

**ANTI-STRESS AND NOOTROPIC ACTIVITY OF ETHANOLIC
EXTRACT OF *CORIANDRUM SATIVUM* AERIAL PARTS**

ABSTRACT

The antistress effect of a seven-day treatment (200 and 400 mg/kg, *p.o.*) of the ethanolic extract of *Coriandrum sativum* (EECS) aerial parts was evaluated by using the swimming endurance test, anoxic tolerance test and cognitive enhancing effect using Basal activity by Actophotometer. *In vitro* antioxidant activity was performed by using Hydroxyl radical scavenging assay. Both the doses of *Coriandrum sativum* showed antistress activity in all the tested models. The EECS treated animals showed an increased swimming time, increased duration stay on the rotarod and an increase in anoxic tolerance time in anoxic tolerance test and swimming endurance test respectively. *The in vitro* antioxidant activity was determined based on the ability of the *C. sativum* to scavenge free radicals which further enhanced the cognitive effect. The results have clearly depicted that *in vitro* antioxidant potential of the extract was responsible for *in vivo* adaptogenic performance and cognitive enhancement. The EECS treated animals showed increase locomotor scores in basal activity by Actophotometer. The enhanced swimming endurance stress tolerance as well as overall performance in the animals may be due to the plant secondary metabolites that are flavonoids, glycosides, triterpenoids and phenolic compounds. This study provided evidence for antioxidant, anti-stress and nootropic activity activities of the ethanolic extract of *Coriandrum sativum* and that use of them by humans as nutraceuticals is beneficial and scientific. Antioxidant effect provides the mechanistic basis in relieving stress and memory by way of combating stress in both the models.

Keywords: Antistress activity, Nootropic activity, *Coriandrum sativum*, anoxic tolerance test, swimming endurance test, Basal activity score.

1.INTRODUCTION:

Biological stress is a response to physical, chemical, biological and emotional changes, consisting of a pattern of metabolic and behavioural reactions that helps to strengthen the organism. During stressful situations, the energy requirement of the organism is increased, resulting in enhanced generation of free radicals. Free radicals cause oxidation of nucleic acids and proteins. Free radicals also damage bio-membranes, reflected by increased lipid peroxidation, thereby compromising cell integrity and function. During this process, the ability of the body's **defense** system to combat the oxidative stress may diminish due to reduced anti-oxidants. If the stress level increases beyond the threshold limit of an individual, it results in decreased performance and stress-induced disorders. The management of unusual stress therefore has acquired enormous significance in day-to-day life [1].

Temporal prolongation of adaptation response to discrete stressful events is characterized by hyperactivity of hypothalamic–pituitary–adrenal (HPA) axis. Sustained hyperactivity of the stress system (HPA axis) results in various pathophysiological states that cut across the traditional concept of disease and include a range of disorders like hypertension, coronary heart disease, gastric ulcers, immunosuppression, metabolic disorders like diabetes, reproductive dysfunction, mental depression, memory loss and host of other diseases [2].

Similarly increased physical and psychological stress leads to increased incidence of amnesia. There is increasing evidence that Alzheimer's disease 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. Supplementation with higher ascorbic acid and beta-carotene was associated with better memory performance which indicates the role of potential antioxidants in brain aging and cognitive impairment. Literature also indicates that the role of free radicals in the pathogenesis of cancer, aging, Alzheimer's disease, diabetes and the compounds having capacity to scavenge these free radicals has great potential in mitigation of these disorders [3].

Adaptogens were once defined as substances that enhance the “state of non-specific resistance” of an organism against stress. However, Panossian *et al.*, 1999 [4] referred to adaptogens as “new class of metabolic regulators which increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors”. The stress-protective effect of adaptogens has been linked with the regulation of homeostasis via several mechanisms that are associated with the hypothalamic–pituitary–adrenal (HPA) axis and key mediators of stress responses. The beneficial effects of adaptogens have also been linked to their capacity to inhibit the formation of free radicals and to modulate other processes involved in the adaptation of the organism to stress. Moreover, adaptogens are generally

believed to boost energy or resilience in the face of stress and to enhance the defense mechanisms of the body [4].

