASR on learning performance by Morris water maze model

Group No.	Morris water maze performance		Memory percentage Retention
	After scopolamine		
	Average time taken on 7th day (Seconds)	Average time taken on 8th day (Seconds)	
Control (5 ml/kg)	59.10±0.20	62.05±0.95	96.70
Amnesic control (AC) Scopolamine (1mg/kg)	21.40±1.30	60.09±13.85	38.93
AC+HAEASR (200 mg/kg)	30.80±1.15	63.40±7.55*	48.30
AC+HAEASR (400 mg/kg)	29.70±1.05	57.10±5.32**	52.65
Bacopa monnieri (100mg/kg)	44.45±1.16	52.95±9.46**	81.95

All values are mean ± SEM. Statistical analysis of data was carried out by one-way ANOVA followed by Dunnett's test. ns = not significant, *P<0.05 and **P<0.01 when compared with AC group.

Escape latency (EL) is the time to locate the hidden platform in MWM is noted as an index of acquisition or learning. In case the animal is unable to locate the hidden platform within 120 sec, it is gently guided by hand to the platform and allowed to remain there for 20 sec. On the 9th day, 60 min after the last dose, platform is removed and time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval 18 Grouping & treatment was same as EPMT.

RESULTS

Effect HAEASS test of (SET): In swimming endurance test, HAEASS (200 mg/kg) did not significant increased the swimming time whereas, HAEASS (400 mg/kg) showed significant effect (0.01) and compound standard drug *Withania somnifera* (100 mg/kg) more significantly ($p<0.001$) increased the swimming time compared to the control group as the results are shown in table 1.

Effect of HAEASS and WS in swimming endurance test

Effect of HAEASR test of (SET): In swimming endurance test, HAEASR (200 mg/kg) showed significant effect ($p<0.01$) increased the swimming time whereas, HAEASR (400 mg/kg) showed significant effect (0.001) and compound standard drug *Withania somnifera* (100 mg/kg) more significantly ($p<0.001$) increased the swimming time compared to the control group as the results are shown in table 2.

Effect of HAEASS test of (ASTT): The results are represented that the HAEASS (200 mg/kg) showed significant effect ($p<0.05$) increased the anoxia time. HAEASS (400 mg/kg) showed significant effect ($p<0.001$) and compound standard drug *Withania 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 compound standard drug (100 mg/kg) were found to be comparable effective in this test as the results are shown in table 3.

Effect of HAEASR tests (ASTT): The results are represented that the HAEASR (200 mg/kg) did not significant effect HAEASR (400 mg/kg) produced and compound standard drug *Withania 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 compound standard drug (100 mg/kg) were found to be comparable effective in this test as the results are shown in table 4.

Nootropic activity

Elevated plus maze (EPM): Effect of HAEASS of elevated plus maze test (EPM): In this test, HAEASS (200 & 400 mg/kg p.o) were significantly ($p<0.05$) decreased the transfer latency (TL) the memory (increased the memory retention 67.00 and 74.38% respectively) and standard group *Bacopa monieri* (100 mg/kg) showed more significantly decreased the TL (increased the memory retention 82.70%) compared to amnesic Control group (memory retention 33.10%) as the results are shown in table 5.

Effect of HAEASR of Elevated plus maze test (EPM): In this test, HAEASR (200 to 400 mg/kg p.o) were significantly ($p<0.5$) decreased the transfer latency (TL) the memory increased the memory retention (68.00and73.38% respectively) and standard group *Bacopa monnieri* (100 mg/kg) showed were significantly decreased the TL(increased the memory retention 83.70%) compared to amnesic control group (memory retention 34.10%) as the results are shown in table 6.

Morris water maze (MWM)

Effect of HAEASS of Morris water maze test (MWM): In this test, HAEAS (200 mg/kg) was significantly ($p<0.05$) decreased the transfer latency Transfer latency (TL) (increased the memory retention 50.09%) whereas HAEASS (400 mg/kg) and standard group *Bacopa monnieri* (100mg/kg) more significantly decreased the TL (increased the memory retention 51.85 and 80.93% respectively) compared to AC group (memory retention 37.80%) as the results are shown in table 7.

