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Neuroprotective effect of *Adiantum capillus-veneris* Linn. on scopolamine induced memory impairment in rats

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ABSTRACT

Alzheimer's disease is an age old disorder occurs due to loss of neurons. It is characterized by the loss of cognitive and motor skills, memory loss and behavioral changes. The currently available FDA approved pharmacological treatments are very limited and offer only symptomatic relief. Simultaneously, they are also associated with adverse effects. Current study aimed to evaluate the nootropic potential of the 50% hydroalcoholic extract of *Adiantum capillus-veneris*, and its isolated compound quercetin against scopolamine-induced amnesia in Wistar rats using MWM and rotarod tests. The study is designed to assess the effects of the extract and quercetin on spatial learning and memory and motor functions. Biochemical analysis quantified the levels of acetylcholinesterase markers of oxidative stress and neuroinflammation in the rat brain. Treatments significantly improved the spatial memory and motor performance by restoring the altered biochemical parameters compared to the control. The findings support the potential of *A. capillus-veneris* as a therapeutic agent for cognitive enhancement and neuroprotection, highlighting its role in managing Alzheimer's disease. This can overcome the rate limiting factor of drug failure in the drug development pipe-line as it act by multi-model-mechanisms. Further research is needed to validate the mechanisms underlying these effects and to assess the clinical applicability of this herbal remedy.

Key Words: Acetylcholinesterase, *Adiantum capillus-veneris*, Alzheimer's Disease, Oxidative stress, Scopolamine.

INTRODUCTION

Alzheimer's disease is a highly prevalent, devastating and progressive neurodegenerative disorder characterized by the decline in cognitive and motor skills, memory loss and behavioral changes. Alzheimer's disease significantly shortens the life span and decreases quality of life among the elderly (Khan et al., 2019; Qiu et al., 2009). Advancing age, genetic factors, vascular risk factors, and psychosocial determinants are certain risk factors which play the crucial roles in the epidemiology of disease (Mayeux et al., 2021; Qiu et al., 2009; Tahami Monfared et al., 2022). Amyloid-beta (Aβ) peptides accumulation and tau proteins hyperphosphorylation is one of the major hallmarks of AD which leads to synaptic dysfunction and neuronal death in the brain of AD patient (Pattanashetti et al., 2017; Bhat et al., 2022). Death tolls with Alzheimer's disease had increased to 146.2% since 2000 to 2018 in the United States, making it the fifth-largest cause of death in American older adults (Zhang et al., 2021).

The cholinergic hypothesis plays one of the crucial roles in the pathophysiology of Alzheimer's disease (AD). The cholinergic neurons which are responsible for the production of Ach, significantly lost in the brain, especially in the basal forebrain of AD patients. Ach is a neurotransmitter which is responsible for memory and learning (Chen and Mobley, 2019). In addition, ACh is also significantly reduced due to over activity of acetylcholinesterase (AChE), the enzyme responsible for ACh breakdown (Pattanashetti et al., 2017; Bhat et al., 2022).

Scopolamine is an anti-muscarinic drug which blocks the neurotransmission in cholinergic neurons, resulting in to memory impairment. Several study reported that scopolamine results in increased oxidative stress and neuroinflammation leading to memory loss (Yadang et al., 2020). The effect of any lead can be tested on cholinergic pathway by administration of scopolamine which will induce cognitive impairment by mimicking the effects that are observed in AD, and treatment will be aimed at restoring the activity of the cholinergic system by inhibiting acetylcholinesterase enzyme and reducing the markers of oxidative stress and neuroinflammation.

Currently, available FDA approved medicines of AD are AChE inhibitors (e.g., donepezil, rivastigmine, and galantamine) and NMDA receptor antagonists (e.g., memantine), provide only the symptomatic relief and have limited efficacy. However, these drugs are associated with

adverse side effects, highlighting the need for the development of safer and more effective therapeutic alternatives (Pattanashetti et al., 2017; Bhat et al., 2022). Surprisingly, no new drug has been approved by FDA for the treatment of AD since 2003. Although, drug-pipeline for the AD is full of plethora of molecules which may either modify or provide symptomatic relief to the AD patient. But the rate of failure in clinical trials is crucial and rate limiting factor for the development of new molecule.