Coriandrum sativum Linn. belongs to *Apiaceae/Umbelliferae* family, commonly known as coriander, is a widely cultivated spice all over the world. Coriander has been used since roughly 1550 BC, making it one of the world's oldest spice crops. Traditionally, *C. sativum* is used in the disorders of digestive, respiratory, urinary, convulsions, insomnia and anxiety. Pharmacologically, *C. sativum* was reported to possess anti-diabetic, hepatoprotective, anti-mutagenic, antihypertensive, antioxidant, anxiolytic, antimicrobial activity and heavy metal detoxification. Due to its multifunctional uses and protective and preventive action against various chronic diseases, this herb is rightly called the "herb of happiness". The Major chemical components identified in *Coriandrum sativum* are flavonoids, polyphenols, and carotenoids [5, 6].

2. MATERIALS AND METHODS

2.1 Collection and preparation of plant extract

Aerial parts (leaves, stems, twigs and flowers) of *Coriandrum sativum* were collected from garden during the month of December 2021. This material was identified and authenticated by botanist Government Degree College, Kukatpally.

2.2 Preparation of ethanolic aerial part extract of *Coriandrum sativum*:

The aerial parts were shade dried for a week and coarsely powdered in a mixer grinder. The powdered material was stored taken up for concurrent extraction through maceration with 99.9 % ethanol for 7 days and filtrate was collected and evaporates to dryness to obtain thick extract [7].

2.3 Phytochemical evaluation

The Aerial parts of ethanolic extract of *Coriandrum sativum* were subjected to various preliminary phytochemical tests to detect the phytoconstituents present in plant extract [8, 9, 10, 11, and 12].

2.4 Experimental animals

Swiss albino mice (approx. 20 to 25 gms) were procured from Albino research, Hyderabad. Present studies were carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of pharmacy, Bachupally, Hyderabad, India (Reg. No. 1175/PO/Re/S/08/CPCSEA). The animals were housed in poly acrylic cages with not more than six animals per cage, with 12 hour-light /12 hour dark cycle. Mice have free access to standard diet and drinking water ad libitum. The mice were allowed to acclimatize the laboratory environment for a week before the start of the experiment. The care and maintenance of the animals were carried out

as per the approved guidelines of the committee for the purpose of control and supervision of experiments on animals.

2.5 Acute Toxicity Study

Acute toxicity studies were carried out in order to check the toxic effects for ethanolic extract of *Coriandrum sativum* aerial parts. The studies were performed as per Organization for economic cooperation and Development (OECD). The method is used to evaluate the acute oral toxicity is up and down procedure (OECD guideline-425). Up and down procedure (OECD guideline-425) acute toxicity studies were carried out as per the OECD 425 guidelines. Animals are observed with a special attention given during the first 4 hours and daily thereafter, for a total of 14 days generally.

2.6 In vivo Methods for Evaluation of Adaptogenic and Cognitive enhancement activity

In vivo evaluation of Anticonvulsant activity of the ethanolic aerial parts extract of *Coriandrum sativum* was carried out using the following models.

2.6.1 In-vivo Methods for Evaluation Adaptogenic Activity

2.6.1.1 Swimming endurance and post-swimming motor function test:

Swiss Albino mice were divided into four groups, Group I normal control that received saline water, Group II & Group III received ethanolic extract of *Coriandrum sativum* (EECS) 200 & 400 mg/kg, bd.wt. *p.o* and Group IV received standard Geriforte tablets (Himalaya drugs) 50 mg/kg, bd.wt., *p.o* used as a standard adaptogenic drug for seven days continuously. All the drugs were administered orally to all the animals. Stress was induced in mice by carrying out forced swimming endurance test. All the animals in different groups were subjected to stress on last day after 1hr drug administration by placing them in polypropylene tank filled with water to a height of 25cm at a room temperature of (30±2°C). Mice were allowed to swim until complete exhaustion and end point was taken when animal starts drowning. The mean swimming time for each group was calculated and allowed to recover for about 5min. All the animals were subsequently tested for post swimming muscle coordination on rota rod rotating at 15rpm and the duration of stay on the rod was recorded [13].

Table 1: Experimental study design for scheduled drug treatment in Swimming endurance and post-swimming motor function test

GROUPS	TREATMENT
Group-I	Control received vehicle
Group-II	EECS 200 mg/kg,bd.wt., <i>p.o</i> for 7days +Stress

Group-III	EECS 400 mg/kg, bd.wt., <i>p.o</i> for 7days + Stress
Group-IV	Geriforte 50 mg/kg, bd.wt., <i>p.o</i> for 7days + Stress

