RESEARCH ARTICLE

Pre-clinical Evaluation and *In-silico* Docking of *Coriandrum sativum* on Stress and Cognitive Deficits in Rodents

N.V.L.S.R. Vanukuru[1*], Ganga R. Mudunuri[1], Prathyusha Bhimasani[1], Niharika Malipatel[1], Mahesh Thorat[2], Harshal Tare[3]

[1]*Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Affiliated to Osmania University, Hyderabad, Telangana, India*

[2]*Department of Pharmacology, Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara, Affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Dist. Raigad, Maharashtra, India.*

[3]*Department of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India*

Received: 28th December, 2022; Revised: 18th January, 2023; Accepted: 15th February, 2023; Available Online: 25th March, 2023

ABSTRACT

Ethanolic extract aerial portions of *Coriandrum sativum* (EECS) were tested for their anxiolytic effects on the cold-resistant stress test after seven days of treatment and their cognitive boosting effects after eight days of therapy (EECS). Total phenolic content and flavonoid compound levels were calculated. *C. sativum* showed anti-stress efficacy in a cold-resistant stress animal at 200 and 400 mg/kg doses. The plasma glucose, triglyceride, cholesterol levels, as well as the weight of the adrenal glands are all affected by the stimulation of the HPA in stressful situations. Pre-treatment with EECS and Geriforte, which contain secondary metabolites such as flavonoids, glycosides, triterpenoids, and phenolic compounds, significantly reduced stress-induced changes in these biochemical levels in cold stress animals. Screening for acetylcholinesterase inhibition activity *in-vitro* using Ellman's approach increased AChE inhibition dose-dependently in the brains of mice. The data proved that the extract's potential to reduce pain *in-vitro* is what led to the observed cognitive benefits *in-vivo*. Animals given EECS at 200 and 400 mg/kg had their inflexion ratio improved as a result of the memory deficit being reversed. The scopolamine-induced amnesia group of mice showed degeneration of vacuolated cells, pyramidal cells, pyknosis, loss of architecture and the creation of lesions in the hippocampus; however, these characteristics were restored by EECS and standard therapy. Effective docking scores in mcule software were observed for interactions with receptors PDB: 4K5Y (CRF-1) for adaptogenic and PDB: 1E66 (AChE) for cognition, according to *in-silico* research. The ethanolic extract of *C. sativum* has been shown to have positive and scientifically-supported effects on human health when used as a nutraceutical, with the *in-vitro* acetylcholinesterase Inhibition assay, anti-stress, and cognitive enhancement activities, and *in-silico* studies all corroborated by this study.

Keywords: Adaptogen, Cognition, *Coriandrum sativum*, Cold stress.

International Journal of Pharmaceutical Quality Assurance (2023); DOI: 10.25258/ijpqa.14.1.08

How to cite this article: Vanukuru N.V.L.S.R., Mudunuri GR, Bhimasani P, Malipatel N, Thorat M, Tare H. Pre-clinical Evaluation and *In-silico* Docking of *Coriandrum sativum* on Stress and Cognitive Deficits in Rodents. International Journal of Pharmaceutical Quality Assurance. 2023;14(1):36-44.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Stress disturbs normal physiological conditions and results in a state of threatened homeostasis. Despite enormous investigations and rigorous study by medical professionals, therapy to develop non-specific resistance to aging, various illness, recovery from diseases, and environmental changes is nevertheless unattainable. Stress disrupts physiological homeostasis and exerts an effect on several biological systems. Multiple visceral, behavioral, and endocrinological abnormalities emerge from the breakdown of adaptational processes, which complex mechanisms would cause. According to theories, stress has a role in the etiopathogenesis of many disorders, such as peptic ulcers, hypertension, ulcerative colitis, immunosuppression, endocrine problems, male libido, and cognitive dysfunction. For controlling stress physiology, HPA axis and adrenal glands are essential.[1] Central nervous system (CNS) Involvement in their stress physiology has been postulated more recently. Numerous studies have shown that excessive stress can impair the immunological function necessary for such stress-induced immune modulation, and

**Author for Correspondence: suvarchalakiran@gmail.com*

complex neuroendocrine mechanisms such as damaging the cholinergic system in the brain lead to memory loss depending on duration and the type of the stressor.[2]

The areas of the brain responsible for thinking, remembering, and communicating are particularly vulnerable to the effects of Alzheimer's disease (AD). As the disease progresses, patients become increasingly disoriented and becoming incapable of carrying out regular tasks. In the end, they lose all the mental functions that make life enjoyable and fulfilling. The symptoms of this condition include memory loss and dementia. When the systems that produce reactive oxygen radicals are out of whack, this is called oxidative stress. Increasing acetylcholine levels by blocking acetylcholinesterase (AChE) is one of the key therapeutic approaches for AD[3].