Natural products, particularly plant-derived compounds, have gained increasing attention as potential sources for the discovery of novel anti-AD agents (Bhat et al., 2022; Bakhsh et al., 2024). *Adiantum capillus-veneris* (ACV), commonly known as the maidenhair fern, is an herb that has been traditionally used as a 'Rasayana' in Ayurvedic system of medicine (Dehdari and Hajimehdipoor, 2018; Olimat, 2020; Das et al., 2022). Rasayana refers to a class of herbs, minerals, and other natural substances that are believed to have rejuvenating and restorative properties. Rasayana herbs are considered to promote longevity, enhance physical and mental capabilities, and increase resistance to disease. Rasayana balance the normal physiology of the body by modulating 'Neuro-endocrino-immune' system (Govindarajan et al., 2005). To meet the drastically increased urgent need for the of global burden of Alzheimer's disease study aimed at evaluating the memory enhancing potential of 50% hydroalcoholic extract of Rasayana herb, *A capiluus-veneris* and its isolated compound quercetin against scopolamine-induced amnesia in rats.

MATERIALS AND METHODS

Animals

Adult male Wistar albino rats (250-300 gm) were used in the study. Animals were obtained from the National Institute of Pharmaceutical Education and Research, Mohali. Rats were randomly distributed into different experimental groups and kept at comfortable temperature of 25 ±1°C and 45–55% RH, with a 12-h light/dark cycle. The animals were given free access to food and tap water ad libitum. Experiments were conducted between 9:00 and 14:00 h. The behavioural tests were performed during the day time. Animals were acclimatized to the laboratory conditions for at least 7 days prior using them for experiments and exposed only once to every

experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) IAEC/HIP/2022-23/1032(A).

Chemicals and Reagents

Scopolamine hydrobromide, piracetam, acetyl thiocholine iodide (ATCI), 5,5-dithiobis-2-nitrobenzoate ion (DTNB), bovine serum albumin (BSA), carboxy methy cellulose (CMC) trisfamino methane hydrochloride (Tris-HCl), ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide phosphate (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), trichloroacetic acid (TCA), thiobarbituric acid (TBA) and trichloroacetic acid all were purchased from Sigma-Aldrich, USA. Unless otherwise specified, all other chemicals were of analytical grade.

Plant Material

The aerial parts of *Adiantum capillus-veneris* were collected from Kempty falls, Mussoorie, Uttarakhand in the month of July and authenticated by Botanical Survey of India, Dehradun with herbarium sheet no. 115873.

Preparation of Extract

The aerial parts of *Adiantum capillus-veneris* were air dried at room temperature and powdered. The powdered plant material (25 g) was placed in a Soxhlet apparatus and sequentially extracted with petroleum ether, hexane and 50% hydroalcohol. The extracts were concentrated under reduced pressure and stored in the refrigerator until further use. Based on the results obtained from the activity-guided fractionation of 50% hydroalcoholic extract using in vitro antioxidant and acetylcholinestrase inhibitory activity, quercetin was isolated (results are not given here) and further projected for the evaluation of anti-amnesic potential.

Experimental Protocol and Drug Administration

Male Wistar albino rats were used in each group in the number of six. 50% Hydroalcoholic extract of ACV was administered as aqueous solution and quercetin was administered as 0.5% CMC solution orally as given in the Table 1, once in daily for ten consecutive days (7 days as pretreatment and 3 days as posttreatment to scopolamine). Piracetam (500 mg/kg, i.p.) was used

as the standard nootropic agent and was administered to one group of rats, 30 min before experiments (Day 8th) for comparison. Control rats were treated with the 0.5% CMC solution. Scopolamine was given to produce amnesia in 30 min before subjecting to behavior tests (Kumar *et al*., 2000).