2.6.1.2 Anoxia stress tolerance test:

Healthy swiss Albino mice of either sex weighing 20-25gm. The animals were divided into four groups, Group I normal control that received saline, Group II & Group III received EECS 200 & 400 mg/kg, bd.wt., *p.o* and Group IV received Geriforte tablets (Himalaya drugs) 50 mg/kg, bd.wt., *p.o* used as a standard adaptogenic drug for 3 weeks continuously. All the drugs were administered orally to the animals. Every week after 1hr of drug administration each animal was placed in the conical flask (hermetic vessel) of 250 ml were used to induce stress. These flasks made airtight by using rubber cork before the conduct of experiment. Each animal kept in airtight vessel and time was recorded. The moment at which the animal shows first convulsion immediately it was removed from conical flask. The time duration from the entry of animal in the hermetic vessel to the appearance of first convulsion was taken as the time of “anoxic stress tolerance”. The mean time of convulsion was recorded, and animal was removed from flask and taken as end point. Mean duration of anoxia tolerance time in mice (min) was recorded for three weeks [13, 14].

Table 2: Experimental study design for scheduled drug treatment in Anoxia stress tolerance test

GROUPS	TREATMENT
Group-I	Control received vehicle
Group-II	EECS 200 mg/kg, bd.wt., <i>p.o</i> for 3 weeks + Anoxic Stress for every week
Group-III	EECS 400 mg/kg, bd.wt., <i>p.o</i> for 3 weeks + Anoxic Stress for every week
Group-IV	Geriforte 50 mg/kg, bd.wt., <i>p.o</i> for 3 weeks + Anoxic Stress for every week

2. 6.2 *In vivo* Methods for evaluation of cognitive enhancement activity:

2.6.2.1 Basal activity by Actophotometer:

This study will be carried out by taking healthy 30 Albino mice of either sex weighing having 20-25 gm. Group I normal control group that received saline. Group II served as disease

control received Midazolam (2 mg/kg, bd.wt., *i.p*), Group III & Group IV received ethanolic extract of *Coriandrum sativum* (200 & 400 mg/kg, bd.wt, *p.o*) and Group V received Standard drug Donepezil (1mg/kg, bd.wt., *i.p*) will be injected followed by Midazolam (2 mg/kg, bd.wt., *i.p*) after 30 min. Each animal will be placed individually in the actophotometer and the basal activity score of all the animals will be recorded after 30, 60 and 120 min of drug treatment. The activity of each animal will be tested for 10 min. The difference in the activity will be recorded considering standard drug treatment score and extract treatment score [15, 16].

Table 3: Experimental study design for scheduled drug treatment in Basal activity by actophotometer

GROUPS	TREATMENT
Group-I	Control received Normal saline
Group-II	Disease control received Midazolam 2 mg/kg, bd.wt., <i>i.p</i>
Group-III	EECS 200 mg/kg, bd.wt., <i>p.o</i> + Midazolam 2 mg/kg, bd.wt., <i>i.p</i>
Group-IV	EECS 400 mg/kg, bd.wt., <i>p.o</i> + Midazolam 2 mg/kg, bd.wt., <i>i.p</i>
Group-V	Donepezil 1 mg/kg, bd.wt., <i>i.p</i> + Midazolam 2 mg/kg, bd.wt., <i>i.p</i>

2.6.3 In-vitro Determination of hydroxyl radical scavenging activity:

A free radical is an atom or molecule that has an unpaired electron and is therefore unstable. This unstable radical has the tendency to become stable through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells, thus causing protein and DNA damage. All biological systems have innate antioxidant defense mechanisms that remove damaged molecules, but these mechanisms can be inefficient. Therefore dietary intake of antioxidants is imperative to protect cells from damage caused by free radicals. Antioxidants also turn free radicals into waste by-products, which are eliminated from the body.

Procedure:

In hydroxyl radical scavenging assay, the reaction mixture was prepared by adding 100 ml of 2-deoxy- D ribose (28 mM in 20 mM KH₂PO₄-KOH buffer, pH 7.4), 500 ml of EECS at different concentrations (10, 20, 30, 40, 50 mg/mL), 200 ml EDTA (1.04 mM) and 200 mM FeCl₃, 100 mL of H₂O₂ (1 mM) and 100 mL ascorbic acid (1mM), and incubated at 37 C for 1 h. 1mL thiobarbituric acid (1%) and 1mL of trichloroacetic acid (2.8%) was added to

resultant mixture and again incubated at 100 C for 20 min. After cooling, absorbance of resultant solution was measured at 532 nm, against a blank sample [17].

$$\text{Hydroxyl radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{sample}}} \times 100$$

Where, $\text{Abs}_{\text{sample}}$ = Absorbance of the test sample

$\text{Abs}_{\text{control}}$ = Absorbance of control.