Natural and plant-based products have been crucial for treating and preventing human disease since ancient times. Due to their structural variety, natural products are increasingly used as valuable sources of positive leads in searching for innovative treatments. Modern techniques for isolating, identifying, structurally elucidating, and synthesizing in a combinatorial fashion have increased their potential. Some of the most important bioactive molecules found in chemicals found in plants such as alkaloids, flavonoids, tannins, and phenolic acids.[4] Herbal medicine is based on the teachings of traditional Eastern systems of medicine and encompasses a broad spectrum of approaches to health and healing. It has always been the approach of indigenous people of the East to utilize herbal plants as part of their traditional medical and spiritual practices. Several portions of the plant, especially the seeds, are used as a food supplement across Asia and the Middle East due to their high levels of petroselinic acid and linalool. Medicines derived from herbs can come from either natural sources or be manufactured through chemical synthesis. Herbal remedies have been utilized extensively for centuries as a means of disease prevention, treatment, and cure. In the modern age, herbal medicines have become a significant part of our holistic health care. The *Coriandrum sativum* belongs to the Apiaceae family (Umbelliferae).[5] *C. sativum* is an herb that is a staple in many cuisines across the world. Coriander helps prevent stomach ulcers by decreasing acid secretion in the stomach, stimulating prostaglandin formation in the stomach, and stimulating the production of protective mucus in the stomach. The pharmacological uses of *C. sativum* are antiseptic, antispasmodic, antifungal, hepatoprotective, antihistaminic, hypoglycemic, diuretic, anti-depressant, anti-hypertensive, antiproliferative, cardiotonic, antimicrobial, antioxidant, appetizer, anti-helmintic properties. It was also used for fainting and memory loss (Cognition).[6]

METHODS

Collection and Preparation

C. sativum leaves, stems, twigs, and flowers were harvested from the garden in the month of December 2021. Botanists from the Government Degree College in Kukatpally were able to positively identify and verify the authenticity of this plant material.

Extraction of *C. sativum* from its Aerial Parts using Ethanol

The plant's tops were air-dried in the shade for a week, then ground into a coarse powder in a blender. Maceration in 99.9% ethanol was used to extract the powdered material simultaneously; the filtrate was collected and evaporated to dryness to provide a concentrated extract.[7]

Phytochemical Analysis

C. sativum's ethanolic extract from its aerial parts was put through a battery of assays to identify any active phytoconstituents present in the extract.[8,9]

Quantitative and Qualitative Analysis

TPC Estimated using the FC Reagent for EECS Aerial Parts: A Quantitative Study

To calculate how much phenolic compounds were in the ethanol extract, the Folin-Ciocalteau reagent was used. A test tube included extract in 100, 200, and 300 L volumes, 2 mL of 20% Na_2CO_3, and 0.5 mL of Folin-Ciocalteau (1:1). The absorbance was measured at 650 nm after a 40°C incubation period. The total phenolic content was expressed in mg per milligram of dry weight, and the gallic acid equivalent was used to do so. TPC was performed and computed in Excel by means of regression equations. The formula y=mx+c was used to find the value, which was then compared to the concentrations of *C. sativum* L. extracts.[10,11]

Analyzing Data Qualitatively Paper Chromatography for Flavonoid Separation

Standard procedures for isolating flavonoids using paper chromatography were followed. In a mortar and pestle, 2 g of the tissue were thoroughly homogenized with 20 mL of 80% methanol in 1% HCl. Methanol was used to get the volume up to 25 mL. The homogenate was placed in the dark for 24 hours to improve flavonoid extraction. For another 20 minutes at 4,000 rpm, the extract was centrifuged. Next, the samples were placed in vials and kept at 20°C until chromatography could be performed.

Paper Chromatography for Flavonoid Separation

Ascending paper chromatography employed an n-butanol/acetic acid/water solvent combination (4:1:5). Flavonoids were spotted with the use of a UV transilluminator. Ammonia fumes were then used to expose the chromatogram for a full night in order to improve the clarity of the separated flavonoids in the photographs.[11]

Animals used in Research

Albino research in Hyderabad provided the swiss albino mice (20–25 gm in weight). These experiments were conducted in an animal facility at GRCP in Bachupally, Hyderabad, India, that has been licenced by the CPCSEA. No more than six animals were housed in each poly acrylic cage, and each cage had a light/dark cycle of 12 hours daily. Mice can eat a regular mouse meal and drink tap water whenever they like. The mice were given seven days so any new noises or smells

wouldn't stress them. Animals used in tests were cared for and maintained in accordance with protocols established by an animal experimentation ethics committee.

Studies of the Acute Toxicity of EECS

Acute toxicity tests investigated the potential toxicity of an EECS. As per the OECD 425 recommendations, experiments were conducted using the up-and-down approach. Animals are typically watched for a total of 14 days, with a focus on the first 4 hours and then daily thereafter.

Ellman's Assay for AChE Inhibition *In-vitro*

Rapidly after decapitation, the brains of the mice were taken and homogenized in a 10-fold dilution of ice-cold 10mM phosphate buffer (pH 7.4) on a sterile plate. Centrifuged at 3500 rpm the tissue homogenates and the resultants were collected for further use as a source of the enzyme. The aforementioned procedures all took place at a temperature of 4ºC. AChE activity was measured with a variant of the Ellman test. The brain homogenates were mixed with 10–50 µg/mL of EECSin a 10 mM phosphate buffer, yielding a final 150 µL/mL volume. 2100 µL of 1 mM DTNB was added 300 µL of 3 mM ATCI in 10 mM phosphate buffer (pH 7.4) subsequentat 37°C a 5 minutes pre-incubation. The UV spectrophotometer was used to record the rise in absorbance over 1-minute at 405 nm and at 37°C formation of the 5-thio-2-nitrobenzoate anion. Donepezil, a commonly used anticholinesterase medication, was employed as a comparison. We were able to determine the percentage of inhibition of AChE activity.[12,13] Adjustments in absorbance per minute were used to calculate AChE activity. The tests were done in triplicate according to the following protocol:

$$(E\text{-}S)/E \times 100$$

where E and S represent the enzyme's activity before (without the test sample) and after (with the test sample).

