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Albiflorin Alleviates Neurocognitive Impairment in L-Methionine-Induced Vascular Dementia through FoxO1/SIRT1/RANKL/BDNF Pathway

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- Albiflorin
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- VaD

Abstract

Objectives: To assess the possible neuroprotective impacts of albiflorin (ALB) on VaD induce methionine (L-MET) and elucidate the potential underlying mechanisms responsible for these **Background:** Vascular dementia (VaD) has the second highest prevalence rate after Alzheimer's Inflammation, endothelial dysfunction, and oxidative stress are critical factors in the pathogenesis diseases. **Methods:** A total of 30 male albino rats were separated into three groups, each involving Control, VaD, and VaD+ALB groups. Following the trial, the researchers assessed the rats'c function and measured many factors, including homocysteine (Hcy), superoxide dismutase (SOI profile (triglyceride levels and total cholesterol), IL-10, malondialdehyde (MDA), and Furthermore, the levels of forkhead box protein O1 (FoxO1), brain-derived neurotropic factor (receptor activator of nuclear factor kappa B ligand (RANKL), and SIRT1 were evaluated using r PCR. The histological and immunohistochemical research of the thoracic aorta and left cerebral hen involved examining eNOS, GFAB, and Bax immunoreactivity. **Results:**The study revealed that administration led to the impairment of cognitive performance, a significant increase in Hcy, MDA, and lipid profile, and a decrease in SOD and IL-10. Moreover, it resulted in downregulating hipp FoxO1, SIRT1, BDNF, and eNOS immunoreaction, along with the upregulation of the RANK expression and GFAB and Bax immunoreaction. ALB administration reversed the changes induce MET. **Conclusion:** ALB ameliorated cognitive impairment and endothelial dysfunction in VaD antioxidant, anti-inflammatory, and antiapoptotic effects and improved neurogenesis and gli modulating the FoxO1/SIRT1/RANKL/BDNF signaling pathway.

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Introduction

VaD is the second most common kind of dementia, frequently manifesting after the onset of Alzheimer's disease. It places significant burden on healthcare systems worldwide**[1]**. VaD is responsible for roughly 15%–20% of dementia cases in North America and Europe, as well as around 30% in Asia and emerging countries **[2]**. It is related to multiple risk factors, such as cardiovascular disease, dyslipidemia, smoking, diabetes mellitus, and hyperhomocysteinemia (HHcy) **[3]**.

The main clinical features of VaD are cognitive dysfunction and impairment of memory and executive function **[4]**. Dementia and cerebrovascular illness contribute to each other's development **[5]**. Hypoxia and ischemia, brought on by reduced cerebral blood flow, lead to cell death in the brain tissue **[6]**.

L-Methionine (L-MET) is a precursor of homocysteine, a neurotoxic sulfur amino acid intermediate in the methylation. Some neurological and mental illnesses are related to problems with methionine metabolism **[7]**.

Increased oxidative stress in brain tissue and inhibition of nitric oxide (NO) due to the buildup of NO synthase inhibitors are two ways in which HHcy contributes to neurological dysfunction **[8]**. Another critical function of NO produced by endothelial nitric oxide synthase (eNOS) is preserving vascular tone **[9]**.

Essential components of the pathogenesis of vasodilation involve oxidative stress and endothelial dysfunction. Moreover, the brain tissue is particularly susceptible to oxidative stress because it contains a large amount of polyunsaturated fatty acids, requires a high amount of oxygen, and has significantly lower levels of antioxidants **[10]**.

An observed increase in inflammatory cytokine levels in the brains of VaD patients so that inflammation might contribute to VaD**[11]**.

Limiting VaD progression markedly is challenging. Therefore, FoxO1/SIRT1/RANKL/BDNF gene pathways could be a promising approach to combating cognitive loss. Alterations of these genes can lead to neuronal cell pathology; trials with new treatment regimens in neurodegenerative disorders can delay the onset in addition to the progression of dementia.

The mammalian FoxO1 gene is associated with a big group of forkhead transcription factors regulating different physiological processes, and their genetic deletion affect behaviors **[12]**.

In addition, SIRT1 is a deacetylase crucial for aging, cell life span, and antioxidant defense. It influences the mitochondrial electron transport cycle, enhancing aging cells' oxidative resistance. It requires the FoxO1 gene for its activation **[13]**.

One of the most well-studied transcription factors is receptor activator of nuclear factor kappa-B ligand (RANKL). Numerous genes, including FOXO1, SIIRT1, and BDNF, are regulated by its expression and ubiquitously expressed **[14]**.

Learning, neurogenesis, and synaptic plasticity are all impacted by BDNF. Also, BDNF controls neuronal survival and affects cognitive functions**[15]**.

ALB, a monoterpenoid glycoside, is one of the active substrates of *Paeonialactiflora*, a widely used Chinese medication. It has antidepressant, anticonvulsant, analgesic, anti-inflammatory, and antiapoptotic effects **[16]**. Furthermore, previous clinical studies revealed that ALB may have anti-

inflammatory and antioxidant effects in neurocognitive disorders and can modulate BDNF expression **[17]**.

As far as we know, no one has investigated the possible involvement of ALB in L-MET-induced VaD. The aim of this study was to assess the possible neuroprotective impacts of albiflorin (ALB) on VaD induced by L-methionine (L-MET) and elucidate the potential underlying mechanisms responsible for these effects.

Methods

The research was conducted according to the Guiding Principles in the Use and Care of Animals, as outlined in the National Institutes of Health (NIH) Publication No. 85–23, Revised 1996. The Ethics Committee of the Faculty of Medicine at Menoufia University approved the care and use of animals. The Institutional Review Board number is 6/2024 BIO 4.

Animals

A total of 30 male wistar rats were acquired from a nearby animal procurement facility. The rats were given 10 days to acclimate before the study commenced. Rats were provided unrestricted access to a standard chow meal and water in a temperature-controlled chamber with alternating 12-hour periods of light and darkness. After completion of the experiment, the rats were euthanized by cervical dislocation.