Table 1: Treatment Protocol

Group	**Treatment**
Group 1 (Control)	0.5% CMC
Group 2 (Negative Control)	Scopolamine (1 mg/kg s.c.)
Group 3 (Test 1a)	50% HA extract of ACV at 100 mg/kg p.o. + Scopolamine
Group 4 (Test 1b)	50% HA extract of ACV at 150 mg/kg p.o. + Scopolamine
Group 5 (Test 2a)	Isolated quercetin at 50 mg/kg p.o. + Scopolamine
Group 6 (Test 2b)	Isolated quercetin at 100 mg/kg p.o. + Scopolamine
Group 7 (Standard)	Piracetam at 500 mg/kg, i.p. + Scopolamine

Morris Water Maze Test

The MWM task has been widely used to study learning and memory in rats (Morris et al., 1984; Davoodi et al., 2009). Maze was designed as semi spherical pool (150 cm in diameter, 43.18 cm deep) filled with water to a depth of approximately 31.48 cm and water was made opaque with the colour. It was divided into four equal quadrants and a platform (10.5 cm^2) was submerged 1 cm below the opaque surface in the centre of one of the quadrants. The pool was placed in a test room where many cues (e.g., pictures, lamps, etc.) were kept external to the maze they were visible from the pool, which animals can use for spatial orientation. The position of the cues was not changed through the entire task. Escape latency and time spent in target quadrant was recorded by live scoring. Video tracking system was not used.

a) Habituation

All the rats subjected to MWM test were habituated to the pool 24 hours prior (day 7th) to the start of training by allowing them to perform a 60 seconds swim without the platform.

b) Spatial reference memory (SRM) testing in the Morris water maze

On day 8, prior subjecting the rats to SRM version of MWM task, rats were treated with Piracetam (500 mg/kg, i.p), Scopolamine (1 mg/kg, s.c), two doses of 50% hydroalcoholic extract of ACV (100 and 150 mg/kg, p.o) and quercetin (50 and 100 mg/kg, p.o). After treatment, all the rats were placed in the water individually by hand facing the wall of the pool and were allowed for 60 seconds to find the hidden platform on 8th day. The successful rat was given 20 sec on the platform to observe the spatial cues. If the rat failed to find the platform within 20 sec, it was guided there by hand. Before releasing the rat from the next start position, the animal was rotated. Animals had six trials per day (separated by 10 min), for three consecutive days (day 8 to 10). In each trial the animal was released from a different start point in the pool, but the escape platform was kept on the same position (at the center of the 4th quadrant). After completion of three days of training (day 8 to 10), the animals were returned to their home cages until the retention testing (probe trial) 24 hours later on day 11. The probe trial consisted of a 60 seconds free swim period without a platform in which the time spent in the target quadrant was recorded by live scoring as shown in Figure 1 (Rubio et al., 2007; Solanki et al., 2012).

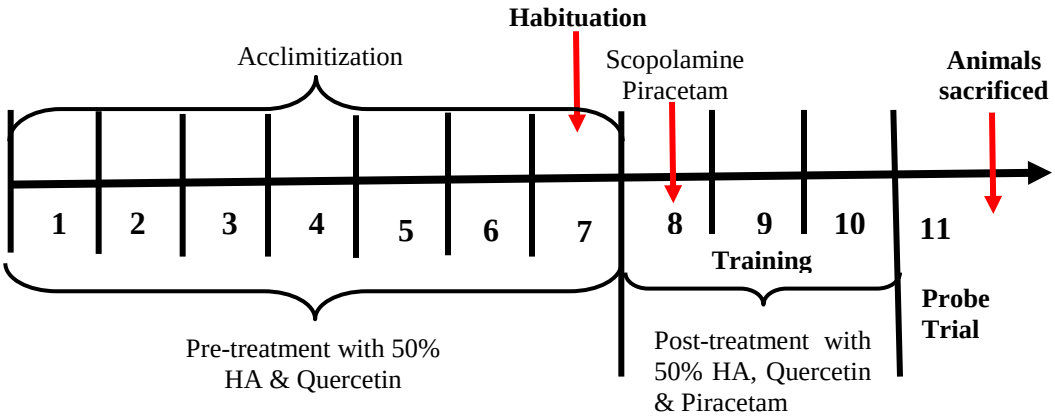

Figure 1: Schematic Representation of Experimental Protocol.