Adaptogenic Activity Evaluation *In-vivo*

Cold-resistant Stress Test

Group I got saline. Groups II and III received an ethanolic extract of *C. sativum* (EECS) 200 and 400 mg/kg, bd.wt.,*p.o*., and Geriforte tablets (Himalaya medicines) 50 mg/kg, bd.wt., *p.o*. as a standard adaptogenic drug for seven daysto group IV. The animal received all medications *via* a ball-ended feeding needle for one week. Exposing these animals to 4 ± 10°C for 2 hours everyday was a cold stress test. All animal groups underwent this for seven days. Plasma was obtained by centrifuging heparinized blood samples at 5000 g for 10 minutes. Mouse blood was collected to measure all biochemical parameters and adrenal gland weight.[14,15]

***In-vivo* Cognitive Enhancement Evaluation Methods**

Elevated Plus Maze Transfer Latency (Learning and Memory)

Group I received distilled water, Scopolamine (0.4 mg/kg, bd.wt., *i.p*) to group II, and EECS (200 & 400 mg/kg, bd.wt, *p.o*) to group III and IV received, and piracetam (200 mg/kg, bd.wt, *p.o*) and scopolamine (0.4 mg/kg, bd.wt, *i.p*) to group V. Each group receives scopolamine (0.4 mg/kg, bd.wt.,*i.p.*) after 30 minutes of treatment to cause cognitive deficit in mice. Transfer latency time (TLT) was 90 seconds for each mouse to move to the closing arm. L0 was the transfer latency (TL) of a mouse moving inside an enclosed arm with all four legs. Mice are free to explore the equipment for 10 minutes on the first day of the trial. If the mice do not enter either closed arm within 90 seconds, they are gently forced into one and TLT is 90 seconds. On the eighth day (8^{th} day), 24 hours after the experiment, the TL was L1. TL influence was expressed by inflexion ratio (IR). TLT reduction improves memory [16]

IR = (L1−L0)\L0, where L0 is the initial TL (s) on the first day and L1 is the second day's TL.

Scopolamine-induced Mouse Amnesia Histopathology

Tissue processors were processed in 10% buffered formalin-fixed tissues. A rotary microtome cut 5 µm sections from paraffin-embedded tissue. Routine hematoxylin-eosin staining was used on these sections. Vacuolated cells, increased inflammatory infiltration, necrosis, and pyknosis were evaluated microscopically on the slides. Hippocampal lesions were examined at 100× magnification.[17]

***In-silico*/Moleculardocking**

Molecular docking, which simulates how protein-ligand complexes interact by calculating the shape complementarities between the two molecules, has been applied to a variety of problems ranging from ligand design to drug discovery. The basic idea of the docking procedure is that ligands binding with high affinity to protein-binding sites tends to possess a molecular shape complementary to the shape of the binding site. Which is usually described by the shape and the spatial distribution of the hydrophobic and hydrogen bonding residues that surround the binding site.[18] PDB ID: 4K5Y (Corticotropin-releasing factor receptor-1(CRF-1)) and PDB ID:1E66 (acetylcholinesterase enzyme) proteins are docked against 16 ligands (Phytocompounds) obtained from GC-MS studies for adaptogenic or anti-stress and cognitive enhancement activity.

Docking Simulations 4K5Y

Depression, anxiety, sleep, eating, and stress disorders, as well as their associated neurological and psychiatric illnesses, may all have a potential new target in corticotropin-releasing factor receptor-1(CRF-1). Therefore, CRF antagonists have initially considered candidate drugs for treating stress-related disorders.[19]

Docking Simulation on 1E66

AChE inhibitors or anti-cholinesterases inhibits are a type of medication that prevents this from preventing acetylcholine breakdown, thereby increasing its levels and its action in the brain. Common treatments for Alzheimer's disease and other diseases affecting memory and behavior involve blocking the activity of AChE. Blocking AChE has been shown to positively affect cognitive decline and memory loss. Other symptoms of neurodegenerative diseases like dementia, Alzheimer's disease, cerebellar ataxia, and muscle-wasting disorders can be alleviated by acetylcholinesterase inhibitors.[20]

Ligand Preparation

Chemical structures of molecules are drawn and ligand preparation was created. The 2D ligands sketched in Mcule docking in the ligand imported side.

Protein Preparation

PDB ID: 4K5Y (Corticotropin-releasing factor receptor-1(CRF-1)) and PDB ID:1E66 (acetylcholinesterase enzyme) proteins are initially were downloaded from the RCSB protein bank website in PDB format and were prepared by removing extra chains. SBD site sphere attributes collected from the discovery studio visualizer are prepared and annotated.

Protein-ligand Interactions

In docking simulations, the binding orientation of drug candidates to their protein targets is predicted. In order to create docking simulation experiments, Mcule was used.

Ligand Docking and Scoring

Proteins are uploaded with sphere attributes for 4K5Y (X=-40.442375, Y=-20.809792,Z=35.251250);1E66(X=-19.209643, Y=85.031286, Z=50.795071). Docking indicated that some of the compounds have the good binding ability with both 4K5Y and 1E66 proteins. Protein ligand interactions were stimulated through flexible glide-ligand docking withm CULEdocking allowed. The compounds docked display a docking score. Following are the ligand interactions of compounds present in *C. sativum* with 4K5Y and 1E66 proteins.

Visualization and Analysis

The discovery studio visualizer was used to see the generated docking poses. Their interactions were depicted to gain insight into how ligands bind to proteins. Glide score was used to determine the best-docked structures. If the rating is lower, the binding will be more to your advantage. It was also possible to see the docked ligand poses and investigate the various ligand-receptor interactions.

STATISTICAL ANALYSIS

Mean standard error of the Mean (n=6) is presented for all values. Every group was measured against a "control," "disease," and "standard" group. ANOVA and posthoc tests for statistical analysis.