Experimental design

Following acclimatization, 10 rats were randomly assigned to one of the three groups described as follows:

Group I (Control): The rats were given 2mL of 0.9% normal saline oral gavage once daily for 32 days.

Group II (L-MET-induced VaD group) [VaD]: L-MET, obtained from Sigma in the form of powder, was dissolved in distilled water and administered orally at a dosage of 1700 mg/kg once a day for a duration of 32 days to develop VaD **[18]**.

Group III) [VaD + ALB]: The 32-day course of L-MET administration began with the oral administration of 20 mg/kg of ALB (Chengdu HerbpurifyCo., Ltd.; purity: >98%; molecular weight: 480.46 g/mol), which was produced daily in 2 mL of 0.9% normal saline **[19]**.

Over the course of the experiment's last five days, the animals' cognitive function was evaluated. Following the behavioral tests, the rats were anesthetized (intraperitoneal injection of 60 mg/kg phenobarbital) and sacrificed by cervical dislocation. Blood samples were taken from the retroorbital venous plexus for a biochemical analysis. The cerebral hemispheres and thoracic aorta were dissected to evaluate neurodegenerative alterations and vascular affection, respectively. After being dissected, the right hippocampi were quickly frozen at -80°C to facilitate additional biochemical research. The left cerebral hemispheres underwent processing to study the hippocampal histology and immunohistochemistry (CA1 area).

Cognitive Performance Assessment

Novel Object Recognition: In this experiment, we tested the rats' ability to recognize an unfamiliar object within a controlled environment. Every rat

underwent a three-day testing process, including habituation, training, and testing. During the habituation phase, rats were given 10 minutes to adjust to their environment in an open-field apparatus of 50 cm × 50 cm × 40 cm. For the first five minutes of training, we kept each rat in a room with two identical items. As part of the testing phase, after the 24-hour interval, each rat was kept in the chamber for five minutes following the object exchange. We utilized the stopwatch. The discrimination index = [(novel object exploration time–familial object exploration time)/total exploration time X 100%]. Alcohol was used to disinfect the open field **[20]**.

Elevated Plus Maze (EPM) Test: As previously described by **[21]**, a plus-sign apparatus was used to assess the rats' anxiety-like behavior. Individual rats were placed in the middle of the gadget and allowed to explore the maze for 10 min. A surveillance camera positioned above was employed to monitor the locomotion of the animals. The duration of time spent within the expansive boundaries of the maze was meticulously documented. The duration of time was inversely correlated with the level of anxiety-like behavior.

Morris Water Maze (MWM) Test: This test aimed to evaluate the rats' memory and spatial learning abilities over the last five days of study. An equal number of portions were cut out of the circular pool at MWM. During the initial four days, a platform was placed in one of the corners, one centimeter below the water's surface. Every day of the acquisition session, consisting of three trials each, each rat was randomly given one of the three spots in the pool. The experiment termination and determination of the mean escape latency occurred when the rat successfully navigated to the platform. The maximum time allowed for the test was sixty seconds. We gently positioned the rat on the platform and set the timer for 60 seconds. If it didn`t climb up within that time, we removed it. On the fifth day, a "probe trial" was done to see how well the rats could remember the spot of the hidden platform within a minute. In this time, the platform was extracted from the pool **[22]**.

Blood Sampling and Biochemical Analysis: After the experiment, the animals were not given food for the night and blood samples were taken. After 30 min at room temperature, clotting of the samples was permitted. The next step was centrifugation at a speed of 2,000 revolutions per minute for 15 min. Before analysis, the serum was extracted and stored at a temperature of -80°C,adhering to the directions provided by the manufacturer; serum Hcy levels were determined using rat ELISA kits (Hcy: 201-11-1646, Shanghai Sunred Biological Technology Co., Ltd, China). Colorimetric kits were employed to assess serum lipids, such as total cholesterol and triglycerides (Biodiagnostic Company, Dokki, Giza, Egypt).

Tissue Homogenate Preparation: The samples of the weighted hippocampus were ground into a homogeneous paste at 4,000 rpm for 15 min using a tissue homogenizer (MPW120, MPW Medical Instruments, China). The resulting liquid was stored at a temperature of -80°C until the MDA and SOD levels could be measured. This was done using a standard colorimetric assay from Bio Assay Systems (USA) and the hippocampal tumor necrosis factor-alpha (TNF-α) as well as IL-10 assay kits from Quantikine (Abcam, UK),

following the instructions given by the manufacturers.

Analysis of Gene Expression Quantitative RT-PCR

We used a real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) technique to review the hippocampal mRNA expression levels of the regulatory genes FoxO1, SIRT1, RANKL, and BDNF. TRI reagent (Sigma-Aldrich, UK) was used to extract total RNA. The next step was reverse transcribing the hippocampus RNA using a high-capacity RNA-to-cDNA kit (Applied Biosystems, California, USA). Next, the cDNA produced was used to evaluate the mRNA expression of the chosen genes. Primer Express Software 2.0, created by Applied Biosystems, was used to synthesize the gene-specific primers. Furthermore, GAPDH, a housekeeping control gene, was employed **(Table 1) [23]**.

RT- PCR assays were performed in duplicate for all target and housekeeping genes were performed on Applied Biosystems 7500 FAST 96-well PCR machines (USA). The determination of the relative mRNA expression of the target gene was conducted by employing the comparative Ct ($2^{-\Delta\Delta Ct}$) approach, with GAPDH serving as the endogenous control. The information was supplied in the form of a ratio between the target gene and GAPDH. This ratio represented the average value plus or minus the standard deviation of the mean, which was derived from a minimum of three consecutive comparisons.

Table (1): Primers were utilized to determine the expressions of RANKL, SIRT1, FoxO1, BDNF, and GAPDH genes [24].