Rota rod Test

Rotarod apparatus (Orchid Scientific, India) with an automatic timer was used for the motor co-ordination and motor memory test. The diameter of the rod was 3 cm with a rippled surface. The animals were allowed to adjust their posture on the rotating rod with 4 to 20 rpm (rotation per minute). Over the course of five minutes, each rat received three trials, with the experiment's end time of 180 s being maintained. The latency to fall in a test session of 180 s was taken as a measure of motor coordination and motor memory. The training session was of 3 days divided into two parts based on the speed of rotating rod: training days 1 and 2 (day 8 and 9 of experiment); and training day 3 (day 10 of experiment). The training was started by placing the rats on the rotating rod at the speed of 4 to 12 rpm (training days 1, 2) or from 4 to 20 rpm (day 3). Animals were allowed to maintain their posture on the rotating rod. The cut off time was of 180 s. It was followed by the probe testing on day 4 by placing the trained animals on the rotating rod at the speed of 20 rpm. The time to fall from the rotating rod was noted (Janakiraman et al. 2016; Pant et al., 2024).

Estimation of Biochemical Parameters

Brain Tissue Preparation

Twenty-four hours after the end of treatment, biochemical analysis was performed. The animals were sacrificed by the cervical decapitation. Brain was removed rapidly and rinsed with 0.1 M/L phosphate buffer saline (pH 7.4) and homogenized in the same buffer. The homogenate was centrifuged at 10,000×g for 20 min at 4 °C, and aliquots of supernatant were separated and used for the following biochemical estimations:

Acetylcholinestrase Assay in Brain

The effect of HACV and isolated quercetin on AChE enzyme activity was calculated as as per the modified method of Ellman et al., (1961) in supernatant of brain homogenate. The rate of development of colour to yellow was used to measure the activity of AChE. Change in the absorbance per minute due to the development of the colour in the aliquot was measured at 410 nm. The enzyme activity was expressed as the 'n' moles of substrate hydrolyzed/minute/mg of

protein (Ahemed et al., 2009). The protein contents were determined in the brain samples using Lowry (1951) method.

$$R = \delta OD/E \times \text{mg of protein}$$

where R is the rate of enzyme activity in 'n' mole of acetylthiocholine iodide hydrolyzed per minute per mg of protein. δOD is the change in absorbance per minute and E is the extinction coefficient, which is 13600 M^{-1} cm^{-1} .

MDA level in Brain

Malondialdehyde (MDA) is the product of lipid peroxidation in the biological system. The MDA level in brain homogenate was measured by Buege and Aust method by measuring the thiobarbituric acid reactive substances (TBARS) formed by (Fleischer and Packer 1978). The pink colour developed was read immediately at 532 nm by using a systronic-117 UV–visible spectrophotometer. TBARS concentration was calculated by using the molar extinction coefficient of chromophore (1.56× 105 (mol/l)−1 cm−1) and the values are expressed in µmoles/mg protein (Rao et al., 2021)**.**

GSH level in Brain

The level of Glutathione (GSH) in brain homogenate was measured by the method given by Ellman, (1959). 1 ml of supernatant was precipitated with% sulfosalicylic acid (1 ml). It was then cold digested at 4 °C for 1 h and centrifuged at 4 °C for 10 min at 2000×*g* after 5 min. the reacted was further followed by the addition of 0.2 ml DTNB (0.1 mM, pH 8.0) and 2.7 ml phosphate buffer (0.3 M, pH 8.0) to the 0.1 ml of the supernatant. The absorbance was read at λ_{max} = 412 nm using a UV-visible spectrophotometer, and GSH was quantified using a molar extinction coefficient:

$$\varepsilon = 1.36\times10^4\ M^{-1}cm^{-1}$$

of the chromophore and expressed as micromoles of GSH per milligram of protein (Khurana et al., 2021).

GST level in Brain

The glutathione-S-transferase (GST) activity was measured in brain homogenate by the method reported by Habig et al., 1974. Briefly, the assay was initiated by mixing 1.75 ml of phosphate buffer, 0.1 ml of reduced GSH, 0.05 ml of CDNB as substrate and 0.1 ml of brain homogenate. A blank sample was run same as above reagents except the brain homogenate. The increase in the absorbance was recorded at 340 nm for 5 min at 1-min interval using spectrophotometer (Model UV-1650PC). The results were expressed as nmol of CDNB conjugated/min/mg protein (Khan et al., 2013).

SOD level in Brain

The level of superoxide dismutase (SOD) enzyme was measured by the method of autoxidation of pyrogallol by Marklund & Marklund in brain homogenate. The assay was started by treating the 0.1 ml of brain homogenate with 2.5 ml of Tris–HCl buffer (pH 8.2), 0.1 ml of 1 mM EDTA (ethylene diamine tetra acetic acid), 0.5 ml of 1 mM DTPA (diethylene triamine penta acetic acid) and 0.1 ml of 0.02 mM pyrogallol. Kinetic end point method was used for measuring the SOD activity, where change in the autoxidation of pyrogallol was observed for 3 min for each sample at 420 nm. The control sample was run same as above without tissue homogenate (Rao et al., 2021).