RESULTS

A Preliminary Screening for Phytochemicals

Alkaloids, glycosides, steroids, flavonoids, phenols, terpenoids, anthocyanins, coumarins, and volatile oils were found in a preliminary phytochemical analysis of EECS.

Study of Acute Toxicity

Aerial parts of *C. sativum* have been demonstrated to be non-toxic in acute toxicity trials, with a dose of 2000 mg/kg of EECS causing no fatalities or other ill effects. These results indicate that *C. sativum*, at doses up to 2,000 mg/kg, is safe for human consumption.

Selection of Doses

Based on toxicology data, we know that a dose of 2,000 mg per kg of body weight per day provides enough protection and that the effective dose is ten times less, at 200 mg per kg of body weight per day. Pharmacological evaluations in the current study were conducted with doses of 200 and 400 mg/kg.

Analyzing *C. sativum* through GC-MS

Following GC-MS analysis, the extract of EECS's aerial portions was shown to contain the beneficial phytochemical substances listed in Table 1.

Total Phenolic Content of EECS at a Quantitative Level

Calculations for the linear correlation coefficient and the analysis of correlation were performed in Microsoft Office Excel 2010. Researchers used the aforementioned regression equation to determine how concentrated an extract would be. Using a standard calibration curve in Table 2 and Figure 1, we calculated that EECS contained 139.770 ± 3.9220 mg/gallic acid equivalent/g extract.

Flavonoids are Separated using Chromatography

Table 3 displays the outcomes of paper chromatography. Standard retention factor (Rf) values were used to identify flavonoids. Biflavonyl (Rf-95) and flavonols (myricetin) (Rf-47)

Table 1: Phytoconstituents of aerials parts of *C. sativum*

S. No.	*RT*	*Compound Name*	*Mol. Wt*	*%Area of the peak*
i	27.956	n-Hexadecanoic acid	256.40	0.150
ii	22.653	Caffeic acid	180.160	3.900
iii	23.067	Camphor	152.230	6.030
iv	23.296	α-Linolenic acid	206.330	3.210
v	10.039	Scopoletin	192.160	1.690
vi	3.861	Luteolin	286.240	0.140
vii	12.474	Chlorogenic acid	354.3110	4.660
viii	2.474	Linalool	154.250	28.210
ix	7.258	Catechin	290.260	1.010
x	3.715	Carvone	150.220	5.290
xi	22.988	Ferulic acid	194.180	2.490
xii	1.453	Quercetin (Rutin)	302.2360	15.030
xiii	3.157	Gallic acid	170.120	0.550
xiv	32.086	γ-Sitosterol	414.70	5.590
xv	8.043	Kaempferol	286.230	1.170
xvi	11.235	Geranyl acetate	196.290	0.830

Table 2: Gallic acid's reference curve for standardization purposes

S. No	*Concentration (µg/mL)*	*Absorbance*
1.	100.0	0.0670
2.	200.0	0.1540
3.	300.0	0.1980
4.	400.0	0.2980
5.	500.0	0.3820

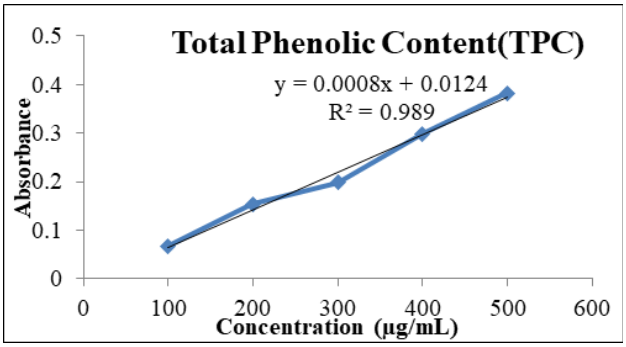


Figure 1: Gallic acid standard calibration curve.

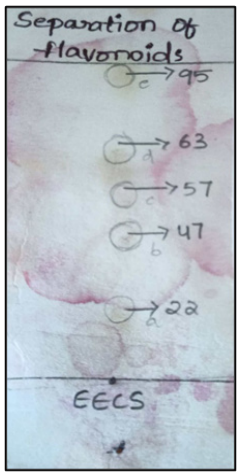


Figure 2: Flavonoids are separated using paper chromatography.

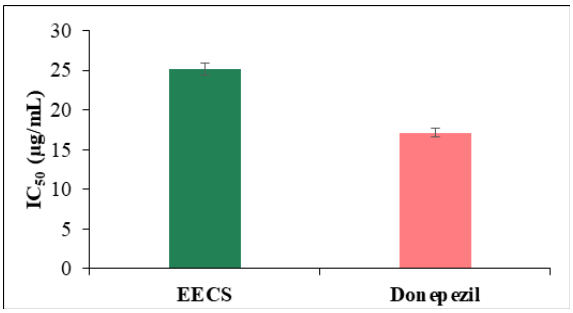


Figure 3: IC_{50} value of EECS and standard donepezil in *in-vitro* AChE inhibitory activity.

were found to be among the flavonoid components present in the extract of *C. sativum's* aerial parts. The chromatographic separation of flavonoids using ammonia is depicted in Figure 2.

Expression of AChE Inhibitory Activity Using the Ellman Method *In-vitro*

With Ellman's method, we tested the AChE inhibitory activity of an EECS. *C. sativum* was tested for its ability to inhibit AChE from 10 to 50 µg/mL. The percentage of AChE activity was inhibited by *C. sativum*. Table 4 shows that its IC_{50} value was determined to be 25.17 µg/mL. The extract's IC_{50} value of 17.14 µg/mL was equivalent to that of normal donepezil (positive control) (Figure 3).