Gene	Primer sequence
FoxO1	Forward CACCTTGCTATTCGTTTGC Reverse CTGTCCTGAAGTGTCTGC
SIRT1	Forward AGA AACAATTCCTCCACCTGA Reverse GCTTTGGTGGTTCTGAAAGG.
RANKL	Forward GACAGGCACGGACT CGTA Reverse CGCTCATGCTAGTC GTCTA.
BDNF	Forward GCTGCCTTGATGTTTACTTTG. Reverse ATGGGATTACACTTGGTCTCGT.
GAPDH	Forward TGCACCACCAACTGCTTAGC. Reverse GGCATGGACTGTGGTCATGAG.

Histological and Immunohistochemical Methods:

Thoracic aorta and cerebral hemisphere tissues were prepared for light microscopy after being fixed in ten percent neutral formaldehyde.

- For routine histological investigation, H&E staining was applied to paraffin slices that were 5 µm thick. After being rinsed with PBS, 5 µm slices deparaffinized and rehydrated were placed in a 3% H_2O_2 solution to decrease endogenous peroxidase

activity. This was done for the immunohistochemistry procedure. Following a PBS rinse, the microwave antigen retrieval procedure was used.

-For measurement of endothelial dysfunction, an antibody called eNOS [1:1000, mouse monoclonal, Abcam ab76198] was applied as the primary antibody for the thoracic aorta sections.

- For measurement of hippocampal degeneration, primary antibodies were used to incubate the brain sections with specific proteins. These proteins consisted of GFAP [1:300, mouse monoclonal, Lab vision MS-1376-R7], Bax [1:200, rabbit monoclonal, Abcam ab16667], and more. The slices were incubated at room temperature for one hour after being coated with various primary antibodies. After washing with PBS, sections were incubated with a secondary biotinylated antibody for twenty minutes. After a 10min PBS wash, slices were treated with "Streptavidin Horseradish peroxidase" enzyme conjugate. The binding of the secondary antibody could be seen with the help of 3,3-diaminobenzoic acid (DAB).Specific proteins were incubated with brain slices using primary antibodies. GFAP and Bax were among the proteins that made up this set. The slices were incubated at room temperature for one hour after being coated with various primary antibodies. Sections were incubated with a secondary biotinylated antibody for 20 min after being washed with PBS. An enzyme conjugate solution called "Streptavidin Horseradish peroxidase" was applied to the sections after a 10 min wash in PBS. Utilizing 3,3-diaminobenzoic acid (DAB), the secondary antibody's binding could be observed. After giving the sections a final PBS wash, two drops of hematoxylin were added to the slides as a counterstain.

Morphometric Study

IMT was measured by light microscopic inspection of the Hx&E stain photographs ×400. Four measurements of IMT per segment were taken at 0°, 90°, 180°, and 270°. To get the value corresponding to the single segment, the acquired measurements were averaged. In the end, the average of the three evaluated aortic sections was applied to estimate the IMT for each animal **[18]**.

The percentage of endothelial cells that exhibited positive results for eNOS was determined by conducting a light microscopic analysis of the immune-stained images at a magnification of ×400. We employed an image analyzer (Leica Q 500 MC software, Wetzlar, Germany) in the anatomy department of the College of Medicine at Menoufia University to quantify all of the measurements taken across five different slides for each group. Every single slide included five different fields.

Three non overlapping fields per slice were used to record the percentage of deteriorated neurons from H&E-stained sections, calculated as the number of degenerated cells/total cells × 100.

We evaluated the percentage of the area of GFAP immunoreaction and the percentage of Bax immunopositive cells in three non overlapping fields/sections for the immunohistochemistry quantitative evaluation.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software, version 23, was utilized to tabulate and analyze the data. The Shapiro–Wilk test was used to perform a normal distribution analysis of the data sets. The quantitative data expression was the mean plus or minus the standard deviation.

Following the completion of a one-way analysis of variance (ANOVA), we utilized a post hoc Tukey`s test to determine whether the distinctions between the groups were statistically significant. A P value below or equal to 0.05 is considered statistically significant. Microsoft Excel was used to create graphs that showed all the results.

Results

In VaD group, there were significant increase in serum homocysteine, hippocampal MDA, hippocampal TNF-α, serum cholesterol, and serum triglyceride with concomitant decrease in hippocampal SOD, IL10 when compared with the control group **(Table 2).**

In the VaD + ALB group, there were significant decrease in serum homocysteine, hippocampal MDA, hippocampal TNF-α, serum cholesterol, and serum triglyceride with concomitant increase in hippocampal SOD, IL10 when compared with the VaD group but still significantly differ than the corresponding values in the control group **(Table 2).**

Table (2): The measured Serum homocysteine, oxidative stress markers (Hippocampal MDA &SOD), inflammatory markers (Hippocampal TNF-α&IL10), and lipid profile (Serum cholesterol&triglycerides) in the studied groups.∗P < 0:05 versus control group; # P < 0:05 versus VaD group.

	Control group	**VaD group**	**VaD+ALB group**
Serum homocyteine (nmol/ml)	4.6±0.37	18.3±0.79*	13.3±0.55$^{*\#}$
Hippocampal MDA (nmol/mgprotein)	42.2 ±1.72	77.5± 1.87*	56.8± 1.94$^{*\#}$
Hippocampal SOD (U/mg protein)	31.7±1.5	10.8±1.1*	22±0.69$^{*\#}$
Hippocampal TNF-α (pg/mg protein)	16.9±0.96	75.1±1.5*	42.6±1.4$^{*\#}$
Hippocampal IL10 (pg/mg protein)	166.3±2.1	82.5±1.6*	123.1±1.6$^{*\#}$
Serum cholesterol (mg/dl)	87.3±4.6	139±2.6*	103.3±1.9$^{*\#}$
Serum triglycerides (mg/dl)	49.3±3.3	93.3±1.1*	76.1±1.3$^{*\#}$

The mean values of BDNF, FoxO1, and sirtuin 1 (SIRT1) gene expressions were considerably lower in the VaD group (0.36±0.04; 0.49±0.01; 0.58±0.02, correspondingly) than those in the

control group. The results were considerably higher in the VaD + ALB group than in the VaD group (0.63 ± 0.03; 0.8 ± 0.01; 0.88 ± 0.01, respectively), but they were still lower than the control group. Within the VaD group, the RANKL gene's expression was considerably higher (3.4±0.05 versus 1) than that inthe control group. Even though the VaD + ALB group had a significant decrease compared to the VaD group (2±0.07), it continued to be much greater than the control group **(Fig. 1)**.