CAT level in Brain

The Catalase (CAT) activity was measured in the supernatant as per the method described by Claiborne (1985). The assay was initiated by mixing 0.05 ml supernatant (10%), 1 ml hydrogen peroxide (H_2O_2) (0.02 M, prepared in 0.05 M phosphate buffer), and 1.95 ml phosphate buffer (0.05 M, pH 7.0) in a final volume of 3 ml. Change in absorbance was noted at λ_{max} = 240 nm using spectrophotometer for 3 min at 30 s interval. Enzymatic activity (micromole H_2O_2 decomposed/min/mg protein) was determined using the molar extinction coefficient of ε = 43.6 $M^{-1}cm^{-1}$ (Khurana et al., 2021).

Total Nitrites level in Brain

Total nitrites are measured as the indicator of inflammation in brain and were estimated as per the method described by Sastry et al. (2002). The assay was started by mixing 0.4 ml of

carbonate buffer (pH 9.0), 0.1 ml of supernatant of brain homogenate, and 150 mg Cu–Cd alloy. The mixture was incubated for 1 h at room temperature resulting in reduction of nitrate to nitrite. It was followed by addition of 0.1 ml sodium hydroxide (0.35 M) for stopping the reaction. Further, for the deproteination, 0.4 ml of zinc sulfate solution (120 mM) was added, and allowed to stand for 10 min. The mixture was then centrifuged at 4000×*g* for 10 min. To 0.1 ml of supernatant, 0.5 ml of Griess reagent (1:1 solution of 1% sulfanilamide in 3.0 M HCl and 0.1% *N*-1-napthylethylenediamine dihydrochloride in water) was added and incubated for 10 min at room temperature in the dark. This step converts nitrite into colored azo compound (R–N = N–R'). The absorbance was noted at λ_{max} = 548 nm wavelength using a spectrophotometer. The standard curve of sodium nitrite (10–100 μM) was prepared to quantify the concentration of the test sample of nitrite, and the results were reported as micromoles per milligram of protein (Khurana et al., 2021).

Protein Estimation

Measurement of protein in the brain homogenate was measured by slight modification in the method provided by Lowry et al (1951). The tissue homogenate was 10X diluted with water. To the diluted tissue homogenate, 4.5 ml of alkaline copper reagent was mixed and allowed to rest at room temperature for 20 min. It was then followed by addition of 0.5 ml Folin's phenol reagent and mixture was well agitated. The standards and blank were handled in the same way. After 15 minutes, the blue complex was developed and it was measured at 640 nm. The amount of protein in a sample is given as mg/mL. Bovine Serum Albumin (BSA) was used as standard at 100 μg/ml concentration.

Statistical Analysis

The experimental results were expressed as the mean ±SEM of triplicate measurements. The data were subjected to one-way analysis of variance with level of significance $P \leq 0.05$ which was calculated by Graph Prism pad version is 10.3.0.

RESULTS AND DISCUSSION

Effect of HACV and Quercetin on Spatial Learning and Memory of Rats in MWM Test

The MWM model is used for testing the spatial memory in animals (Ruan and Yao 2020). The 50% hydroalcoholic extract of the *A capillus-veneris* and isolated quercetin exhibited significant ($P<0.05$) memory enhancing potential against scopolamine-induced amnesia in MWM test in rats by restoring the increased transfer latency to target quadrant during acquisition trial (training) on day 8^{th}, 9^{th} and 10^{th} as shown in Table 2. Transfer latency is the time taken by rats to locate the platform placed in the target quadrant. Transfer latency is considered as the indicator of spatial learning as the animals take the help of spatial clues kept in the room for locating the hidden platform placed in the target quadrant of MWM. Food was placed as a reward on the platform to encourage the rats. Scopolamine is a muscarinic acetylcholine receptor antagonist which produces cognitive decline as a result of cholinergic dysfunction caused by increased amyloid β deposition (Chen and Yeong 2020).