2.7 Statistical analysis:

All the values were expressed as arithmetic mean \pm SEM & were analysed by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test $p < 0.05$ way the criterion for statistical significance.

3. Results

3.1 Preliminary phytochemical screening

Preliminary phytochemical investigation of ethanolic extract *Coriandrum sativum* aerial parts showed the presence of alkaloids, glycosides, steroids, flavonoids, phenols, terpenoids, anthocyanins, coumarins and volatile oils.

3.2 Acute toxicity study

In our acute toxicity studies, the administration of EECS at the dose of 2000 mg/kg has not shown mortality or evidence of adverse effects implying that an aerial part of *Coriandrum sativum* is non-toxic. No changes observed in behavioural pattern, clinical signs and body weight of mice throughout the 14 days of observation. This shows that *Coriandrum sativum* was safe up 2000mg/kg.

3.3 Dose Selection

From poisonousness studies, a portion of 2000 mg/kg bd. wt. was distinguished to be protected, and the functioning portion was considered as 1/10th i.e. 200 mg/kg, bd. wt. In the current review pharmacological assessments were finished utilizing 200 mg/kg. bd. wt. further 100 mg/kg, bd. wt.

4.4 Swimming endurance and post-swimming motor function test:

The swimming capabilities of mice treated with EECS at 200 & 400 mg/kg and geriforte over control group of mice shown in table 4. The control group of mice swam for 133.5 ± 1.257 s, EECS treated mice at a dose of 200 and 400 mg/kg/day swam for 218.83 ± 1.45 and 342.83 ± 0.94 s whereas geriforte treated mice swam for 425.16 ± 1.42 at a dose of 50 mg/kg. Table 5 shows that the duration of stay on rota rod which was significantly increased from 8.66 ± 0.494 s in control group to 16.66 ± 0.66 s and 22.5 ± 0.763 s in the group treated with EECS at dose of 200 and 400 mg/kg/day compared to geriforte treated group at 50 mg/kg/day that is 28 ± 0.577 s.

Table 4: Effect of EECS on swimming endurance test in mice

GROUPS	Treatment	Mean±SEM
I	Control Vehicle	133.5±1.257
II	EECS 200 mg/kg bd.wt, p.o	218.83±1.45 ^{*A}
III	EECS 400 mg/kg bd.wt, p.o	342.83±0.94 ^{*A}
IV	Geriforte 50mg/kg bd.wt, p.o	425.16±1.42 [*]

The values are expressed as mean±SEM, n=6. Statistical analysis was performed by using one-way (ANOVA) followed by Dunnett's multiple comparison test by comparing with control (^{*}p<0.0001), and standard (^Ap<0.0001) were considered as statistically significant.

Table 5: Effect of EECS on swimming endurance test in mice

GROUPS	Treatment	Mean±SEM
I	Control Vehicle	8.66±0.494
II	EECS 200 mg/kg bd.wt, p.o	16.66±0.66 ^{*A}
III	EECS 400 mg/kg bd.wt, p.o	22.5±0.763 ^{*A}
IV	Geriforte 50mg/kg bd.wt, p.o	28±0.577 [*]

The values are expressed as mean±SEM, n=6. Statistical analysis was performed by using one-way (ANOVA) followed by Dunnett's multiple comparison test by comparing with control (^{*}p<0.0001), and standard (^Ap<0.0001) were considered as statistically significant.

4.5 Anoxia stress tolerance test:

It was observed that EECS 200 & 400 mg/kg and standard drug Geriforte significantly enhanced the anoxia tolerance time (p< 0.05). The anoxia tolerance effect was increased with dose and duration of treatment as it is depicted in table 6. Pre-treatment with EECS (200 & 400 mg/kg) observed that increase in anoxia stress tolerance time indicating the significant adaptogenic activity.