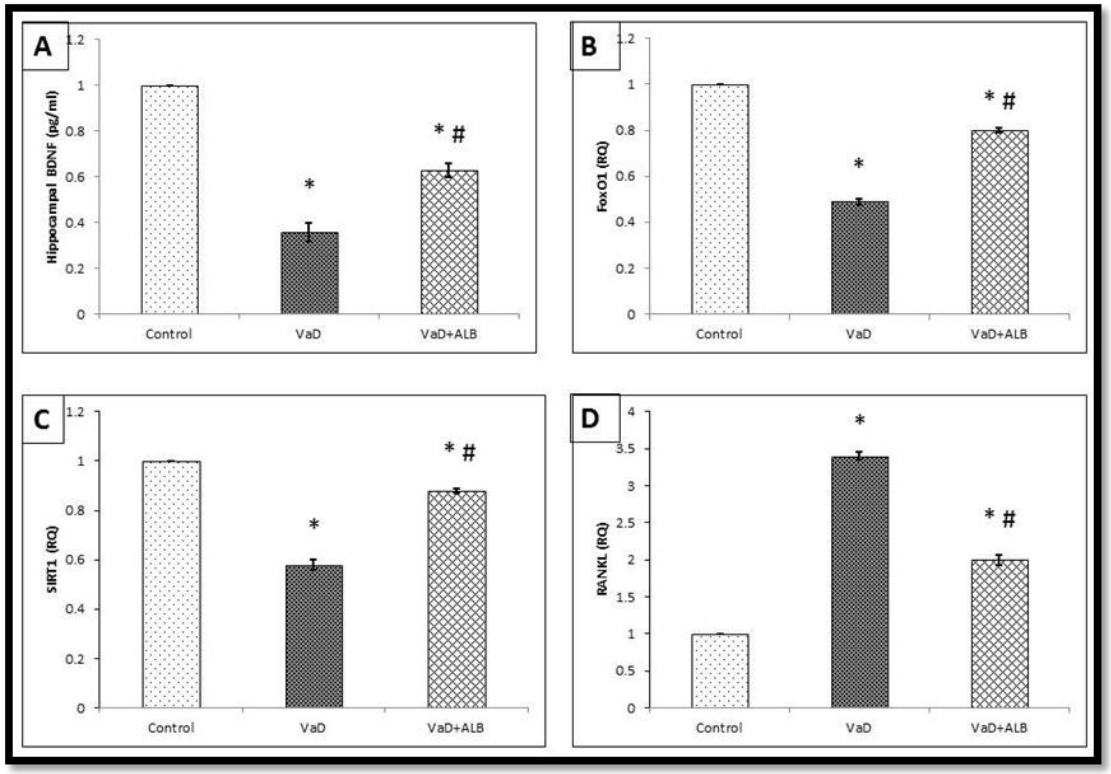

Figure 1: The impact of Albiflorin on the expression of BDNF, FoxO1, SIRT1, and RANKL genes in VaD induced by L-MET. Each group, consisting of 10 rats, is represented by the mean value ±the standard deviation. (A)The hippocampus BDNF.(B)The FoxO1 protein in the hippocampus.(C)The SIRT1 protein in the hippocampus.(D) The hippocampal RANKL.∗P < 0:05 versus control group; # P < 0:05 versus VaD group**.**

The VaD group had a significantly decreased mean discrimination index than the control group (41.8±2.3 vs. 56.3±2.1, respectively). The value in the VaD+ALB group was 28.2 ± 2.5, substantially more significant than in the VaD group but still significantly less than in the control group **(Fig.2A)**.

The control group's rats spent 114±2.6 minutes in the EPM test, while the VaD group's rats spent 51.8±2.3 minutes, which is a significant variance.

Although it was much lower in the control group, the VaD+ALB group had an even higher value of 83.2 ± 1.5 than that in the VaD group **(Fig.2B)**.

In day 1, there was insignificant difference in mean escape latency time in MWM test between the studied groups. In days 2,3,4,5, there was significant increase in mean escape latency time in VaD group compared to control group. Rats in the VaD+ALB group showed significant decrease in mean escape latency time than those in VaD group,

however, it was still significantly higher than that in the control group **(Fig.2C)**.