Table 3: *C. sativum* Rf values

S. No	*Name of the plant*	*Spot No*	*Rf value*	*Active constituent*
1.	Ethanolic extract of *C. sativum* aerial parts	a	22	Unidentified
		b	47	Flavonols (Myrcetin)
		c	57	Unidentified
		d	63	Unidentified
		e	95	Biflavonyl

Table 4: Ellman's Method Impact on the Inhibitory Activity of Endogenous Acetylcholinesterase (EECT)

S. No	*Compound*	*Concentration (µg/mL)*	*Percentage Inhibition (Mean ± SEM)*	*IC_{50} value (µg/mL)*
1	Donepezil	10.0	31.05 ± 0.2620	17.14
		20.0	58.32 ± 0.2460	
		30.0	63.05 ± 0.3500	
		40.0	72.61 ± 0.7420	
		50.0	79.23 ± 1.7350	
2	EECS	10.0	28.04 ± 2.656	25.17
		20.0	36.05 ± 1.855	
		30.0	59.58 ± 1.908	
		40.0	68.97 ± 1.868	
		50.0	73.95 ± 0.795	

Table 5: Docking Scores with PDB: 4K5Y

S. No	*Ligands*	*Docking score*
1.	Quercetin	-8.6
2.	Kaempferol	-8.5
3.	Catechin	-8.0
4.	α-linolenic acid	-7.3
5.	Luteolin	-7.3
6.	Diazepam	-8.3

Table 6: Docking Scores with PDB: 1E66

S. No	*Ligands*	*Docking score*
1.	Catechin	-6.8
2.	Luteolin	-6.8
3.	Chlorogenic acid	-6.8
4.	Kaempferol	-6.7
5.	Quercetin	-6.7
6.	STD Donepezil	-6.4

The Role of EECS in a Cold Resistant Stress Test

When compared to the normal group, stress (disease) group experiences arise in blood glucose (98.83 ± 0.833), cholesterol (98.83 ± 0.833), and triglycerides (85.33 ± 0.881), as well as an increase in adrenal gland weight (10.41 ± 0.1137) due to cold stress. Blood glucose was reduced by 86.5 ± 0.882, 78.33 ± 1.053, and 72.83 ± 0.748 in the EECS 200 mg/kg, EECS 400 mg/kg, and geriforte groups. Cholesterol was reduced by 86.5 ± 0.882, 78.33 ± 1.053, and 72.83 ± 0.748; triglycerides were reduced by 75 ± 0.9309, 62.5. As can be seen in Figures 4 and 5, therapy with EECS reversed the changes in all the parameters produced by cold restraint stress.

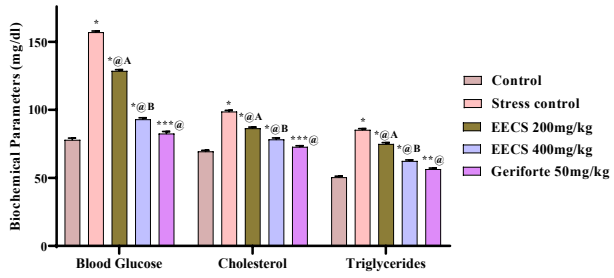


Figure 4: Biochemical parameters affected by cold-induced stress in mice treated with EECS.

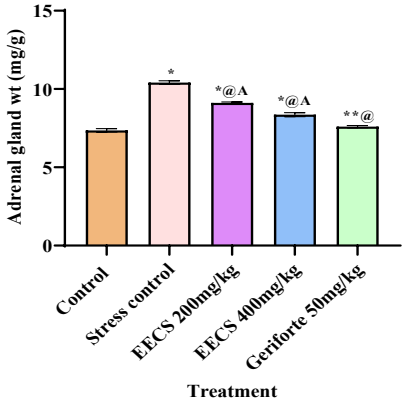


Figure 5: Effect of EECS on the adrenal gland in the cold-induced stress model.

Mean standard error of the mean (n=6) is provided. ANOVA was used, and then post hoc test was used to determine statistical significance in comparisons to the disease control group (@=p<0.0001), control group (*=p<0.0001, **=p<0.05), and the gold standard (A=p<0.0001).

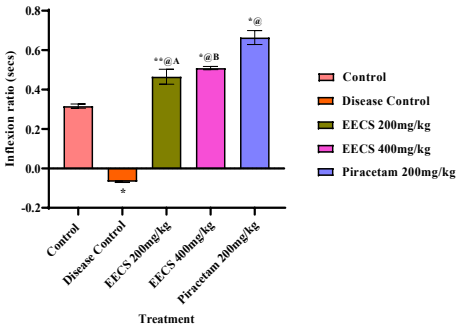
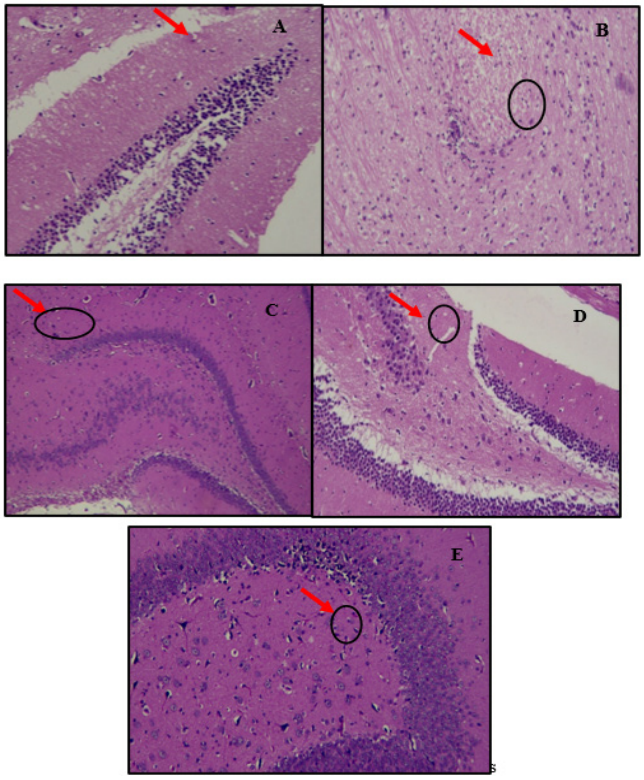


Figure 6: Changes in inflexion ratio caused by EECS in mice exposed to scopolamine to induce amnesia.