Table 6: Effect of EECS on Anoxia stress tolerance time in mice

GROUPS	Treatment	Mean duration of anoxia tolerance time in mice (min)			
		0 Week	1 st Week	2 nd Week	3 rd Week
I	Control	14.83±0.401	16.33±0.49	17±0.447	18.16±0.600
II	EECS 200 mg/kg bd.wt, <i>p.o</i>	16.66±0.477	23.16±0.40 ^{*A}	25.33±0.494 ^{*A}	26.5±0.562 ^{*A}
III	EECS 400 mg/kg bd.wt, <i>p.o</i>	15.5±0.670	26.83±0.60 ^{*A}	31.5±0.444 ^{*B}	32±0.447 ^{*A}
IV	Geriforte 50mg/kg bd.wt, <i>p.o</i>	16.33±0.666	32.16±0.40 [*]	34±0.365 [*]	36.5±0.427 [*]

The values are expressed as mean±SEM, n=6. Statistical analysis was performed by using one-way (ANOVA) followed by Dunnett's multiple comparison test by comparing with control (^{*}=p<0.0001), and standard (^A=p<0.0001, ^B=p<0.005) were considered as statistically significant.

4.6 Basal activity by Actophotometer:

The locomotor or basal activity scores were measured at 0, 30, 60, 120 mins to evaluate the effect of EECS on memory loss in swiss abino mice. EECS at 200 & 400 mg/kg, standard group mice showed significant increase in BAS at 30, 60, and 120 mins when compared to disease control that showed decreased BAS which is shown in below table 7.

Table 7: Effect of EECS on basal activity scores in mice

GROUPS	Treatment	Locomotor Activity scores in 5 mins			
		0 min	30 min	60 min	120 min
I	Control	332.33±1.0 53	340.5±1.0877	374.5±1.6885	386.5±1.543
II	Disease control	352.5±0.95 7	217.83±1.166 [*]	183.16±1.6414 [*]	173.33±1.45 3 [*]
III	EECS 200 mg/kg bd.wt, <i>p.o</i>	343.33±1.0 85	346.83±1.077 [*] ^{*@A}	355.33±1.115 [*] ^{@A}	366.33±1.83 7 ^{*@B}
IV	EECS 400	363.66±1.1	383.5±1.335 ^{*@}	391.66±1.475 [*]	406.66±2.34

	mg/kg bd.wt, <i>p.o</i>	15	A	@A	7* ^{@ns}
V	Donepazil 1 mg/kg bd.wt, <i>i.p</i>	372.16±0.9 45	407.66±1.475* @	421.83±0.869* @	437.83±1.24 9* [@]

The values are expressed as mean±SEM, n=6. Statistical analysis was performed by using one-way (ANOVA) followed by Dunnett's multiple comparison test by comparing with control (*=p<0.0001, **=p<0.001), Disease control (@=p<0.0001) and standard (A=p<0.0001, B=p<0.05) ns =non-significant were considered as statistically significant.

3.6 *In-vitro* Hydroxyl radical Scavenging assay:

Table 8: Effect of EECS on Hydroxyl radical Scavenging assay

S.No	Compounds	Concentration (µg/mL)	% Inhibition (Mean±SEM)	IC ₅₀ Value (µg/mL)
1.	EECS	10	21.27±1.76	25.60
		20	41.93±1.45	
		30	69.45±1.07	
		40	75.56±2.10	
		50	81.67±0.19	
2.	Ascorbic acid	10	21.62±1.40	19.51
		20	51.25±0.47	
		30	62.56±2.15	
		40	74.80±0.78	
		50	79.27±2.07	

Free radicals are generated spontaneously as by products in biological systems during metabolic processes that can

cause oxidative damage to tissues and biomolecules leading to various severe clinical implications particularly diabetes mellitus, chronic inflammation, neurodegenerative disorders and cancer.

Oxidative stress is a state of imbalance between the production of reactive oxygen species (ROS) and the ability of the body's antioxidant defense system to neutralize them. ROS are highly reactive and can damage DNA, proteins, and lipids, leading to cell death and tissue damage. Oxidative stress is a key factor in the pathogenesis of many chronic diseases, including cardiovascular disease, cancer, and neurodegenerative disorders.

extensive damage to tissues and biomolecules leading to various severe clinical implications particularly diabetes mellitus, chronic inflammation, neurodegenerative disorders and cancer.

Oxidative Stress

5. CONCLUSION Further study is required for isolation & identification of active constituents and to confirm exact mechanism.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee of GRCP approved the research entitled “Anti-Stress and Nootropic activity of

The present study suggested that ethanolic extract of *Coriandrum sativum* aerial parts showed significant adaptogenic activity in rodents.

ETHICAL APPROVAL Ethanolic extract of *Coriandrum Sativum* aerial parts” with Regd number. 1175/PO/Re/S/08/CPCSEA. All animal experiments were approved by the Institutional Animal Ethics Committee of GRCP.

The Institutional Animal Ethics Committee of GRCP approved the research entitled “Anti-Stress and Nootropic activity of

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