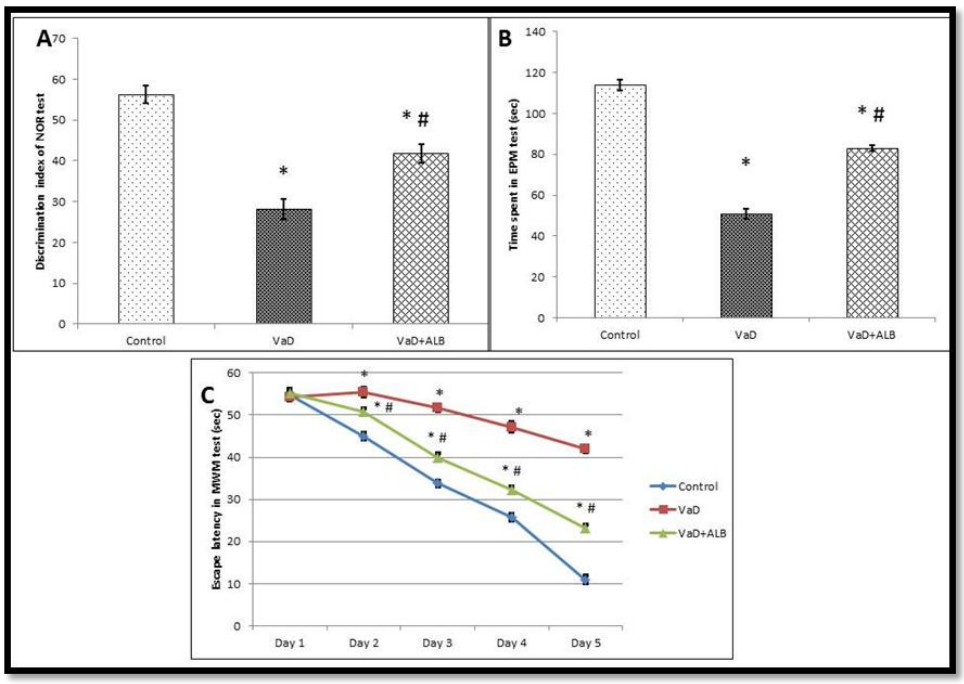

Figure 2: Effect of Albiflorin on cognitive functions in L-MET induced VaD.(A) Discrimination time in NOR test. (B) Time spent in EPM test. (C) Escape latency in MWM test. ∗P < 0:05 versus control group; # P < 0:05 versus VaD group**.**

Histological examination of the H&E–stained sections showed the normal structure of tunica intima, media, and adventitia in the control group.VaD group showed endothelial denuding, numerous blood cells, vacuolation of some smooth muscle cells, and deposition of perivascular adipose tissue and inflammatory infiltrates in tunica adventitia. The VaD + ALB group showed nearly normal aortic structure except for vacuolation of some smooth muscle cells. Statistically, there was a significant increase in the aortic IMT in the VaD group compared with the control group (63.4±2.7 vs 35.8±1.9, p<0.05). The aortic IMT was significantly lowered in the VaD + ALB group compared with the VaD group (43.8±2.3 vsVaD, p<0.05) but still significantly higher than the control group. Immunohistochemical assessment of the endothelial dysfunction revealed a significant decrease in the percentage of eNOS positive cells in the VaD group compared with the control group (51±2.3 vs 88.2±0.8, p<0.05). VaD + ALB group showed significant upregulation in eNOS immunoreaction when compared with the VaD group (64±2.5 vs VaD, p<0.05), but still significantly lower than that of the control group **(Fig. 3).**

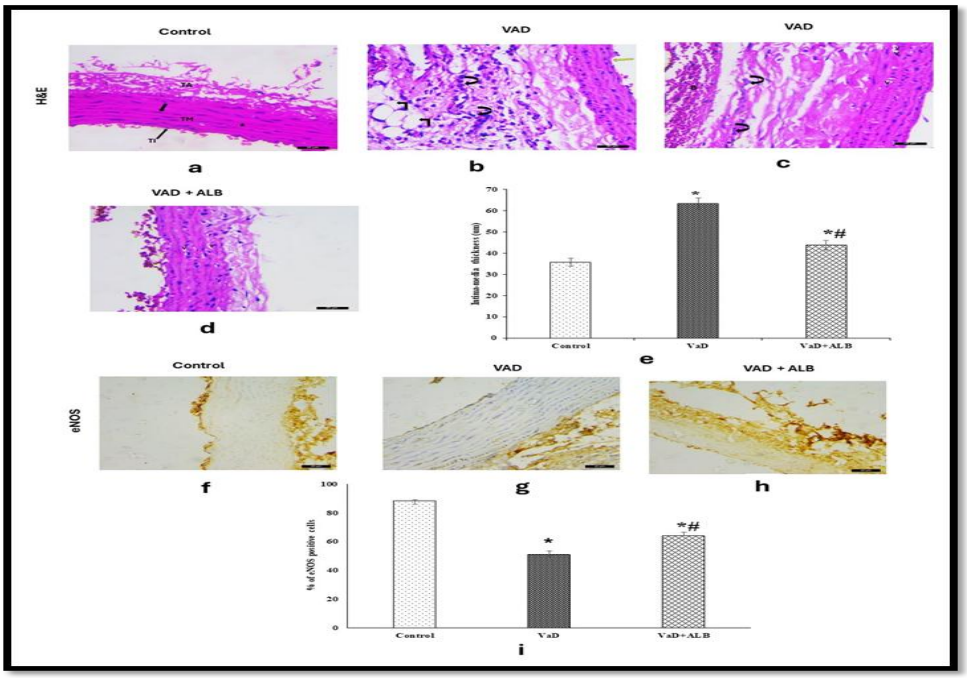

Figure 3: Representative photomicrographs of H&E and eNOS-stained aortic stained aortic of the different groups (×400, scale bar = 20 μm) and measurement of the intima media thickness and % of eNOS positive cells. H&E stain: (a) Control group: intact tunica intima (TI), normal endothelial lining (black arrow), regular arrangement of elastic lamina (asterisk), and normal smooth muscle cells (notched arrow) in the tunica media (TM), tunica adventitia (TA). (b, c): VaD group: endothelial denudation (yellow arrow), accumulated blood cells (B), vacuolation of smooth muscle cells (V), perivascular deposition of adipose tissue (bent arrows), and inflammatory cell infiltration within the tunica adventitia (arched arrows). (d): VaD + ALB group: vacuolation of some smooth muscle cells (V). (e) Intima media thickness. eNOS-stain: (f) Control group, (g) VaD group, (h) VaD + ALB group. VaD group showing downregulation of eNOS immunoreaction compared to the control group and upregulation of the immunreaction in the VaD + ALB group compared to the VaD group. (i) Percentage of the eNOS endothelial positive cells. Each group (n = 10) represents as mean ± SD. ∗P < 0:05 versus control group; # P < 0:05 versus VaD group (VaD: vascular dementia; VAD + ALB: vascular dementia +Albiflorin; H&E: haematoxylin and eosin).