Mean standard error of the mean (n=6) is provided. ANOVA was followed by posthoc test to determine statistical significance for comparisons to the disease control group (@=p<0.0001), control group (*=p<0.0001, **=p<0.0005), and the standard (A=p<0.0001, B=p<0.001).

Effect of EECS on Transfer Latency by Elevated Plus Maze

Figure 6 displays the impact of EECS on the transfer delay and inflexion ratio in the elevated plus maze. On the eighth day of medication treatment, the initial latency's transfer latency



= Vaculoated cells (VC); = Pyramidal cells (PC)

Figure 7: Histopathology of mice brain in scopolamine induced amnesia.

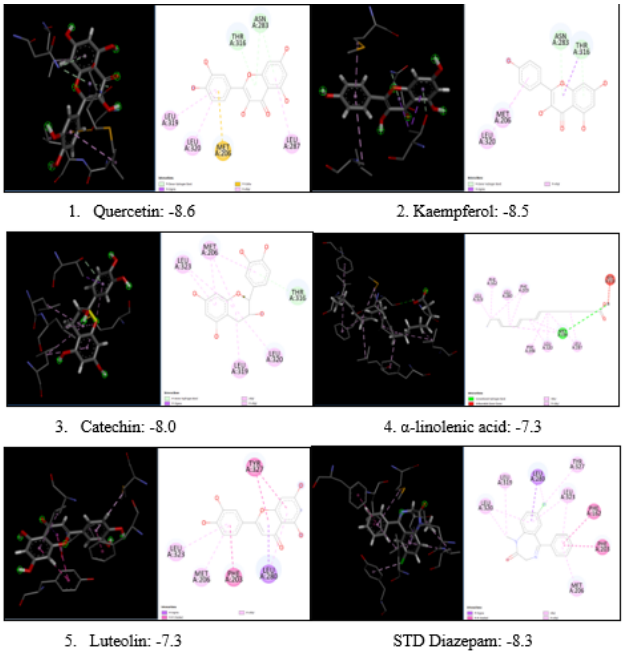


Figure 8: Docking interaction with corticotropin-releasing factor receptor-1

(TL) showed the animals' ability to learn. In dissimilarity, the final latency (9 days later) mirrored the ability to remember what was learned. The inflexion ratio was calculated using transfer times. The inflexion ratio of untreated animals in

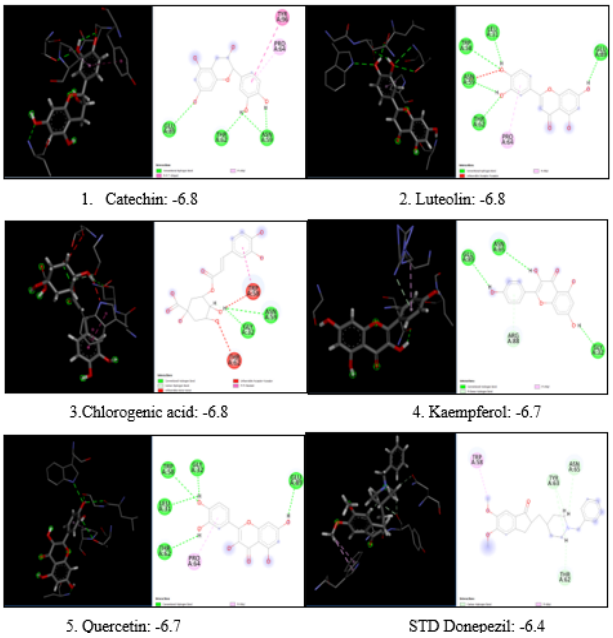


Figure 9: Docking interactions with acetylcholinesterase enzyme

the current study was 0.316 ± 0.0108. The retention transfer latency of animals in group II, who were given 0.4 mg/kg *i.p.* scopolamine, was significantly longer than that of animals in the control group. The inflexion ratio was found to be significantly lower (-0.06806 ± 0.0033) in group II animals compared to group I. Scopolamine, at a dose of 0.4 mg/kg, dramatically affected memory recall in group II rats, as seen by a decreased inflexion ratio, suggesting the induction of amnesia. Mice in groups III and IV that were pre-treated with EECS at 200 and 400 mg/kg, respectively, for eight days prior to the administration of scopolamine showed a significant increase in inflexion ratio compared to group II mice that had amnesia produced by scopolamine. Animals given extract at 200 mg/kg body weight orally had an inflexion ratio of 0.465 ± 0.0378; those given extract at 400 mg/kg body weight orally had an inflexion ratio of 0.509 ± 0.00816. An improved inflexion ratio is an indicator of better memory. A higher inflexion ratio after extract treatment was associated with enhanced cognition and memory, and this effect was dose-dependent. Mice in group V which were given piracetam 200 mg/kg body weight orally had an inflexion ratio of 0.664 ± 0.03556, which was statistically significant when compared to scopolamine-induced mice.