Table 2: Effect of HACV and isolated Quercetin on Escape Latency of Rat in MWM Test

Treatment	Escape latency (sec) (Mean ±SEM)		
	Day 1 (8^{th} Day)	Day 2 (9^{th} Day)	Day 3 (10^{th} Day)
Control (Group 1)	35.50±0.78	33.40±0.67	29.87±0.92
Scopolamine (Group 2)	58.17±0.44	55.17±0.44	50.17±1.17
Scopolamine+HACV-100 mg/kg (Group 3)	46.7±0.66	41.7±1.12	35.37±0.90
Scopolamine+HACV-150 mg/kg (Group 4)	38.37±0.62	33.37±0.67	28.37±2.75
Scopolamine+quercetin-50 mg/kg (Group 5)	28.03±0.69	26.37±0.43	22.37±0.38
Scopolamine+quercetin-100 mg/kg (Group 6)	25.83±0.73	24.17±0.60	16.50±1.76
Scopolame+Piracetam-500mg/kg (Group 7)	26.1±0.78	25.43±0.56	23.77±0.43

Similarly, the time spent by the rats in the target quadrant in search of platform was taken as the indicator of spatial memory during probe trial on day 11^{th}. Both the extract and isolated quercetin significantly increased the time spent by rats in the target quadrant dose-dependently which was

reduced by the administration of scopolamine as shown in the Table 3. The highest time spent in the target quadrant was reported with piracetam followed by quercetin and HACV.

Table 3: Effect of HACV and isolated Quercetin on TSTQ by Rat in MWM Test

TREATMENT	**Time spent in target quadrant (sec) on Day 4 (Day 11th) (mean±SEM)**
Control (Group 1)	31.67±0.88
Scopolamine (Group 2)	15.33±0.88
Scopolamine+HACV-100 mg/kg (Group 3)	40.10±1.07
Scopolamine+HACV-150 mg/kg (Group 4)	45.23±0.96
Scopolamine+quercetin-50 mg/kg (Group 5)	48.07±0.58
Scopolamine+quercetin-100 mg/kg (Group 6)	51.67±0.88
Scopolame+Piracetam-500mg/kg (Group 7)	53.3±1.35

Effect of HACV and Quercetin on Motor Function of Rats in Rotarod Test

Rotarod test is widely used to study motor coordination in rodents. Poor motor function is often associated with dementia (Nichols and Rabin 2024). Rotarod test is a simple, sensitive and quantifiable test for screening the neuroprotective potential (Ruan and Yao 2020). Both HACV and isolated quercetin attenuated the scopolamine-induced motor incoordination in rats by increasing the fall of time of animals from the rotating rod during acquisition and probe trials in dose-dependent manner. Highest motor activity was reported with piracetam followed by isolated quercetin and HACV extract as shown in the Table 4.

Table 4: Effect of HACV and isolated Quercetin on Fall of Time of Rat in Rotarod Test

Treatment	**Fall of Time (sec) (Mean ±SEM)**		
	Day 1 & 2 (8th & 9th Day)	**Day 3 (10th Day)**	**Day 4 (11th Day)**
Control (Group 1)	85.58±0.71	86.25±0.63	87.92±1.04
Scopolamine (Group 2)	19.03±2.00	22.37±0.69	26.37±0.33
Scopolamine+HACV-100 mg/kg (Group 3)	39.03±0.22	43.37±2.77	50.03±1.45
Scopolamine+HACV-150 mg/kg (Group 4)	45.37±1.72	48.37±3.64	60.03±1.19
Scopolamine+quercetin-50 mg/kg (Group 5)	49.03±0.92	55.36±0.99	73.37±1.45
Scopolamine+quercetin-100 mg/kg (Group 6)	55.83±0.73	59.17±0.60	78.50±0.50
Scopolame+Piracetam-	81.10±1.00	82.43±0.50	82.43±0.50

500mg/kg (Group 7)			

Effect of HACV and Quercetin on Biochemical Parameters in Rat Brain

Study of biochemical parameters provides an idea of probable mechanism of action of the given treatment. All the treatments significantly restored the scopolamine- induced alterations in the activity of AChE, GST and SOD enzymes; and levels of MDA, GSH and nitrites in the rat brain homogenate in dose-related manner as shown in Table 5. The highest restoration was noted with piracetam followed by quercetin and 50% hydroalcoholic extract. The results suggest that the both extract and isolated quercetin exhibited anti-Alzheimer's potential through cholinergic, oxidative and inflammatory pathways by affecting their mediators in brain.