Morris water test

Effect of HAEASR of morris water maze test (MWM): In this test, HAEASR (200 mg/kg) was significantly ($p<0.05$) decreased the transfer latency (TL) (increased the memory retention 48.30%) whereas HAEASR (400 mg/kg) and standard group *Bacopa monnieri* (100 mg/kg) more significantly decreased the TL (increased the memory retention 52.65 and 81.95% respectively) compared to AC group (memory retention 38.93%) as the results are shown in table 8.

DISSCUSION

In the recent work, it was observed that *Amaranthus spinosus* stem & root (200 and 400 mg/kg) possessed the anti-stress and nootropic activity in dose dependent manner.

In the current effort it was observed that the *Amaranthus spinosus* stem & root both showed the presence of flavonoids, alkaloids, tannins, carbohydrates, proteins, amino acids steroids and sterols.

The *Amaranthus spinosus* stem & root was studied for acute toxicity at doses of 2000mg/kg. The stem & root was found to be devoid of mortality and any type of toxicity in treated animals. Therefore, (200 and 400 mg/kg) were selected for further experimental study. In swimming endurance test, *Amaranthus spinosus* stem & root (200 mg/kg) significantly ($p<0.05$) increased the swimming time whereas, HAEAS (400 mg/kg) and compound standard drug *Withania somnifera* (100 mg/kg) more significantly ($p<0.01$) increased the swimming time compared to the control group. High dose of HAEAS (400 mg/kg) is more effective then low dose of extract. The swim endurance test results indicated clearly that the extract has the properties whereby it increases the physical endurance as well as the overall performance in rats and possess significant anti-stress activity. It may be possibly normalizing the plasma level of catecholamine and monoamine oxidase [18].

The HAEAS (200 mg/kg) significantly ($p<0.05$) increased the anoxia time. HAEAS (400 mg/kg) and compound standard drug *Withania somnifera* (100 mg/kg) more significantly ($p<0.01$) increased the anoxia time on 7th day compared to the control group. Test drug (400 mg/kg) and compound standard drug (100 mg/kg) are found to be more effective in this test.

HAEAS had increased stress tolerance indicating their adaptogenic/anti-stress activity. This may be due to that during stress the HAEAS would be capable of increasing succinate dehydrogenase (SDH) in the brain. This enzyme is responsible for the utilization and conservation of energy in the cellular system of the organism, which helps adaptive processes during stress[19].

This protective action of HAEAS on hypoxia in mice may also be due to another effect on the pituitary adrenal gland axis. The anti-hypoxia effect is related to improved or raised cerebral resistance to hypoxia and reduced cerebral consumption of oxygen in acute hypoxia. When animal are exposed to a hypobaric environment

for a specified period, the mitochondria of heart and brain cells of are seriously damaged and brain neurotransmitters, i.e. norepinephrine, dopamine, serotonin and acetylcholine are significantly decreased. Our results demonstrated that HAEAS (200 and 400 mg/kg) exhibited significant anti-stress activity as indicated by increase in duration of anoxia stress tolerance time (20)**.**In the immobilization stress trial HAEAS (200mg/kg) significantly decreased the level of biochemical parameters like Glucose, cholesterol, BUN and haematological parameters like compared to stress control group. HAEAS (400 mg/kg) and compound standard drug *Withania somnifera* (100 mg/kg) more significantly ($p<0.01$) produced the effect compared to stress control group. Both compound standard drug WS (100 mg/kg) and HAEAS (400 mg/kg) showed almost similar effect. The mechanism by which stress raises serum cholesterol is likely to be related to the enhanced activity of hypothalamo- hypophyseal axis, resulting in increased liberation of catecholamine's and corticosteroids which lead to elevated levels of serum cholesterol since adrenaline mobilizes the lipids from adipose tissues[21]**.**

After treatment with HAEAS cholesterol was reduced in immobilization stress models. The effect of stress on serum has been shown to be variable; probably catecholamine's mobilize lipids from adipose tissues. In the present study immobilization stress models showed an increase level. However, HAEAS were able to suppress the stress-induced increase in levels. Immobilization typically increases total leukocyte and erythrocyte count, during stress heart rate, blood pressure, blood flow rate and oxygen demand increases, to meet these extra demands, RBC and WBC count increases .Plant adaptogens are smooth pro-stressors which reduce the reactivity of host defence system and decrease the damaging effects of various stressors due to increased basal levels of mediators involved in the stress response[22].