Histopathological Studies

Examining scopolamine-induced hippocampal histopathology in a mouse model of amnesia. Haematoxylin and eosin staining revealed pathological alterations shown in Figure 7.

- Healthy control mice have pyramidal or neuronal cells in their hippocampus.
- Scopolamine 0.4 mg/kg treated mice have fewer pyramidal cells, amyloid plaque deposition, inflammatory infiltration, increased pyknosis, necrosis, and vacuolated cells (circle) in the hippocampal area.
- Partial repair of pyknosis, and vacuolated cells (circle), pyramidal cells (mild proliferation of hippocampus neurons) (arrow) and significant inflammatory infiltration, necrosis in the hippocampal region following EECS 200 mg/kg compared to disease control animals.
- Compared to disease control rats, EECS 400 mg/kg in the hippocampus repaired deteriorated mild inflammatory infiltration, pyramidal cells (arrow), necrosis, pyknosis, and vacuolated cells (circle).
- Piracetam (200 mg/kg) reduced necrosis, pyknosis, inflammatory infiltration and vacuolated cells in the hippocampus, exposing a well-organized zone with numerous pyramidal cells ($p < 0.05$).

In-silico or Molecular Docking Studies

Virtual docking studies for the best five hits obtained from the mcule.com server to deepen our knowledge of ligand-target interactions in Table 5, 6 and Figure 8,9.

Corticotropin-releasing Factor Receptor-1(CRF-1) Inhibition

4K5Y (X=-40.442375, Y=-20.809792, Z=35.251250)

Acetylcholinesterase Enzyme (ACHE) Inhibition

1E66 (X=-19.209643, Y=85.031286, Z=50.795071)

DISCUSSION

This research shows that, the evaluation of *in-vitro* AChE inhibition, *in-vivo* cognitive enhancement, and *in-vivo* anti-stress effects of EECS was done along with the *in-silico* studies. Plant-established drugs are obtaining much attention, with the source of unique chemical characterization and pharmacological experimentation techniques. The accumulation of fibrillar amyloid was significantly reduced by polyphenols (mostly flavonoids).[21] In cold stress, EECS is effective in normalizing stress parameters such as hyperglycemia, plasma cholesterol, triglycerides, and adrenal hypertrophy dose-dependently. Excess cortisol synthesis in response to stress (chronic cold restraint stress) raises plasma glucose by stimulating the release of other adrenal hormones, including catecholamines.[22] Flavonoids, glycosides, triterpenoids, phytosterols (sitosterol a steroid), and phenolic compounds are the primary secondary metabolites in plants that alleviate stress.[4] Treatment of stressed animals with EECS at 200 and 400 mg/kg may reduce biochemical levels because quercetin acts on the hypothalamus to reduce CRF.[23] The high concentrations of components in the extracts suggest they may mediate the extracts' anti-stress effects. The binding of cortisol to the glucocorticoid receptor initiates a pathway that regulates the production of specific stress-response proteins. Stress's physiological impacts, such as sickness, inflammation, and anxiety, can be traced back to this downstream expression, which the glucocorticoid receptor signs.

High quantitative and qualitative correlations were found between total phenolic and flavonoid levels and acetylcholinesterase inhibitory activity. The EECS was

effective in AChE inhibition in different concentrations when compared with standard donepezil dose-dependently. Phytochemical analysis of EECS revealed the presence of triterpenes, steroids, phenolics, flavonoids, and coumarins. Scopoletin, kaempferol, and quercetin are three of these chemicals that have been found to have numerous biochemical actions. A variety of plant chemicals, including alkaloids, xanthones, and flavonols like quercetin, have been discovered to decrease acetylcholinesterase activity. Polyphenol-rich food consumption has been intriguingly associated to a reduced risk of age-related neurodegenerative illnesses such macular degeneration and dementia.[3,4] Phenolic compounds have powerful antioxidant properties that can donate hydrogen, chelate metal ions, scavenge free radicals, and interrupt radical chain reactions, all of which contribute to lowering stress and improving memory and learning. Quick recall can be evaluated with EPM. The EPM method has been widely utilized to study memory and learning in rodents. In mice, the effectiveness of memorizing and remembering was measured by estimating their transfer latency time (TLT) by utilizing elevated plus-maze that can serve as the exteroceptive and interoceptive behavioral model. Scopolamine induces amnesia in experimental mouse models by muscarinic (cholinergic) ACh receptor antagonists that can impair cholinergic neurotransmission and learning and memory. As shown in Figure 1, scopolamine impairs learning and memory in the EPM by increasing TL and lowering IR.[16] Eight days of EECS pretreatment superior retention of information in normal mice, as measured by an increase in IR and a decrease in TL (without scopolamine). *In-silico* docking studies displayed the most promising antistress and cognitive effect in clue by indicating the highest interaction docking scores of ligands with CRF-1 and AChE proteins.

CONCLUSION

The present data suggest EECS has shown effectiveness in reducing stress (as Adaptogen) and amnesia by enhancing cognition studied in rodent models significantly and along with *in-silico* studies. Further isolation of active constituents, identification, and confirmation of exact mechanism, additional research is needed.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee of GRCP approved the research entitled "Pre-clinical evaluation and *in-silico* docking of *C. sativum* on stress and cognitive deficits in rodents" with Regd number. 1175/PO/Re/S/08/CPCSEA.