Table 5: Effect of HACV and isolated Quercetin on Biochemical Parameters of Rat Brain

Treatment	AChE	MDA	GSH	GST	SOD	CAT	Nitrites
Control (Group 1)	11.63±0.30	0.11±0.01	20.33±0.70	122.00±1.67	13.46±0.52	12.42±0.94	14.83±4.64
Scopolamine (Group 2)	28.14±0.60	0.61±0.01	12.43±0.53	78.96±1.33	07.46±0.26	07.33±0.30	12.43±0.53
Scopolamine+HACV-100 mg/kg (Group 3)	23.17±0.37	0.53±0.01	14.4±0.45	85.23±0.85	08.63±0.29	08.37±0.34	09.40±0.54
Scopolamine+HACV-150 mg/kg (Group 4)	20.17±0.49	0.47±0.00	17.23±0.92	95.83±0.56	09.20±0.14	09.30±0.33	07.73±0.49
Scopolamine+quercetin-50 mg/kg (Group 5)	18.23±0.32	0.36±0.01	19.30±0.43	98.47±0.27	11.97±0.66	09.90±0.68	06.60±0.12
Scopolamine+quercetin-100 mg/kg (Group 6)	16.10±0.54	0.26±0.00	22.5±0.63	111.47±1.18	12.21±0.42	10.9±0.50	05.30±0.31
Scopolame+Piracetam-500mg/kg (Group 7)	12.38±0.26	0.22±0.00	26.23±0.59	124.07±1.28	12.66±0.38	11.13±0.66	04.50±0.26

The values are expressed as: AChE (nmol/min/mg protein), MDA (µmoles/gm protein), GSH & Nitrites (µmoles/mg protein), GST (nM/min/mg protein), SOD & CAT (U/mg protein**)**

Cholinergic system in brain is responsible for cognitive function. Activity of acetyltransferase (ChAT) and acetyl cholinesterase (AChE) is altered in the brain of AD patient leading to altered cholinergic transmission. AChE is responsible for breakdown of acetylcholine (ACh), a key neurotransmitter of cholinergic system, leading to reduced cholinergic system activity in the brain (Orta-Salazar et al., 2014). Both, HACV and isolated quercetin restored the scopolamine-induced increased activity of AChE enzyme in rat brain suggesting its mechanism via cholinergic system.

Several studies suggested that scopolamine increases oxidative stress in the brain of animals (Ton et al., 2020). As all the treatments increased the levels of GSH, GST, CAT, SOD and reduced the level of MDA in the brain homogenate of treated rats suggests that the HACV and isolated quercetin acts by reducing the oxidative stress (Rao et al., 2021).

Previous studies suggests that scopolamine elevates levels of nitrites due to the increased activity of nitric oxide synthase (NOS) especially iNOS in the brain resulting in the activation of neuroinflammatory cascade. In the present study, total nitrite content was significantly increased in the brain of rat after scopolamine administration. All the treatments significantly restored the elevated level of nitrites suggesting their mechanism of action by suppressing neuroinflammatory pathway (Khurana et al., 2021).

CONCLUSION

The study concluded that the 50% hydroalcoholic extract of *Adiantum capillus-veneris* and its isolated compound, quercetin, showed significant memory-enhancing and neuroprotective potential against scopolamine-induced cognitive impairment in rats using MWM and Rotarod tests. The effects thus observed may be due to the inhibition of acetylcholinesterase enzyme activity and the reduction of oxidative stress and neuroinflammatory markers, supporting the cholinergic, oxidative and neuroinflammatory hypothesis of Alzheimer's disease. These findings validate the one of traditional use of *A. capillus-veneris* as Rasayana in memory enhancing potential. The currently used three US FDA approved drugs for the treatment of AD act only by single mechanism and have many side effects. However, this ‘Rasayana’ plant can target multiple pathways resulting in development of AD. Thus, it can prove to be an asset in the treatment of AD. Future research should focus on re-validation of these mechanisms of action of Rasayana through different models; so that memory enhancing potential of this Rasayana can be successfully translated in humans.

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