Pre-treatment with HAEAS reduced the stress-induced elevated levels of haematological parameters in Immobilization stress, since the stress-induced increased by HAEAS, hence it would be said that the plant possessed anti-stress activity. Liver and adrenal glands weights were significantly increased in immobilization stress models. Stress induces adreno-medullary response in man to release adrenaline which in turn stimulates β2 receptors on the pituitary gland. It leads to greater release of ACTH that can stimulate the adrenal medulla as well as cortex resulting in further release of adrenaline and increase in weight of adrenal gland to a greater extent. The adrenal hypertrophy takes place in response to the secretion of ACTH from the pituitary for increased corticosterone from cortical cells to combat stress. The level of corticosterone was found to be elevated during the immobilization stress in the experiment. Pre-treatment with HAEAS prevented the stress-induced increase in weight of liver and adrenal glands and spleen, indicating the protective effect against stress. Interestingly, it can be inferred that the anti-stress activity of HAEAS (400 mg/kg) was equal to that of compound standard drug *Withania somnifera.* HAEAS showing significant antistress activity against stress-induced models. Flavonoids, tannins and phenolic glycosides were reported to possess a variety of biological activities including adaptogenic activity[23].

Learning has been defined as the process acquiring the knowledge while memory is the retention of the acquired knowledge that can be retrieved[24].The elevated plus maze is used to measure the anxiety state in animals, however transfer latency i.e. the time elapsed between the movement of the animal from an open to an enclosed arm was markedly shortened if the animal had previously experienced entering open and closed arms, and this shortened transfer latency has been shown to be related with memory processes. Recent studies of several nootropic and amnesic agents on EPM made this model a widely accepted paradigm to study learning and memory processes in rodents in elevated plus maze and Morris water maze, acquisition (learning) can be considered as transfer latency on 7^{th} day trials and the retention/consolidation (memory) is examined 24 h later that is on 8^{th} day. After 7^{th} day of treatment, Scopolamine treated group has shown decrease in percentage retention as compared to normal control group indicated the induction of amnesia. After 7^{th} day of treatment, HAEAS (400 mg/kg) and compound standard drug Vitamin C (200mg/kg) more significantly and HAEAS (200 mg/kg) significantly increased memory percentage retention which is an indication of the cognitive enhancer effect of test drug in rodents.

Among the various approaches attempted to increase cholinergic activity, the inhibition of Acetyl-cholinesterase (AChE) is the most successful one[25].Cholinesterase Inhibitors (ChEI) are the only class of compounds consistently proven to be efficacious in treating the cognitive and functional symptoms in patients with neurodegenerative disorders such as AD, Parkinson's disease, senile dementia, ataxia and myasthenia gravis etc[26]. In addition, new findings show that both AChE and Butyric cholinesterase (BChE) are involved in the breakdown of acetylcholine in the brain and, thus, dual inhibition of these enzymes may prove efficient in treating dementia[27]**.** The drug of HAEAS the chemical constituents present in the drug may be responsible for inhibition of Acetylcholine esterase which would be helpful for nootropic activity. Brain senescence plays an important role in cognitive dysfunction and neurodegenerative disorders.

A new study in adults age 50-74, without existing dementia, showed that a comprehensive blend of antioxidants taken for four months improved memory function. Another study showed that a key antioxidant directly inhibited the formation of beta-amyloid plaque. Beta-amyloid gets a bad name, like LDL cholesterol, because when systems malfunction it piles up and excess levels are a common feature of dementia and Alzheimer's. Antioxidants prevents brain aging and may have implications for prevention of progressive cognitive impairments[28-29]**.**

The brain and nervous system are relatively more susceptible to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. Free radical damage of nervous tissue may be involved in normal aging and neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases. This *Amaranthus spinosus* effect may be due to antioxidant effect of drug[30-31], It is likely that the presence of these above active constituents especially flavonoids & saponins etc, may perhaps account for pharmacological effects demonstrated by *Amaranthus spinosus* [32-33].

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