The H&E-stained sections of the control group showed the normal structure of the hippocampus formed of molecular, pyramidal, and polymorphic layers. Pyramidal cell layer contains pyramidal cells with large rounded vesicular nuclei. The VaD group showed many dispersed neurons in the pyramidal layer with deeply stained pyknotic nuclei and perineuralvacuolation. Dilated blood vessels were also noticed. VaD + ALB group revealed nearly normal structure of hippocampus CA1 region, except for slight neurodegeneration in the form of vacuolation and few deeply stained pyknotic nuclei in the pyramidal layer. Statistically, the VaD group showed a highly significant increase in the percentage of degenerated neurons compared with the control group (26.4±2.8 vs 3.4±0.5, $p<0.05$).The percentage of the degenerated neurons was significantly decreased in the VaD + ALB group compared with the VaD group (9.4±0.5 vs VaD, $p<0.05$) but still higher than the control group **(Fig. 4).**

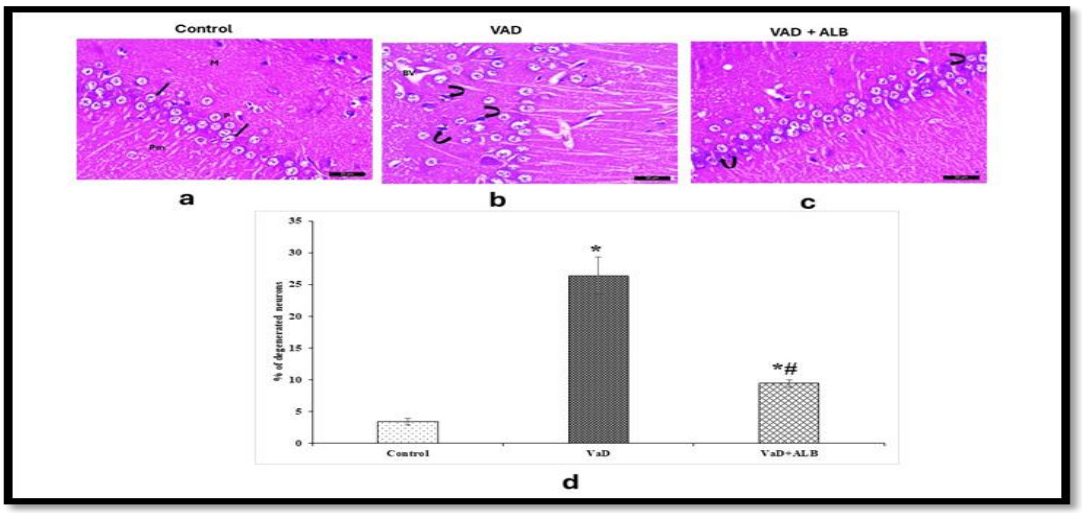

Figure 4: Representative photomicrographs of H&E-stained hippocampal CA1 sections of the different groups (×400, scale bar = 20 µm) and % of degenerated neurons. (a) Control group: molecular (M), pyramidal (P), and polymorphic (Pm) layers. Pyramidal cells are compactly arranged and have large vesicular nuclei (arrows) are noted in the pyramidal layer. pyramidal cells contain large rounded vesicular nuclei. (b) VaD group: dispersed neurons in the pyramidal layer with deeply stained pyknotic nuclei (arched arrows), perineuralvacuolation (V) and dilated blood vessels (BV). (c) VaD + ALB group: few deeply stained pyknotic nuclei (arched arrow). (d) Percentage of degenerated neurons in the hippocampal CA1 region. Each group (n = 10) represents as mean ± SD. ∗P < 0:05 versus control group; # P < 0:05 versus VaD group (VaD: vascular dementia; VaD + ALB: vascular dementia +Albiflorin).

Immunohistochemical Assessment of GFAP and Bax Immunoreaction: There was a significant increase in the percentage of the GFAP (26.2±2.5 vs 4.8±0.8, p<0.05) and Bax (78.8±1.3 vs 4.8±2.1, p<0.05) immunoreaction of the VaD group compared with the control group. On the other hand, the VaD + ALB group showed a significant decrease in GFAP (11±1 vsVaD, p<0.05) and Bax immunoreaction (40.2±1.4 vsVaD, p<0.05) compared with the VaD group but still higher than that of the control group **(Fig. 5).**

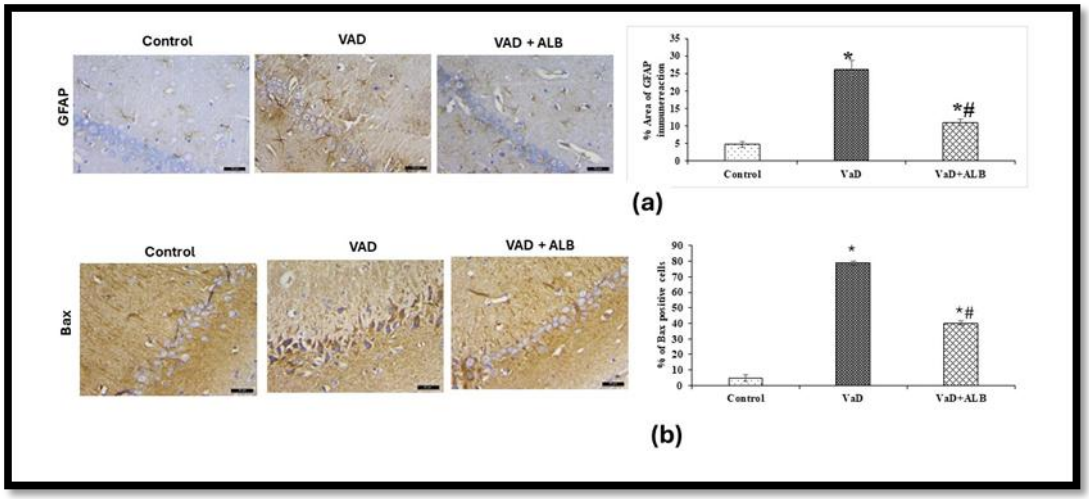

Figure 5: Representative photomicrographs of the hippocampal CA1 region immunostaining of the different groups (×400, scale bar = 20 µm) and quantitative analysis of positive immunoreaction. (a) GFAP, (b): Bax. VaD group showing upregulation of GFAP and Bax immunoreaction compared to the control group. Downregulation of GFAP and Bax are noted in VaD + ALB group compared to VaD. Each group (n = 10) represents as mean ± SD. ∗P < 0:05 versus control group; # P < 0:05 versus VaD group (VaD: vascular dementia; VaD + ALB: vascular dementia +Albiflorin; GFAP: glial fibrillary acidic protein).