REFERENCES

1. Kulkarni MP, Juvekar AR. Attenuation of Acute and chronic restraint stress induced perturbations in experimental animals by *Nelumbo nucifera Gaertn*. Indian Journal of Pharmaceutical Science, 2007;70 (3):327-33.
2. Siripurapu KB, GuptaP, Bhatia G, MauryaR,NathaC, PalitG. Adaptogenic and anti-amnesic properties of *Evolvulusalsinoides* in rodents. Pharmacology Biochemistry and Behavior.2005;81(3): 424-32.
3. AbulHMd, PervinM, LimBO. Acetylcholinesterase Inhibition and *invitro* and *in vivo* antioxidant activities of *Ganoderma lucidum* grown on germinated brown rice. Molecules. 2013;18:6663-6678.
4. Singh SK, Patra A. Evaluation of the adaptogenic potential of *Polygonatum cirrhifolium*(Wall.) Royle: *In vitro*, *in vivo,* and *in silico* studies. South African Journal of Botany.2019;121:159- 177.
5. Shyamapada M, Manisha M. Coriander (*Coriandrum sativum* L.) essential oil: Chemistry and biological activity. Asian Pacific Journal of Tropical Biomedicine.2015;5(6): 421–428.
6. Dr EsmailAA. A review on chemical constituents and pharmacological activities of *Coriandrum sativum*. IOSR Journal of Pharmacy. 2016;6(7):17-42.
7. Kadek SA, Elisabeth A, Kadek NBP, Fiorenza JT, Gusti NJAP. Antibacterial activity testing of hand sanitizer gel extract of *Coriandrum sativum* seeds against *Staphylococcus aureus*, Journal of Pharmaceutical Science and Application.2021;3(2):98-107.
8. Dharmalingam R and Nazni P. Phytochemical evaluation of *Coriandrum sativum* flowers. International journal of food and nutritional science.2013;2(4):34-39.
9. Nimish P, KastureSB, BhattNM. Phytochemical screening of *Coriander sativum L*inn. International Journal of Pharmaceutical Sciences Review and Research.2011;9(2):159-163.
10. Priyanka S, Geetha S, Virendra S, Kshipra M &Shashi BS. *In vitro* antioxidant profiling of sea buck thorn varieties and their adaptogenic response to high altitude-induced stress), International Journal ofBiometeorol.2015;59(8):1115-26.
11. Sangeeta S, Vrunda V.Quantitative and Qualitative Analysis of Phenolic and Flavonoid Content in *Moringaoleifera Lam* and *Ocimum tenuiflorum L*. A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products. 2016;8(1):16-21.
12. Xinchi F, Xin W, Youping L and Xin D. *Linarin* Inhibits the Acetylcholinesterase activity *In-vitro* and Ex-vivo. Iranian Journal of Pharmaceutical Research.2015;14(3): 949-954.
13. Suganthy N and Pandima DK. *In vitro* antioxidant and anti-cholinesterase activities of *Rhizophora mucronate*. Pharmaceutical Biology.2016;54(1):118-129.
14. Prafulla PA, Pranita PJ, Shirishkumar DA, Bhaskar VH, and Tushar S. Adaptogenic Activity of Lyophilized Hydroethanol Extract of *Pandanus odoratissimus* in Swiss Albino Mice. Hindawi Publishing Corporation, International Scholarly Research Notices.2014;Article ID 429828:1-10.
15. Arif M, Sheeba F, Talib H, Ali M. Adaptogenic activity of lanostane triterpenoid isolated from *Carissa carandas* fruit against physically and chemically challenged experimental mice. Pharmacognosy Journal.2013;5:216-220.
16. Pahaye DB, Elisabeth NB, Germain ST, Gwladys TN, Neteydji S, Fleur COM, Nadège K, Stephanie JKN, Gisele N, Antoine K, Jean PO, Veronique M, and Josiane LO. Neuroprotective and Antiamnesic Effects of *Mitragyna inermis*Willd (*Rubiaceae*) on Scopolamine-Induced Memory Impairment in Mice. Behavioural Neurology. 2017; 5952897.
17. LoizzoMR, Tundis R, MenichiniF, BonesiM, StattiGA, DeguinB, TillequinF, MenichiniFand HoughtonPJ. Acetyl-cholinesterase Inhibition by Extracts and Isolated Flavones from *Linaria reflexa* Desf. (*Scrophulariaceae*). Natural Product Communications.2007;2(7):759 – 763.
18. Vidal-LimonA, Aguilar-ToaláJE, LiceagaAM.Integration of Molecular Docking Analysis and Molecular Dynamics

Simulations for Studying Food Proteins and Bioactive Peptides. Journal of Agricultural and Food Chemistry.2022;70(4): 934-943.

19. Kehne JH. The CRF1 receptor, a novel target for the treatment of depression, anxiety, and stress-related disorders. CNS Neurological Disorders Drug Targets. 2007;6(3): 163-82.
20. Čolović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, and Vasić VM. Acetylcholinesterase Inhibitors: Pharmacology and Toxicology.Current Neuropharmacology, 2013;11(3): 315–335.
21. Dominik S. Anticholinesterase Activities of Selected Polyphenols – a Short Report. Polish Journal of Food and Nutrition Sciences. 2013;64(1):59-64.
22. Owolabi TA, Ezenwa KC, Olayioye EY, Iyorhibe OC, Amodu E, Aferuan OF, PC Okubor, Ayinde BA and Okogun JI. International Journal of Current Microbiology and Applied Sciences. 2019;8(10):2558-2565.
23. Haleagrahara N, Radhakrishnan A, Lee N, Kumar P. Flavonoid quercetin protects against swimming stress-induced changes in oxidative biomarkers in the hypothalamus of rats. European journal of pharmacology. 2009 Oct 25;621(1-3):46-52.