Discussion

VaD is a progressive cerebrovascular disease that worsens over time causing a continuous and irreversible deterioration in the quality of life **[25]**. Memory loss, anxiety problems, and endothelial dysfunction were observed in the VaD group after supplementation with L-MET, along with a notable rise in serum Hcy levels, which is in line with Moretti et al.'s results **[26]**. There is evidence that increased ROS and structural and functional alterations in the cerebral vasculature are associated with elevated Hcy**[27]**. Supplementing the VaD group with ALB significantly improved cognitive impairments and a marked reduction in serum Hcy levels. This is due to its ability to modulate inflammation and oxidative stress, which agrees with previous research **[19]**. VaD group showed memory, cognitive impairment, and less interest in exploring new objects on the NOR test. This agrees with research by Li et al. **[28]**, who reported that L-MET enhanced brain inflammation and impaired neurogenesis. Moreover, Alachkar et al. **[29]** demonstrated that HHcy-induced neuroinflammation and neurogenesis had a role in mediating cognitive deficits. Furthermore, during the EPM test, the VaD group spent less time in the open arms than the control group. Animals exhibited less anxiety-inducing behavior the longer they lingered in the open arms. Our outcomes match those of Koladiya et al. **[30]**, who also noted comparable phenomena. Consequently, the anxiety markers in the EPM test showed a considerable increase when administered L-MET. In this work, the MWM test was used to identify the hippocampal function involved in spatial learning and memory **[8]**. According to the data, the VaD group performed substantially lower on the MWM test than the control group, suggesting a significant decline in memory acquisition and retrieval abilities **[31].**

ALB supplementation to VaD revealed significant improvement of cognitive deficits proved by ameliorative RI in the NOR test, with better performance in EPM and MWM tests in the VaD group, indicating its role in improving cognitive deficit and anxiety induced by HHcy.The findings agreed with previous studies by Xu et al. **[19]**, who stated that ALB had a role in managing obesity, memory deficit, cognitive function, and inflammation. Moreover, ALB was found to enhance cognitive function and neurodegenerative changes associated with dementia and depression by elevation of hypothalamic monoamine levels **[32]**.One potential new medicine that could protect against VaD is ALB.

Fayez et al.**[10]** confirmed the link between HHcy and oxidative stress. In the VaD group, we found a marked rise in MDA levels, a marker of lipid peroxidation, and a drop in SOD concentration compared to the control group. This agreed with the findings of Wang et al. **[33]** and Dragomanova et al. **[34]**, who also found that oxidative stress and the severity of neurodegenerative alterations were linked triggered by an excess of ROS in the VaD group's hippocampi.

ALB treated group showed reduced oxidative stress markers, as evidenced by a significant decline in MDA levels and a rise in SOD activity. These findings align with those of earlier research **[16]**.The free radical scavenging and oxidative stress reduction capabilities of ALB, as well as its capacity to upregulate the Nrf2/HO1 system with antioxidant effects by regulating antioxidant gene expression **[35]**. Restoring mitochondrial

membrane function and reducing neuronal oxidative stress were both observed by Zhou et al. **[36]** in ALB-treated rats.

The VaD group exhibited a significant rise in the production of proinflammatory cytokines TNF-α in the hippocampus and a significant decrease in interleukin 10 (IL-10) compared with the control group. This coincides with the results of Fayez et al. **[10]**. Exacerbated Inflammation was often linked to learning, memory, and cognition impairment in VaD.

In contrast, treatment with ALB significantly decreased the hippocampal TNF-α content and significantly increased the IL-10 level due to its anti-inflammatory and free radical scavenger effects, which coincides with Chen et al. **[16]**.

ALB inhibits neuroinflammation, fibrosis, and apoptosis by targeting many intracellular mitochondrial signaling cascades, as demonstrated by Jiao et al. **[37]**.Furthermore, ALB targets toll-like receptor-mediated signaling in brain tissue and immune cells, particularly dendritic cells (DC), and macrophages. Moreover, ALB could reduce TNF-α by activating the PI3K/Akt anti-inflammatory pathway and influencing NF-κB signaling **[38]**.

Most cognitive impairments observed in the VaD group were thought to be caused by vasculopathy, which is associated with impending neurodegenerative alterations that disrupt CA1 hippocampal neurons and malfunction of endothelial cells **[39]**. Significant increases in triglyceride levels and total cholesterol in the VaD group corroborated the morphological and immunohistochemical alterations in the thoracic aorta and hippocampal CA1 of the VaD group, proving HHcy-induced vascular dysfunction in the present investigation, which was first demonstrated by Zarrouk et al. **[40]**. HHcy may enhance cholesterol production and lead to lipid peroxidation by blocking N-methyl-D-aspartate (NMDA) receptors **[41].** Besides this, there were concomitant histopathological alterations of the aorta, which agreed with a previous study by Tan et al. **[42]**. Aortic histopathological findings showed significant vascular endothelial dysfunction and distorted elastic lamellae reflecting atherosclerotic changes, which was postulated by Othman et al. **[43].**In the current experiments, the VaD group was associated with significantly decreased aortic eNOS by immunohistochemical staining, resulting in vasoconstriction, which agrees with Luzzi et al.'s results **[31]**.

A deficiency in eNOS leads to endothelial dysfunction, resulting in significant brain damage and cognitive impairments **[44]**. One of the risk factors for cerebral vasculopathy is HHcy. By targeting endothelial cells, HHcy reduces eNOS activity and cell growth **[45]**.

ALB in this study significantly ameliorates dyslipidemia and atherosclerosis in accordance with Chen et al.**[46]**. ALB has a beneficial effect on lipid metabolism through its impact on the GALNT2-ANGPTL3-LPL pathway **[47]**. VaD + ALB revealed upregulation in eNOS immunoreaction in the aorta, which contrasted with the VaD group, improving endothelial dysfunction. This was in line with Li et al. **[48]**.

Pathology in the CA1 region in the VaD group corroborated our findings and mirrored the presence of transitory ischemia events followed by neurodegenerative alterations, as explained by other research **[49]**. Moreover, the VaD group showed neurodegenerative alterations, such as an increase in the percentage of deteriorated neurons

and the appearance of strongly pigmented pyknotic nuclei with perinuclearvacuolation. This is in line with what Khodir et al. **[18]** found. In this study, the considerable overexpression in the Bax immunoreaction revealed that enhanced cellular death might be the cause, which agrees with the outcomes of Xu et al. **[19]**. The presence of oxidative stress was linked to apoptosis, according to Kizilay et al. **[50]**. Compared to the VaD group, ALB supplementation decreased the number of deteriorated neurons i.eantiapoptotic activity, as corroborated by the significant downregulation of Bax. ALB alleviated cerebral ischemic reperfusion injury by enhancing neuronal cell viability and attenuating apoptosis **[51]**.Moreover, Xu et al.**[19]**declared that ALB suppressed the mitochondrial pathway of programmed cell death by reducing the amounts of Bax protein in the hippocampus.

Astrocyte marker, GFAP, and immunoreaction were significantly upregulated in the VaD group's hippocampus, suggesting that gliosis plays a role in the pathophysiology of VaD. Previous results have shown this to be true **[52]**. Glial cell sensitization produced by HHcy has been discovered. In contrast to the VaD group, however, the VaD + ALB group outcomes showed a substantial decrease in GFAP immunoreactivity. These results are consistent with what Zhou et al. **[53]** had previously reported. This could be because of ALB's anti-inflammatory and antioxidant properties.

Limiting VaD progression markedly is challenging; however, the FoxO1/SIRT1/RANKL/BDNF signaling pathway offers an exciting strategy to address cognitive impairment. Our research showed that modifying the expressions of these genes using ALB can lead to improvements in neuronal cell pathology.

In this experiment, the VaD group significantly decreased FoxO1/SIRT1/BDNF gene expression in hippocampal tissue with a significantly increased RANKL gene. This pathway disruption is associated with brain damage, cognitive deficit, and cerebrovascular changes related to VaD. This was revealed in previous studies by Zang et al. **[54]**.

Multiple studies showed that FoxO1 transcription factors stimulated the expression of genes related to oxidative stress tolerance, cell cycle arrest, cell survival, and cell differentiation and FoxO1-null mice dying from impaired vasculogenesis **[18]**.

In the adult brain, synaptic plasticity, learning, and neurogenesis are all areas where BDNF plays a significant role. Moreover, BDNF was discovered to control the survival of neurons and impact mental operations **[15]**. Evidence suggests that BDNF level might affect FoxO1 activity **[17]**.

SIRT1 is a histone deacetylase dependent on NAD+ with several metabolic and stress-tolerance features **[55]**. In addition to regulating the circadian rhythm, SIRT1 influences inflammation, cellular glucose tolerance, and memory decline. Furthermore, mitochondrial NAD+ pools are associated with oxidative stress, and SIRT1 can control their production **[13].** By influencing neuronal survival, synaptic plasticity, cognitive function, and neurogenesis, SIRT1 is recognized to influence the fight against neurodegenerative illnesses positively. A decrease in NAD^+-dependent SIRT1 activity disrupts mitochondrial homeostasis, leading to oxidative stress, which could further lead to neurological diseases **[56]**.

SIRT1 overexpression was related to an increase in the nuclear level of the FOXO1 protein.By bringing

FOXO1 into the nucleus and activating CAT and SOD, SIRT1 may protect against oxidative damage **[57]**. Moreover, molecular research demonstrated that the SIRT1/RANKL signaling pathway can suppress the development of amyloid β plaques when SIRT1 is activated **[58]**. Stapledon et al. **[59]** reported that RANKL is a well-known transcription factor, a bone turnover marker that strongly correlates with CNS genes related to dementia.

Our study findings may offer fresh perspectives on the FOXO/SIRT1/BDNF signaling pathway, which controls the expression of several genes **[14]**. Many of these genes produce proteins that are crucial in the mechanisms of neuroinflammation and oxidative stress, leading to cognitive impairments **[60]**.SIRT1 adds acetyl groups to ADP-ribose molecules by transferring them from substrates like NF-kB, FOXO1, and PARP1 with the help of NAD+, an enzyme cofactor **[61]**. Additionally, SIRT1 has a neuroprotective function and therapeutic promise in neurodegenerative disorders in animal and neuronal culture investigations **[62]**.

Our study found that ALB could significantly improve cognitive behavior in rats, which agrees with a previous study **[63]**. That may be achieved by regulating the FoxO1/SIRT1/RANKL/BDNF signaling pathway in the hippocampus, Moreover, ALB promotes the phosphorylation of membrane receptors, which is attributed to the increased expression of BDNF in the hippocampus region, alleviating plaque deposition and cognitive impairment **[64]**. The antioxidant, anti-inflammatory, and neuronal autophagy-regulating characteristics of ALB may explain its function in neurogenesis.

To the best of our knowledge, this is the first study to investigate the effect of ALB on the FoxO1/SIRT1/RANKL/BDNF signaling pathway in the hippocampus. This new pathway offers promise in the search for drugs that can mitigate the progression of the VaD-associated cognitive deficit.

Conclusion

The current research found that ALB may alleviate L-MET-induced dementia in adult male rats. This consequence might be explained by ALB's capacity to limit the progression of oxidative stress, apoptosis, inflammation, gliosis and improve normal brain homeostasis by its effect on the expression of a novel studied FoxO1/SIRT1/RANKL/BDNF signaling pathway. The findings of this research support the application of ALB as a potential medicine in the treatment of dementia.

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